COBAFISH FINAL REPORT

ANNEX I

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List of species names and abbreviations used for stomach analyses.

Order	Family	Species	Abbreviation
OSTEOGLOSSIFORMES			
	Mormyridae		
		Cyphomyrus psittacus	Cyp psi
		Gnathonemus petersii	Gna pet
		Hippopotamyrus cf. psittacus	Hip cf psi
		Hippopotamyrus psittacus	Hip psi
		Hippopotamyrus weeksii	Hip wee
		Marcusenius greshoffi	Mar gres
		Marcusenius leopoldianus	Mar leo
		Marcusenius schilthuisiae	Mar schi
		Marcusenius cf. stanleyanus	Mar cf sta
		Mormyrops anguilloides	Mor ang
		Mormyrops mariae	Mor mar
		Mormyrops nigricans	Mor nig
		Petrocephalus arnegardi	Pet arn
		Petrocephalus binotatus	Pet bin
		Petrocephalus christyi	Pet chr
		Petrocephalus grandoculis	Pet gra
		Petrocephalus cf. micropthalmus	Pet cf mic
		Petrocephalus micropthalmus	Pet mic
		Pollimyrus isidori	Pol isi
		Pollimyrus nigripinnis	Pol nig
		Stomatorhinus sp.	Sto cf
	Notopteridae		
		Xenomystus nigri	Xen nig
	Pantodontidae		
		Pantodon buchholzi	Pan buch
CLUPEIFORMES			
	Clupeidae		
		Microthrissa royauxi	Mic roy

Orde	Familie	Soort	Afkorting
CHARACIFORMES			
	Alestidae		
		Alestopetersius brichardi	Al bri
		Alestopetersius caudalis	Al cau
		Alestopetersius cf. compressus	Al cf com
		Alestopetersius compressus	Al com
		Bathyaethiops caudomaculatus	Ba cau
		Brachypetersius altus	Bra al
		Brycinus imberi	Bry imb
		Brycinus prognathosi	Bry pro
		Bryconaethiops boulengeri	Bryco b
		Bryconaethiops macrops	Bryco ma
		Bryconaethiops microstoma	Bryco mi
		Hydrocynus vittatus	Hyd vit
		Micralestes acutidens	Mic acu
		Micralestes humilis	Mic hum
		Micralestes sardina	Mic sar
		Micralestes stormsi	Mic sto
		Phenacogrammus interruptus	Phe int
	Citharinidae		
		Citharinus gibbosus	Cit gib
	Distichodontidae		
		Distichodus affinis	Dis aff
		Distichodus antonii	Dis ant
		Distichodus fasciolatus	Dis fas
		Distichodus lusosso	Dis lus
		Distichodus sexfasciatus	Dis sex
		Distichodus teugelsi	Dis teu
		Eugnatichthys macroterolepis	Eug mac
		Eugnatichthys virgatus	Eug vir
		Mesoborus crocodilus	Mes cro
		Phago boulengeri	Pha bou
		Xenocharax spilurus	Xen spi
CYPRINIFORMES			
	Cyprinidae		
		Barbus miolepis	Bar mio
		Barbus sp.	Bar sp
		Clypeobarbus pleuropholis	Cly pleu
		Labeo lineatus	Lab lin
		Labeo longipinnis	Lab lon

Orde	Familie	Soort	Afkorting
SILURIFORMES			
	Clariidae		
		Clarias cf angolensis longmaxbarbels	Cla cfa
	Claroteidae		
		Chrysichthys longibarbis	Chry lon
		Chrysichthys ornatus	Chry lon
		Chrysichthys punctatus	Chry pun
		Chrysichthys sharpii	Chry shar
		Parauchenoglanis punctatus	Par pun
	Mochokidae		
		Synodontis aterrimus	Syn at
		Synodontis congicus	Syn cong
		Synodontis contractus	Syn cont
		Synodontis dorsomaculatus	Syn dor
		Synodontis flavitaeniatus	Syn fla
		Synodontis cf. greshoffi	Syn cf gre
		Synodontis nigriventris	Syn nig
		Synodontis nummifer	Syn num
		Synodontis pleurops	Syn pleu
	Schilbeidae		
		Parailia congica	Para con
		Pareutropius debauwi	Pare deb
		Schilbe grenfelli	Sch gren
		Schilbe intermedius	Sch int
		Schilbe laticeps	Sch lat
		Schilbe marmoratus	Sch mar
PERCIFORMES			
	Anabantidae		
		Aspercauda acutirostre	Aso acu
		Aspercauda ocellata	Aso oce
	Cichlidae		
		Lamprologus congoensis	Lam con
		Lamprologus mocquardi	Lam moc
		Lamprologus sp.	Lam sp
		Tylochromis lateralis	Tyl lat
SYNBRANCHIFORMES			
	Mastacembelidae		
		Mastacembelus congicus	Mas con
Totaal			

List of species found in the Cobafish area studied

Species	Row Labels	Congo	Lobaye	Lobilo	Lomami
Alestes liebrechtsii	AlesLieb	0	0	0	1
Alestopetersius brichardi	AlestBri	0	0	1	1
Alestopetersius caudalis	AlestCau	0	0	1	1
Alestopetersius compressus	AlestCom	0	1	1	1
Bathyaethiops caudomaculatus	BathCaud	0	0	1	0
Belonoglanis brieni	BeloBrie	0	0	0	1
Brachypetersius altus	BrachAlt	1	1	1	1
Brycinus bimaculatus	BryBima	0	1	0	0
Bryconaethiops boulengeri	BrycBoul	1	1	1	1
Bryconaethiops macrops	BrycMacr	1	0	0	1
Bryconaethiops microstoma	BrycMicr	1	0	1	1
Brycinus imberi	BryImbe	1	1	1	1
Brycinus "new parvus"	BryNparv	1	0	1	1
Brycinus "prognathosi"	BryProgn	1	0	0	1
Campylomrmyrus rynchophorus	CampRync	1	0	0	0
Campylomormyrus tamandua	CampTama	0	0	0	1
Chrysichthys longipinnis	ChrLongP	1	0	0	0
Chrysichthys congicus	ChryCong	1	0	0	1
Chrysichthys habereri	ChryHabe	1	0	0	0
Chrysichthys longibarbis	ChryLong	1	0	0	1
Chrysichthys oratus	ChryOrat	0	1	0	1
Chrysichthys punctatus	ChryPunc	0	1	1	0
Chrysichthys sharpii	ChryShar	1	0	0	1
Chrysichthys thonneri	ChryThon	0	0	0	1
Citharinus gibbosus	CithGibb	0	1	0	1
Citharinus macrolepis	CithMacr	0	1	0	1
Clarias cf. angolensis long maxillary barbels	ClarAnLb	0	0	1	0
Clarias cf. angolensis "verry long barbels"	ClarAnVL	0	0	1	1
Clarias buthopogon	ClarButh	0	0	1	0
Clarias gabonensis	ClarGabo	0	1	1	0
Clypeobarbus congicus	ClypCong	1	0	0	1
Clypeobarbus pleuropholis	ClypPleu	1	0	0	1
Ctenopoma acutirostre	CtenAcut	0	0	1	0
Ctenopoma gabonense	CtenGabo	0	1	0	0
Ctenopoma ocellata	CtenOcel	1	0	1	0
Ctenopoma weeksii	CtenWeek	0	0	1	0
Distichodus affinis	DistAffi	1	1	1	1
Distichodus antonii	DistAnto	1	1	0	0
Distichodus fasciolatus	DistFasc	1	1	0	1
Distichodus lusosso	Distluso	1	0	0	1
Distichodus nobili	DistNobi	0	1	1	0
Distichodus sexfasciatus	DistSexf	1	0	0	1

Distichodus teugelsi	DistTeug	0	1	1	0
Enteromius brazzai	EnteBraz	1	1	1	0
Enteromius sp. humeralis	EnteHume	0	0	0	1
Enteromius miolepis	EnteMiol	0	0	1	0
Enteromius stanleyi	EnteStan	0	0	1	0
Eugnatichthys macroterolepis	EugnMacr	1	1	1	1
Eugnatichthys virgatus	EugnVirg	0	1	1	1
Gnathonemus echidnorhynchus	GnatEchi	0	0	1	0
Gnathonemus petersii	GnatPete	0	1	1	1
Hemichromis elongatus	HemiElon	0	1	1	0
Hemichromis lifalili	HemiLifa	0	1	0	0
Hepsetus microlepis	HepsMicr	0	0	1	0
Hippopotamyrus macrops	HippMacr	0	0	0	1
Hippopotamyrus psittacus	HippPsit	1	0	0	1
Hippopotamyrus weeksii	HippWeek	0	1	1	1
Hydrocynus vittatus	HydrVitt	1	0	0	1
Labeo cyclorhynchus	LabeCycl	1	0	0	1
Labeo lineatus	LabeLine	0	0	1	1
Labeo longipinnis	LabeLong	1	0	0	1
Labeo parvus	LabeParv	1	0	0	0
Lamprologus congoensis	LampCong	0	0	0	1
Lamprologus moquardi	LampMoqu	1	0	0	1
Leptocypris modestus	LeptMode	1	0	0	0
Malapterurus gossei	MalaGoss	1	0	0	0
Marcusenius greshoffi	MarcGres	1	1	0	1
Marcusenius kutuensis	MarcKutu	1	1	1	0
Marcusenius leopoldianus	MarcLeop	0	0	1	0
Marcusenius monteiri	MarcMont	1	0	1	1
Marcusenius moorii	MarcMoor	0	0	1	0
Marcusenius schilthuisiae	MarcSchilt	1	1	1	1
Marcusenius stanleyanus	MarcStan	1	1	1	1
Mastacembelus congicus	MastaCem	1	0	0	0
Mesoborus crocodilus	MesoCroc	1	1	1	0
Micralestes acutidens	MicraAc	1	0	0	1
Micralestes congicus	MicraCo	0	0	1	0
Micralestes humilis	MicraHu	1	1	1	1
Micralestes lualabae	MicraLu	1	0	1	1
Micralestes stormsi	MicraSt	0	0	1	0
Microthrissa congica	MicroCo	1	0	0	1
Microthrissa royauxi	MicroRo	1	0	1	1
Mormyrops anguiollidens	MormAngu	1	0	0	0
Mormyrops attenuatus	MormAtte	0	0	0	1
Mormyrops furcidens	MormFurc	0	0	1	0
Mormyrops nigricans	MormNigr	0	0	1	0
Mormyrops sirenoides/marsuianus	MormSire	0	0	1	0
Oxymormyrus boulengeri	OxymBoul	0	0	1	0

Oxymormyrus zanclirostris	OxymZanc	0	0	1	0
Pantodon bucholzi	PantBuch	1	1	1	1
Paralia congica	ParaCong	1	1	0	1
Parachanna obscura	ParaObsc	0	1	1	0
Parauchenoglanus punctatus	ParauPun	0	0	1	0
Pareutropius debauwi	PareuDeb	1	1	1	1
Pelmatochormis nigrofasciatus	PelmaNig	0	1	0	0
Petrocephalus arnegardi	PetroArn	0	0	1	0
Petrocephalus balayi	PetroBal	0	0	1	1
Petrocephalus binotatus	PetroBin	1	1	1	1
Petrocephalus christyi	PetroChr	1	0	1	1
Petrocephalus grandoculis	PetroGra	1	1	1	1
Petrocephalus micropthalmus	PetroMic	1	1	1	1
Petrocephalus pulsivertens	PetroPul	0	0	1	1
Petrocephalus sauvagii	PetroSau	1	0	1	1
Petrocephalus schoutedeni	PetroSch	0	1	0	0
Petrocephalus simus	PetroSim	1	0	0	1
Phago boulengeri	PhagBoul	1	1	1	1
Phago intermedius	PhagInte	1	0	0	0
Phenacogrammus interruptus	PhenInter	1	1	1	1
Pollimyrus isidori	PollIsid	1	1	1	1
Pollimyrus nigripinnis	PollNigr	1	0	1	1
Pollimyrus plagiostoma	PollPlag	1	0	0	0
Pollimyrus cf. pulverulentus	PollPulv	1	0	0	0
Pollimyrus cf schreyeni	PollSchr	0	1	0	0
Polypterus polli	PolypPol	0	1	0	0
Potamothrissa acutirostris	PotamAcu	1	0	0	1
Rhabdalestes cf. yokai	RhabYoka	0	1	1	0
Schilbe grenfelli	SchilGren	1	0	1	1
Schilbe intermedius	SchilInte	1	0	0	1
Schilbe laticeps	SchilLati	0	0	1	0
Schilbe marmoratus	SchilMarm	1	1	1	1
Schilbe yangambianus	SchilYang	0	0	1	0
Stomatorinus cf. kunguensis	StomKung	1	0	0	0
Stomathorinus patrizii	StomPatri	1	0	0	0
Synodontis alberti	SynoAlbe	1	0	0	0
Synodontis angelicus	SynoAnge	1	0	0	1
Synodontis atterimus	SynoAtte	0	0	1	1
Synodontis centralis	SynoCent	0	0	0	1
Synodontis congicus	SynoCong	1	0	0	1
Synodontis contractus	SynoCont	1	0	1	0
Synodontis dorsomaculatus	SynoDors	0	0	0	1
synodontis flaevitaeniatus	SynoFlae	0	1	1	0
Synodontis greshoffi	SynoGres	1	0	1	1
Synodontis nigriventris	SynoNigr	1	1	1	1
Synodontis notatus	SynoNota	0	1	0	0

Synodontis nummifer	SynoNumm	0	0	0	1
Synodontis pleurops	SynoPleu	1	0	0	1
Synodontis schoutedeni	SynoScho	0	0	1	0
Tilapia zilli	TilaZill	0	1	0	0
Tylochromis lateralis	TyloLate	1	0	0	1
Xenomystus nigri	XenomNig	1	1	1	1
Xenocharax spilurus	XenoSpil	0	1	1	1

Appendix 1 Faunal inventory of ostracods associated with *E. crassipes* recorded in Amazon River catchment

Family Cyprididae
Cypricercus centrura (Klie 1940) Martens and Behen 1994
Diaphanocypris meridana (Furtos 1936) Würdig and Pinto 1990
Strandesia lansactohai Higuti and Martens in Higuti et al. 2013
S. mutica (Sars 1901) GW Müller 1912
S. psittacea (Sars 1901) Roessler 1990
S. tolimensis Roessler 1990
S. trispinosa (Pinto and Purper 1965) Broodbakker 1983
S. velhoi Higuti and Martens in Higuti et al. 2013
Chlamydotheca deformis Farkas 1958
C. iheringi (Sars 1901) Klie 1930
Cabelodopsis hispida (Sars 1901)
Cypridopsis vidua OF Müller 1776
C. cf. *vidua* sp. 2
"*Cypridopsis*" n. gen. 1 n.sp.
Neocypridopsis nana (Sars 1901) Klie 1940
Cypretta sp. 1 n.sp.
Cypretta sp. 2 n.sp.
Cypretta sp. 3 n.sp.
Stenocypris major (Braid 1859) Daday 1898
Family Candonidae
Candobrasilopsis brasiliensis Sars 1901
Candobrasilopsis rochai Higuti and Martens 2012
Pseudocandona agostinhoi Higuti and Martens 2014
Physocypria sp.1 n.sp.
Physocypria sp. 2 n.sp.
Family Limnocytheridae
Cytheridella ilosvayi Daday 1905

Appendix 2 Faunal inventory of ostracods associated with native *V. cuspidata* and invasive *E. crassipes* recorded in Congo River catchment

	V. cuspidata	*E. crassipes*
Family Cyprididae		
Strandesia elliptica sp. 1 n.sp.	x	x
Strandesia elliptica sp. 2 n.sp.	x	
Strandesia sp. 3 n.sp.	x	x
Strandesia sp. 4 n.sp.		x
Strandesia sp. 5 n.sp.	x	x
S. cf. *variegata*	x	
Cypridopsis sp. 1 n.sp.	x	x
Cypridopsis sp. 2 n.sp.	x	x
Cypridopsis sp. 3 n.sp.		x
Cypridopsis sp. 4 n.sp.	x	x
Cypridopsis sp. 5 n.sp.	x	
Cypridopsis sp. 6 n.sp.		x
Zonocypris sp. 1 n.sp.	x	x
Zonocypris sp. 2 n.sp.	x	x
Zonocypris sp. 3 n.sp.	x	x
Zonocypris sp. 4 n.sp.	x	
Zonocypris sp. 5 n.sp.	x	
Zonocypris sp. 6 n.sp.		x
Acocypris capillata (Vavra 1895) Müller 1898	x	x
Acocypris sp. 2 n.sp.	x	x
Chrissia sp. 1 n.sp.	x	x
Chrissia sp. 2 n.sp.	x	x
Chrissia sp. 3 n.sp.		x
Chrissia cf. *caesia*	x	
Stenocypris major (Braid 1859) Daday 1898	x	x
Stenocypris sp. 2 n.sp.		x
Family Candonidae		
Candonopsis sp. 1 n.sp.	x	x
Candonopsis sp. 2 n.sp.	x	x
Allocypris n.sp.		x
Physocypria n.sp.		x
Family Limnocytheridae		
Cytheridella sp. 1 n.sp.	x	
Cytheridella sp. 2 n.sp.	x	
Cytheridella sp. 3 n.sp.		x
Elpidium sp.		x
Gomphocythere sp. 1 n.sp.		x
Gomphocythere sp. 2 n.sp.		x
Gomphocythere sp. 3 n.sp.	x	

	V. cuspidata	*E. crassipes*
Family Darwinulidae		
Alicenula serricaudata (Klie 1935)	x	x
Vestalenula n.sp.	x	x
Penthesilenula n.sp.		x

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Technical Note: Large overestimation of pCO_2 calculated from pH and alkalinity in acidic, organic-rich freshwaters

G. Abril[1,2], S. Bouillon[3], F. Darchambeau[4], C. R. Teodoru[3], T. R. Marwick[3], F. Tamooh[3], F. Ochieng Omengo[3], N. Geeraert[3], L. Deirmendjian[1], P. Polsenaere[1], and A. V. Borges[4]

[1]Laboratoire EPOC, Environnements et Paléoenvironnements Océaniques et Continentaux, CNRS, Université de Bordeaux, France
[2]Programa de Geoquímica, Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil
[3]Katholieke Universiteit Leuven, Department of Earth & Environmental Sciences, Leuven, Belgium
[4]Unité d'Océanographie Chimique, Université de Liège, Belgium

Correspondence to: G. Abril (g.abril@epoc.u-bordeaux1.fr)

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Abstract. Inland waters have been recognized as a significant source of carbon dioxide (CO_2) to the atmosphere at the global scale. Fluxes of CO_2 between aquatic systems and the atmosphere are calculated from the gas transfer velocity and the water–air gradient of the partial pressure of CO_2 (pCO_2). Currently, direct measurements of water pCO_2 remain scarce in freshwaters, and most published pCO_2 data are calculated from temperature, pH and total alkalinity (TA). Here, we compare calculated (pH and TA) and measured (equilibrator and headspace) water pCO_2 in a large array of temperate and tropical freshwaters. The 761 data points cover a wide range of values for TA (0 to 14 200 µmol L^{-1}), pH (3.94 to 9.17), measured pCO_2 (36 to 23 000 ppmv), and dissolved organic carbon (DOC) (29 to 3970 µmol L^{-1}). Calculated pCO_2 were $> 10\,\%$ higher than measured pCO_2 in 60 % of the samples (with a median overestimation of calculated pCO_2 compared to measured pCO_2 of 2560 ppmv) and were $> 100\,\%$ higher in the 25 % most organic-rich and acidic samples (with a median overestimation of 9080 ppmv). We suggest these large overestimations of calculated pCO_2 with respect to measured pCO_2 are due to the combination of two cumulative effects: (1) a more significant contribution of organic acids anions to TA in waters with low carbonate alkalinity and high DOC concentrations; (2) a lower buffering capacity of the carbonate system at low pH, which increases the sensitivity of calculated pCO_2 to TA in acidic and organic-rich waters. No empirical relationship could be derived from our data set in order to correct calculated pCO_2 for this bias. Owing to the widespread distribution of acidic, organic-rich freshwaters, we conclude that regional and global estimates of CO_2 outgassing from freshwaters based on pH and TA data only are most likely overestimated, although the magnitude of the overestimation needs further quantitative analysis. Direct measurements of pCO_2 are recommended in inland waters in general, and in particular in acidic, poorly buffered freshwaters.

1 Introduction

Inland waters (streams, rivers, lakes, reservoirs, wetlands) receive carbon from terrestrial landscapes, usually have a net heterotrophic metabolism, and emit significant amounts of CO_2 to the atmosphere (Kempe 1984; Cole et al., 1994; Raymond et al., 2013). This terrestrial–aquatic–atmosphere link in the global carbon cycle is controlled by complex biogeographical drivers that generate strong spatial and temporal variations in the chemical composition of freshwaters and the intensity of CO_2 outgassing at the water–air interface (e.g. Tamooh et al., 2013; Dinsmore et al., 2013; Abril et al., 2014; Borges et al., 2014). Hence, large data sets are necessary in order to describe the environmental factors controlling these CO_2 emissions and to quantify global CO_2 fluxes from inland waters (Sobek et al., 2005; Barros et al., 2011; Raymond et al., 2013). Dissolved inorganic carbon (DIC) concentration and speciation in freshwaters greatly depend

on the lithological nature of watersheds (Meybeck 1987). For instance, rivers draining watersheds rich in carbonate rocks have a high DIC concentration, generally well above 1000 µmol L^{-1}. Bicarbonate ions contribute to most of the total alkalinity (TA) in these waters, which have high conductivities and high pH. In these hard waters, dissolved CO_2 represents a minor fraction (5–15 %) of the DIC compared to bicarbonates. In rivers draining organic-rich soils and non-carbonate rocks, DIC concentrations are lower (typically a few hundred µmol L^{-1}) but dissolved organic carbon (DOC) concentrations are higher, and commonly exceed the DIC concentrations. Organic acid anions significantly contribute to TA of these soft waters (Driscoll et al., 1989; Hemond 1990), which have low conductivities and low pH. Dissolved CO_2 represents a large, generally dominant, fraction of DIC in these acidic, organic-rich waters.

Fluxes of CO_2 between aquatic systems and the atmosphere can be computed from the water–air gradient of the concentration of CO_2 and the gas transfer velocity (Liss and Slater, 1974) at local (e.g. Raymond et al., 1997), regional (e.g. Teodoru et al., 2009), and global scales (e.g. Cole et al., 1994; Raymond et al., 2013). The partial pressure of CO_2 (pCO_2) is relatively constant in the atmosphere compared to surface freshwaters pCO_2 that can vary by more than 4 orders of magnitude spatially and temporally (Sobek et al., 2005; Abril et al., 2014). Consequently, water pCO_2 controls the intensity of the air–water flux, together with the gas transfer velocity. At present, both measured and calculated water pCO_2 data are used to compute CO_2 fluxes from freshwater systems, although calculated pCO_2 is overwhelmingly more abundant than directly measured pCO_2 (e.g. Cole et al., 1994; Raymond et al., 2013). pCO_2 can be calculated from the dissociation constants of carbonic acid (which are a function of temperature) and any of the following couples of measured variables: pH/TA, pH/DIC, DIC/TA (Park, 1969). In a majority of cases, calculated pCO_2 is based on the measurements of pH/TA and water temperature. These three parameters are routinely measured by many environmental agencies, and constitute a very large database available for the scientific community. Calculation of pCO_2 from pH and TA was initiated in world rivers in the 1970s (Kempe, 1984) and relies on the dissociation constants of carbonic acid, and the solubility of CO_2, all of which are temperature-dependent (Harned and Scholes, 1941; Harned and Davis, 1943; Millero, 1979; Stumm and Morgan, 1996). Measured pCO_2 is based on water–air phase equilibration either on discrete samples (headspace technique, e.g. Weiss, 1981) or continuously (equilibrator technique, e.g. Frankignoulle et al., 2001) using various systems and devices, followed by direct, generally infrared (IR), detection of CO_2 in the equilibrated gas. Commercial IR gas analysers are becoming cheaper and more accurate, stable and compact, and provide a large range of linear response well adapted to variability of pCO_2 found in freshwaters.

A limited number of studies have compared directly measured pCO_2 to computed pCO_2. Earlier examples provided a comparison between pCO_2 measured by headspace equilibration coupled to gas chromatography (GC) and pCO_2 calculated from pH and DIC (Kratz et al., 1997; Raymond et al., 1997). Reports by these authors in Wisconsin lakes and the Hudson River show that the pCO_2 values were linearly correlated but showed a variability of ±500 ppmv around the 1 : 1 line, over a range of measured pCO_2 from 300 to 4000 ppmv. Later, Frankignoulle and Borges (2001) reported the first comparison of pCO_2 calculated from pH and TA and pCO_2 measured by equilibration coupled to an IR analyser in an estuary in Belgium. In this high TA (2500–4800 µmol L^{-1}) and high pH (> 7.4) system, they found a good agreement between the two approaches, calculated pCO_2 being either overestimated or underestimated, but always by less than 7 %. In 2003, concomitant measurements of pH, TA and pCO_2 were performed in acidic, humic-rich ("black" type) waters of the Sinnamary River in French Guiana (Abril et al., 2005, 2006). Calculation of pCO_2 from pH (∼ 5) and TA (∼ 200 µmol L^{-1}) gave unrealistically high values compared to those measured directly with a headspace technique (typically 30000 ppmv vs. 5000 ppmv). Direct measurements of CO_2 and CH_4 outgassing fluxes with floating chambers and the computation of the respective gas transfer velocities of these two gases (Guérin et al., 2007) confirmed that pCO_2 values calculated from pH and TA were overestimated compared to direct measurements in the Sinnamary River. More recently, Hunt et al. (2011) and Wang et al. (2013) provided evidence that organic acid anions in DOC may significantly contribute to TA in some rivers and generate an overestimation of calculated pCO_2. Butman and Raymond (2011) reported higher calculated than measured pCO_2 in some US streams and rivers, but no information was available on the potential role of organic acids in this overestimation. These authors concluded that the low number of samples in their study reflected the need for more research on this topic.

With the growing interest on pCO_2 determination in freshwaters globally, and given the apparent simplicity and low cost of pH and TA measurements, the number of publications that report calculated pCO_2 in freshwaters has increased dramatically in the past decade. Some of these publications report extremely high and potentially biased pCO_2 values in low-alkalinity and high-DOC systems. It has thus become necessary to pay attention to this issue and investigate the occurrence of such potential bias and its magnitude in the different types of freshwaters. Here, we present a large data set of concomitant measurements of temperature, pH, TA, pCO_2, and DOC in freshwaters. This is the first comprehensive data set to investigate the magnitude of the bias between calculated and measured pCO_2, as it covers the entire range of variation of most parameters of the carbonate system in freshwaters. The objective of this paper is to alert the scientific community to the occurrence of a bias in pCO_2

Table 1. Summary of the presented data set. Average, minimum, and maximum values of temperature, DOC, pH (measured on the NBS scale), total alkalinity (TA), and measured partial pressure of CO_2 (pCO_2) in the different freshwater ecosystems.

Country	Watersheds	Temperature (°C)			DOC (µmol L^{-1})			pH (NBS scale)			TA (µmol L^{-1})			Measured pCO_2 (ppmv)			N
		Av.	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	
Brazil	Amazon	30.3	27.4	34.3	352	118	633	6.60	4.53	7.60	385	30	1092	4204	36	18400	155
Kenya	Athi-Galana-Sabaki	25.9	19.8	36.0	307	29	1133	7.69	6.49	8.57	2290	407	5042	2811	608	10405	44
DRC	Congo	26.3	22.6	28.2	1002	149	3968	6.01	3.94	7.22	212	0	576	6093	1582	15571	97
DRC/Rwanda	Lake Kivu	24.0	23.0	24.7	162	142	201	9.05	8.99	9.17	13037	12802	13338	660	537	772	53
France	Leyre	12.5	7.9	19.2	588	142	3625	6.20	4.40	7.41	280	38	1082	4429	901	23047	92
France	Loire	15.5	8.8	19.3	195	167	233	8.70	8.07	9.14	1768	1579	1886	284	65	717	18
Belgium	Meuse	18.1	13.3	25.9	229	102	404	7.89	6.95	8.59	2769	360	7141	2292	176	10033	50
Madagascar	Rianila and Betsiboka	25.4	20.2	29.5	138	33	361	6.84	5.83	7.62	233	76	961	1701	508	3847	36
Kenya	Shimba Hills	25.1	21.9	31.8	214	36	548	7.37	6.22	8.93	1989	227	14244	2751	546	9497	9
French Guiana	Sinnamary	27.1	24.1	28.7	419	213	596	5.50	5.08	6.30	143	66	290	7770	1358	15622	49
Kenya	Tana	26.6	25.0	27.9	321	193	651	7.65	7.32	8.02	1619	1338	2009	2700	845	6014	51
Zambia/Mozambique	Zambezi	26.9	18.8	31.8	252	103	492	7.59	5.06	9.08	1245	52	3134	2695	151	14004	107
Entire data set		24.6	7.9	36.0	408	29	3968	7.00	3.94	9.17	1731	0	14244	3707	36	23047	761

calculation from pH and TA in acidic, poorly buffered and organic-rich freshwaters, to briefly discuss its origin in terms of water chemistry, and to provide the range of pH, TA, and DOC values where pCO_2 calculation should be abandoned and the range where it still gives relatively accurate results.

2 Material and methods

2.1 Sample collection

Our data set consists of 761 concomitant measurements of temperature, pH, TA, water pCO_2, and DOC in 12 contrasting tropical and temperate systems in Europe, Amazonia, and Africa (Fig. 1; Table 1). These samples were obtained in the Central Amazon River and floodplains system in Brazil, the Athi-Galana-Sabaki River in Kenya, the Tana River (Kenya), small rivers draining the Shimba Hills in southeastern Kenya, the Congo River and tributaries in the Democratic Republic of the Congo (DRC), Lake Kivu in Rwanda and DRC, the Leyre River and tributaries in France, the Loire River in France, the Meuse River in Belgium, the Rianila and Betsiboka rivers in Madagascar, the Sinnamary River downstream of the Petit Saut Reservoir in French Guiana, and the Zambezi River in Zambia and Mozambique (Fig. 1). Details on some of the sampling sites can be found in Abril et al. (2005, 2014), Borges et al. (2012, 2014), Marwick et al. (2014a, b), Polsenaere et al. (2013), Tamooh et al. (2013), Teodoru et al. (2014). These watersheds span a range of climates and are occupied by different types of land cover, which include tropical rainforest (Amazon, Congo, Rianila), dry savannah (Tana, Athi-Galana-Sabaki, Betsiboka, Zambezi), temperate pine forest growing on podzols (Leyre), mixed temperate forest, grassland, and cropland (Meuse), and cropland (Loire). Lithology is also extremely contrasted as it includes for instance carbonate-rocks-dominated watershed as for the Meuse, sandstone-dominated silicates (Leyre), and precambrian crystalline magmatic and metamorphic rocks with a small proportion of carbonate and evaporite rocks for the Congo river.

2.2 Field and laboratory measurements

Although pH measurements might seem almost trivial, highly accurate and precise pH data are in fact not easy to obtain, especially in low-ionic strength waters, where electrode readings are generally less stable. Even though pH measurements in the laboratory might be more accurate, it is crucial to measure pH in situ or immediately after sampling, as pH determination several hours or days after sampling will be affected by CO_2 degassing and/or microbial respiration (Frankignoulle and Borges, 2001). In this work, water temperature and pH were measured in the field with different probes depending on the origin of the data set. However, all the pH data were obtained with glass electrodes and rely on daily calibration with two-point United States National Bureau of Standards (NBS) standards (4 and 7). Measurements were performed directly in the surface water, or in collected water immediately after sampling.

Several techniques were used to measure water pCO_2. Water–gas equilibration was performed with a marbles-type equilibrator (Frankignoulle et al., 2001) for the Amazon, Loire, Leyre, Sinnamary, and Congo rivers (December 2013) as well for Lake Kivu, or with a Liqui-Cel MiniModule membrane contactor equilibrator (see Teodoru et al., 2009, 2014) for the Zambezi and some sites within the Congo basin (December 2012): water was pumped either continuously from a ship, or on an ad hoc basis from the bank of the rivers after waiting $\sim$ 15 min for complete equilibration; air was continuously pumped from the equilibrator to the gas analyser (see e.g. Abril et al., 2014 for a more detailed description of the system). A syringe-headspace technique (Kratz et al., 1997; Teodoru et al., 2009) was used in the field in all African rivers and in the Meuse River: 30 mL volume of atmospheric air was equilibrated with 30 mL volume of river water by vigorously shaking during 5–10 min in four

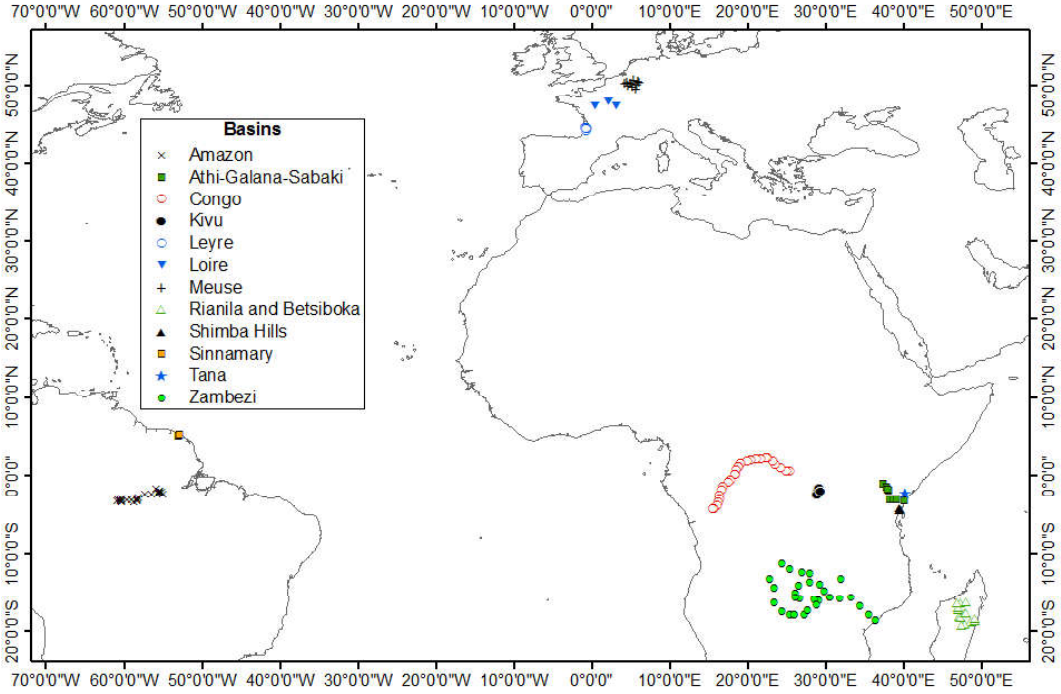


Figure 1. Location of the sampling sites in Africa, Amazonia, and Europe.

replicate gas-tight syringes. The four replicates 30 mL of equilibrated gas and a sample of atmospheric air were injected in an IR gas analyser (Li-Cor® models 820 or 840, or PP systems® model EGM-4); the first gas injection served as a purge for the air circuit and cell and the three other injections were used as triplicate pCO_2 determination (average repeatability of ±1 %). The pCO_2 in the river water was deduced from that measured in the headspace accounting for the initial pCO_2 in the air used for equilibration, water temperature in the river and in the water at equilibrium in the syringe, and based on Henry's law. Comparison between syringe-headspace and marbles or membrane equilibrator was made during two cruises on the Congo River and three cruises in the Zambezi basin and gave very consistent results, deviation from the 1 : 1 line being always less than 15 % (see Fig. 2). This highlights the consistency of the present data set of direct pCO_2 measurements although different techniques were used. A serum bottle-headspace technique (Hope et al., 1995) was also used on the Sinnamary River; surface water was sampled in 120 mL serum bottles that were poisoned with $HgCl_2$ and sealed excluding air bubbles. Back in the laboratory, a 40 mL headspace was created with pure N_2 (Abril et al., 2005). The CO_2 concentration of equilibrated gas in the headspace was analysed by injecting small volumes (0.5 mL) of gas in a gas chromatograph calibrated with certified gas mixtures.

Immediately after water–gas phase equilibration, CO_2 was detected and quantified in most samples with non-dispersive IR gas analysers (Frankignoulle et al., 2001; Abril et al., 2014). The gas analysers were calibrated before each field cruise, with air circulating through soda lime or pure N_2 for zero and with a certified gas standard for the span. Depending on the cruises and expected pCO_2 ranges, we used gas standard concentration of 1000–2000 ppmv, or a set of calibration gases at 400, 800, 4000 and 8000 ppmv. Stability of the instrument was checked after the cruise, and deviation of the signal was always less than 5 %. These instruments offer a large range of linear response, depending on manufacturer and model: 0–20 000 ppmv or 0–60 000 ppmv. The linearity of an Li-COR® Li-820 gas analyser was verified by connecting it to a closed circuit of gas equipped with a rubber septum to allow injection of pure CO_2 with a syringe. Linearity was checked by injecting increasing volumes of CO_2 in order to cover the whole range of measurement and was excellent between zero and ∼20000 ppmv. In addition to the IR analysers generally used in this work, in the Sinnamary River, pCO_2 was also measured with an INNOVA® 1312 optical filter IR photoacoustic gas analyser (range 0–25 000 ppmv) connected to an equilibrator and with a Hewlett Packard® 5890 gas chromatograph equipped with a thermal conductivity detector (TCD); both analysers were calibrated with a gas mixture of 5000 ppmv of CO_2. Both methods gave results consistent at ±15 % in the 0–13 000 ppmv range (Abril et al., 2006). Sinnamary data reported here are from headspace and GC determination.

TA was analysed by automated electro-titration on 50 mL filtered samples with 0.1N HCl as titrant. Equivalence point was determined with a Gran method from pH between 4

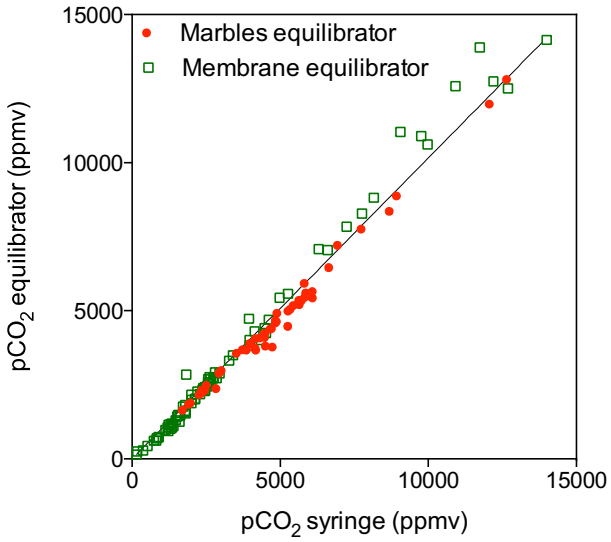


Figure 2. Comparison of results of different water–air equilibration designs for direct pCO_2 measurements; pCO_2 measured with a marbles equilibrator (Congo) and with a membrane equilibrator (Congo and Zambezi) are plotted against pCO_2 measured with a syringe headspace technique. Detection was made with an IR gas analyser.

and 3 (Gran, 1952). Precision based on replicate analyses was better than $\pm 5\,\mu mol\,L^{-1}$. TA measurements should be done on filtered samples; otherwise some overestimation would occur in turbid samples, which may contain significant amounts of acid-neutralizing particles (e.g. calcium carbonate). In contrast to TA measurements based on titration to an endpoint of 5.6 (e.g. Wallin et al., 2014), the Gran titration method allows the determination of TA values in samples with in situ pH down to ~ 4.5, i.e. very close to the dissociation constant of HCO_3^- / H_2CO_3. In most acidic samples with low TA, reproducibility was improved by slightly increasing the pH by up to 0.2 units by vigorously stirring during ~ 15 min in order to degas as much CO_2 as possible before starting the titration. DOC was measured on samples filtered through pre-combusted (490 °C) glass fibre filter with a porosity of 0.7 µm and stored acidified with ultrapure H_3PO_4 in borosilicate vials capped with polytetrafluoroethylene stoppers. Analysis was performed with a Shimadzu TOC5000 analyser based on high-temperature catalytic oxidation, after removal of dissolved CO_2 for samples from Amazon, Loire, Leyre, and Sinnamary rivers. DOC concentrations were measured with a customized wet oxidation TOC analyser (Thermo HiperTOC, or IO Analytical Aurora 1030W) coupled to a Delta+XL or Delta V IRMS.

2.3 pCO_2 calculation from pH and TA

We calculated pCO_2 from TA, pH, and temperature measurements using carbonic acid dissociation constants of Millero (1979) (based on those of Harned and Scholes, 1941 and Harned and Davis, 1943) and the CO_2 solubility from Weiss (1974) as implemented in the CO2SYS program. Hunt et al. (2011) reported discrepancy lower than 2 % for pCO_2 computed this way with those obtained with the PHREEQC program (Parkhurst and Appelo, 1999). Differences in software or dissociation constants cannot account for the large bias in calculated pCO_2 compared to measured pCO_2 we report in this paper.

3 Results

3.1 Data ranges and patterns in the entire data set

Measured pCO_2 varied between 36 ppmv in a floodplain of the Amazon River and 23 000 ppmv in a first-order stream of the Leyre River (Table 1). Minimum values of pH and TA occurred in the Congo River (pH = 3.94 and TA = 0) and maximum values in Lake Kivu (pH = 9.16 and TA = 14200 $\mu mol\,L^{-1}$). Highest DOC concentrations ($> 3000\,\mu mol\,L^{-1}$) were observed in small streams in the Congo basin and in first-order streams draining podzolized soils in the Leyre basin. Lowest DOC concentrations ($< 40\,\mu mol\,L^{-1}$) occurred in some tributaries of the Athi-Galana-Sabaki, in the Rianila and Betsiboka rivers, and in the Shimba Hills streams. When considering the whole data set, measured pCO_2 and DOC were negatively correlated with pH, whereas TA was positively correlated with pH (Fig. 3, $p < 0.0001$ for the three variables). This illustrates the large contrast in acid–base properties between acidic, organic-rich, and poorly buffered samples on one hand, and basic, carbonate-buffered samples on the other.

3.2 Comparison between measured and calculated pCO_2

Calculated pCO_2 was more than 10 % lower than measured pCO_2 in 16 % of the samples; the two methods were consistent at ±10 % in 24 % of the samples; calculated pCO_2 was more than 10 % higher than measured pCO_2 in 60 % of the samples and more than 100 % higher in 26 % of the samples. Absolute values, as expressed in ppmv, were largely shifted towards overestimation, calculated vs. measured pCO_2 data being well above the 1 : 1 line, and calculated minus measured pCO_2 values ranging between −6180 and +882 022 ppmv (Fig. 4). The largest overestimation of calculated pCO_2 occurred in the most acidic samples, whereas underestimations of calculated pCO_2 occurred in neutral or slightly basic samples (Fig. 4b). Ranking the data according to the pH, TA and DOC reveals that overestimation of calculated pCO_2 compared to measured pCO_2 increased in acidic, poorly buffered waters in parallel with an increase in the DOC concentration (Table 2). Discrepancies between calculated and measured pCO_2 were very different from one system to another, depending on the chemical status of the waters. On average at each sampled site, the

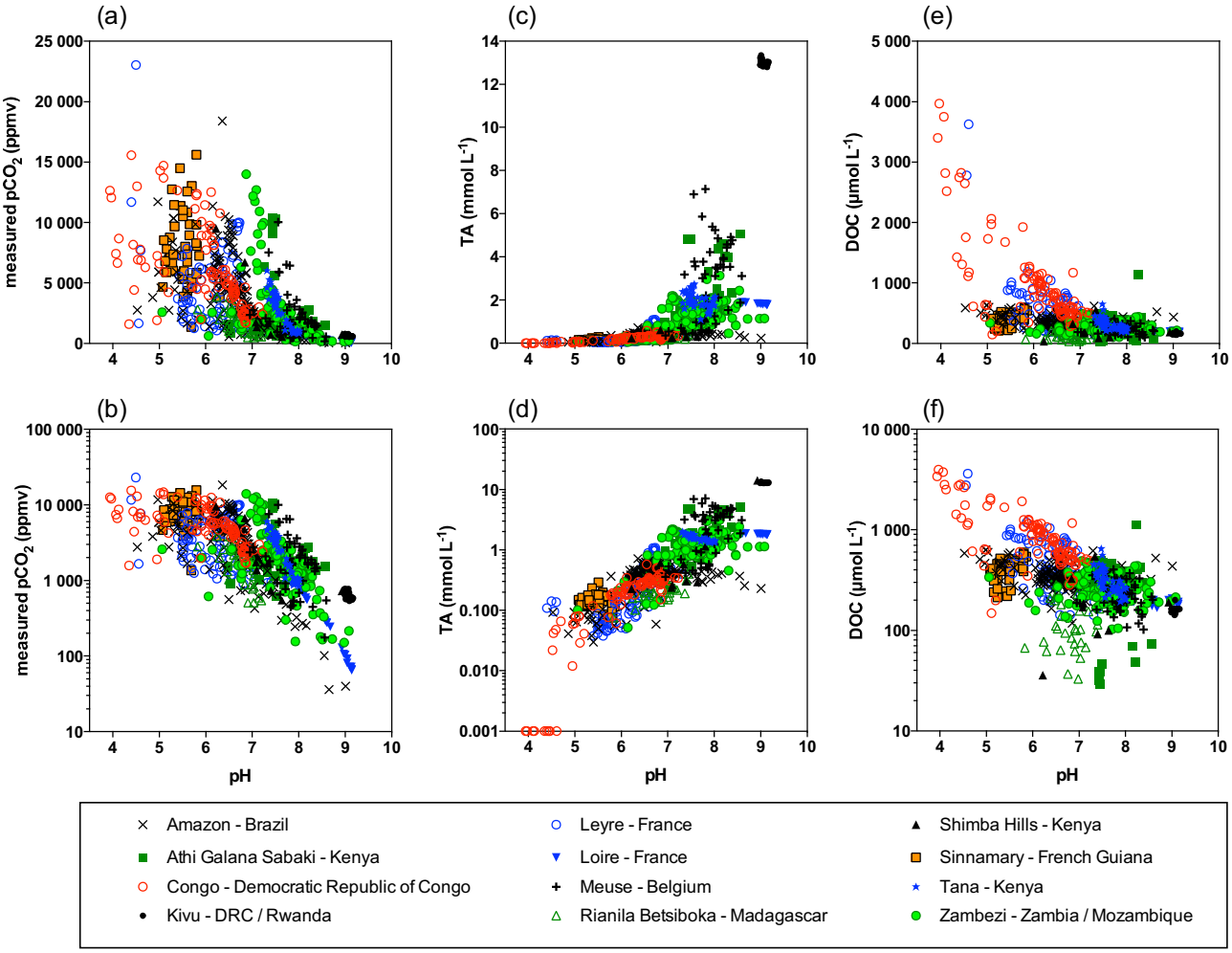


Figure 3. Plot of carbon variables vs. pH in the studied freshwater systems. Top panels are shown with a linear scale and bottom panels with a logarithmic scale; **(a, b)**: measured pCO_2; **(c, d)** total alkalinity; **(e, f)** DOC. Zero TA values are plotted as 0.001 in order to be visible on the log pCO_2 scale. Rianila and Betsiboka are plotted together although they belong to different watersheds in Madagascar.

relative overestimation of calculated pCO_2 decreased with pH and TA and increased with DOC (Fig. 5). Overestimation of calculated pCO_2 was on average $< 10\,\%$ in the Kivu Lake, and the Meuse, Loire, Shimba Hills and Tana rivers, which all have neutral or basic pH, TA $> 1000\,\mu mol\,L^{-1}$ and low to moderate DOC concentrations ($< 400\,\mu mol\,L^{-1}$) (Fig. 5). In contrast, calculated pCO_2 was overestimated by $> 200\,\%$ on average in the Congo, Leyre, Sinnamary and Amazon rivers, which have acidic pH, TA $< 500\,\mu mol\,L^{-1}$ and highest DOC concentration, reaching $1000\,\mu mol\,L^{-1}$ on average in the Congo. The cases of Athi-Galana-Sabaki, Rianila, Betsiboka, and Zambezi rivers were intermediate in pH, TA and DOC, and with average overestimations of calculated pCO_2 of 50–90 % (Fig. 5).

4 Discussion

4.1 Origin of overestimation of calculated pCO_2

Our data set (Fig. 3; Table 1) probably covers the full range of conditions of carbon speciation that can be encountered in continental surface waters. A pCO_2 overestimation negatively correlated with pH ($p = 0.001$) and TA ($p = 0.005$) and positively correlated with DOC ($p < 0.001$) (Fig. 5) is consistent with the observations of Cai et al. (1998) in the freshwater end-members of some estuaries in Georgia, USA, and of Hunt et al. (2011) in rivers in New England (USA) and New Brunswick (Canada). These authors performed NaOH back-titration in order to measure non-carbonate alkalinity (NCA). They found that NCA accounted for a large fraction (in some cases the greater part) of TA; in addition, the contribution of inorganic species other than carbonate was assumed negligible and most of the NCA was attributed to organic

Table 2. Median and average values of DOC, pH (measured on the NBS scale), total alkalinity (TA), and calculated minus measured pCO_2 in the data set.

	N	% of samples	cal – meas pCO_2 (ppmv)		cal – meas pCO_2 (% of meas pCO_2)		pH		TA ($\mu mol\,L^{-1}$)		DOC ($\mu mol\,L^{-1}$)	
			Med.	Av.	Med.	Av.	Med.	Av.	Med.	Av.	Med.	Av.
All samples	761	100 %	+611	+10692	+23%	+194%	6.94	7.00	467	1731	315	408
Ranked by calculated – measured pCO_2 as % of measured pCO_2												
< −10 %	122	16 %	−540	−890	−34 %	−36 %	7.89	7.85	1269	1766	259	275
±10 %	174	23 %	+15	+50	+2%	+1%	7.67	7.78	1576	3735	228	273
> +10 %	465	61 %	+2430	+17710	+72%	+327%	6.52	6.49	308	972	360	497
> +50 %	280	37 %	+5490	+28660	+162%	+526%	6.18	6.14	192	460	375	567
> +100 %	199	26 %	+9080	+39120	+270%	+710%	5.89	5.96	166	364	389	602
Ranked by pH												
pH > 7	368	48 %	+1	+82	+1%	+15%	7.82	7.92	1572	3284	231	255
pH < 7	393	52 %	+3280	+20630	+71%	+362%	6.30	6.13	232	277	413	558
pH 6–7	256	34 %	+1580	+2710	+40%	+96%	6.58	6.55	334	370	350	427
pH < 6	136	18 %	+18410	+54486	+308%	+864%	5.50	5.35	93	101	487	828
pH < 5	25	3 %	+115580	+209910	+1645%	+3180%	4.53	4.53	41	45	1427	1,843
Ranked by TA												
TA > 2000 $\mu mol\,L^{-1}$	110	14 %	+20	+340	+2%	+12%	8.58	8.47	7023	8326	163	202
TA 1000–2000 $\mu mol\,L^{-1}$	157	21 %	−8	−163	−2 %	−9 %	7.81	7.83	1566	1534	271	295
TA 500–1000 $\mu mol\,L^{-1}$	99	13 %	+1307	+1900	+28%	+72%	6.97	7.11	651	697	304	318
TA < 500 $\mu mol\,L^{-1}$	395	52 %	+2070	+20090	+64%	+350%	6.30	6.24	222	232	400	538
TA < 100 $\mu mol\,L^{-1}$	82	11 %	+6840	+60560	+230%	+1040%	5.50	5.35	59	56	603	988
Ranked by DOC												
DOC < 200 $\mu mol\,L^{-1}$	179	24 %	+40	+776	+5%	+62%	7.89	7.92	1579	4807	163	149
DOC 200–300 $\mu mol\,L^{-1}$	167	22 %	+102	+2755	+5%	+69%	7.56	7.37	1132	1259	258	252
DOC 300–400 $\mu mol\,L^{-1}$	165	22 %	+887	+4473	+25%	+101%	6.90	6.93	499	866	341	344
DOC > 400 $\mu mol\,L^{-1}$	250	33 %	+3070	+27197	+59%	+434%	6.15	6.14	200	415	555	765
DOC > 800 $\mu mol\,L^{-1}$	79	10 %	+4995	+62784	+92%	+886%	5.80	5.62	94	180	1099	1438

acid anions. Hunt et al. (2011) also showed that in the absence of direct titration of NCA, which is labour-intensive and whose precision may be poor, this parameter could be calculated as the difference between the measured TA and the alkalinity calculated from measurements of pH and DIC and the dissociation constants of carbonic acid. Using the latter approach, Wang et al. (2013) obtained a positive correlation between NCA and DOC concentrations in the Congo River, evidencing the predominant role of organic acids in DIC speciation and pH in such acidic system. Because we did not directly measure DIC in this study, we could not calculate NCA with the same procedure as these studies. We attempted to calculate TA from our measured pH and pCO_2 with the CO2SYS program. However, TA values calculated this way were inconsistent with other measured variables (with sometimes negative values). Indeed, because pH and pCO_2 are too interdependent in the carbonate system, very small analytical errors on these variables lead to large uncertainties in the calculated TA (Cullison Gray et al., 2011). A second attempt to correct our TA data from NCA consisted in calculating organic alkalinity using pH and DOC as input parameters. We compared the model of Driscoll et al. (1989), which assumes a single pK value for all organic acids, and the triprotic model of Hruska et al. (2003), which assumes three apparent pK values for organic acids. These two models applied to our pH and DOC gave very similar organic alkalinity values, which could be subtracted from the measured TA. In the most acidic samples (e.g. some sites from the Congo basin), modelled organic alkalinities were larger than measured TA and the difference was thus negative. Nevertheless, we then recalculated pCO_2 from the measured pH and the TA corrected from organic alkalinity. Calculated pCO_2 values corrected with that method were, however, still very different from those measured in the field, being sometimes higher and sometimes lower than the measured pCO_2, without any meaningful pattern (indeed, corrected pCO_2 was negatively correlated ($p < 0.001$) with measured pCO_2). Consequently, we were unable to derive any empirical relationship to correct for the bias in pCO_2 calculation from pH and TA. Nevertheless, the negative correlation between pH and DOC and positive correlation between pH and TA (Fig. 3) confirm a

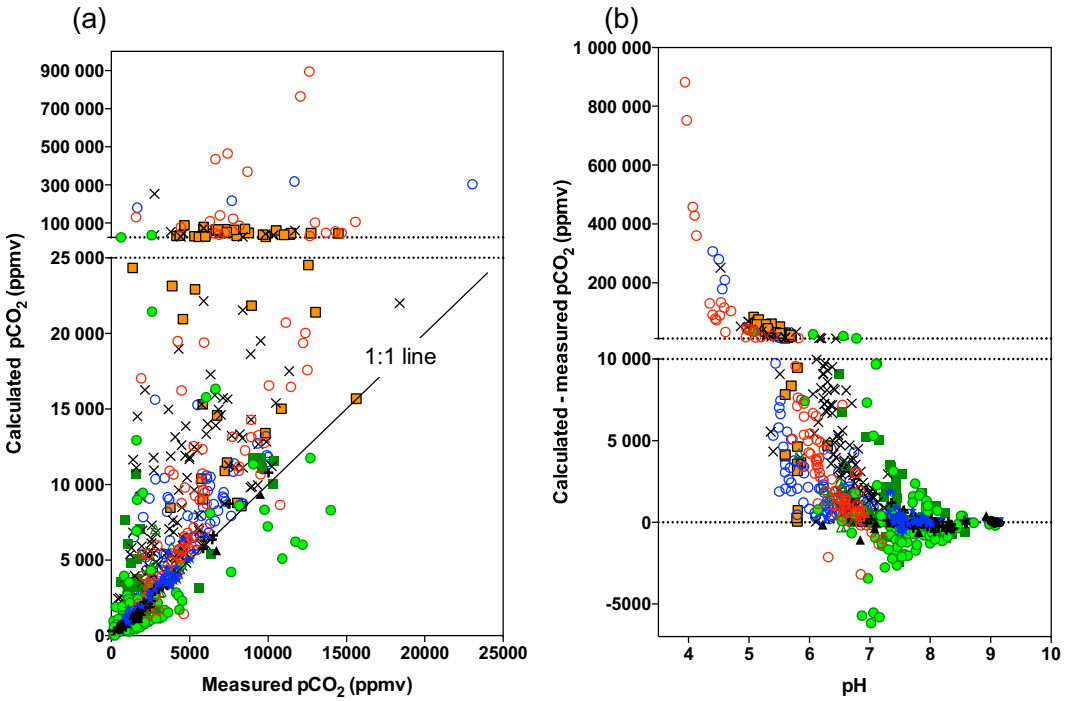


Figure 4. Comparison between measured and calculated pCO_2 for the whole data set: **(a)** calculated vs. measured pCO_2, the line shows when measured pCO_2 equals calculated pCO_2; **(b)** the difference between calculated and measured pCO_2 as a function of pH; same symbols as in Fig. 3.

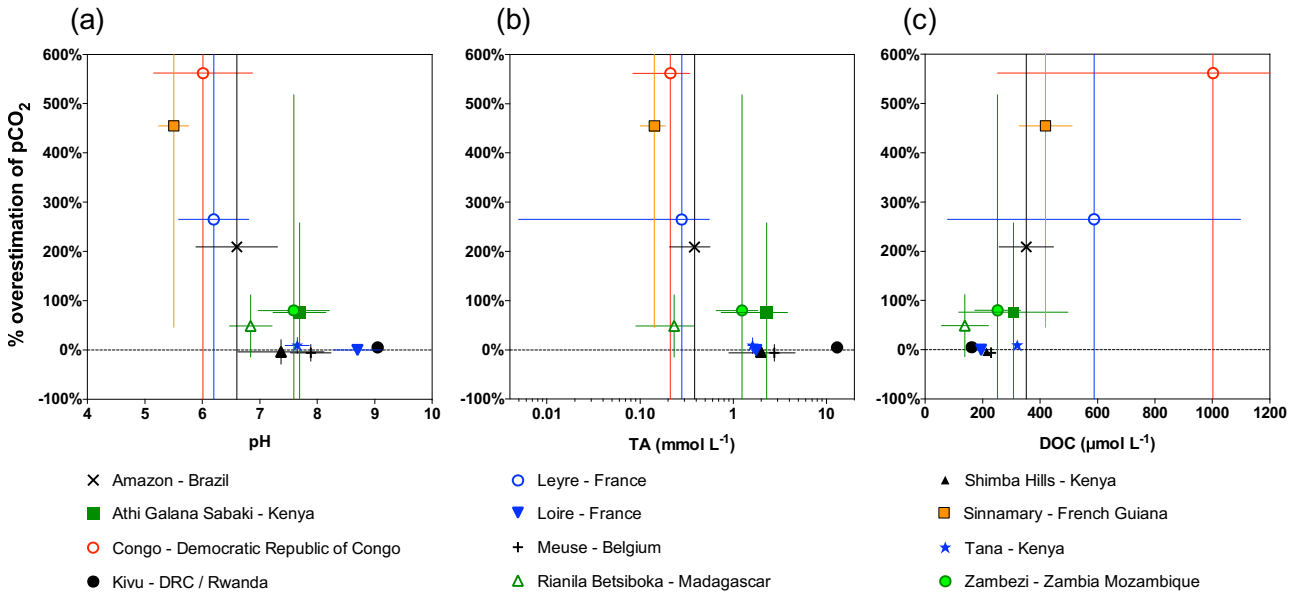


Figure 5. Average percentages of pCO_2 overestimation, calculated as 100 × (calculated pCO_2 – measured pCO_2)/measured pCO_2, as a function of **(a)** pH, **(b)** TA, and **(c)** DOC, for the 12 studied sites. Error bars indicate the standard deviation from the mean for each freshwater system.

strong control of organic acids on pH and DIC speciation across the entire data set.

As discussed by Hunt et al. (2011), a significant contribution of organic acids to TA leads to an overestimation of calculated pCO_2 with the CO2SYS program, or with any program that accounts only for the inorganic species that contribute to TA. It is thus obvious that the observed increase in pCO_2 overestimation when pH decreases (Figs. 4b and

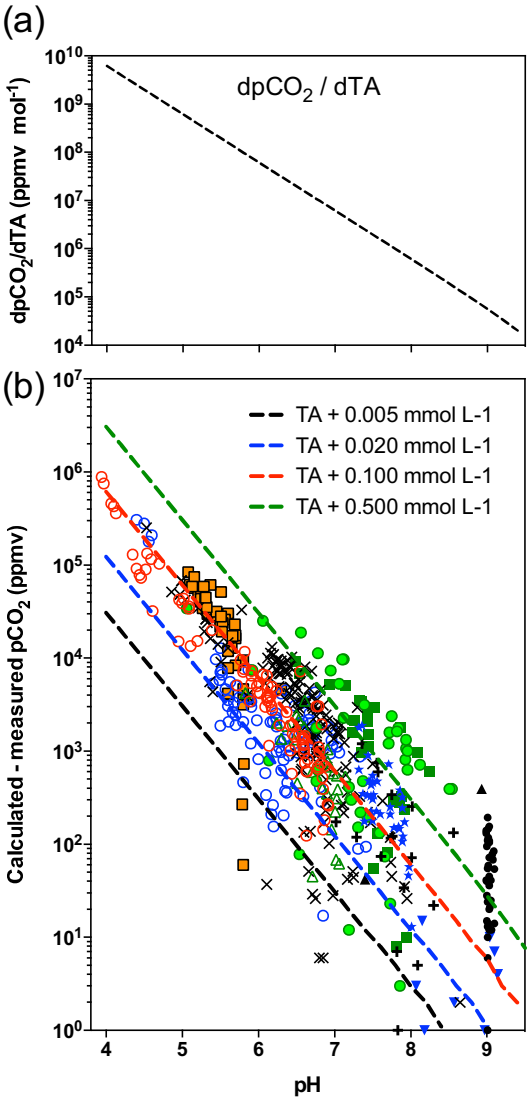


Figure 6. Sensitivity of pCO_2 overestimation to pH: **(a)** theoretical factor $dpCO_2$ / dTA, which describes the sensitivity of calculated pCO_2 to the TA value; **(b)** the solid lines show the increase in calculated pCO_2 induced by various increases in TA, as functions of pH; these lines mimic the overestimation of calculated pCO_2 generated by increasing contributions of organic alkalinity to the TA; field data (as calculated – measured pCO_2) have been plotted for comparison; same symbols as in Fig. 3. Note that negative values do not appear in the logarithmic scale.

5; Table 2) is due to an increasing contribution of organic acid anions to TA. However, this effect is not the only driver of the observed overestimation of pCO_2, which is also due to a decrease in the buffering capacity of the carbonate system at acidic pH. To investigate the magnitude of this second effect, we calculated the factor $dpCO_2$ / dTA (in ppmv mol^{-1}), which describes the change in calculated pCO_2 induced by a change in TA. This factor, which is the opposite of a buffer factor as it reflects the sensitivity of pCO_2 calculation to the TA, increases exponentially when pH decreases (Fig. 6a), i.e. it is proportional to the H^+ concentration. To go further in this theoretical analysis, we computed the difference between the pCO_2 calculated at a given TA value and the one calculated at a slightly higher TA value (TA+X µmol L^{-1}). These calculations reveal an extreme sensitivity of calculated pCO_2 to TA at acidic pH (Fig. 6b). For instance, increasing TA by 5 µmol L^{-1} (a value close to the precision of TA titrations) increases the calculated pCO_2 by 31 ppmv at pH 7, by 307 ppmv at pH 6 and by 3070 at pH 5. Increasing TA by 100 µmol L^{-1} (a typical value of NCA found in freshwaters; Driscoll et al., 1994; Cai et al., 1998; Hunt et al., 2011), increases the calculated pCO_2 by 615 ppmv at pH 7, by 6156 ppmv at pH 6 and by 61560 ppmv at pH 5. Note that this increase in calculated pCO_2 is independent of the chosen initial TA value. The difference between calculated and measured pCO_2 from our data set shows that an NCA contribution around 100 µmol L^{-1} is sufficient to explain the overestimation of calculated pCO_2 of most samples at pH < 6, whereas an NCA contribution higher than 500 µmol L^{-1} would be necessary for several samples at circumneutral and slightly basic pH (Fig. 5b). Samples requiring this high NCA contribution are from the Athi-Galana-Sabaki and Zambezi watersheds, and correspond to TA values well above 1000 µmol L^{-1}. An NCA value of 500 µmol L^{-1} in these samples is thus plausible.

We have no definitive explanation for lower calculated than measured pCO_2, which is observed mainly at neutral to slightly basic pH, for example in the Zambezi River (Fig. 4). In most of these samples, owing to the relatively high TA value, an overestimation of pH of less than 0.2 units is sufficient to account for the low calculated pCO_2 compared to measured values. In general, it is not easy to judge the accuracy of pH measurements, especially when data come from environmental agencies. Thus, one factor of variability throughout the data set as well as in literature data is the accuracy of pH measurements – despite the care taken (e.g. calibrations with NBS buffers for each day of measurements), we cannot rule out that drift or malfunction of pH electrodes contributes to the observed variability, constituting an additional disadvantage compared to direct pCO_2 measurements with very stable gas analysers.

4.2 Impact on estimates of CO_2 emissions from freshwaters

According to our analysis, overestimation of calculated pCO_2 is largest in acidic, poorly buffered and organic-rich waters. Consequently, the overestimation of regional and global CO_2 emissions computed from calculated pCO_2 depends on the relative contribution of these types of waters worldwide. In their analysis, Raymond et al. (2013) have discarded all calculated pCO_2 values with a pH value of less than 5.4, as well as all pCO_2 values above 100 000 ppmv. These criteria would exclude only 8 % of samples from our

data set. Indeed, from our analysis, it appears that overestimation of calculated pCO_2 occurs at pH much higher than 5.4 (Figs. 4, 5 and 6; Table 2). The two techniques were consistent at ±10 % on average in only 5 of the 12 studied systems, which combine a circumneutral to basic pH with a TA concentration well above 1000 µmol L^{-1} (Fig. 5). Although it would not be sufficient for the cases of the Zambezi and Athi-Galana-Sabaki rivers, where overestimation is still significant, a TA value above 1000 µmol L^{-1} appears as a more robust criterion than a pH threshold to separate calculated pCO_2 values affected by bias from those consistent with measured pCO_2 (Table 2). In fact, pCO_2 calculation from pH and TA in freshwaters historically relies on theoretical background and validation data in high-alkalinity waters (Neal et al., 1998), including karstic waters (Kempe, 1975). At the global scale, high TA typically occurs in rivers draining watersheds with a significant proportion of carbonate rocks, typically > 30 % of their surface area if the criterion of TA > 1000 µmol L^{-1} is chosen and the normalized weathering rates of Meybeck (1987) are applied. According to Meybeck (1987), the average and discharge-weighted TA is around 900 µmol L^{-1} for world rivers and around 600 µmol L^{-1} for tropical rivers. Among the 25 largest rivers in the world, 15 have a TA > 1000 µmol L^{-1} according to Cai et al. (2008). The two largest rivers in the world in terms of discharge, the Amazon and the Congo, are also well below this limit of 1000 µmol L^{-1} and have large overestimation in calculated pCO_2 (on average 200 and 360 %, respectively). Very low TA and pH and high DOC values have also been reported in boreal streams and rivers (Humborg et al., 2010; Dinsmore et al., 2012; Wallin et al., 2014).

In lakes, the highest pCO_2 values in the literature come from tropical black water lakes and were also calculated rather than directly measured (Sobek et al., 2005). Calculated pCO_2 was 65 250 ppmv in Lago Tupé in the Brazilian Amazon, a Ria lake connected to the Rio Negro, where, according to our own data set, pH is below 5 and TA is around 70 µmol L^{-1}. It was 18 950 ppmv in Kambanain Lake in Papua New Guinea, corresponding to a pH value of 6.1 and a TA value of 350 µmol L^{-1} (Vyverman, 1994). This suggests a widespread overestimation of calculated pCO_2 that significantly impacts the estimation of global CO_2 emissions from inland waters. However, a precise analysis based on exact quantitative information on the relative contribution of acidic and high- and low-alkalinity waters to the total surface area of inland waters is necessary in order to evaluate the exact magnitude of the overestimation.

5 Conclusions

From our analysis, it appears that the validity of calculating pCO_2 from pH, TA and temperature is most robust in freshwaters with circumneutral to basic pH and with TA exceeding 1000 µmol L^{-1}. At lower TA and pH, however, calculated pCO_2 (and hence, CO_2 degassing rates) are overestimated by 50 to 300 % relative to direct, in situ pCO_2 measurements. Since a large majority of freshwater systems globally have characteristics outside the range of applicability of pCO_2 calculation, it appears reasonable to assume that recent estimates of global CO_2 emission from lakes and rivers, which are based exclusively on calculated pCO_2 data, are too high. We propose that while TA and pH measurements remain useful to describe the aquatic chemistry, data on pCO_2 should in the future rely on direct measurements of pCO_2. Even if some studies report relatively robust calculation of pCO_2 from pH and DIC measurements (Raymond et al., 1997; Kratz et al., 1997; Aberg and Wallin, 2014), direct pCO_2 values in the field are stable, precise and straightforward and do not depend on the quality of pH measurements, which are often uncertain. Further, high-quality DIC measurements are very time-consuming, fairly complicated to set up and do not allow continuous measurements to be carried out in a simple and straightforward fashion. Although there are some practical limitations to their use in the field, submerged IR sensors, which allow high temporal resolution, are also promising (Johnson et al., 2010). Long-term instrument stability and accuracy based on newly developed off-axis integrated cavity output spectroscopy and cavity ring-down spectroscopy technologies seem to improve in comparison to traditional IR instruments, although the latter are more affordable, more compact and have lower power requirements. Joint international efforts are necessary to define the most appropriate protocols for the measurement of DIC parameters in freshwaters.

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ARTICLES

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Globally significant greenhouse-gas emissions from African inland waters

Alberto V. Borges[1]*, François Darchambeau[1], Cristian R. Teodoru[2], Trent R. Marwick[2], Fredrick Tamooh[2,3], Naomi Geeraert[2], Fredrick O. Omengo[2], Frédéric Guérin[4], Thibault Lambert[1], Cédric Morana[2], Eric Okuku[2,5] and Steven Bouillon[2]

Carbon dioxide emissions to the atmosphere from inland waters—streams, rivers, lakes and reservoirs—are nearly equivalent to ocean and land sinks globally. Inland waters can be an important source of methane and nitrous oxide emissions as well, but emissions are poorly quantified, especially in Africa. Here we report dissolved carbon dioxide, methane and nitrous oxide concentrations from 12 rivers in sub-Saharan Africa, including seasonally resolved sampling at 39 sites, acquired between 2006 and 2014. Fluxes were calculated from published gas transfer velocities, and upscaled to the area of all sub-Saharan African rivers using available spatial data sets. Carbon dioxide-equivalent emissions from river channels alone were about 0.4 Pg carbon per year, equivalent to two-thirds of the overall net carbon land sink previously reported for Africa. Including emissions from wetlands of the Congo river increases the total carbon dioxide-equivalent greenhouse-gas emissions to about 0.9 Pg carbon per year, equivalent to about one quarter of the global ocean and terrestrial combined carbon sink. Riverine carbon dioxide and methane emissions increase with wetland extent and upland biomass. We therefore suggest that future changes in wetland and upland cover could strongly affect greenhouse-gas emissions from African inland waters.

Climate predictions necessitate a full and robust account of natural and anthropogenic greenhouse-gas (GHG) fluxes, especially for CO_2 (refs 1–3), CH_4 (ref. 4) and N_2O (ref. 5), which together accounted for 94% of the anthropogenic global radiative forcing by well-mixed GHGs in 2011 relative to 1750 (ref. 6). Inland waters (streams, rivers, lakes and reservoirs) are increasingly recognized as important sources of GHGs to the atmosphere, with global CO_2 and CH_4 emissions estimated at 2.1 PgC yr^{-1} (ref. 3) and 0.7 PgC yr^{-1} (CO_2-equivalents; CO_2e) (ref. 4) (1 Pg = 10^{15} g), respectively. Considering that the oceanic and land carbon (C) sinks correspond to ~1.5 and ~2.0 PgC yr^{-1} (ref. 7), respectively, the GHG flux from inland waters is significant in the global C budget.

In a recent global compilation of inland CO_2 data[3], <20 data points (out of 6,708, that is, <0.3%) represented African inland waters (with the exception of South Africa, which has been densely sampled), even though they account for ~12% of both global freshwater discharge[8] and riverine surface area[3], and include some of the largest rivers and lakes in the world. Equally for the global CH_4 database, there is a strong under-representation of tropical inland waters, whereby a recent synthesis[4] resorted to extrapolating CH_4 fluxes from temperate rivers.

The prevailing large uncertainty involved in GHG flux estimates for inland waters, essentially due to the paucity of available data, is coupled to a poor understanding of underlying processes, both of which preclude gauging of future fluxes in response to human pressures. In particular, there is a need to further understand the link between inland water GHG fluxes and catchment characteristics, in particular regarding their connectivity with upland terrestrial[9,10] and wetland[11–13] C production and stocks. The CO_2 emissions from inland waters have been traditionally interpreted as fuelled by organic C from upland terrestrial biomass[1,14]. In the Amazon basin, CO_2 emissions from floodplain lakes[11] and from river channels[12,13] have been attributed to organic and inorganic C from wetlands (flooded forest and macrophytes). Finally, recently recognized biases in computed CO_2 data traditionally used in inland water studies[15,16] highlight the requirement for careful data-quality checking and future emphasis on high-quality direct CO_2 measurements.

In this study, we report an extensive compilation of dissolved CO_2, CH_4 and N_2O concentrations (Supplementary Table 1) gathered in 12 river basins in sub-Saharan Africa (SSA; Fig. 1 and Supplementary Fig. 1). The rivers range from 7.8×10^3 to 3.7×10^6 km^2 in watershed area and from 1.9 to 1.3×10^3 km^3 yr^{-1} in discharge, including the largest in Africa (Supplementary Table 2). A wide size range was sampled, from <1 m width (headwaters) to ~10 km width (mainstem Congo).

Variability of GHG concentrations and fluxes

The partial pressure of CO_2 (p_{CO_2}) values spanned two orders of magnitude, ranging between 300 and 16,942 ppm, CH_4 concentrations varied over five orders of magnitude, ranging between 2 and 62,966 nmol l^{-1}, whereas N_2O spanned three orders of magnitude, ranging between 0.2 and 85.4 nmol l^{-1} (Supplementary Fig. 2). The Congo River was the most variable in GHG concentrations, with distinct differences between streams (<100 m width) and rivers (>100 m width): p_{CO_2} and CH_4 values were higher in streams than rivers, whereas the opposite was observed for N_2O and oxygen saturation level

[1]Université de Liège, Unité d'Océanographie Chimique, Institut de Physique (B5), B-4000, Belgium. [2]Katholieke Universiteit Leuven, Department of Earth and Environmental Sciences, Celestijnenlaan 200E, B-3001 Leuven, Belgium. [3]Kenyatta University, Department of Zoological Sciences, PO Box 16778-80100, Mombasa, Kenya. [4]Geosciences Environnement Toulouse UMR 5563 & UR 234 IRD, Université Paul-Sabatier, Avenue Edouard Belin 14, F-31400 Toulouse, France. [5]Kenya Marine and Fisheries Research Institute, PO Box 81651, Mombasa-80100, Kenya.
*e-mail: alberto.borges@ulg.ac.be

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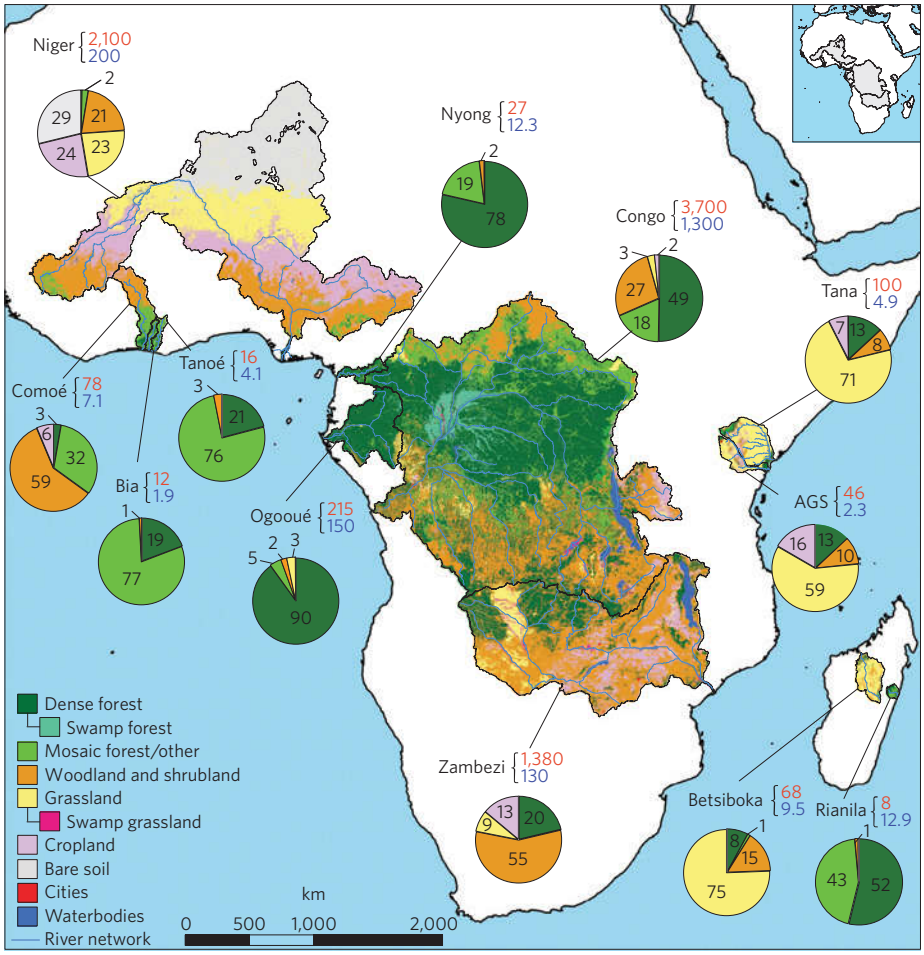


Figure 1 | The 12 studied rivers cover a wide range of discharge, catchment size and land cover. The red numbers correspond to the catchment surface area (10^3 km^2) and the blue numbers correspond to the annual freshwater discharge (km^3 yr^{-1}). Refer to Methods for data sources. AGS, Athi-Galana-Sabaki River.

(%O_2) (Supplementary Fig. 2). The overall average p_{CO_2} and CH_4 in rivers was 6,415 ppm and 2,205 nmol l^{-1}, respectively, corresponding to large over-saturation of surface waters with respect to atmospheric equilibrium of CO_2 and CH_4 (on average ~395 ppm and ~2 nmol l^{-1}, respectively). The N_2O values oscillated between under- and over-saturation, with an overall average of 9.2 nmol l^{-1}, only slightly above the atmospheric equilibrium of ~6.6 nmol l^{-1}.

The fluxes were computed from the gas transfer velocity (k) using two approaches. The Aufdenkampe *et al.* (Auf) approach[2] relies on constant k values across basins separated into streams and rivers (<100 m and >100 m width, respectively). The Raymond *et al.* (Ray) approach[3] uses basin-specific k values computed from hydraulic equations and basin characteristics. We present both estimates to provide a range of flux values, allowing a comparison with published fluxes. An error analysis is provided in the Supplementary Methods and Methods. Among river systems, the air–water CO_2 fluxes computed with the Auf approach (FCO_{2Auf}) ranged between 186 ± 9 and 1,149 ± 53 mmol m^{-2} d^{-1}, the air–water CH_4 fluxes (FCH_{4Auf}) ranged between 0.5 ± 0.1 and 18 ± 1 mmol m^{-2} d^{-1}, and the air–water N_2O fluxes (FN_2O_{Auf}) ranged between 2.0 ± 0.1 and 16 ± 1 μmol m^{-2} d^{-1} (Supplementary Table 3). The fluxes computed using the Ray approach were ~1.34 times higher, as it accounts for lower-order streams that are more turbulent and have higher k values. The average FCO_2 for all rivers was 11 times higher than FCH_4 (in CO_2e) and 273 times higher than FN_2O (in CO_2e). For individual basins, the FCO_2:FCH_4 ratio ranged between 4 (Zambezi) and 39 (Tana). This is in contrast to other concurrent FCO_2, FCH_4 and FN_2O estimates in temperate rivers (Supplementary Table 4), where FCO_2:FCH_4 ratios are higher (between 9 and 411, average 108) and FCO_2:FN_2O ratios are lower (between 10 and 71, average 35). This reflects the less extensive flooded areas in these temperate rivers (floodplains promote FCH_4 compared to FCO_2), and higher inputs of fertilizer- or wastewater-derived nitrogen, which promote FN_2O (ref. 5) compared to FCO_2.

The FCO_{2Ray} we report were 1.1–4.7 times (on average 2.6 times) higher than those given by Raymond *et al.*[3] for the same basins (Supplementary Fig. 3), suggesting that the reliance of the latter on a very limited African p_{CO_2} database resulted in underestimating the riverine FCO_2 for the African continent. When comparing the surface-area-integrated FCO_2 per basin (Supplementary Table 3) to the flux of total organic C (TOC) and dissolved inorganic C (DIC) to the ocean for the Congo, the Zambezi and the Tana (data are unavailable for other rivers sampled), FCO_2 was, respectively, 8.6, 2.1 and 9.9 times higher than the TOC+DIC export to the ocean, or 13.3, 16.5 and 23.0 times higher than the TOC export to the ocean. By comparison, in the Amazon River, the total FCO_2 is 6.6 and 13.1 times higher than the TOC+DIC and TOC export to the ocean[14]. This clearly highlights the significance of vertical C fluxes from inland waters to the atmosphere.

Regional and global significance of GHG fluxes

The FCO_2 integrated for all SSA rivers ranged between 0.27 ± 0.05 (Auf) and 0.37 ± 0.07 (Ray) PgC yr^{-1}. Such values are of the same

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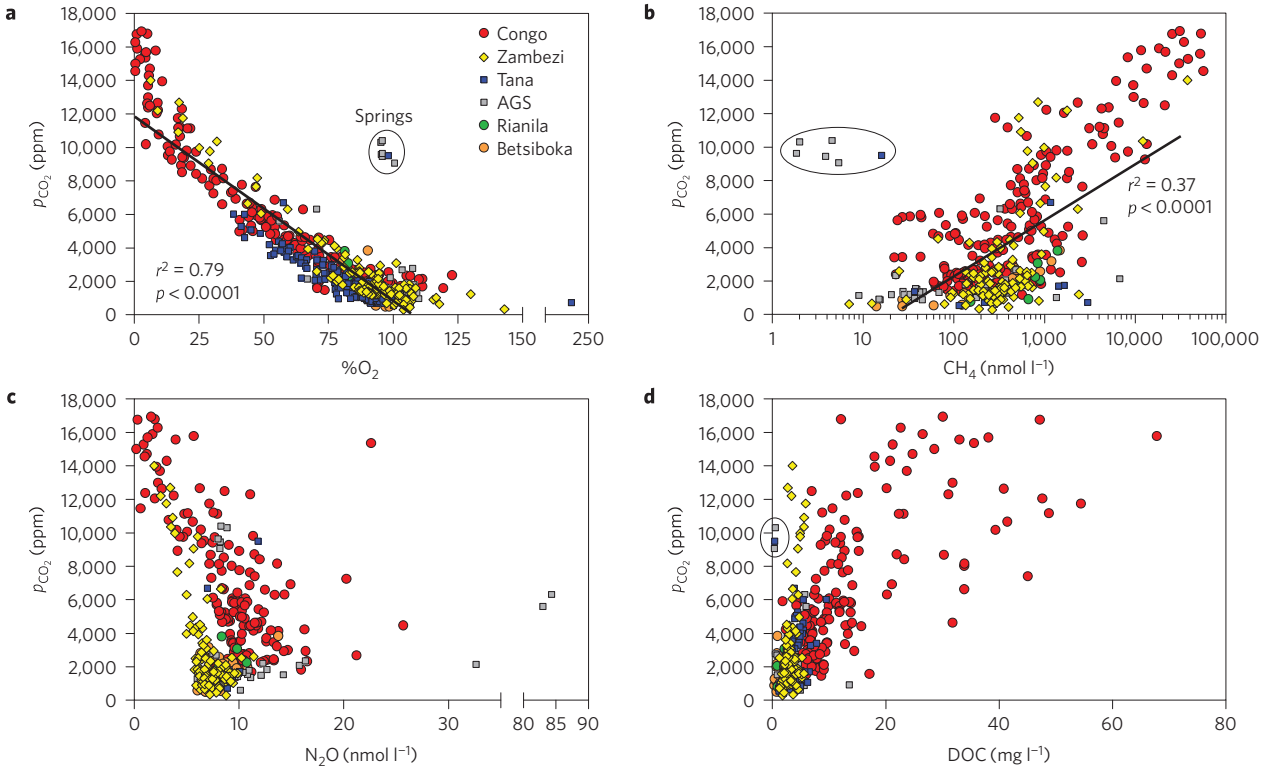


Figure 2 | Complex interplay of biogeochemical processes is revealed by GHGs property–property relations. p_{CO_2} as functions of %O_2 (**a**), log CH_4 (**b**), N_2O (**c**) and DOC (**d**) in six African river systems. The encircled data points correspond to water from springs that are enriched in CO_2, depleted in CH_4 and DOC owing to bacterial removal in groundwaters and at O_2 saturation owing to rapid equilibration with the atmosphere. Lines correspond to a linear regression fit.

order of magnitude as the integrated FCO_2 for the Amazon River of 0.47 PgC yr^{-1} (ref. 14), although the latter estimate included wetlands that accounted for 86% of flooded land whereas river channels only occupied 14% (ref. 13). The FCH_4 integrated for all rivers in SSA ranged between 2.9 ± 0.1 (Auf) and 3.9 ± 0.1 (Ray) TgC yr^{-1}. This is roughly five times higher than the FCH_4 recently attributed for all tropical rivers (0.7 TgC yr^{-1}) in a recent data synthesis[4], where, in the absence of empirical data from the tropics, the average CH_4 flux from temperate rivers was upscaled. The sum of integrated FCO_2 and FCH_4 ranged between 0.29 ± 0.05 (Auf) and 0.39 ± 0.07 (Ray) $PgCO_2e$ yr^{-1}. These values are highly significant when compared to other fluxes at the scale of the African continent given by the most recent overarching synthesis[8], such as the C emission from fossil fuel (~1.25 PgC yr^{-1}), terrestrial net ecosystem production (NEP) (1.2–2.9 PgC yr^{-1}), or TOC+DIC export to the ocean (0.06 PgC yr^{-1}). The emission of CO_2 and CH_4 in CO_2e from SSA rivers would thus balance two-thirds of the reported net C sink for continental Africa (0.6 ± 0.6 PgC yr^{-1}; ref. 8). Note that the net C sink estimate of the latter did not account for the fluxes from inland waters.

We propose that our emission estimates of CO_2 and CH_4 from SSA rivers may be conservative for two reasons. First, the FCH_4 values correspond only to diffusive emissions and do not account for CH_4 ebullition, which can be highly significant in tropical aquatic environments[17–19]. Based on floating chamber flux measurements in the Congo and Zambezi rivers ($n = 68$), we found CH_4 ebullition rates to be on average 0.25 times the diffusive CH_4 flux (Supplementary Fig. 4). This compares well with data from six Amazon river channels, where CH_4 ebullition was 0.33 times the diffusive CH_4 flux[19]. Applying a factor of 0.25 to estimate the CH_4 ebullition, the sum of integrated FCO_2 and FCH_4 would then range between 0.30 ± 0.05 (Auf) and 0.40 ± 0.07 (Ray) $PgCO_2e$ yr^{-1}. Second, our estimates do not account for extensive tropical wetlands, which in the Amazon are known to show CH_4 fluxes two orders of magnitude higher than river channels[17]. The Congolese 'Cuvette Centrale', with a flooded surface area of 360×10^3 km^2 (ref. 20), is the second largest tropical wetland area after the Amazon. The sum of FCO_2 and FCH_4 from the 'Cuvette Centrale' would correspond to 0.48 ± 0.08 $PgCO_2e$ yr^{-1} (based on the scaling of a subset of fluxes from the rivers, streams and navigation channels draining the 'Cuvette Centrale' and computed with the k recommended for flooded areas[2]). The above FCH_4 estimate for the 'Cuvette Centrale' does not include the ebullition of CH_4; using a reported ebullition:diffusion ratio of 0.73 for tropical wetlands[18] would bring the total FCO_2 and FCH_4 from SSA rivers and wetlands to range between 0.85 ± 0.10 (Auf) and 0.95 ± 0.11 (Ray) $PgCO_2e$ yr^{-1}. These fluxes are significant beyond the African continental scale, considering that the global net CO_2 sink is at present estimated at 1.5 and 2.0 PgC yr^{-1} for the oceans and the terrestrial biosphere, respectively[7].

Processes underlying GHG dynamics

Although it is crucial to quantify GHG fluxes, unravelling the underlying processes is equally important. This can be attempted with a correlation analysis with other biogeochemical variables and catchment characteristics. The p_{CO_2} data were well correlated to %O_2 and to the log of CH_4 (Fig. 2a,b), suggesting that the dynamics of the three variables were driven by net heterotrophy[1]. The lowest N_2O values (below atmospheric equilibrium) were observed at the highest p_{CO_2} (Fig. 2c) and lowest %O_2 levels (Supplementary Fig. 5), suggesting the removal of N_2O by denitrification, as also reported in the Amazon floodplains[21]. No distinct relationship could be established between N_2O and either nitrate (NO_3^-), ammonium (NH_4^+), or dissolved inorganic nitrogen ($DIN = NO_3^- + NH_4^+$)

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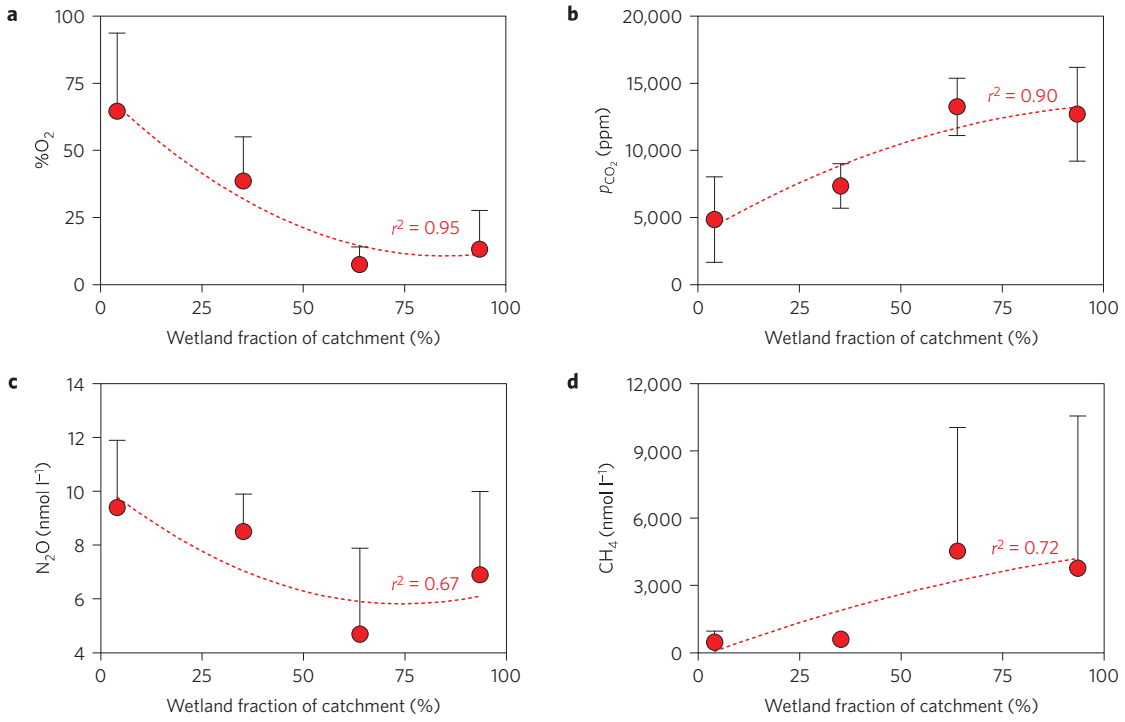


Figure 3 | Wetland presence drives the pattern of GHGs and O_2 in the Congo River. $\%O_2$ (**a**), p_{CO_2} (**b**), N_2O (**c**) and CH_4 (**d**) in 46 rivers of the Congo basin as a function of wetland fraction of catchment surface. Data were bin-averaged in intervals of 25%. Two extreme CH_4 values (>25,000 nmol l^{-1}) were removed from the analysis. Error bars show mean ± s.d. Lines correspond to a second-degree polynomial fit.

(Supplementary Fig. 5), unlike in some temperate rivers[5]. In three of the sampled rivers, data sets from long-term fixed station monitoring showed a positive relationship between N_2O and NO_3^- (Supplementary Fig. 6). The sampled rivers had relatively low DIN values[22], as fertilizer use is minimal within the catchments studied[23], and samples were not directly impacted by wastewaters from major cities; the only exception being the upper reaches of the Athi River, which are strongly influenced by wastewater inputs from Nairobi[22] and where the highest N_2O concentrations were recorded (Supplementary Fig. 3).

No relationship could be established between GHGs and elevation or water temperature (Supplementary Fig. 7). There was a general positive relationship between p_{CO_2} and dissolved organic C (DOC) (Fig. 2d) that has been used to infer the role of terrestrial organic matter inputs in sustaining net heterotrophy and CO_2 production within inland waters[9], although this can also be interpreted as an indication of concurrent DOC and CO_2 inputs from soils or wetlands. The p_{CO_2} in the Congo River levelled off for DOC concentrations >15 mg l^{-1}, which may indicate a limitation of bacterial growth (and subsequent CO_2 production) by the low pH in these very acidic organic environments (so called 'black waters', with pH between 3.6 and 5.9 and averaged 4.4), O_2 availability ($\%O_2$ ranged between 0.3% and 93.5% and averaged 23.4%; Supplementary Fig. 8), or phosphorus availability as reported in other tropical black water rivers[24].

Concentrations of GHGs increased as the wetland fraction of the catchment surface increased for individual catchments (Congo River) and across river systems (Figs 3 and 4). In the Congo River, p_{CO_2} and CH_4 were positively related to wetland fraction, whereas $\%O_2$ and N_2O were negatively related to the latter (Fig. 3). The CO_2, CH_4 and $\%O_2$ patterns in the Congo tributaries along a 1,700 km river stretch (Supplementary Fig. 9) and in the Zambezi River[25] also follow the presence of wetlands. Similarly, a relationship between CH_4, p_{CO_2} and $\%O_2$ with wetland fraction was also found across river systems (Fig. 4), consistent with findings in the Amazon basin, where CO_2 emissions from wetland lakes and river channels have been attributed to organic C from wetlands[11–13] that also sustain intense CH_4 evasion[17].

However, basins that are virtually devoid of wetlands, such as the Tana (<0.5% of the catchment), were also found to be sources of CO_2, although admittedly lower than other SSA rivers. This suggests that part of the CO_2 emissions from SSA rivers are partly sustained by upland biomass. The increase of CO_2 and CH_4 and decrease of $\%O_2$ in the Congo tributaries also follow the downstream increase of aboveground biomass and of the relative dense forest cover (Supplementary Figs 9 and 10). These patterns were also observed across river systems, although the Rianila catchment deviated from the general pattern owing to much steeper slopes within the basin (Supplementary Fig. 11). This highlights the role of relief, whereby gentle slopes increase water residence time and the extent of flooded areas, and steeper slopes enhance k, which drives dissolved GHG concentrations closer to saturation[26]. Precipitation has been previously used to model river CO_2 regionally[26]. We indeed found a positive relationship between p_{CO_2} and CH_4 and precipitation across SSA rivers, with the exception of the two Malagasy rivers (Supplementary Fig. 11). For the Rianila, this might also be related to the steeper slope. In the Betsiboka, nearly all of the yearly precipitation occurs from December to March, the rest of the year being extremely dry, leading to vegetation typical of a semi-arid climate. This suggests that models to predict p_{CO_2} from precipitation should also account for the regional differences in seasonal patterns.

Lateral inputs versus *in situ* respiration as CO_2 drivers

In addition to our GHG concentration measurements, we acquired 812 aquatic community respiration (R) data (Supplementary Fig. 12). The R values ranged between 0.3 and 404.0 mmolC m^{-3} d^{-1}, with a median of 20.0 mmolC m^{-3} d^{-1}, which is close to the median value of pelagic bacterial respiration reported in a recent data synthesis in tropical inland waters[27]. On individual paired mea-

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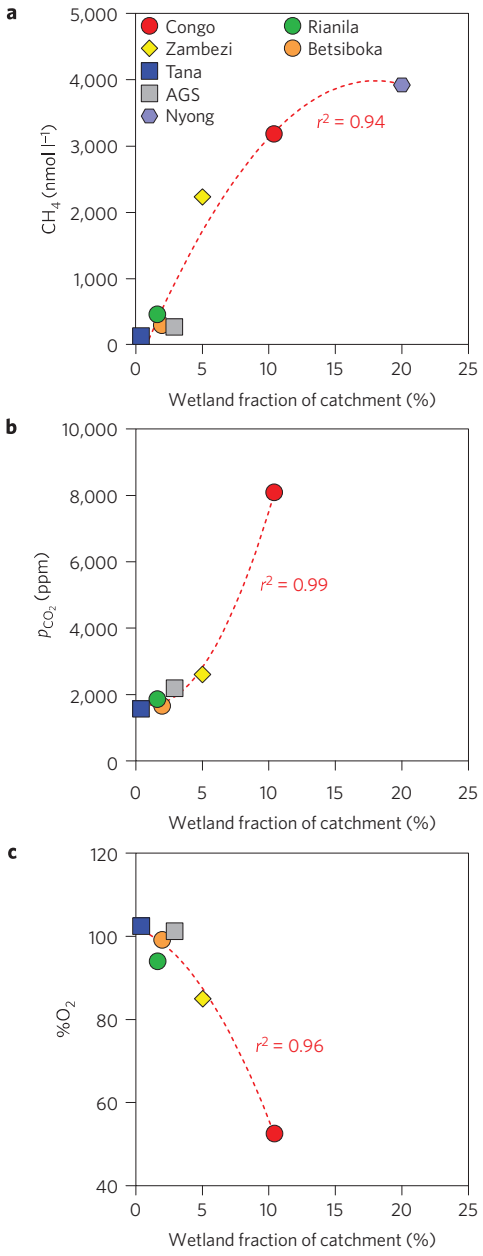


Figure 4 | Wetland presence drives the pattern of CO_2 and CH_4 concentrations across sub-Saharan African Rivers. p_{CO_2} (**a**), CH_4 (**b**) and $\%O_2$ (**c**), as a function of the wetland fraction of catchment surface (%) in seven African rivers. Only the data sets that capture spatial variations were included in the analysis (excluding fixed time series in the mainstem of rivers). Lines correspond to a second-degree polynomial fit.

surements, the FCO_2 was on average 8 times (Auf) to 12 times (Ray) higher than vertically integrated R ($n = 602$). The median R was 99.4 mmolC m^{-2} d^{-1}, which, when upscaled to all SSA rivers, can account only for 11% of the FCO_2 (0.27 PgC yr^{-1}). Although benthic respiration was not measured during our study, using the average value from a recent data synthesis for tropical rivers and streams (50.8 mmolC m^{-2} d^{-1}; ref. 28), we find that pelagic and benthic R combined still account for only <14% of the FCO_2. Furthermore, these estimates do not account for pelagic and benthic aquatic primary production, which would decrease the dissolved CO_2 concentration. These calculations imply that lateral inputs of CO_2 from soils, groundwaters and wetlands would be the largest contributors of the CO_2 emitted from rivers and streams.

Implications for understanding global C fluxes

Large CO_2 fluxes from inland waters have significant implications for our understanding of overall C fluxes and cycling at the landscape or catchment scale. The CO_2 emitted to the atmosphere from inland waters can be considered as a component of the respiration of upland and wetland vegetation, whether it is related to lateral transport of soil/wetland CO_2 (that is, respiration taking place in terrestrial or wetland habitats) or lateral transport of soil or wetland DOC and POC that is mineralized to CO_2 within the aquatic domain. Hence, the lateral transport of C from the upland terrestrial biosphere and wetlands to inland waters and its subsequent emission to the atmosphere offsets the estimates of terrestrial NEP. Furthermore, lateral transport of soil DOC leads to an underestimation of NEP based on biomass accumulation techniques, whereas the lateral transport of soil CO_2 leads to an overestimation of NEP derived from atmospheric measurements (eddy-covariance or flux towers). The significant FCO_2 from African inland waters at the continental scale is particularly important in the context of the mitigation of, and accounting for, GHG emissions, as C emissions related to tropical deforestation are only partially offset by C sequestration from forest regrowth[29]. Accounting for the additional C emissions from inland waters could further offset this balance. Alternatively, if most of the CO_2 emissions from SSA river channels are derived from wetland C, the net balance would be nearly neutral as it is balanced by the atmospheric CO_2 fixation by the emerged vegetation, as shown in the Amazon lowland areas[11–13]. Untangling the relative contributions of wetland and upland C in sustaining CO_2 and CH_4 emissions from inland waters is essential to better understand the role of tropical inland waters in the global C cycle and related potential feedbacks on a warming climate. Our results in SSA rivers show that this relative contribution is variable both within individual catchments and across catchments.

Methods

Methods and any associated references are available in the online version of the paper.

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Author contributions

A.V.B. and S.B. conceived and designed the study and coordinated the project and fieldwork. Field data collection was carried out by all co-authors. T.L. carried out the geographical system information (GIS) analysis. A.V.B. drafted the manuscript, which was substantially commented upon and amended by S.B., C.R.T., T.R.M., N.G., T.L. and F.G. All co-authors approved the manuscript.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to A.V.B.

Competing financial interests

The authors declare no competing financial interests.

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Methods

We acquired 1,880 data for CH_4, 1,625 data for N_2O and 693 data for p_{CO_2} from June 2006 to September 2014 in 12 river basins (Supplementary Table 1). The dissolved gases were measured with a uniform method based on the headspace technique, either directly in the field by infrared gas analysis (IRGA; for CO_2), or on return in the laboratory by gas chromatography (GC; for CH_4 and N_2O). Our analysis excludes p_{CO_2} data computed from pH and total alkalinity, because of strong biases due to the interference from organic acids, in particular in organic C rich black waters[15,16].

Sampling and field measurements. Two approaches were used during this study (Supplementary Table 1). First, a 'survey' approach aimed at sampling the mainstem of a river network over the longest possible stretch, as well as a maximum number of contributing tributaries, typically during a short period of time (2–60 d). In most cases, the surveys were done by car, with sampling from boats, bridges, or from the shore. In other cases, travel and sampling were carried out by boat (Congo River). Second, a 'monitoring' approach was applied at various fixed stations during a 1–2 yr period, with sampling taking place at monthly or fortnightly intervals in the mainstem of one or several rivers. The two approaches are highly complementary as the 'survey' approach provides a snapshot of the spatial variability, whereas the 'monitoring' approach provides the range of seasonal variability, but with little or no description of the spatial heterogeneity.

Water was collected with a Niskin bottle just below the surface (<0.5 m) and two serum bottles (50 ml) for the determination of CH_4 and N_2O were filled through tubing, allowed to overflow, poisoned with a saturated solution of $HgCl_2$ (50–100 µl), sealed with butyl stoppers, crimped with aluminium caps, and stored at ambient temperature in the dark. Samples for the determination of p_{CO_2} were directly collected in surface waters in four plastic 60 ml syringes and 30 ml of sample water was equilibrated with 30 ml of ambient air (5–10 min of vigorous shaking). The p_{CO_2} in ambient air and in the equilibrated gas phase were determined with a portable IRGA, from which the *in situ* p_{CO_2} was computed using the temperature values *in situ* and in the equilibrated water (that is, in the syringe), and Henry's constant. The IRGAs were calibrated with N_2 and a CO_2:N_2 commercial mixture (Air Liquide Belgium) with a mixing ratio of 1,017 ppm of CO_2 (PP Systems EGM-4 and Li-Cor Li-820) or with N_2 and a suite of CO_2:N_2 commercial mixtures (Air Liquide Belgium) with mixing ratios of 388, 813, 3,788 and 8,300 ppm CO_2 (Li-Cor Li-840). The Li-840 was used on the Congo in December 2013 and June 2014, the EGM-4 was used on the Congo in December 2012 and September 2013 and on the Zambezi, whereas the Li-820 was used on the Malagasy, Tana and Athi-Galana-Sabaki rivers. In the Congo in March 2013, the headspace was injected in pre-evacuated 12 ml Exetainer (Labco) vials and the CO_2 content was analysed by GC back in the laboratory (see hereafter). The overall precision of p_{CO_2} measurements was ±2.0% (n = 447 replications of three to four measurements).

Water temperature, conductivity, $\%O_2$ and pH were measured *in situ* with portable field probes calibrated using standard protocols (in most cases using an YSI Proplus probe). Pelagic R was determined from the decrease of O_2 in 60 ml biological oxygen demand bottles over ~24 h incubation periods. The bottles were kept in the dark and close to *in situ* temperature in a cool box filled with *in situ* water. The O_2 decrease was determined from triplicate measurements at the start and the end of the incubation with an optical O_2 probe (YSI ProODO), and R data were converted into carbon units using a respiratory quotient of 1.3 (ref. 30). Samples for the DOC determination were preserved in 40 ml borosilicate vials with polytetrafluoroethylene-coated stoppers and preserved with H_3PO_4 (85%) after filtration through 0.2 µm pore size polyethersulphone (PES) syringe filters. Samples for nitrate (NO_3^-) and ammonium (NH_4^+) were filtered on a 0.2 µm PES syringe filter, collected in 50 ml plastic vials, to which was added 200 µl of H_2SO_4 5N.

Laboratory chemical analysis. Concentrations of CH_4 and N_2O were determined via the headspace equilibration technique (20 ml N_2 headspace in 50 ml serum bottles) and measured by GC (ref. 31) with flame ionization detection (GC-FID) and electron capture detection (GC-ECD) with a SRI 8610C GC-FID-ECD calibrated with CH_4:CO_2:N_2O:N_2 mixtures (Air Liquide Belgium) of 1, 10 and 30 ppm CH_4 and of 0.2, 2.0 and 6.0 ppm N_2O, and using the solubility coefficients of CH_4 (ref. 32) and N_2O (ref. 33). For the Nyong, the CH_4 was determined with a SRI 8610C GC-FID calibrated with CH_4:N_2 mixtures (Air Liquide France) of 2, 10 and 100 ppm CH_4. For the Ivory Coast rivers, the CH_4 was determined by GC-FID, as described elsewhere[34]. The overall precision of measurements was ±3.9% (n = 1,057 duplicate measurements) and ±3.2% (n = 900 duplicate measurements) for CH_4 and N_2O, respectively. DOC was analysed according either on a Thermo HiperTOC-isotope ratio mass spectrometer (IRMS) or with an Aurora1030 TOC analyser (OI Analytical) coupled to a Delta V Advantage IRMS, with a precision better than ±5% (ref. 35). NO_3^- and NH_4^+ concentrations were estimated by spectrophotometry, using the dichloroisocyanurate-salicylate-nitroprussiate colorimetric method for NH_4^+ (ref. 36) and the sulphanilamide colorimetric method for NO_3^- (refs 37,38). The detection limits were 0.30 and 0.15 µmol l^{-1} for NH_4^+ and NO_3^-, respectively.

GHG flux computations. The air–water gas flux (F) was computed according to

$$F = k\Delta C$$

where k is the gas transfer velocity and ΔC is the air–water gas concentration gradient[39], whereby a positive value corresponds by convention to an emission of gas from the water to the atmosphere.

We used the monthly average corresponding to time of sampling of atmospheric p_{CO_2} from Mount Kenya (Kenya, −0.05° N 37.80° E), retrieved from the GLOBALVIEW-CO2 database (Carbon Cycle Greenhouse Gases Group of the National Oceanic and Atmospheric Administration (NOAA), Earth System Research Laboratory (ESRL)) and of N_2O from Mauna Loa (Hawaii, 19.54° N–155.85° E) from the NOAA/ESRL Chromatograph for Atmospheric Trace Species (CATS) Program. For atmospheric CH_4, a constant mixing ratio of 1.9 ppm was used. Atmospheric mixing ratios were converted from dry air to wet air using the water vapour computed from temperature[33] and into corresponding dissolved concentrations using solubility coefficients of CO_2 (ref. 40) , CH_4 (ref. 32) and N_2O (ref. 33). Fluxes of CH_4 and N_2O were converted into CO_2 equivalents based on the assumption that over a 100-year period the emissions of 1 kg of CH_4 and 1 kg of N_2O correspond to 34 kg and 298 kg of CO_2, respectively[6]. For k, we used two approaches. The first approach[2] uses a constant k normalized to a Schmidt number (Sc) of 600 (k_{600}) of 17.2 cm h^{-1} for streams and small rivers (<100 m width), of 12.3 cm h^{-1} for larger rivers (>100 m width) and of 2.4 cm h^{-1} for wetlands. The second approach[3] provides k_{600} values per river basin corresponding to the average value for the whole river network up to stream order 1 derived from hydraulic equations[41] and a geographical information system (GIS) description of corresponding input hydraulic variables for each catchment. The Sc numbers of CO_2, N_2O and CH_4 were computed from water temperature[42]. The F data were aggregated to derive one value per tributary and per river mainstem, before averaging for a given river system. The global F values were computed as averages weighted by water body surface area for each river catchment derived from the percentage of river/stream effective surface area per catchment given in ref. 3. The F values were similarly upscaled to SSA using the river/stream surface areas given in ref. 3.

FCO_2 and FCH_4 were measured in parallel with a floating chamber in 25 stations in the Congo basin, and 43 stations in the Zambezi basin. The floating chamber consisted of an opaque polyvinyl chloride cylinder 15 cm in height with a 38 cm internal diameter holding a volume of 17 l, and a 7-cm-long underwater skirt. The chamber was deployed for 30 min and the p_{CO_2} change inside the chamber was determined directly with an IRGA (30 s logging), whereas 30 ml gas samples for CH_4 extracted from inside the chamber at 0, 5, 10, 20 and 30 min interval were injected into 50 ml serum vials full of a hyper-saline solution (saturated solution of NaCl) for analysis in the laboratory by GC-FID. The fluxes were computed from the temporal change of the partial pressure of the gases, the law of perfect gases, and the geometry of the chamber[43]. The k_{600} of CO_2 was computed based on the measured FCO_2 and the p_{CO_2} in water and air. Assuming that the FCO_2 is exclusively diffusive (that is, that the CO_2 ebullition flux is negligible), this k_{600} of CO_2 allows the computation of the diffusive CH_4 flux from the dissolved CH_4 concentration. This in turn allows the computation of the ebullition CH_4 flux from the chamber CH_4 measurement that captures both ebullition and diffusive CH_4 fluxes. Chamber measurements have been assumed to provide biased k values[44], although often comparing satisfactorily with atmospheric flux measurements[45,46] or infrared imaging of the water surface[47]. The obtained average k_{600} in the Congo (12 ± 11 cm h^{-1}) was not significantly different from the average in the Zambezi (10 ± 11 cm h^{-1}) (unpaired t-test, $p < 0.05$). These values are distinctly lower than those computed by Raymond *et al.*[3] of 22 and 20 cm h^{-1} for the Congo and Zambezi, respectively. This is related to the fact that Raymond *et al.*[3] provide k_{600} values for the whole basin, including low-order streams that are more turbulent than higher-order streams and rivers, and characterized by higher k_{600} values[41]. Our data were acquired in the mainstem of rivers and adjacent large tributaries, typically high-order systems. The k_{600} values in the highest stream order in the Congo (9) and Zambezi (8) given by Raymond *et al.*[3] are, respectively, 14.5 and 13.3 cm h^{-1}—closer to those derived from our chamber measurements. The Congo and Zambezi streams (<100 m width) had k_{600} values (11 ± 10 cm h^{-1}) that were not significantly different from those (10 ± 7 cm h^{-1}) in rivers (>100 m width) (unpaired t-test, $p < 0.05$). These values were close to the k_{600} value for rivers (>100 m width) of 12 cm h^{-1}, but lower than the value for streams (<100 m width) of 17 cm h^{-1} given by Aufdenkampfe and colleagues[2]. This reflects the fact that our data in the Congo and Zambezi were obtained in high-order streams, mainly in regions with gentle slopes (lowlands). Nevertheless, the actual accuracy of the chamber k values is irrelevant in the present case because we aimed at determining the CH_4 ebullition from the chamber measurements. Yet, these CH_4 ebullition estimates are probably conservative and provide underestimates, as for logistical reasons there was no replication of chambers and the deployment was short relative other studies, where it varies between 1 h (ref. 19) and 24 h (ref. 18). Indeed, CH_4 ebullition is notoriously heterogeneous both in space and time (across different timescales from

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daily to seasonal)[48–51], and adequately capturing this heterogeneity would require a sampling effort and design incompatible with other aims of our research.

GHG flux error analysis. An error analysis on the GHG flux computation and upscaling was carried out by error propagation of the GHG concentration measurements, the *k* value estimates, and the estimate of surface areas of river channels to scale the areal fluxes, using a Monte Carlo simulation with 1,000 iterations. The uncertainty on the GHG concentrations led to an uncertainty of areal fluxes of ±1.2%, ±2.3% and ±5.2% for CO_2, CH_4 and N_2O, respectively. Whereas no information is available in Aufdenkampe *et al.*[2] regarding the uncertainty on *k* values, the uncertainty on *k* given by Raymond *et al.*[3] was estimated to be ±10.0%, based on the errors on slope and constant of the parameterization[41]. This leads to a cumulated uncertainty of areal fluxes of ±4.6%, ±4.9% and ±6.7% for CO_2, CH_4 (diffusive) and N_2O, respectively. Ebullition of CH_4 is highly heterogeneous spatially and temporally[48–51], and it is difficult to quantify this uncertainty. We arbitrarily assigned an uncertainty of ±50% for CH_4 ebullition. This leads to a cumulated uncertainty of total areal fluxes of ±29.4% for CH_4 (diffusive + ebullitive). The river/stream surface areas reported by Raymond *et al.*[3] were estimated using two different hydraulic equations, which allow one to estimate an uncertainty of ±31.0%. The overall uncertainty of integrated fluxes in river channels is ±18.8%, ±18.3%, ±34.2% and ±19.2% for CO_2, CH_4 (diffusive only), CH_4 (diffusive + ebullitive) and N_2O, respectively. The comparison of the wetland mapping of Bwangoy *et al.*[20] and of the Africover vegetation map, allows one to evaluate the uncertainty of the surface area of Congolese 'Cuvette Centrale' to ±10%. The overall uncertainty of integrated fluxes in the Congolese 'Cuvette Centrale' is ±7.0% and ±30.6% for CO_2 and CH_4 (diffusive + ebullitive), respectively.

Other sources of uncertainty that are not straightforward to quantify relate to seasonal and spatial representativeness. For all spatial surveys, we paid particular attention to cover the widest possible spectrum of systems from mainstem to headwaters, to capture spatial variations in the most comprehensive way given logistical constraints. Data from all basins cover different hydrologic conditions and average fluxes capture seasonality. Spatial and seasonal variations have been presented and discussed for some basins in site-specific studies[22,25,34,52–54].

GIS analysis of catchment characteristics. Mean slope and drainage area were extracted from the HYDRO1K global hydrologic data set[55]. The fractional land cover type of the watershed was extracted from the Global Land Cover (GLC) 2000 database of Africa[56]. In total, 27 different land sub-classes are defined in the GLC database, taking into account the dominant vegetation class (namely trees, shrubs and grasses), phenology, seasonality, flooding regime and altitude. These sub-classes were aggregated in seven first-level classes based on vegetation structural categories: dense forest, mosaic forest/other, woodlands and shrublands, grasslands, agricultural lands, bare soils and others (water bodies, urban). Aboveground biomass in Mg km^{-2} (Mg = 10^9 g) was extracted from the Woods Hole Research Center (WHRC) pantropical national level C stock data set[57]. The extent of wetland and floodplain areas was extracted from the Global Lakes and Wetlands Database[58], except for the Nyong[59] and the Tana (Omengo, F. O. Based on A GIS Analysis of Landsat Images). Annual precipitation was extracted from the WorldClim Global Climate Database[60].

Data availability. The full data set of CO_2, CH_4 and N_2O concentrations is available as Supplementary Information.

Data sources. Several publicly available data sources were used:
HYDRO1K global hydrologic data set: https://lta.cr.usgs.gov/HYDRO1K
GLC 2000 database of Africa:
http://forobs.jrc.ec.europa.eu/products/glc2000/products.php
WHRC pantropical national level C stock data set:
http://www.whrc.org/mapping/pantropical/carbon_dataset.html
Global Lakes and Wetlands Database:
https://www.worldwildlife.org/pages/global-lakes-and-wetlands-database
WorldClim—Global Climate Data: http://www.worldclim.org
Data on river/stream surface and *k* values from: http://www.nature.com/nature/journal/v503/n7476/abs/nature12760.html#supplementary-information

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SUPPLEMENTARY INFORMATION

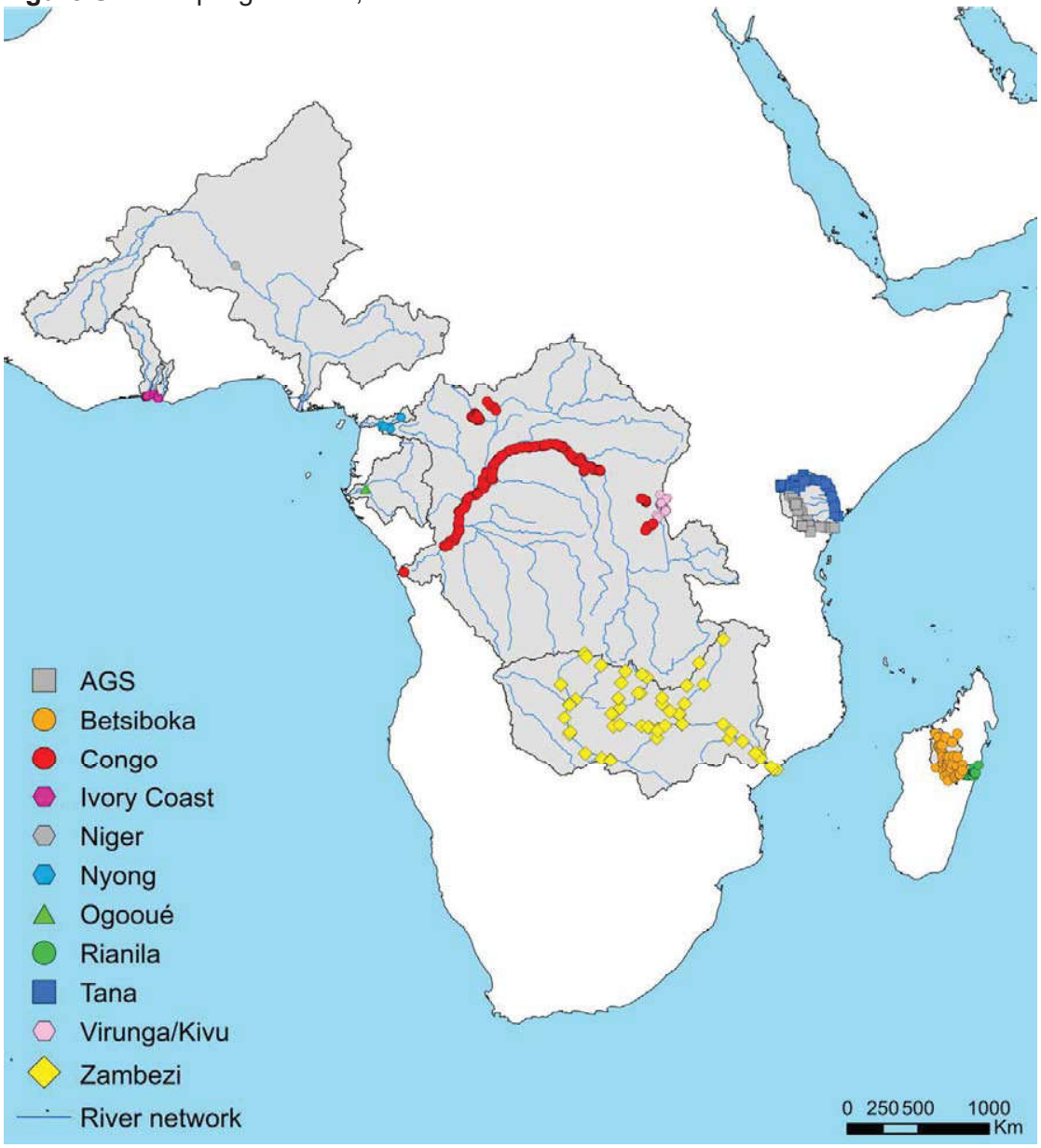
DOI: 10.1038/NGEO2486

Globally significant greenhouse-gas emissions from African inland waters

Alberto V. Borges, François Darchambeau, Cristian R. Teodoru, Trent R. Marwick, Fredrick Tamooh, Naomi Geeraert, Fredrick O. Omengo, Frédéric Guérin, Thibault Lambert, Cédric Morana, Eric Okuku and Steven Bouillon

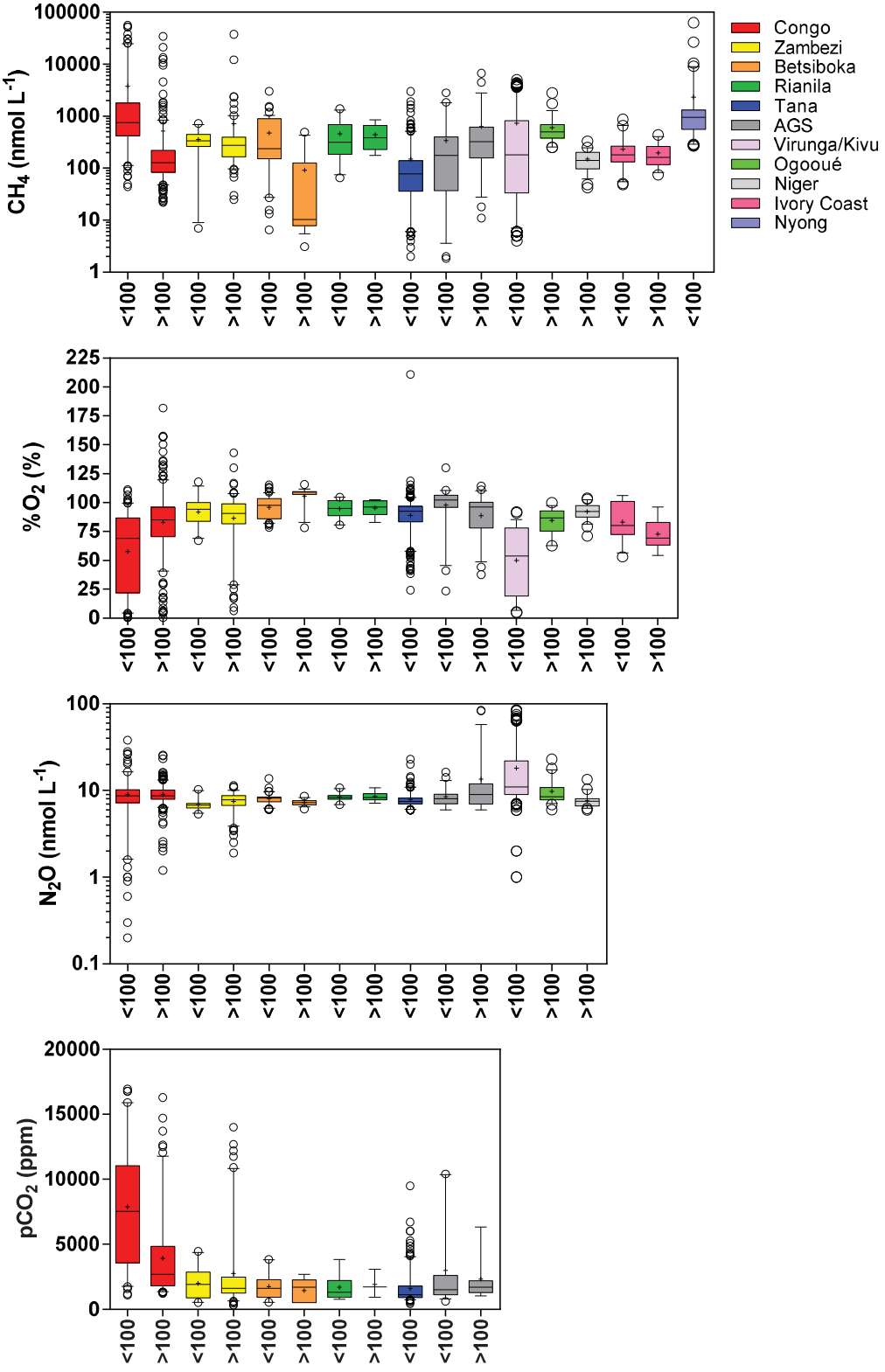
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Figure S1. Sampling stations, catchment and river network of the studied Sub-Saharan rivers.



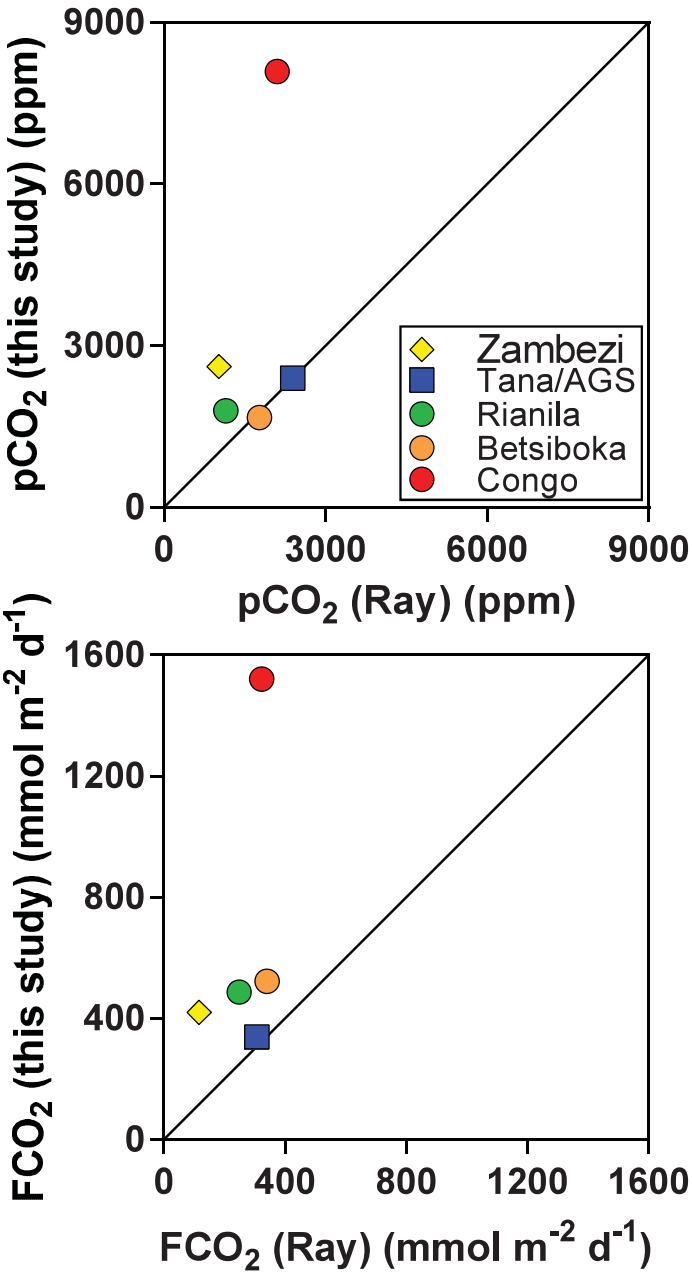
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Figure S2. Box and whisker plots of pCO_2, CH_4, N_2O and $\%O_2$ in the studied Sub-Saharan rivers. Data were categorized into small (<100 m width) and larger (>100 m width) rivers/streams. The horizontal line corresponds to the median, the cross to the average, error bars correspond to the 5 and 95 percentile, symbols to outliers.



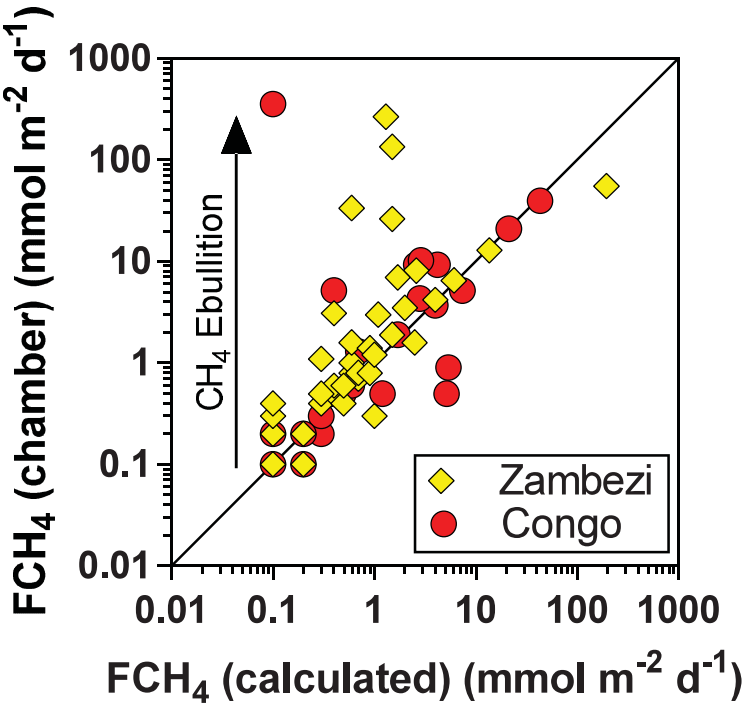
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Figure S3. Comparison of the basin-wide averages of pCO_2 and FCO_2 reported in the present study and by[3] for the Congo, Zambezi, Tana, Rianila and Betsiboka Rivers. The strongest deviations in pCO_2 between data from this study and the modelled data from[3] were observed for the rivers (Zambezi and Congo) with extensive wetland coverage (5 and 10% of the total catchment, respectively).



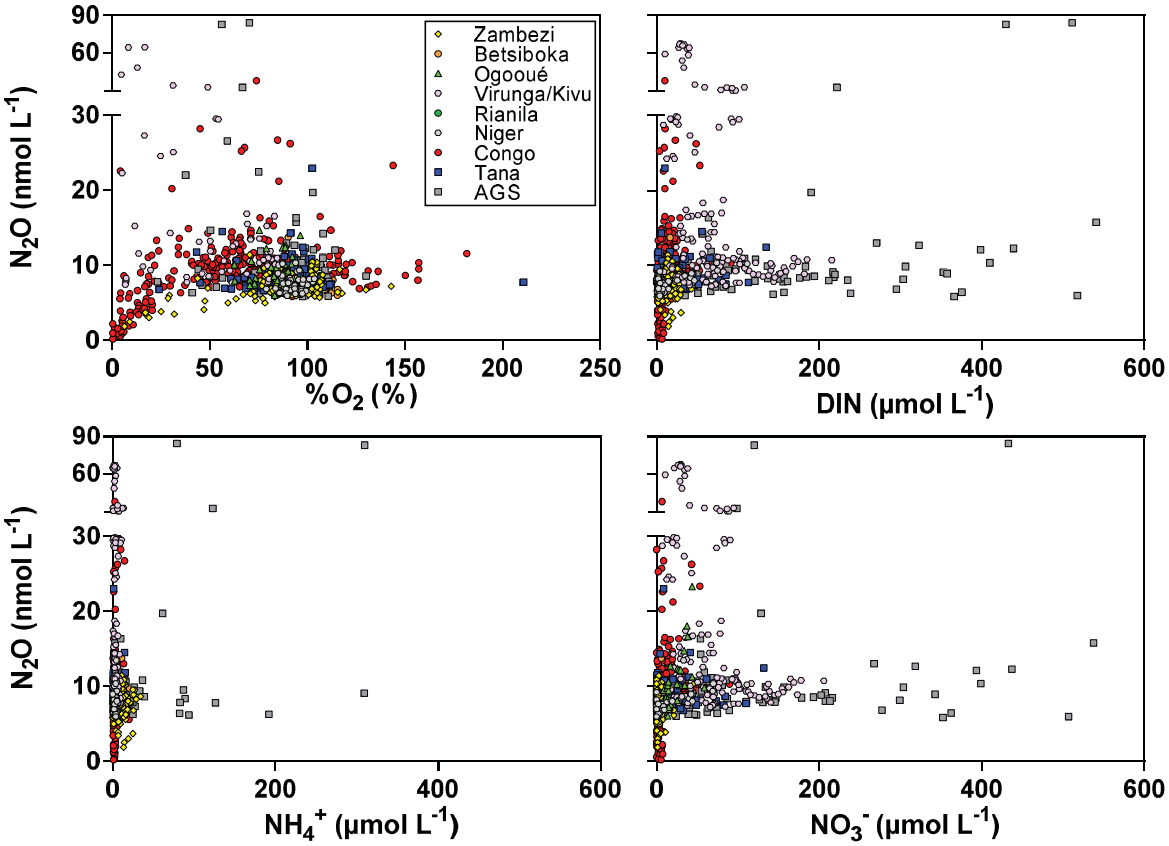
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Figure S4. Comparison of the CH_4 flux calculated from the k_{600} derived from the corresponding chamber FCO_2 (that should correspond exclusively to the diffusive CH_4 flux) and the CH_4 flux measured with the floating chamber (that integrates the ebullition CH_4 flux and the diffusive CH_4 flux). The difference between both approaches corresponds to the ebullition CH_4 flux. The highest ebullition flux (2575 times higher than the diffusive CH_4 flux) was measured in a *Vossia cuspidata* patch in Congo River.



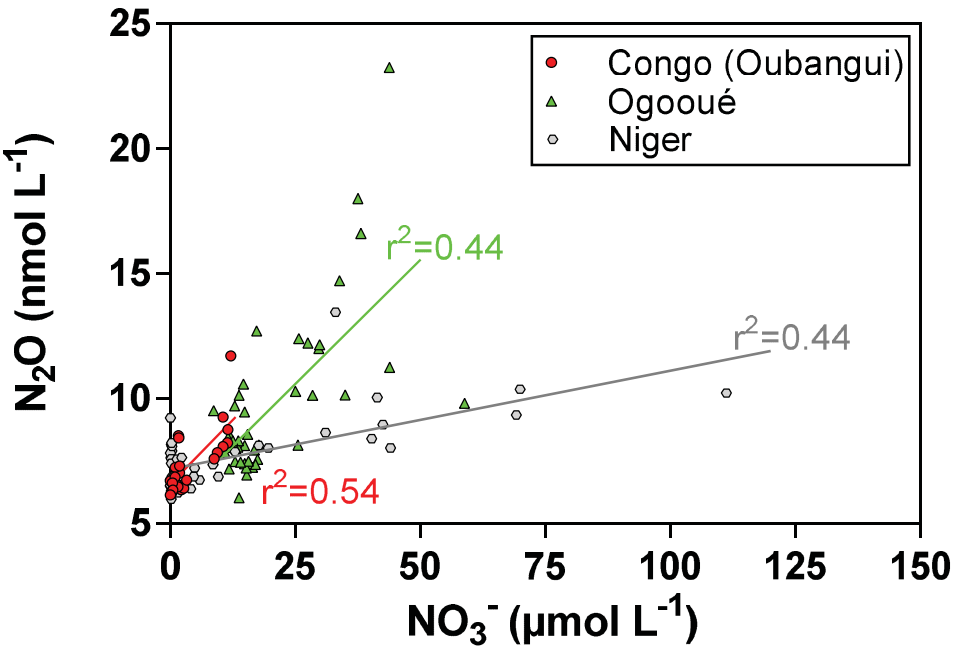
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Figure S5. N_2O as a function of $\%O_2$, NO_3^-, NH_4^+ and DIN in the studied African rivers.



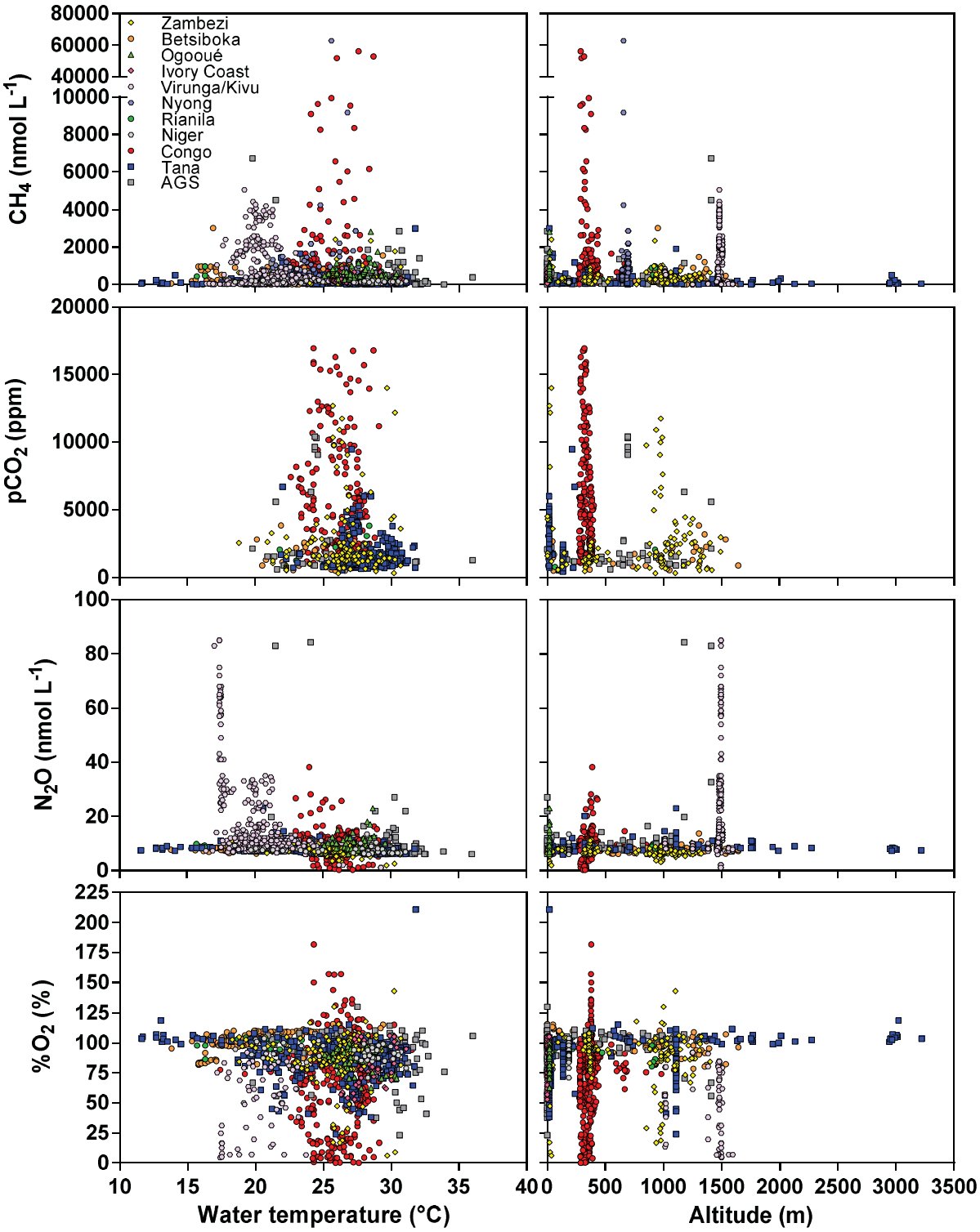
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Figure S6. N_2O as a function of NO_3^- from seasonal monitoring on the mainstem (fixed station) in the Oubangui (Congo River), Niger and Ogooué.



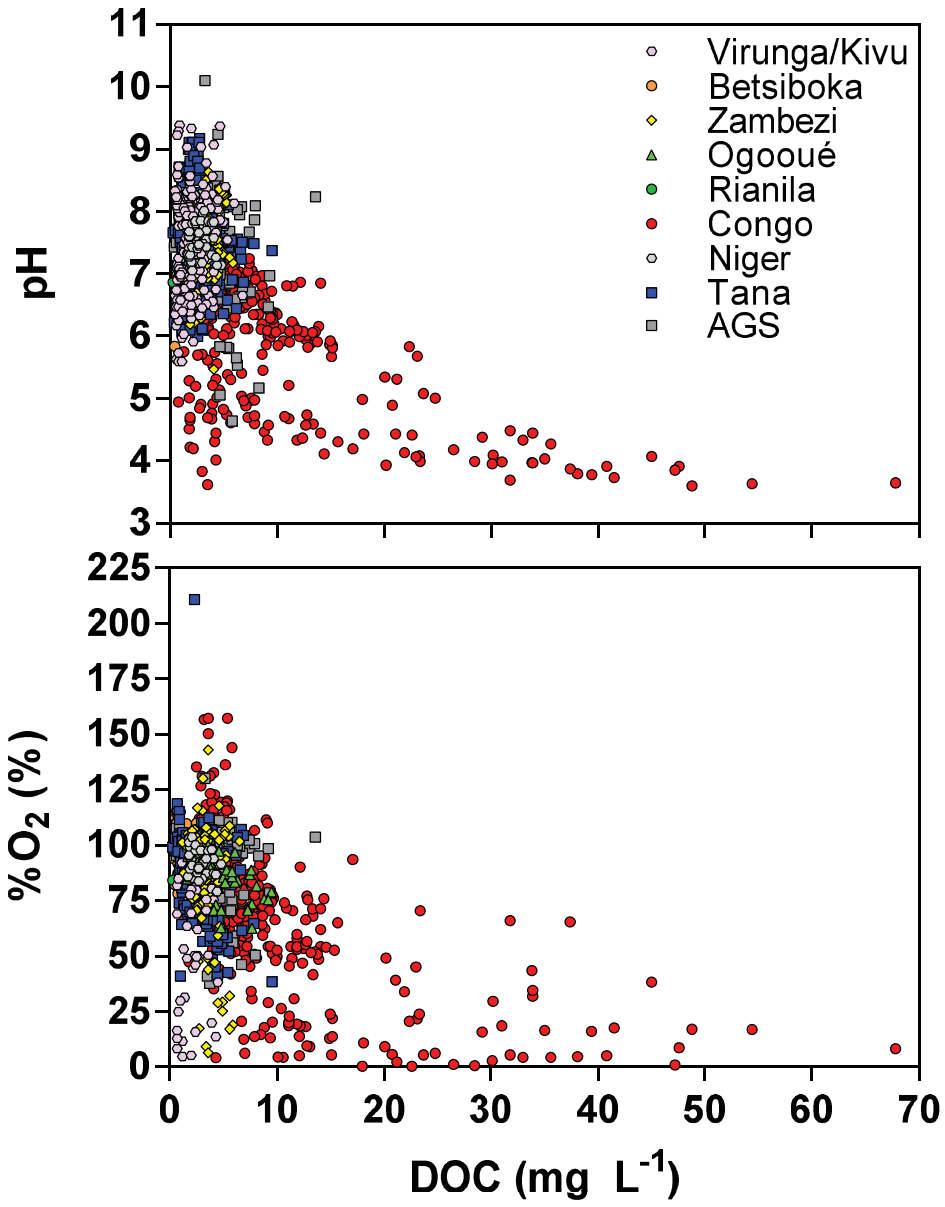
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Figure S7. CH_4, pCO_2, N_2O and $\%O_2$ as a function of water temperature and altitude in the studied African rivers.



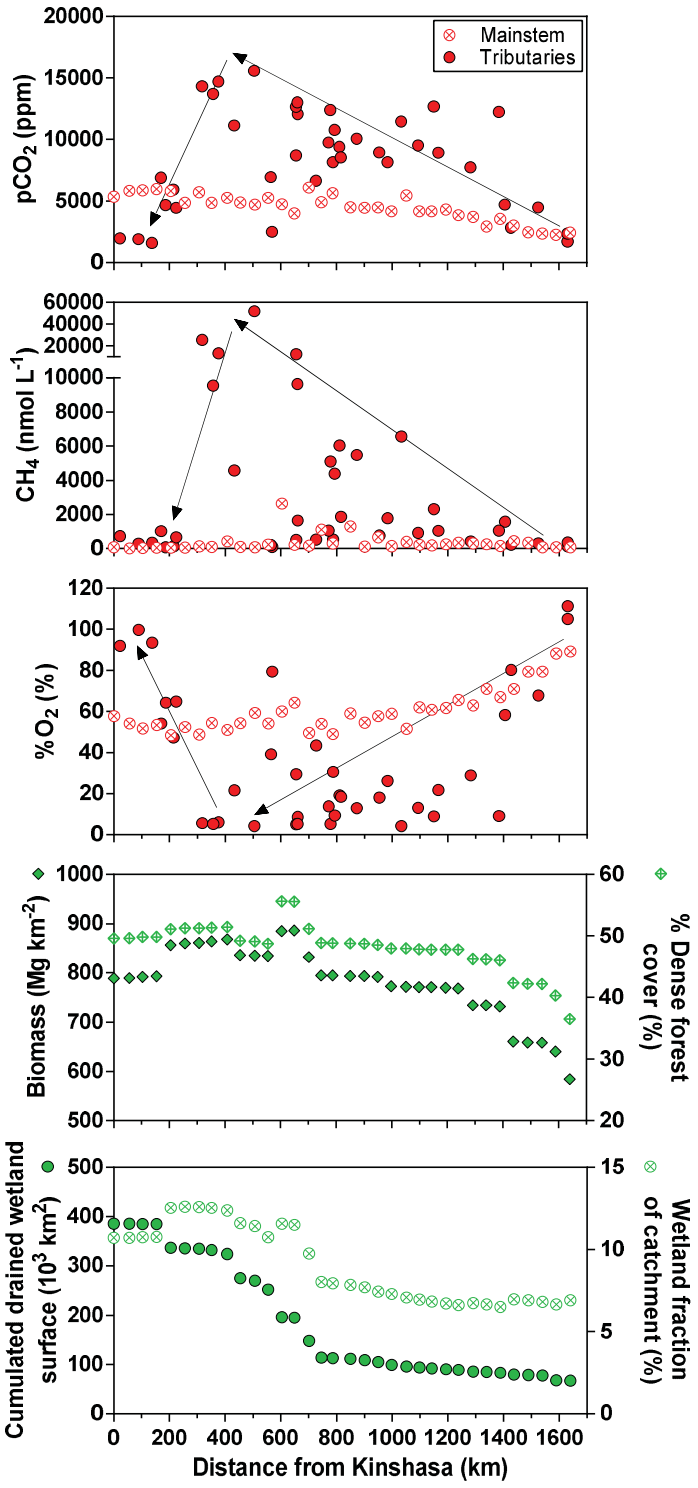
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Figure S8. pH and $\%O_2$ as a function of DOC in the studied African rivers.



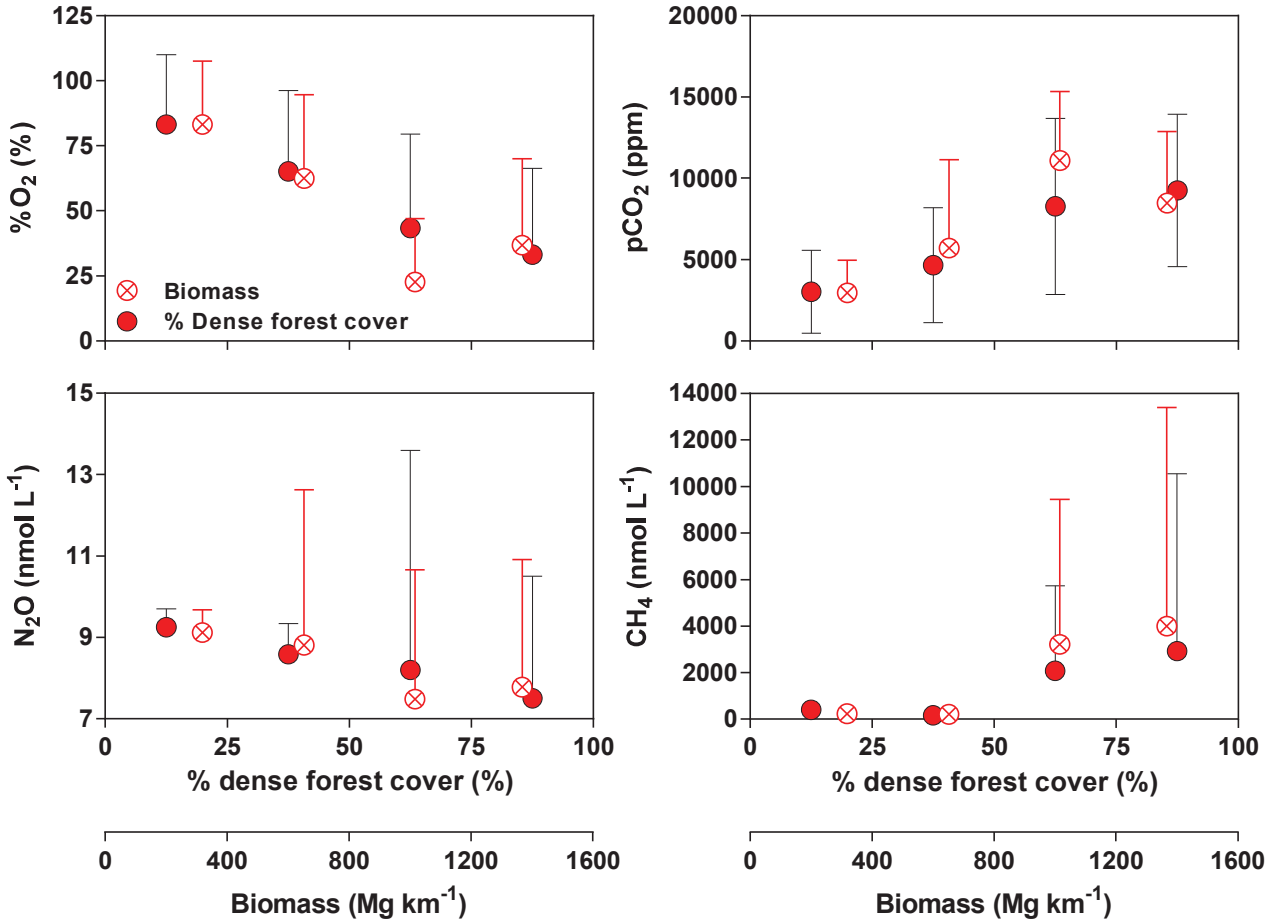
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Fig. S9. Longitudinal variations in the Congo mainstem and tributaries along a transect in December 2013 (high water) from Kisangani to Kinshasa of pCO_2 (ppm), CH_4 (nmol L^{-1}) and $\%O_2$ (%), and aboveground woody biomass (Mg km^{-2})[57], % of dense forest cover (%)[56], and wetland surface (cumulated surface and fraction of catchment)[58]. The strong increase in cumulated wetland surface between 200 and 700 km upstream of Kinshasa corresponds to the core of the "Cuvette Centrale". About 200 km upstream of Kinshasa, the catchment becomes dominated by shrubby savannah. Aboveground biomass and dense forest cover increase gradually along the transect and decrease 200 km above Kinshasa with savannahs becoming more prevalent. The pCO_2 and CH_4 concentration increase downstream towards the "Cuvette Centrale" and collapse in the savannah area upstream of Kinshasa. These patterns are mirrored by $\%O_2$ (%).



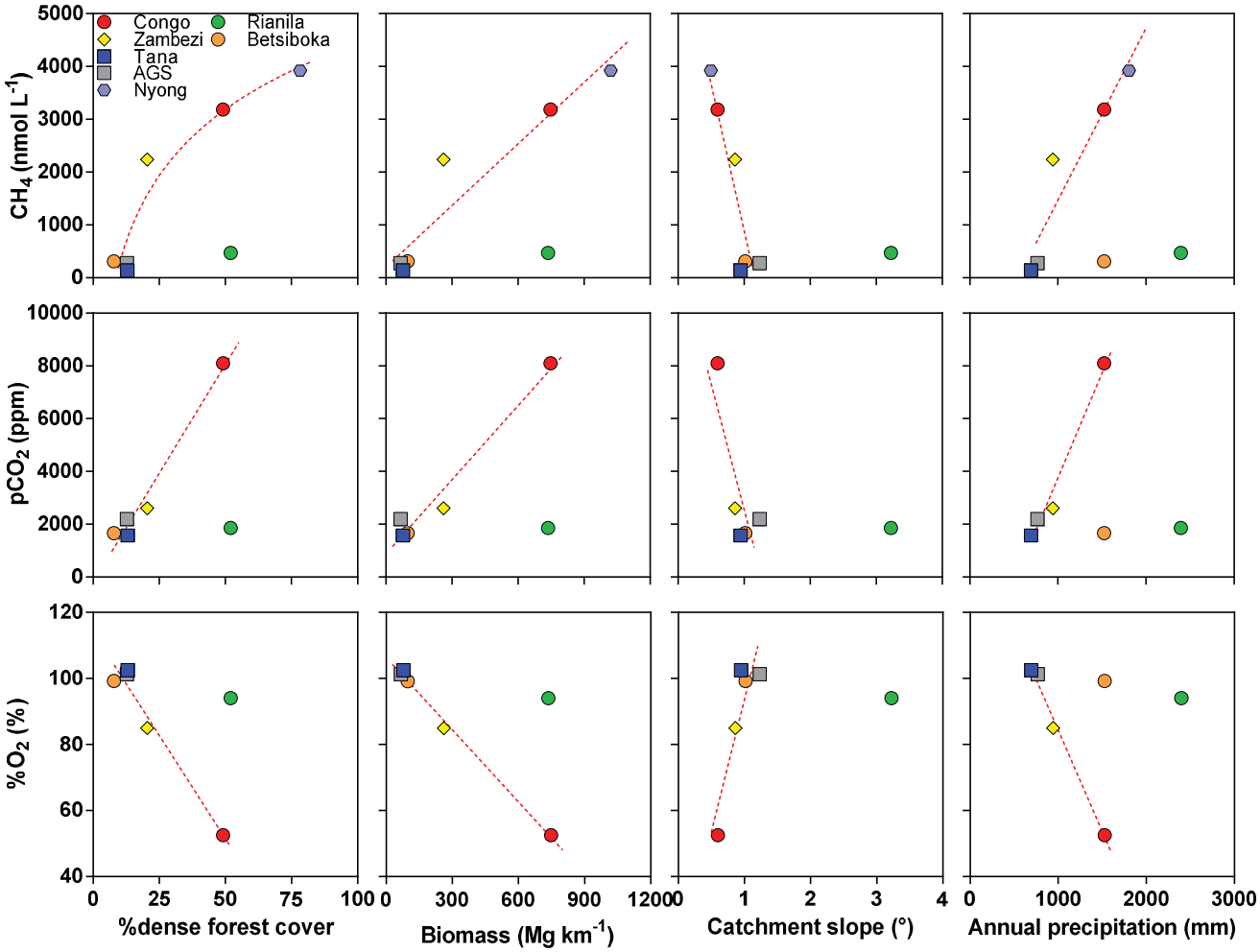
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Fig. S10. pCO_2 (ppm), CH_4 (nmol L^{-1}), N_2O (nmol L^{-1}) and $\%O_2$ (%) in 46 rivers of the Congo basin as function of dense forest cover[56] and aboveground woody biomass (Mg km^{-2})[57] on the catchment[55]. Data were bin-averaged by intervals of 25% of dense forest cover and by intervals of 400 Mg km^{-2} of biomass. Two extreme CH_4 values (>25000 nmol L^{-1}) were removed from the analysis. Error bars correspond to the standard deviation on the mean.



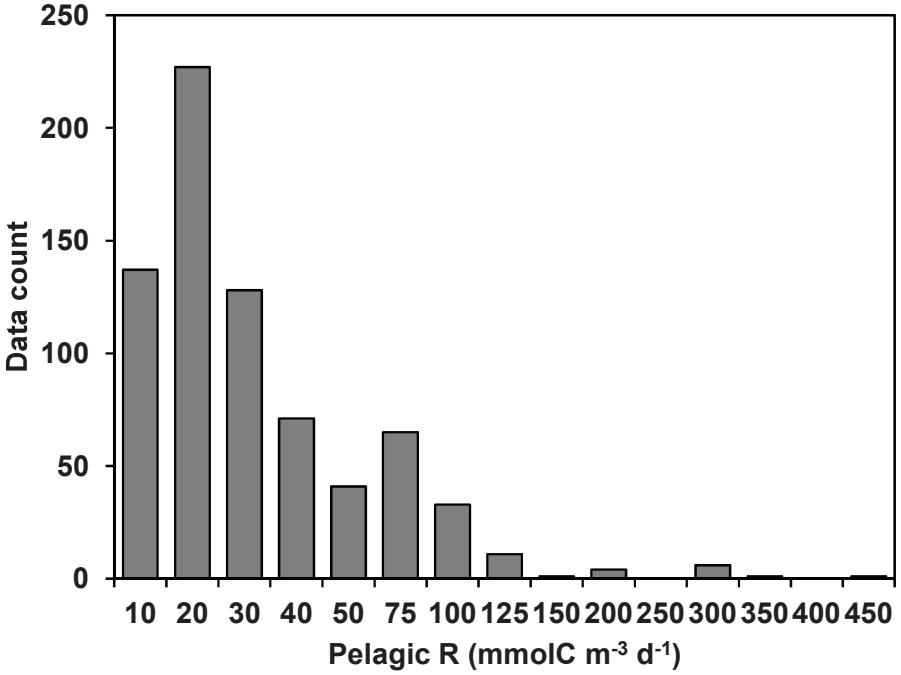
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Figure S11. pCO_2 (ppm), CH_4 (nmol L^{-1}) and $\%O_2$ (%) as function of dense forest cover (%)[56], of aboveground living biomass (Mg km^{-2})[57], average catchment slope[55], and precipitation[61]. Only the data-sets that capture spatial variations were included in the analysis (excluding fixed time-series in the mainstem of rivers).



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Figure S12. Frequency distribution of the pelagic R measurements gathered in the studied Sub-Saharan rivers.



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Table S1. Summary of sample collection for GHGs and pelagic respiration (R) in African rivers and streams. Unpubl. = unpublished

	Approach	Dates	Longitude (°E)	Latitude (°N)	*n* pCO_2	*n* CH_4	*n* N_2O	*n* R	Reference
Congo	Survey	03/12/2013 - 19/12/2013	15.350 ; 25.187	-4.307 ; 2.206	75	75	75	67	Unpubl.
Congo	Survey	10/06/2014 - 30/06/2014	15.357 ; 25.187	-4.306 ; 2.217	89	89	89	69	Unpubl.
Congo	Survey	13/03/2014 - 21/03/2014	24.170 ; 24.604	0.493 ; 0.784	20	20	20	2	Unpubl.
Congo	Survey	17/09/2013 - 26/09/2013	24.169 ; 24.599	0.494 ; 0.775	6	7	7	7	Unpubl.
Congo	Survey	20/11/2012 - 08/12/2012	24.170 ; 25.196	0.490 ; 0.795	32	32	32	21	Unpubl.
Congo	Monitoring	10/12/2012 - 29/05/2014	25.200	0.499		37	37		Unpubl.
Congo	Monitoring	11/12/2012 - 29/05/2014	25.186	0.542		36	36		Unpubl.
Congo	Survey	20/12/2010 - 31/12/2010	27.803 ; 28.574	-3.310 ; -1.283		15	15		Unpubl.
Congo	Survey	21/03/2010 - 23/03/2010	17.025 ; 18.469	3.945 ; 4.919		7	7		[52;53]
Congo	Survey	20/03/2011 - 23/03/2011	17.110 ; 17.529	3.754 ; 4.105		16	16		[52;53]
Congo	Survey	20/11/2012 - 24/11/2012	17.157 ; 17.535	3.755 ; 4.081		14	14		[52;53]
Congo	Monitoring	20/03/2010 - 31/03/2012	18.567	4.350		54	54		[52;53]
Congo	Survey	01/05/2010 - 06/06/2010	22.458 ; 25.181	0.475 ; 2.172		52	52	41	Unpubl.
Congo	Monitoring	22/07/2011 - 23/11/2012	15.276	-4.296		29	28		Unpubl.
Congo	Survey	02/05/2010 - 22/07/2011	15.276 ; 25.181	-4.296 ; 2.172		52	52	40	Unpubl.
Congo	Survey	22/06/2012 - 24/06/2012	12.705 ; 12.733	-5.964 ; -5.956		2	2		Unpubl.
Congo	Survey	22/06/2012 - 24/06/2012	12.705 ; 12.733	-5.964 ; -5.964		2	2		Unpubl.
Virunga Rivers (12)	Monitoring	18/12/2010 - 20/02/2013	29.004 ; 29.478	-1.727 ; -1.028		291	290		Unpubl.
Kivu Rivers	Survey	17/03/2007 - 22/03/2007	28.788 ; 29.411	-2.470 ; -1.991		12			Unpubl.
Kivu Rivers	Survey	28/08/2007 - 10/09/2007	28.788 ; 29.411	-2.505 ; -1.992		13			Unpubl.
Kivu Rivers	Survey	21/06/2008 - 26/06/2008	28.788 ; 29.411	-2.505 ; -1.992		12			Unpubl.
Kivu Rivers	Survey	22/04/2009 - 05/05/2009	28.788 ; 29.411	-2.505 ; -1.991		12	12		Unpubl.
Kivu Rivers	Survey	20/10/2010 - 24/10/2010	28.789 ; 29.072	-2.505 ; -1.574		8	8		Unpubl.
Kivu Rivers	Survey	25/06/2011 - 25/06/2011	28.788 ; 28.837	-2.470 ; -2.362		5	5	5	Unpubl.
Kivu Rivers	Survey	27/01/2012 - 27/01/2012	28.788 ; 28.837	-2.470 ; -2.362		5	5	5	Unpubl.
Ivory Coast (Comoé)	Monitoring	22/06/2006 - 21/03/2007	-3.669	5.241		31			[34]
Ivory Coast (Bia)	Monitoring	08/06/2006 - 08/03/2007	-3.210	5.370		30			[34]
Ivory Coast (Tanoé)	Monitoring	13/06/2006 - 13/03/2007	-2.920	5.123		25			[34]
Ogooué	Monitoring	19/04/2012 - 18/04/2014	10.225	-0.706		55	53		Unpubl.
Niger	Monitoring	03/04/2011 - 17/03/2013	2.006	13.567		49	49		Unpubl.
Zambezi	Survey	04/02/2012 - 27/04/2012	22.688 ; 36.193	-18.549 ; -11.122	50	52	52	44	[25]
Zambezi	Survey	12/01/2013 - 15/03/2013	22.689 ; 36.445	-18.579 ; -8.596	55	45	45	37	[25]
Zambezi	Survey	19/10/2013 - 24/11/2013	22.685 ; 30.414	-17.887 ; -11.350	32	30	30	16	[25]
Zambezi (2 rivers)	Monitoring	19/02/2012 - 24/11/2013	28.858 ; 28.875	-16.035 ; -15.946	16	55	55	57	Unpubl.
Madagascar	Survey	04/07/2010 - 03/09/2010	46.547 ; 49.268	-19.295 ; -16.233		121	121	56	Unpubl.
Madagascar	Survey	21/01/2012 - 01/03/2012	46.547 ; 49.026	-19.295 ; -16.233	36	49	49	43	Unpubl.
Tana	Survey	03/09/2012 - 09/09/2012	39.340 ; 39.472	-4.422 ; -4.158	9	10	10	9	Unpubl.
Tana	Survey	12/02/2008 - 24/02/2008	36.708 ; 40.352	-2.410 ; -0.062		24			[54]
Tana	Survey	03/06/2010 - 22/07/2010	36.674 ; 40.352	-2.410 ; 0.269		98	98		Unpubl.
Tana (1 river)	Monitoring	14/12/2010 - 16/07/2013	37.269	-0.787		51	51		Unpubl.
Tana (5 sites)	Monitoring	25/06/2010 - 02/01/2012	38.311 ; 40.115	-1.851 ; -0.055		78	76		Unpubl.
Tana (2 sites)	Monitoring	11/09/2011 - 16/12/2013	39.636 ; 40.115	-1.851 ; -0.464		106	102		Unpubl.
Tana (2 sites)	Monitoring	13/10/2012 - 20/05/2014	39.636 ; 40.117	-2.269 ; -0.464	247			245	Unpubl.
Athi-Galana-Sabaki	Survey	01/11/2011 - 21/11/2011	37.253 ; 40.108	-3.391 ; -1.078	28	29	30	26	[22]
Athi-Galana-Sabaki	Survey	17/04/2012 - 01/05/2012	37.253 ; 40.108	-3.144 ; -1.078	16	22	22	22	[22]
Athi-Galana-Sabaki	Monitoring	08/09/2011 - 14/12/2013	40.108	-3.144		57	53		Unpubl.
Nyong (6 sites)	Monitoring	26/02/2009 - 15/05/2010	11.283 ; 12.533	3.167 ; 3.900		100			Unpubl.

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Table S2. Characteristics of the 12 studied African rivers. AGS = Athi−Galana−Sabaki River.

	AGS	Betsiboka	Bia	Comoé	Congo	Niger	Nyong	Ogooué	Rianila	Tana	Tanoé	Zambezi
Catchment area (km^2)[55]	40300	68311	11917	78474	3705222	2098640	27415	215216	7844	100608	15608	1378102
Slope (°)[56]	1.23	1.02	0.36	0.48	0.60	0.44	0.50	0.49	3.22	0.95	0.58	0.86
Discharge (km^3yr^{-1})	2.30	9.49	1.86	7.06	1292.98	200.00	12.30	148.22	12.87	4.92	4.16	130.37
Precipitation (mm)[59]	769	1527	1545	1174	1527	646	1809	1794	2397	696	1529	940
River-stream surface (km^2)[3]	359	1105	105	690	26517	4118	222	4261	1125	895	137	7325
Wetland surface (km^2)[58,59,60]	1181	1354	0	150	358756	119734	5483	12612	128	402	126	69254
Aboveground biomass (Mg km^{-2})[57]	67	98	479	279	7488	59	1022	984	736	77	489	262
Land cover[56]												
Dense Forest (%)	12.8	7.9	18.6	2.7	49.1	0.4	78.2	89.8	52.0	12.9	20.7	20.4
Mosaic Forest (%)	0.1	0.9	77.4	32.3	17.8	2.2	19.5	4.4	43.2	0.1	75.6	0.0
Woodland and shrubland (%)	10.2	15.5	0.8	58.7	26.6	21.1	1.6	2.2	1.0	8.1	3.0	54.6
Grassland (%)	59.5	75.3	0.0	0.0	2.9	23.4	0.2	3.2	0.4	71.2	0.0	8.7
Cropland / Bare soil (%)	16.5	0.0	0.0	6.3	1.7	52.2	0.0	0.1	0.0	7.4	0.0	13.4

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Table S3. Average values per river of pCO_2, CH_4, N_2O, FCO_2, FCH_4 and FN_2O computed according to[2] (Auf) and [3] (Ray), integrated at basin and Sub-Saharan continental scaled using the river/stream surface areas reported by[3], and the export of TOC and DIC reported in literature for the Congo, the Zambezi and the Tana. AGS = Athi−Galana−Sabaki River

		Congo	Ivory Coast	Ogooué	Niger	Zambezi	Betsiboka	Rianila	Tana	AGS	Nyong	Total
pCO_2	(ppm)	8092				2612	1665	1861	1571	2197		6415
CH_4	(nmol L^{-1})	3184	223	606	149	2235	305	469	140	277	3922	2205
N_2O	(nmol L^{-1})	10		10	8	7	8	9	8	10		9.2
FCH_4 (Aufd)	(µmol m^{-2} d^{-1})	14296	1003	2115	502	8348	1305	1923	568	1156	18019	9607
FCH_4 (Ray)	(µmol m^{-2} d^{-1})	18534	1667	4668	583	13597	3493	4537	604	1374	28579	13164
FN_2O (Aufd)	(µmol m^{-2} d^{-1})	15		13	4	2	4	5	6	16		11
FN_2O (Ray)	(µmol m^{-2} d^{-1})	19		28	5	2	9	12	6	19		15
FCO_2 (Aufd)	(mmol m^{-2} d^{-1})	1149				283	194	214	186	274		894
FCO_2 (Ray)	(mmol m^{-2} d^{-1})	1520				421	523	509	198	306		1205
FCO_2 (Aufd)	(TgC yr^{-1})	133.41				9.08	0.94	1.06	1.02	0.04		145.5
FCO_2 (Ray)	(TgC yr^{-1})	176.52				13.52	2.53	2.51	1.09	0.05		196.2
TOC export to sea	(TgC yr^{-1})	10.00-14.40 [62;63]				0.55 [25]			0.04 [65]			
DIC export to sea	(TgC yr^{-1})	3.20-3.46 [15;64]				3.69 [25]			0.06 [65]			
TOC+DIC export to sea	(TgC yr^{-1})	15.53				4.24			0.10			

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Table S4. FCO_2, FCH_4 and FN_2O in CO_2 equivalents in temperate rivers, the Ohio[66] and four Belgian[67] (BE) rivers. The sampled African rivers (Fig. 1) were characterized by lower FCO_2:FCH_4(CO_2 equ) ratios and higher FCO_2:FN_2O(CO_2 equ) than temperate rivers.

	FCO_2 mmol m^{-2} d^{-1}	FCH_4 µmol m^{-2} d^{-1}	FN_2O µmol m^{-2} d^{-1}	FCO_2 gC m^{-2} d^{-1}	FCH_4 gC m^{-2} d^{-1} (CO_2 equ)	FN_2O gC m^{-2} d^{-1} (CO_2 equ)
Ohio (USA)	194	1188	23	2.33	0.12	0.08
Ourthe (BE)	67	110	7	0.80	0.01	0.03
Meuse (BE)	224	1060	22	2.69	0.11	0.08
Geer (BE)	1662	22460	550	19.94	2.25	1.97
Blanc Gravier (BE)	275	80	13	3.30	0.01	0.05

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Divergent biophysical controls of aquatic CO_2 and CH_4 in the World's two largest rivers

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Alberto V. Borges[1], Gwenaël Abril[2,3], François Darchambeau[1], Cristian R. Teodoru[4], Jonathan Deborde[2], Luciana O. Vidal[5], Thibault Lambert[1] & Steven Bouillon[4]

Carbon emissions to the atmosphere from inland waters are globally significant and mainly occur at tropical latitudes. However, processes controlling the intensity of CO_2 and CH_4 emissions from tropical inland waters remain poorly understood. Here, we report a data-set of concurrent measurements of the partial pressure of CO_2 (pCO_2) and dissolved CH_4 concentrations in the Amazon (n = 136) and the Congo (n = 280) Rivers. The pCO_2 values in the Amazon mainstem were significantly higher than in the Congo, contrasting with CH_4 concentrations that were higher in the Congo than in the Amazon. Large-scale patterns in pCO_2 across different lowland tropical basins can be apprehended with a relatively simple statistical model related to the extent of wetlands within the basin, showing that, in addition to non-flooded vegetation, wetlands also contribute to CO_2 in river channels. On the other hand, dynamics of dissolved CH_4 in river channels are less straightforward to predict, and are related to the way hydrology modulates the connectivity between wetlands and river channels.

There is an increasing recognition of the importance of inland waters (streams, rivers, lakes and reservoirs) in global budgets of CO_2 and CH_4. According to the most recent estimate, the CO_2 emission from inland waters totals 2.1 PgC yr^{-1} [1] which is equivalent to the ocean or land CO_2 sinks[2]. The global emission of CH_4 to the atmosphere from freshwater ecosystems of 103 $TgCH_4$ yr^{-1} [3] is significant when compared to all other natural (220–350 $TgCH_4$ yr^{-1}) and anthropogenic (330–335 $TgCH_4$ yr^{-1}) CH_4 emissions[4]. Wetlands are among the largest natural CH_4 sources to the atmosphere ranging between 175 and 220 $TgCH_4$ yr^{-1} [4], although pristine freshwater wetlands sequester carbon (C) below ground as organic matter at a rate of ~0.8 PgC yr^{-1} [5]. We adopt, here, the common definition of wetlands as habitats with continuous, seasonal, or periodic standing water or saturated soils[6]. The total estimated CO_2 emission from rivers and streams of 1.8 PgC yr^{-1} [1] is mostly related to tropical areas that account for 1.4 PgC yr^{-1} (78%). However, the CO_2 data distribution is skewed towards temperate and boreal systems in the Northern Hemisphere, and data in several tropical basins (including the Congo) were derived from interpolation from adjacent basins rather than actual measurements. About 49% of the CH_4 emission to the atmosphere from freshwater ecosystems occurs in the tropics, although, there is equally a strong under-representation of tropical inland waters in global estimates, whereby the most recent global synthesis resorted to extrapolating CH_4 fluxes from temperate rivers[3].

[1]Université de Liège, Unité d'Océanographie Chimique, Institut de Physique (B5), B-4000, Belgium. [2]Laboratoire Environnements et Paléoenvironnements Océaniques et Continentaux (EPOC), CNRS, Université Bordeaux 1, Avenue des Facultés, 33405 Talence, France. [3]Programa de Geoquímica, Universidade Federal Fluminense, Outeiro São João Batista s/n, 24020015, Niterói, RJ, Brazil. [4]Katholieke Universiteit Leuven, Department of Earth and Environmental Sciences, Celestijnenlaan 200E, B-3001 Leuven, Belgium. [5]Laboratório de Ciências Ambientais, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense – UENF, Av. Alberto Lâmego, Parque Califórnia, CEP 28013602, Campos dos Goytacazes, RJ, Brazil. Correspondence and requests for materials should be addressed to A.V.B. (email: alberto.borges@ulg.ac.be)

	Amazon	Congo
Catchment area (km^2)[62]	6,025,735	3,705,222
Slope (°)[62]	1.4	0.6
Discharge ($km^3\ yr^{-1}$)[63]	5,444	1,270
Specific discharge ($L\ s^{-1}\ km^{-2}$)	29	11
Precipitation (mm)[64]	2,147	1,527
Air temperature (°C)[64]	24.6	23.7
River-stream surface area (km^2)[1]	74,904	26,517
Wetland surface area (%)[11,58]	14	10
Above ground biomass ($Mg\ km^{-2}$)[65]	909	748
Land cover[60,61]		
Dense Forest (%)	83	49
Mosaic Forest (%)	4	18
Woodland and shrubland (%)	4	27
Grassland (%)	5	3
Cropland/Bare soil (%)	4	2

Table 1. Main characteristics of the Amazon and Congo basins.

The C emissions from inland waters result from complex interactions between hydrology, biogeochemical processing within the aquatic environment and connectivity with riparian zones and the watershed. The CO_2 emissions from inland waters have been traditionally interpreted as mainly resulting from the *in-situ* degradation of organic C from non-flooded land (that is, *terra firme*)[7–15]. Yet, other sources of CO_2 could also contribute to CO_2 emissions from inland waters. In lakes, there is an increasing recognition of the role of hydrological inputs of CO_2 (rivers and groundwaters) in sustaining CO_2 emissions to the atmosphere[16–20]. In rivers, the contribution of groundwater inputs of CO_2 to riverine CO_2 emissions is also recognized as particularly important in headwaters[21,22]. There is also an increasing recognition of the inputs of C from wetlands in sustaining CO_2 and CH_4 emissions to the atmosphere from rivers and lakes. Wetlands contribute to CO_2 emissions through the respiration from flooded roots of vegetation and by providing labile organic C to sustain bacterial degradation[23,24]. In the Central Amazon basin, CO_2 and CH_4 emissions from floodplain lakes[23,25] and from river channels[24,26] have been attributed to C from wetlands (flooded forest and macrophytes) in addition to non-flooded terrestrial organic C. This was established with a mass balance approach of organic C[23,26], high-resolution pCO_2 distributions[24], and stable-isotope signatures of organic C. In African rivers, spatial patterns of pCO_2 and CH_4 relate to the distribution of the fraction of wetland in the catchment within a given system (Congo and Zambezi) and across different basins[27,28]. However, both non-flooded terrestrial biomass and wetlands contribute to CO_2 emissions from inland waters and their relative importance remains uncertain and has not yet been quantitatively resolved[27,29]. This is in part due to the absence of specific molecular tracers for terrestrial organic matter, since numerous plants are common in flooded and non-flooded forests[30]. On the other hand, stable isotopes allow to trace organic matter from floating macrophytes that frequently have a C_4 signature[31], while non-flooded C_4 grasslands have been found to contribute little to organic matter transported by rivers even in catchments where they occupy extensive areas[32]. The relative contribution of flooded and non-flooded biomes to riverine CO_2 emissions will vary from one basin to another as a function of climate[27]. It will also vary within a given basin with a dominance of non-flooded terrestrial inputs in headwaters and highlands and an increased contribution of wetlands in lowlands[24,27,29,31]. In the Amazon basin, wetlands have been conclusively shown to be hotspots of CH_4 emission compared to river channels[25,33,34].

Here, we compare the CO_2 and CH_4 distributions in lowland river channels of the two largest rivers in the World and in the tropics, the Amazon and the Congo (Table 1), using a data-set of concurrent pCO_2 and CH_4 concentration measurements in river channels (Fig. 1, Table 2). We acknowledge that there are several other data-sets of pCO_2 and CH_4 in Amazonian aquatic systems[29] but we focus on direct measurements of pCO_2 (not calculated from pH and TA that are highly biased in acid waters[35]) concurrent with dissolved CH_4 measurements (most other studies are based on either one dissolved gas or the other, but not both). The aim of this study is to determine the extent to which the patterns of CO_2 and CH_4 differ or converge in these two tropical giant water bodies.

Results

The pCO_2 values spanned two orders of magnitude in the Amazon (70 to 16,880 ppm) and one order of magnitude in the Congo (1090 to 22,900 ppm) (Fig. 2a). The CH_4 concentrations spanned four orders

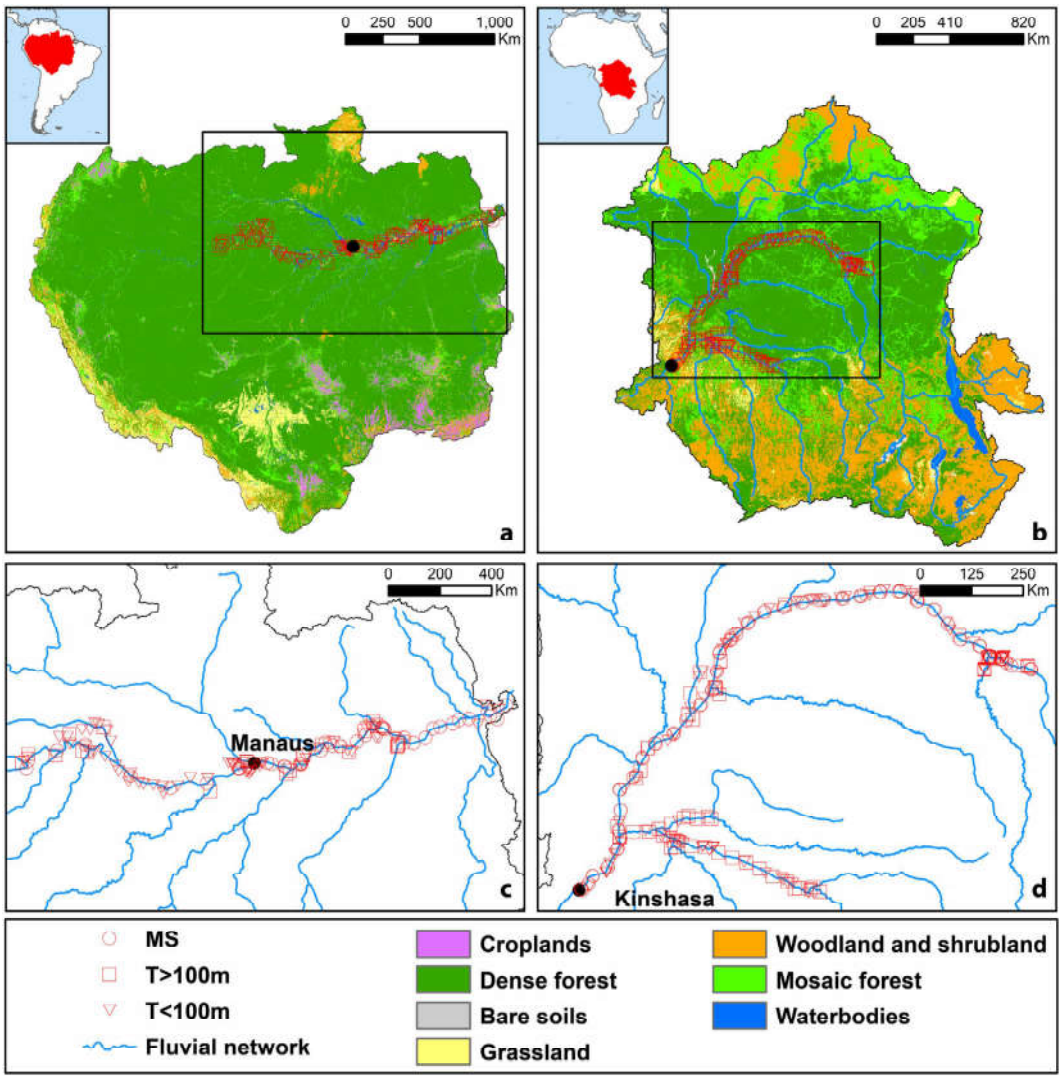


Figure 1. Location of sampling stations in the Amazon and Congo at the scale of the whole basin overlain on the land cover (**a,b**), and a zoom overlain on the main rivers (**d,e**). Maps were generated with ArcGIS using publically available spatial datasets[60,61]. MS = mainstem. T > 100m = large tributaries. T < 100m = small tributaries.

of magnitude in the Amazon (11 to 189,100 nmol L^{-1}) and three orders of magnitude in the Congo (22 to 71,430 nmol L^{-1}) (Fig. 2b). Data were aggregated into mainstem (MS), large and small tributaries (T > 100 m and T < 100 m width, respectively[11,36]). The pCO_2 values significantly increased from the mainstem to the small tributaries in the Amazon (Kruskal-Wallis (KW) test, $p = 0.0001$) and in the Congo (KW test, $p < 0.0001$). The same pattern was observed for CH_4 concentrations in the Amazon (KW test, $p < 0.0001$) and in the Congo (KW test, $p < 0.0001$). In the mainstem, large and small tributaries of both Amazon and Congo, the median pCO_2 and CH_4 (Fig. 2a,b) were distinctly above atmospheric equilibrium of ~390 ppm and ~2 nmol L^{-1}, respectively. The pCO_2 in the Amazon mainstem was significantly higher than in the Congo mainstem, but pCO_2 values were not significantly different in large and small tributaries (Fig. 2a). The CH_4 in the mainstem, large and small tributaries were significantly higher in the Congo than in the Amazon (Fig. 2b). The median CH_4 in the Congo was three to four times higher than in the Amazon, for mainstem/small tributaries and large tributaries, respectively. For a given pCO_2 value, CH_4 concentrations were systematically higher in the Congo than in the Amazon (Fig. 3a–c).

Discussion

The contribution of wetlands to CO_2 emissions in the Amazon, Congo and across tropical rivers. The pattern of higher pCO_2 values in streams compared to rivers in the Amazon and the Congo (Fig. 2) is consistent with an analysis of global averages[36] and also with the regional studies in part of the Congolese "Cuvette Centrale"[37] and in the Oubangui sub-catchment[38]. Higher CH_4 and CO_2 concentrations in tributaries than in the mainstem were also reported in the Paraguay River[39]. The

Dates	River stage	Longitude (°E)	Latitude (°N)	*n*
Amazon				
30/01/2007–09/02/2007	Rising water	−55.769; −51.239	−2.546; −0.116	28
11/05/2008–28/05/2008	High water	−60.920; −60.174	−3.397; −3.076	36
06/10/2008–13/10/2008	Low water	−60.291; −55.288	−3.410; −1.913	14
03/10/2009–20/10/2009	Low water	−60.824; −55.029	−3.467; −1.951	36
25/08/2010–12/09/2010	Falling water	−60.852; −54.988	−3.384; −1.947	22
Congo				
20/11/2012–08/12/2012	High water	24.170; 25.196	0.490; 0.795	32
17/09/2013–26/09/2013	Low water	24.169; 24.599	0.494; 0.775	6
03/12/2013–19/12/2013	High water	15.350; 25.187	−4.307; 2.206	75
13/03/2014–21/03/2014	High water	24.170; 24.604	0.493; 0.784	20
10/06/2014–30/06/2014	Falling water	15.357; 25.187	−4.306; 2.217	89
16/04/2015–06/05/2015	Falling water	15.392; 20.578	−4.394; 2.666	58

Table 2. Dates, river stage, spatial coverage and number (*n*) of paired samples of pCO_2 and CH_4 collected in the Amazon and Congo rivers.

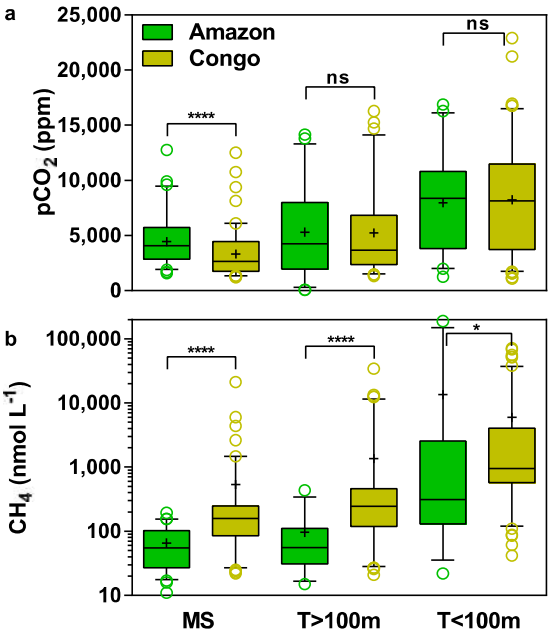


Figure 2. Box and whisker plots of pCO_2 (a) and CH_4 (b) in the Amazon and Congo. The box spans the interquartile range (25–75 percentiles), whiskers correspond to 5–95 percentiles, horizontal bar to median, cross to average, and circles to outliers. Differences were tested with a Mann Whitney test at 0.05 confidence interval level, where **** corresponds to $p < 0.0001$, * to $p = 0.0278$, and ns to not significant. MS = mainstem. T > 100m = large tributaries. T < 100m = small tributaries.

higher pCO_2 in the mainstem of the Amazon than in the Congo in their lowland regions could be due to the higher wetland coverage (Table 1), since organic and inorganic C from wetlands has been shown to partly sustain the CO_2 emission from the Central Amazon mainstem and floodplains[24,26]. In order to expand the range of wetland coverage, we included pCO_2 data acquired in four other African rivers[27] (Fig. 4). In the small and large tributaries and mainstem, pCO_2 was positively correlated to wetland coverage across these six tropical rivers, confirming the contribution of wetland C in partly sustaining CO_2 emissions from lowland tropical river channels[24,26,27]. These positive correlations between pCO_2 and wetland coverage do not necessarily imply that wetlands are the sole drivers of CO_2 in river channels. As previously noted, semi-arid rivers such as the Tana that are virtually devoid of wetlands are CO_2 sources to the atmosphere, although less intense than other tropical rivers, implying that non-flooded land also

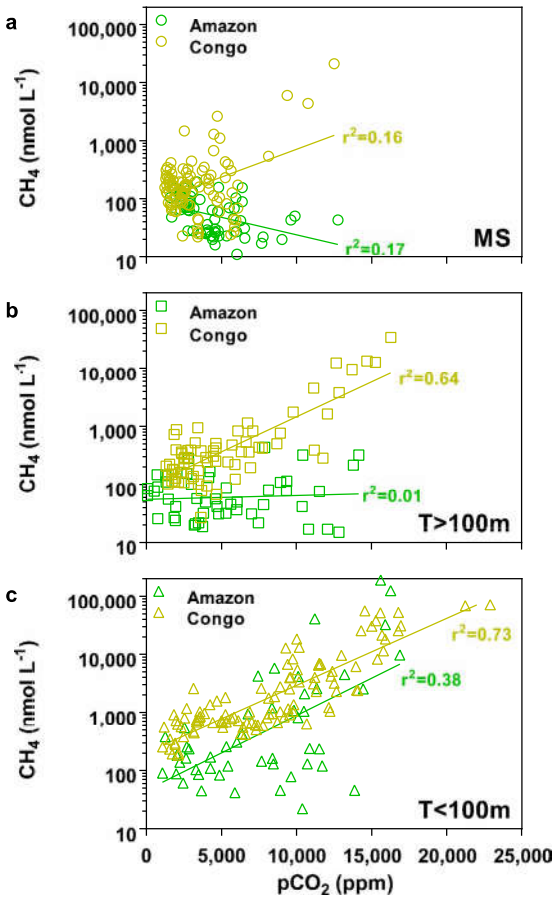


Figure 3. Log of CH_4 concentration as function of pCO_2 in the mainstem (MS) (a), the large tributaries (T > 100 m) (b), and small tributaries (T < 100 m) (c) of the Amazon and Congo basins. Lines correspond to the linear regressions of log transformed CH_4 as a function of pCO_2.

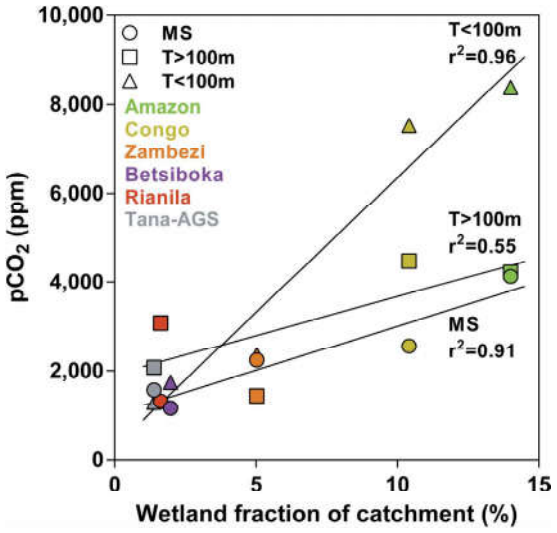


Figure 4. Median river-channel pCO_2 in mainstem (MS), large tributaries (T > 100 m) and small tributaries (T < 100 m) as function of wetland coverage (fraction of the catchment) in the Amazon (n = 136), Congo (n = 280), Zambezi (n = 153), Betsiboka (n = 21), Rianila (n = 9), and Tana and Athi−Galana−Sabaki (Tana/AGS) (n = 442). Solid lines indicate linear regressions, and r^2 are the corresponding coefficient of determination. Amazon and Congo data are from the present study, other data from Borges *et al.*[27].

sustains CO_2 emissions from river channels[27]. The relative importance of non-flooded land and wetlands in sustaining riverine CO_2 emissions remains uncertain and has not yet been quantitatively resolved[29].

Several hypotheses can explain the different behavior of CH_4 in the Amazon and Congo river channels. Although in African rivers average CH_4 concentrations correlate with wetland coverage[27], CH_4 concentrations were significantly higher in the Congo than in the Amazon river channels (Fig. 2), despite the fact that the Amazon has a higher wetland coverage (Table 1). Further, the correlations of CH_4 and pCO_2 are different in the Amazon and Congo river channels (Fig 3). In small streams ($T < 100$ m), the strong positive relationship between CH_4 and pCO_2 in both rivers indicates a common origin. It might indicate a stronger contribution of CO_2 production from anaerobic organic matter degradation compared to aerobic respiration, and that both CO_2 and CH_4 production are related to C processing within wetlands. Small streams receive higher contributions from groundwater that are rich in CO_2[21,22]. However, data in African rivers show that groundwater had an extremely low CH_4 content[27,40]. While groundwater input certainly contributes to high CO_2 in small streams it cannot explain the extremely high CH_4 in small streams. Consequently, the strong correlation between pCO_2 and CH_4 in small streams (Fig. 3b) indicates that groundwater inputs are probably not the major drivers of the high pCO_2 values at our sampling sites in lowland regions. In the mainstem, CH_4 is only weakly positively correlated to pCO_2 in the Congo, while a weak negative relation is observed in the Amazon. This might indicate that in the well mixed and well oxygenated Amazon mainstem, there is a stronger contribution to CO_2 production of aerobic respiration fueled by both non-flooded and wetland organic matter[41], while CH_4 is lost by emission to the atmosphere and bacterial oxidation. In large tributaries ($T > 100$ m) an intermediary situation is observed in the Amazon, while in the Congo, CH_4 and pCO_2 remain strongly correlated. These fundamental differences in the dynamics of CH_4 in these two rivers can be further examined by invoking several hypotheses.

First, the Congo flooded wetland is in majority flooded forest[42] and there are no temporary floodplain lakes but only a handful of relatively large permanent lakes (Mai-Ndombe (2,300 km^2), Tumba (765 km^2)). In the Central Amazon, on the other hand, flooded forest accounts for 80% of the maximum flooded wetland extent, and the remaining 20% corresponds to temporary and permanent lakes (7% of open water and 13% of floating macrophytes). There are 6,500 floodplain lakes from 52.5°W to 70.5°W along the floodplain fringing the Amazon mainstem plus 2,300 lakes on the major tributaries, totaling a surface area of 10,400 km^2 [43]. Floodplain lakes are abundant downstream of the confluence of the Negro and Solimões Rivers, while upstream wetland is dominated by flooded forest. Floodplain lakes are characterized by high gas transfer velocity (k) values[44,45], that promote the evasion of CH_4 to the atmosphere and water oxygenation that will favor bacterial CH_4 oxidation. In the Congolese and Amazonian flooded forest, k values should be low due to wind shielding and moderate diurnal water and air temperature variations below the dense canopy, and the release by the flooded plants of hydrophobic organic matter, which might behave as surfactants. This limits CH_4 loss by evasion to the atmosphere and by bacterial oxidation (low oxygen levels).

Second, local upland runoff is the main source of the wetland water in the Congo, and not flooding by riverine overflow as in the Amazon[46]. This unidirectional flow pattern will promote the transport of the CH_4 produced in the flooded forest towards the small and large river channels of the Congo, unlike in the Central Amazon where during rising water and high water, the water transport is from the river channels towards the wetlands. It is during rising water and high water that floating macrophytes grow and their biomass peaks[47]. This corresponds to the period of highest CH_4 emissions[33], and presumably also highest CH_4 production, when the water transfer from wetlands to the river channels is blocked by flooding. The same applies to flooded forest where CH_4 emissions were also found to be highest during high water[33].

Third, the Congo wetlands are mostly permanently flooded unlike the Amazon floodplains that are seasonally flooded. Permanently flooded wetlands are known to be stronger CH_4 emitters and presumably CH_4 producers than seasonal flooded wetlands[48,49].

Fourth, in the Congo, floating macrophytes (mainly *Vossia cuspidata*) commonly occur along channel edges and within channels, and form large meadows in streams, rivers and mainstem, in all types of waters (white and black). Floating macrophytes are known to host high CH_4 production and emission[25,33,34] that will be directly delivered into the Congo river channels. This does not occur in the Amazon where macrophytes are mainly present in floodplain lakes and do not occur in large tributaries and the mainstem due to important depth and strong currents. This is consistent with the higher CH_4 concentrations in the Congo than in the Amazon mainstem for pCO_2 values >7000 ppm (Fig. 3a). The CH_4 released by floating macrophytes in the Amazonian wetland lakes will be lost locally by evasion to the atmosphere and CH_4 oxidation (see above), and little dissolved CH_4 will be transported to the river channels.

All these differences are related to the smaller water height variations in the Congo mainstem (3–4 m) compared to the Amazon (10–12 m). The Congo basin straddles on the equator, and the dry season on the Northern part of the basin is compensated by the rainy season on the Southern part of the basin, and *vice-versa*, leading to a regulation of seasonal water height variations[50]. These different hypotheses need to be tested and verified although this would require a detailed investigation of the hydrology and wetland habitat mapping that are lacking in the Congo where research on aquatic biogeochemistry and

ecology was largely abandoned since the early 1960's compared to the Amazon that has been the subject of continued investigations for more than five decades.

Re-evaluation of CO_2 emissions from tropical rivers and streams. The total CO_2 emission from river and streams estimated by Raymond *et al.*[1] of 1.8 PgC yr^{-1} is mostly related to tropical areas that account for 1.4 PgC yr^{-1} (78%). However, the data coverage in the tropics was lower than for temperate and boreal regions, and data in several basins (including the Congo) were derived from interpolation from adjacent basins rather actual measurements. Furthermore, only one value of pCO_2 was used for the whole watershed while pCO_2 values increase in lower order streams as shown here (Fig. 2) and across the United States[15]. For African rivers we have previously shown that the Raymond *et al.*[1] dataset underestimated CO_2 fluxes in five basins where new direct pCO_2 measurements were recently made[27]. Although based on a limited number of river basins, we used the regressions in Fig. 4 as a first attempt to re-evaluate CO_2 emissions from tropical rivers and streams globally. The river basins shown in Fig. 4 cover a large range of size, climate, and land and wetland cover typical of those encountered in tropical areas. The resulting flux for the tropics is 1.8 ± 0.4 PgC yr^{-1}, *i.e.* 25% higher than the value originally computed by Raymond *et al.*[1]. While additional data will be required to further refine global estimates, this exercise confirms the importance of CO_2 emissions from rivers in tropical areas.

In conclusion, the analysis of data in river channels in six tropical rivers including the two largest ones (Amazon and Congo) reported here demonstrates that large-scale patterns in pCO_2 across different basins can be apprehended with a relatively simple statistical model related to the extent of wetlands within the basin. Dynamics of dissolved CH_4 in river channels are less straightforward to predict, and appear to be related to the way hydrology modulates the connectivity between wetlands and river channels. The differences we have highlighted in CH_4 concentration in the river channels of the Amazon and Congo should translate into same differences in CH_4 emissions, since in river channels the diffusive CH_4 emission is much higher than CH_4 ebullition flux in both rivers[27,51]. This is not the case in wetlands where ebullition represents the majority of the CH_4 emission to the atmosphere[25,52]. In the Amazon basin, overall aquatic CH_4 emissions are dominated by wetlands[25], while equivalent estimates are unavailable for the Congo basin.

Methods

Study site characteristics. The Amazon and Congo are the first and second largest rivers in the World, respectively, in terms of catchment area and freshwater discharge (Table 1). The Amazon basin is on average ~1 °C warmer and has an annual precipitation about two times higher than in the Congo. This leads to a specific discharge that is also much higher in the Amazon than in the Congo. The higher precipitation can also explain the higher coverage of the basin by evergreen forest (dense and mosaic) in the Amazon (87%) than in the Congo (67%), where conversely savannah (shrubland and grassland) is more abundant (30%), in particular in the northern and southern rims of the catchment (Fig. 1). Consequently, average above ground biomass is higher in the Amazon than in the Congo. The Amazon and Congo basins include the largest tropical wetlands in the World, with annual mean flooded area of 730,000 and 360,000 km^2, respectively[25,42].

Field data collection. Data were acquired during 5 cruises in the Amazon and 6 cruises in the Congo covering different stages of the annual flood cycle (Table 2). The pCO_2 in the Amazon was measured with an equilibrator[53] coupled to an infra-red gas analyzer (IRGA), as described in detail by Abril *et al.*[24]. The pCO_2 in the Congo was measured with both an equilibrator (in the mainstem and largest tributaries) and with a syringe headspace technique (in the mainstem and large and small tributaries) with an IRGA, as described in detail by Borges *et al.*[27]. Both approaches were inter-calibrated and compared very well[35]. Only the data acquired with a syringe headspace technique in the Congo are presented here. Samples for the determination of CH_4, were conditioned in 50 ml serum borosilicate vials, poisoned with a saturated solution of $HgCl_2$ (100 μL) and sealed with gas tight butyl stoppers until analysis by gas chromatography (GC)[54]. The CH_4 partial pressure was measured in a 1 mL subsample of the headspace of 20 mL of N_2 that was allowed to equilibrate about 12h after initial vigorous shaking. The CH_4 concentrations in the Amazon were measured with a flame ionization detector (FID) with a Hewlett Packard 5890A GC calibrated with certified CH_4:N_2 mixtures (Air Liquide France) of 10 ppm and 200 ppm CH_4. The CH_4 concentrations in the Congo were measured with a SRI 8610C GC-FID calibrated with certified CH_4:CO_2:N_2O:N_2 mixtures (Air Liquide Belgium) of 1, 10, 30 and 509 ppm CH_4. The overall precision of measurements was ±2% and ±4% for pCO_2 and CH_4, respectively. Additional data in the Amazon were digitalized with PlotDigitizer© from the plots of Richey *et al.*[55]. Data presented in Richey *et al.*[55] were obtained by headspace technique and GC analysis, from April 1982 to August 1985 during 9 cruises upstream of Manaus, while data reported in the present study were acquired downstream of Manaus.

Computation of tropical river CO_2 efflux and error propagation. The air-water CO_2 flux (F) was computed according to:

$$F = \alpha k \, \Delta pCO_2$$

where α is the CO_2 solubility coefficient, k is the gas transfer velocity and ΔpCO_2 is the pCO_2 air-water gradient, whereby a positive value corresponds by convention to an emission of CO_2 from the water to the atmosphere.

We used the geographical information system (GIS) of Raymond *et al.*[1]. The GIS provides k values, surface areas and width for streams and rivers globally, and the data are structured by stream order into COSCATs (coastal segmentation and its related catchment[56]). The k values themselves are derived from a parameterization as a function of slope and stream velocity[57] included in the GIS. For each of the COSCAT units we derived wetland cover from another GIS, the global database of lakes, reservoirs and wetlands[58]. Based on the wetland coverage and the equations of the regressions in Fig. 4, we computed the water pCO_2 in MS, $T > 100$m and $T < 100$m. Since river/stream surface areas in the GIS are structured by stream order it is not possible to distinguish the surface areas corresponding to MS and tributaries. So, the pCO_2 of MS and $T > 100$m computed from the regressions for each COSCAT were averaged, and computations were further carried for $T < 100$m and for MS and $T > 100$m lumped together. The F values were then computed from the k values derived from the GIS for streams/rivers narrower and wider than 100 m, a constant water temperature of 25 °C to compute α[59] and a constant atmospheric pCO_2 of 390 ppm. The F areal values per COSCAT were scaled to the respective stream/river surface area and the data between 30°N and 30°S were summed to provide a total flux value for tropical areas.

An error analysis on the CO_2 flux computation and upscaling was carried out by error propagation of the pCO_2 computation, the k value estimates, and the estimate of surface areas of river channels to scale the areal fluxes, using a Monte Carlo simulation with 1000 iterations. The uncertainty on the pCO_2 computation was derived from the errors on the slope and Y-intercept of the linear regressions in Fig. 4. The uncertainty on k values from the GIS was estimated to be $\pm 10.0\%$ based on the errors on slope and constant of the parameterization[57]. The river/stream surface areas in the GIS were estimated using two different hydraulic equations, that allow to estimate an uncertainty of $\pm 31.0\%$.

Statistical analysis. The statistical tests were done with GraphPad Prism® Version 6.05 for Windows.

Original data-set. The timestamped and geo-referenced data-set of pCO_2 and CH_4 concentrations (Table 2) are available as a supplementary table.

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Author Contributions

A.V.B., G.A. and S.B. designed the study; A.V.B., G.A., F.D., C.R.T., J.D., L.O.V., T.L and S.B. collected the field data; A.V.B. analyzed the data and drafted the manuscript that was revised and approved by G.A., F.D., C.R.T., J.D., L.O.V., T.L. and S.B.

Additional Information

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Research article

New and interesting *Surirella* taxa (Surirellaceae, Bacillariophyta) from the Congo Basin (DR Congo)

Christine COCQUYT[1] & Jonathan C. TAYLOR[2]

[1, 2] Botanic Garden Meise, Domein van Bouchout, B-1860 Meise, Belgium.
[1] Email: christine.cocquyt@br.fgov.be (corresponding author)
[2] School of Biological Sciences, North-West University, Potchefstroom, South Africa.
[2] South African Institute for Aquatic Biodiversity, Grahamstown, South Africa.

Abstract. Two new diatom taxa belonging to the genus *Surirella*, *S. ebalensis* and *S. congolensis*, are described from material of the Congo Basin, downstream Kisangani, DR Congo. The first taxon is a small, rather common species in the studied material; the second a somewhat larger diatom that was only sporadically observed. The morphology of both taxa is examined with light and scanning electron microscopy. The differences between the new and other closely related taxa such as *S. agonaensis* and *S. bonsaensis*, and *S. takoradiensis*, *S. tenuissima* and *S. pseudotenuissima*, respectively, are discussed.

Keywords. Africa, diatoms, new species, *Surirella.*

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Introduction

Algal reports from for the Democratic Republic of the Congo (DR Congo), formerly Belgian Congo and Zaire, are very limited (e.g., Duvigneaud & Symoens 1949; Hustedt *in* Huber-Pestalozzi 1949; Kufferath 1956a, b; Compère & Symoens 1987, 1988; Compère *et al.* 1989; Golama Swana Kaketa 1992, 1996 and Compère 1995). During the Belgian-Congolese Boyekoli Ebale Congo 2010 Expedition, covering 250 km of the Congo River and some of its tributaries between Kisangani and Bumba (see Nagy *et al.* 2011; Virgilio *et al.* 2011), samples for diatom studies were taken in an unexplored, from an algal point of view, part of the Congo Basin. Some extra samples were taken in this region during two smaller expeditions in the frame of the COBAFISH project. In the sampled area, where people from rural villages live predominantly from subsistence-based agriculture (closely associated with rivers) and fisheries, pristine habitats are rather scarce. The area is characterized by many oil palm and rubber plantations, coffee, cacao, and tea and cotton cultivation, generally farmed by smallholders. The nearest area from where results on diatom studies have been reported in the past is close to Kisangani (Golama Swana Kaketa 1992, 1996; Compère 1995).

From samples taken during the aforementioned Boyekoli Ebale Congo 2010 Expedition, a new diatom, *Cavinula lilandae* Cocquyt, M. de Haan & J.C. Taylor was recently described (Cocquyt *et al.* 2013). The present paper deals with the description of two other new taxa, both belonging to the genus *Surirella* Turpin. The first taxon is a small, rather common species in the studied material sampled in the Congo Basin during the 2010 expedition and during two smaller expeditions in November 2012 and September 2013. The second taxon is a somewhat larger diatom that was only sporadically observed in the studied material.

Material and methods

Material from the Congo Basin, downstream of Kisangani, was collected in May 2010 during the Boyekoli Ebale Congo 2010 Expedition. Supplementary samples were taken in November 2012 and September 2013 on two smaller expeditions in the frame of the COBAFISH project. For the description of the new taxa discussed in the present paper the following samples were examined:
- CCA 2070: Lomami River, 0.49339° N and 24.16960° E, epiphytic on *Nymphaea lotu*s Linnaeus; collected by François Darchambeau and Ernest Tambwe on 24 Nov. 2012; temperature 25.7 °C, pH 6.06, conductivity 22.2 µS cm^{-1}, 0.060 mg l^{-1} NH_4, 0.005 mg l^{-1} NO_2, 0.446 mg l^{-1} NO_3, 0.086 mg l^{-1} soluble reactive phosphorous (SRP).
- CCA 2071: Lomami River, 0.49339° N and 24.16960° E, epiphyton on dead submerged wood; collected by François Darchambeau and Ernest Tambwe on 24 Nov. 2012; temperature 25.7 °C, pH 6.06, conductivity 22.2 µS cm^{-1}, 0.060 mg l^{-1} NH_4, 0005 mg l^{-1} NO_2, 0.446 mg l^{-1} NO_3, 0.086 mg l^{-1} soluble reactive phosphorous (SRP).

In contrast to the samples from the Boyekoli Ebale Congo 2010 Expedition which were fixed *in situ* with 30% formalin, the samples collected on later expeditions in 2012 and 2013 were preserved in ethanol (20% vv.). Part of these samples were oxidized with hydrogen peroxide (30%), rinsed five times with distilled water and embedded in Naphrax® mounting medium. Light microscopic (LM) investigations were carried out with an oil immersion objective (100×) using an Olympus BX 51 microscope, equipped with Nomarski differential interference contrast optics (DIC) and an Olympus UC30 digital camera. For scanning electron microscopy (SEM) aliquots of the oxidized material were filtered through 0.3 µm Millipore® filters and then in turn fixed to aluminum stubs, air-dried and sputter-coated with 50 nm of gold. SEM studied were carried out both at the Botanic Garden Meise (Belgium) and the North-West University (South Africa) with a JEOL-5800LV operating at 25 kV and an FEI Quanta 2000 field emission scanning electron operating at 10 kV respectively.

The raw and the cleaned samples and the permanent slides for diatom investigation are housed at the herbarium of the Botanic Garden Meise (BR). A copy of each permanent slide will be deposited at the Centre de Surveillance de la Biodiversité (CSB) or at the Herbarium of the University of Kisangani, DR Congo.

Water temperature, conductivity and pH were measured using an YSI ProPlus multiprobe field meter equipped with a pH sensor 1001 ProSeries. Samples for nutrient analyses (NH_4, NO_3, NO_2 and SRP) were filtered and acidified *in situ*, and kept frozen until spectrophotometric analysis in the laboratory of the Unité d'Océanographie Chimique, Département d'Astrophysique, Géophysique et Océanographie, University of Liège, Belgium.

Results

Class Bacillariophyceae Haeckel emend. Medlin & Kaczmarska (Medlin & Kaczmarska 2004)
Subclass Bacillariophycidae D.G. Mann *in* Round *et al.* (Round *et al.* 1990)
Order Surirellales D. G. Mann *in* Round *et al.* (Round *et al.* 1990)
Family Surirellaceae Kützing (Kützing 1844)
Genus *Surirella* Turpin (Turpin 1828)

Surirella ebalensis Cocquyt & J.C. Taylor sp. nov.
Figs 1–5

Diagnosis

Valves slightly heteropolar, broadly linear to slightly cuneate with broadly rounded poles and slightly constricted margins in the larger valves, smaller valves not constricted. Apical pole broader than base

pole. Length: 39.4–64.5 µm; width: 16.1–19.4 µm, in the constricted part 18.7 µm. Wing projection very distinct, alar canals smaller than fenestrae, 2.2–3.0 in 10 µm. Fenestral bars distinct in LM, 30 in 10 µm. Striae indistinct in LM, 30 in 10 µm. Valve face covered with numerous silica granules and spines. Spines also present on the keel. Porcae perpendicular to the axial axis, reaching the axial area and becoming radiate near the poles.

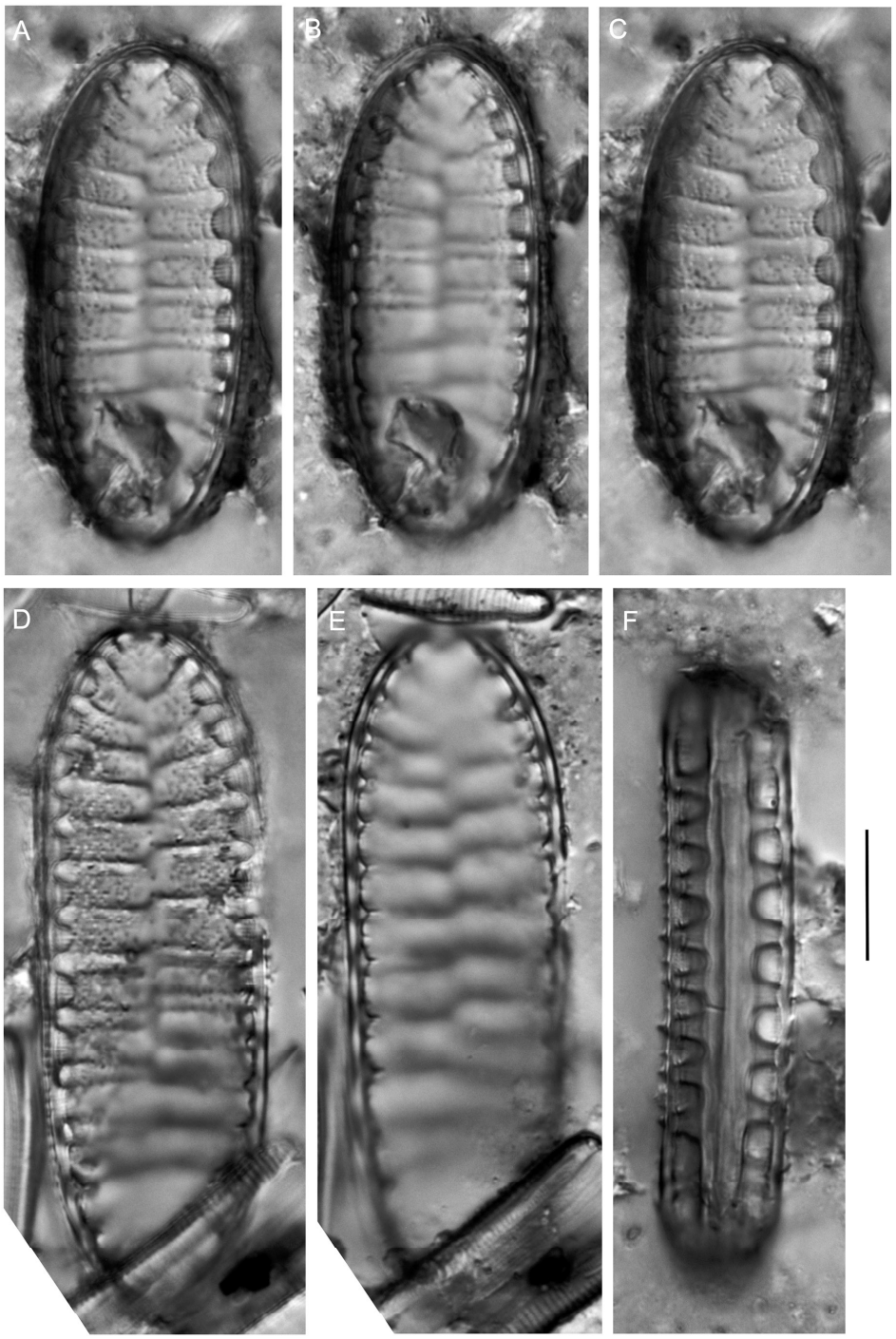


Fig. 1. *Surirella ebalensis* sp. nov., from the holotype slide BR 4398, Lomami River, DR Congo, LM (DIC). **A–C**. Valve representing the holotype, different foci of the same valve. **D–E**. Different foci of the same valve. **F**. Girdle view. Scale bar = 10 µm.

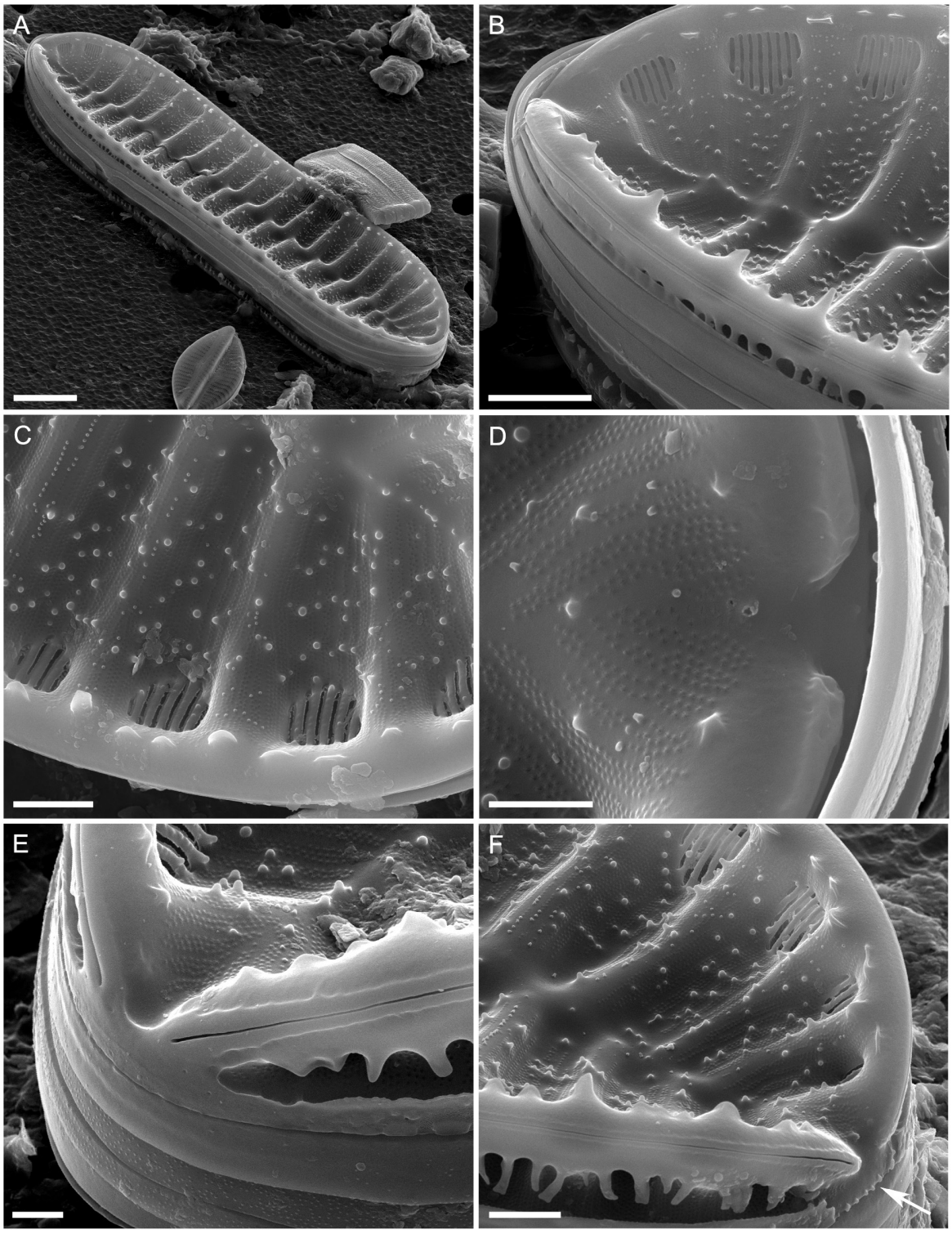


Fig. 2. *Surirella ebalensis* sp. nov., type material from sample CCA 2070, Lomami River, DR Congo, SEM. External view. B, C, F = detail of the raphe keel with blunt spines orientated towards the valve face and which are draped over a large part of the indented mantle side. **A**. Overview. **B–C**. Detail of the valve ornamented with silica granules and blunt spines. **D**. Detail of the apical pole, showing the curved raphe endings. **E–F**. Detail of the foot pole showing the straight raphe endings. **F**. Short spherical shaped silica elements near the pole (arrow). Scale bars: A = 10 μm; B = 4 μm; C, F = 2 μm; D–E = 1 μm.

Etymology

The specific epithet refers to the Lingala word for river which was used in the naming of the expedition, Boyekoli Ebale Congo 2010 (the Study of the Congo River 2010), during which numerous algal samples were collected.

Type material

Holotype

Slide BR 4398 from sample CCA 2070, Botanic Garden Meise, Belgium (BR). The valve representing the holotype is here illustrated in Fig. 1A.

Isotype

Slide Zu10/18 from sample CCA 2070, the Friedrich Hustedt Diatom Collection, Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven, Germany (BRM).

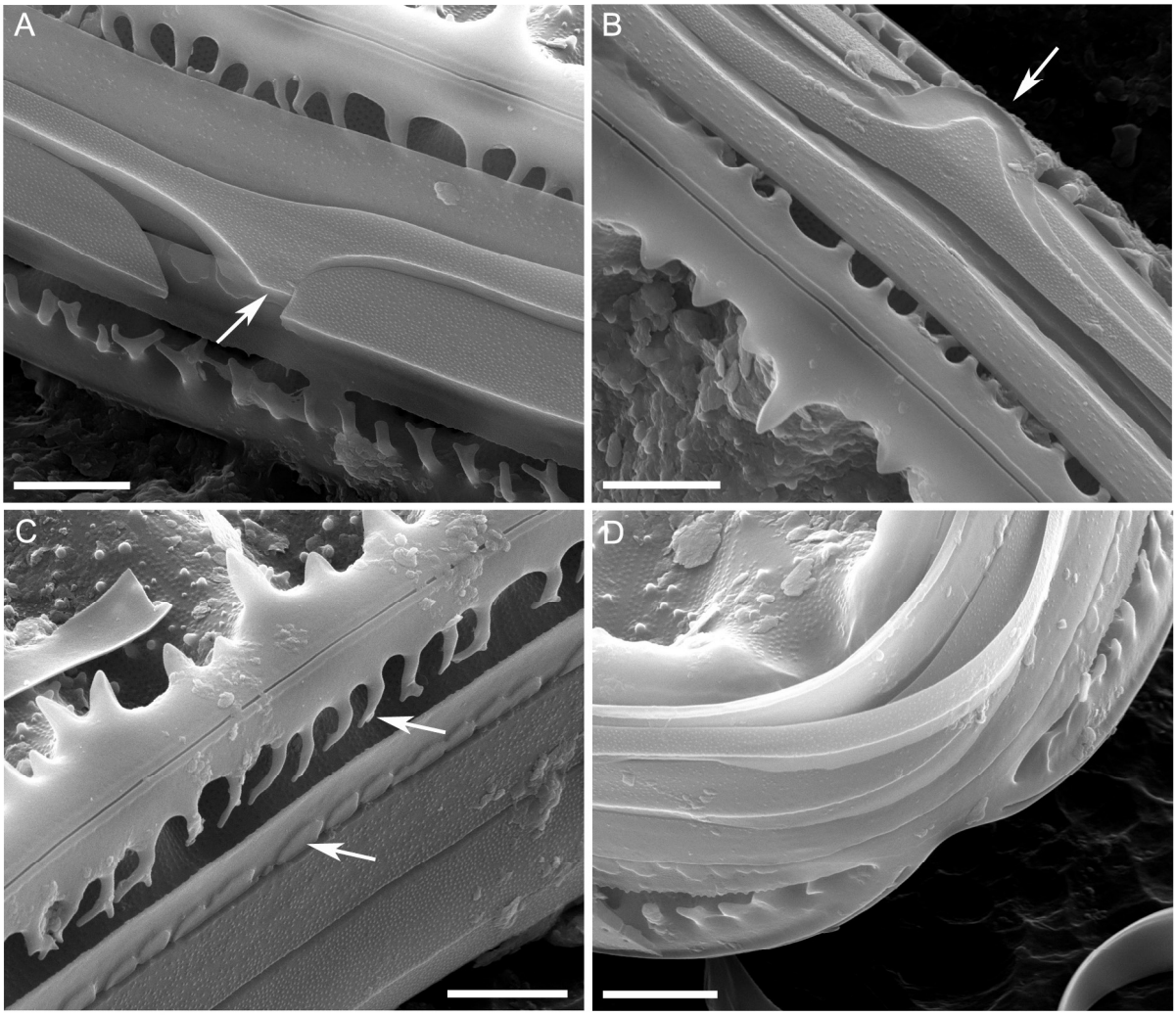


Fig. 3. *Surirella ebalensis* sp. nov., type material from sample CCA 2070, Lomami River, DR Congo, SEM. External view. **A–B**. Detail of the girdle showing a part of a girdle with ligula (arrow) and the neighbouring interrupted band. **C**. Detail of the valve mantle with the draped silica spines (arrow) and the silica plaques (arrow) near the edge of the mantle and the valvocopula. **D**. Detail of the girdle band near the pole. Scale bars = 2 µm.

Type locality

Oriental Province, DR Congo, Lomami River (0.49339° N and 24.16960° E). Epiphyton on *Nymphaea lotus*; collected by François Darchambeau and Ernest Tambwe on 24 Nov. 2012.

Morphological observations using scanning electron microscopy (Figs 2–5)

Axial area narrow to absent near the poles. In the middle of the valve the axial area becomes broader and then larger in the depressions than on the top of the porcae (transapical valve undulations). Porcae perpendicular to the axial axis, becoming radiate near the poles and reaching the axial area but not in the extension of each other near the axial area. Striae biseriate, seldom with uniseriate parts near the axial area (Figs 2C, 4B), about 30 in 10 µm. Near the fenestrae the striae often become triseriate, sometimes quadriseriate (Fig. 2D), and also present for a short distance on the raphe canal just above the fenestrae. Striae composed of 80–110 areolae in 10 µm. Numerous blunt spines scattered over the valve face

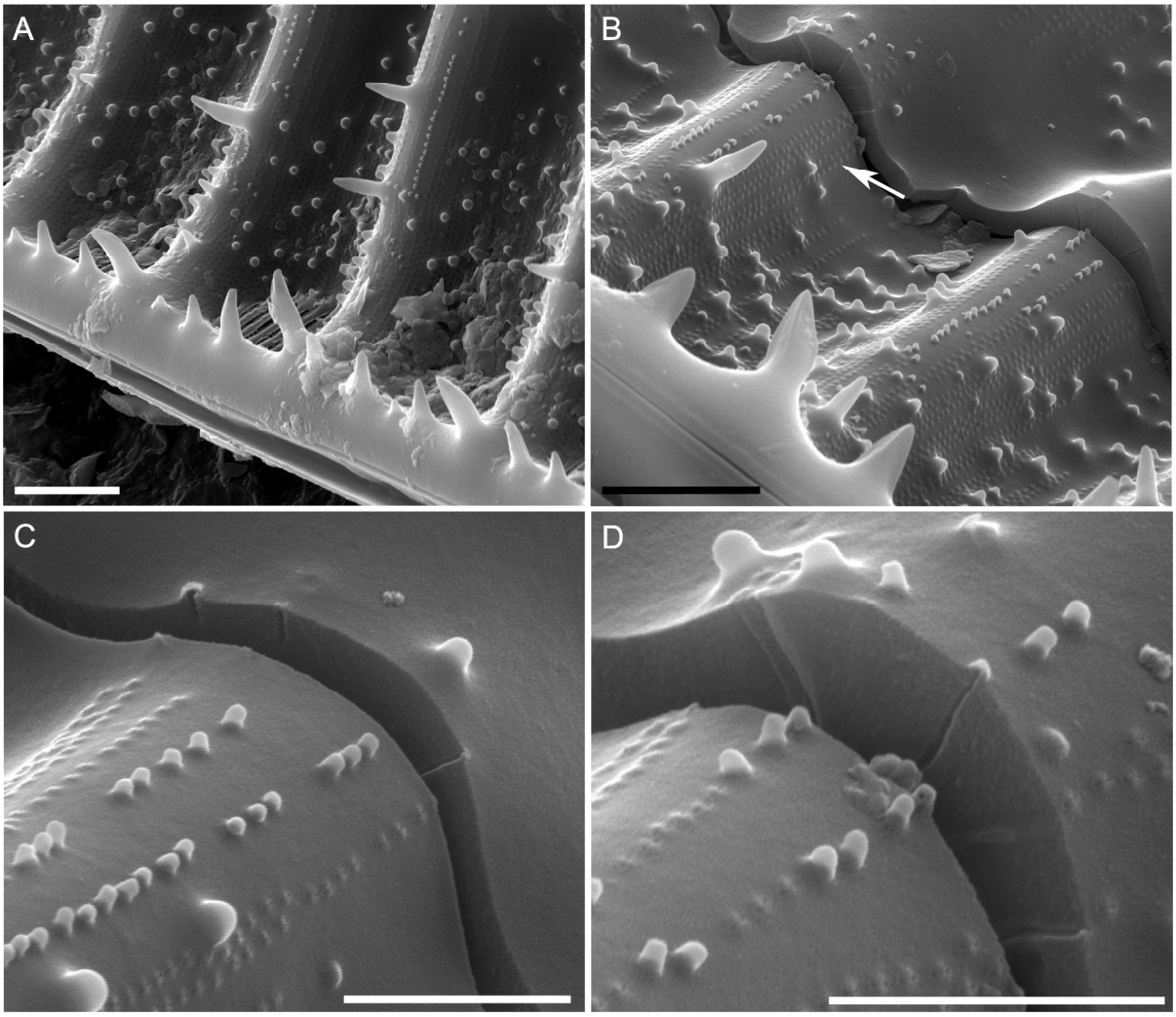


Fig. 4. *Surirella ebalensis* sp. nov., type material from sample CCA 2070, Lomami River, DR Congo, SEM. External view. Details of the various types of spines on the valve face and the keel. **A–B**. Detail of the valve surface with the biseriate striae (arrow) becoming sometimes uniseriate near the axial area. **C–D**. Section of the valve face showing the simple perforation of the silica wall at the areolae. Scale bars: A–B = 2 µm; C–D = 1 µm.

(Fig. 2B–F). On the top of the porcae these spines can become large, up to 1 µm (Fig. 4A–B). In the depressions the spines look more like elongated granules. Another kind of ornamentation is also present on the valve face: small granules located on the striae, composed of an areola that seems to secrete silica on the outside of the valve face (Fig. 4C–D). Sometimes all areolae of almost the entire stria have these granules on the valve face. On the inside the elongated granules are characterized by a perforation of the wall (Fig. 4D) that has no rimmed margins as the normal areolae do (Fig. 5A). Blunt spines are also present on the keel; the spines orientated towards the valve face are of different length and arranged on a single transapical row (Fig. 2B). These spines are often transapically elongated near their base, the shorter ones more triangular in shape (Fig. 4B). On the side towards the mantle the spines on the keel are often bifurcated; they are draped over a large part of the indented mantle side, partially covering the fenestrae (Fig. 3C). The mantle face near the edge of the mantle at the junction with the valvocopula is smooth and bears a single row of small areolae and oval silica plaques (Fig. 3C). At the apical pole these silica plaques are replaced by around 10 short spherical shaped silica elements (Fig. 2F). Bi- to triseriate

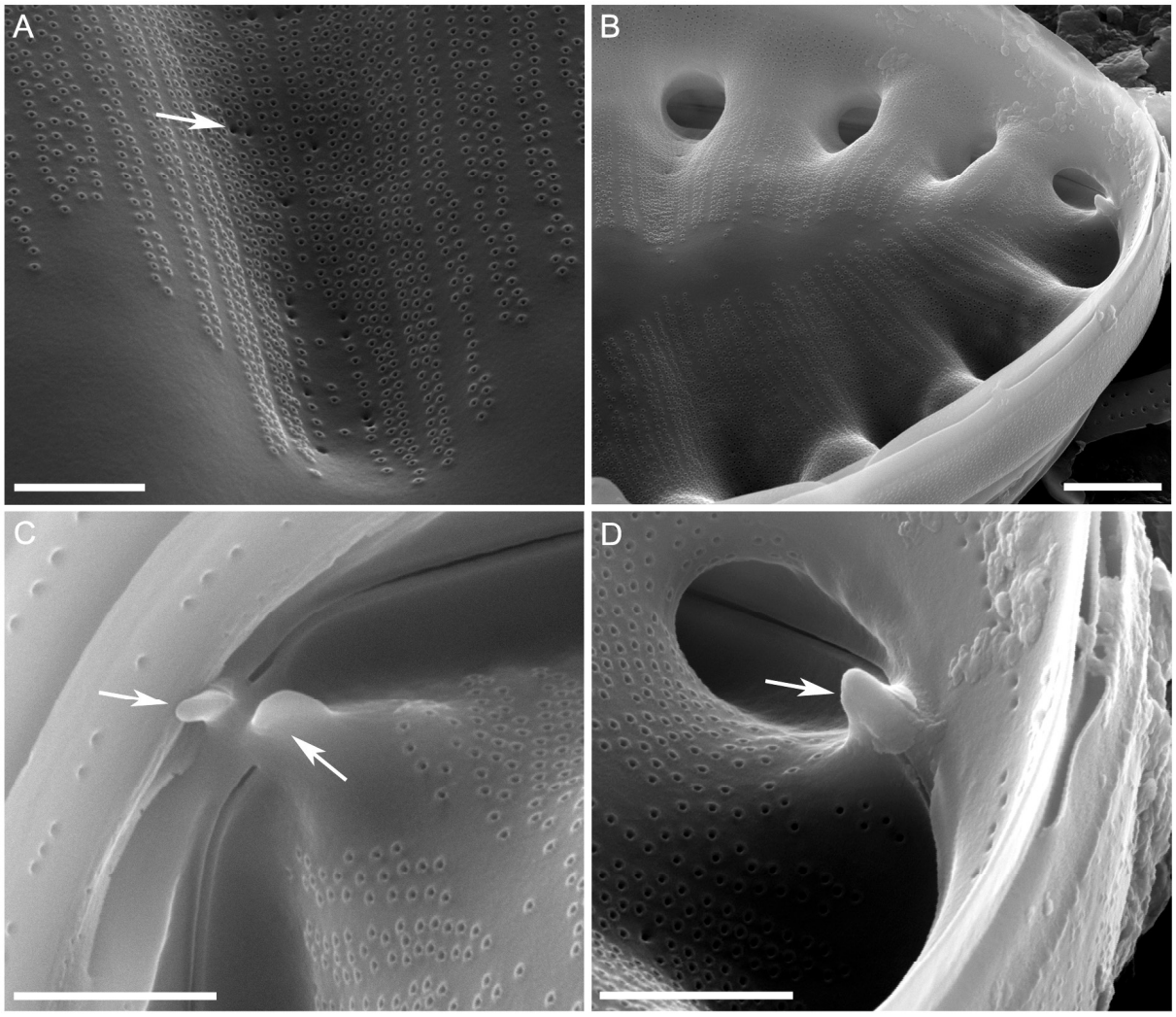


Fig. 5. *Surirella ebalensis* sp. nov., type material from sample CCA 2070, Lomami River, DR Congo, SEM. Internal view. **A**. Detail of the multiseriate striae, and the perforation of the elongated granules without rimmed margin (arrow). **B, D**. Detail of the continuous raphe near the apical pole with a reduced helictoglossa (arrow). **C**. Detail of the straight not expanded raphe endings at the foot pole and their reduced helictoglossae (arrows). Scale bars: B = 2 µm; A, C–D = 1 µm.

striae are present on the indented mantle face at the level of the fenestrae bars. Fenestrae are broader than the alar canals; 30 fenestral bars in 10 µm. External raphe endings are straight, not enlarged at both poles. Internally the raphe is continuous at the head pole while at the base pole the raphe slit is internally interrupted at different levels. Both slits end in a structure resembling a reduced helictoglossa, lacking the typical lip-like structure, and located farther away from the raphe ending (Fig. 5B–D). The girdle is composed of two bands. The open valvocopula band is not smooth but bears tiny granules (Fig. 3C); the second band with a broad ligula and the same tiny ornamentation except on the part of the ligula that is covered by the valvocopula (Fig. 3A–B).

Ecology

Physical and chemical parameters measured at the type locality are as follows: temperature 25.7 °C, pH 6.06, conductivity 22.2 µS cm^{-1}, 0.060 mg l^{-1} NH_4, 0.005 mg l^{-1} NO_2, 0.446 mg l^{-1} NO_3, 0.086 mg l^{-1} soluble reactive phosphorous (SRP).

Surirella ebalensis sp. nov. was observed in a diatom community dominated by *Eunotia bidens* Ehrenberg, *Frustulia* cf. *saxonica* Rabenhorst, *Pinnularia acrosphaeria* W. Smith, *Luticola muticoides* (Hustedt) D.G. Mann, *Encyonema minutum* (Hilse) D.G. Mann, *Stenopterobia anceps* (Lewis) Brébisson, *Eunotia* spp., *Cymbopleura* sp., *Placoneis* sp., *Caloneis* sp. and *Neidium* sp.

Surirella congolensis Cocquyt & J.C. Taylor sp. nov.
Figs 6–9

Diagnosis

Valves heteropolar, lanceolate with broadly rounded apical pole and subacute base pole, becoming gradually narrower from the apical pole to the base pole; larger valves slightly constricted mid-valve. Valve length 18.7–33.0, width (4.2) 5.5–6.9 (7.9) µm. Wing projection distinct. Alar canals short, not reaching the axial area, 5.5–6.0 in 10 µm, much smaller than the fenestrae, and rarely becoming denser near the poles. Striae about 30 in 10 µm.

Etymology

The specific epithet refers to the Congo River and the Democratic Republic of the Congo where the samples were collected.

Type material

Holotype

Slide BR 4399 from sample CCA 2071, Botanic Garden Meise, Belgium (BR). The valve representing the holotype is here illustrated in Fig. 6G.

Isotype

Slide Zu10/19 from sample CCA 2071, the Friedrich Hustedt Diatom Collection, Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven, Germany (BRM).

CCA 2071: Lomami River, 0.49339° N and 24.16960° E, epiphyton on dead submerged wood, collected by François Darchambeau and Ernest Tambwe on 24 Nov. 2012.

Type locality

Oriental Province, DR Congo, Lomami River (0.49339° N and 24.16960° E). Epiphyton on dead submerged wood.

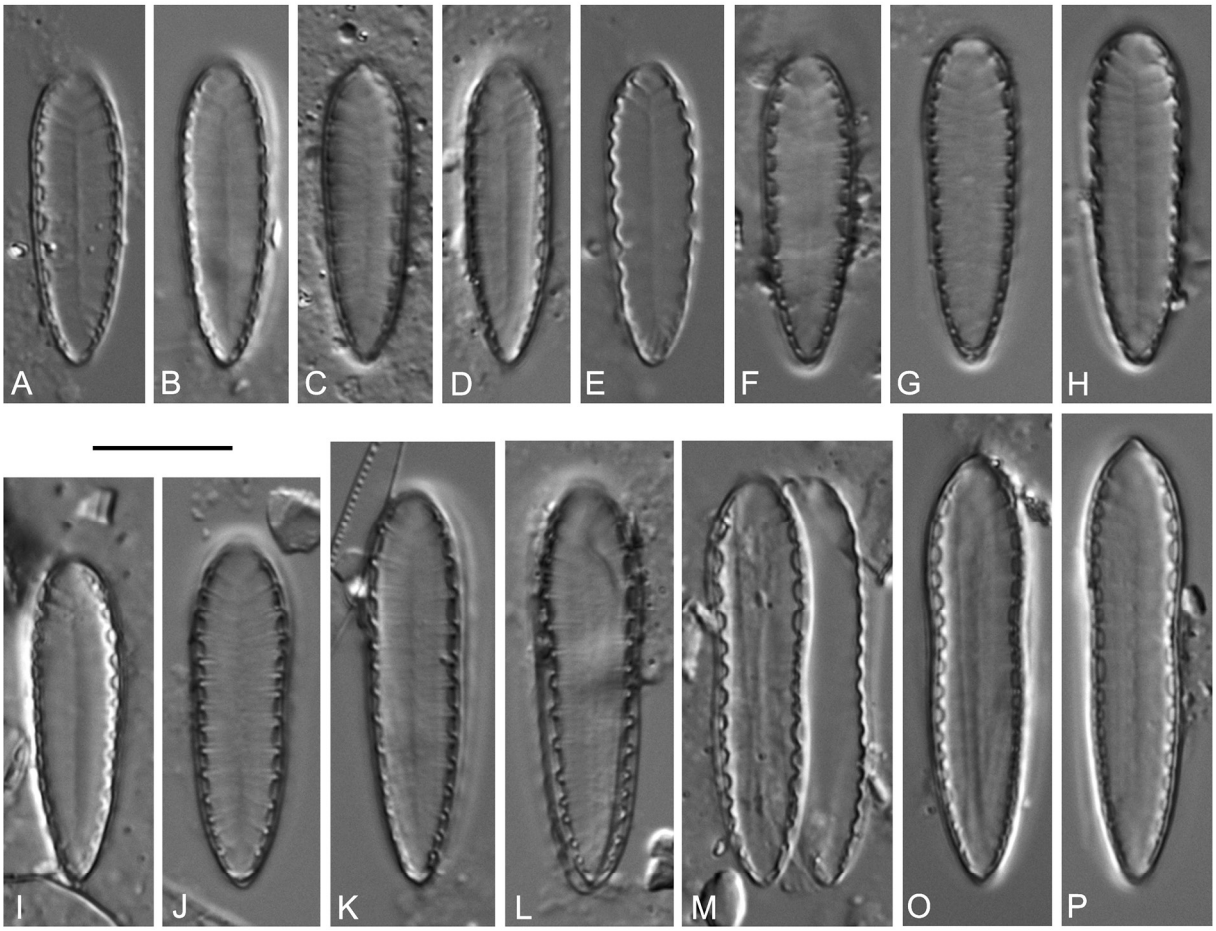


Fig. 6. *Surirella congolensis* sp. nov., from the holotype slide BR 4399, Lomami River, DR Congo, LM (DIC), valve views. **G**. Valve representing the holotype. Scale bar = 10 µm.

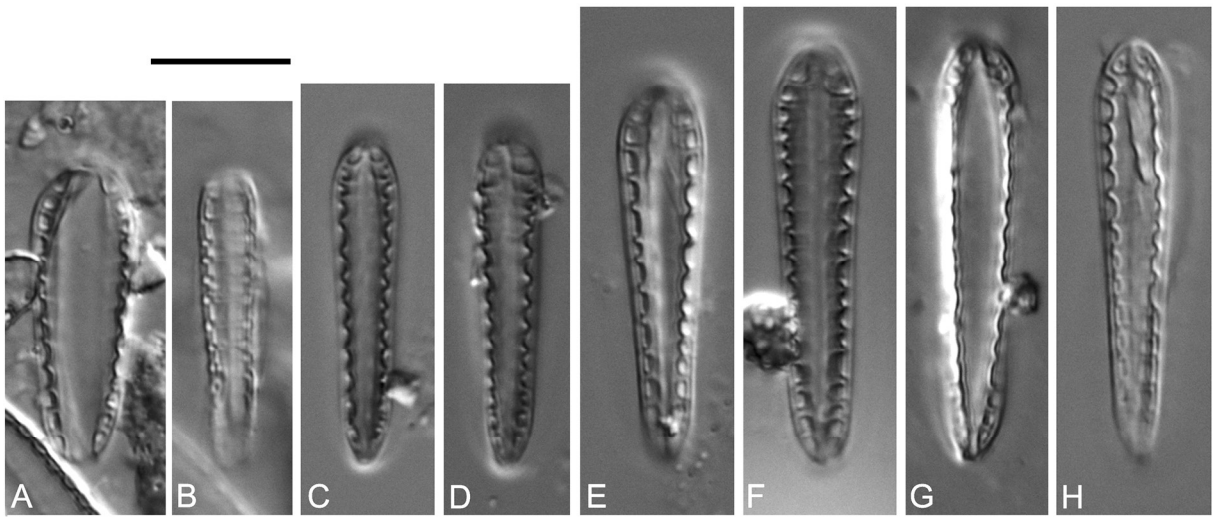


Fig. 7. *Surirella congolensis* sp. nov., from the holotype slide BR 4399, Lomami River, DR Congo, LM (DIC), girdle views. Scale bar = 10 µm.

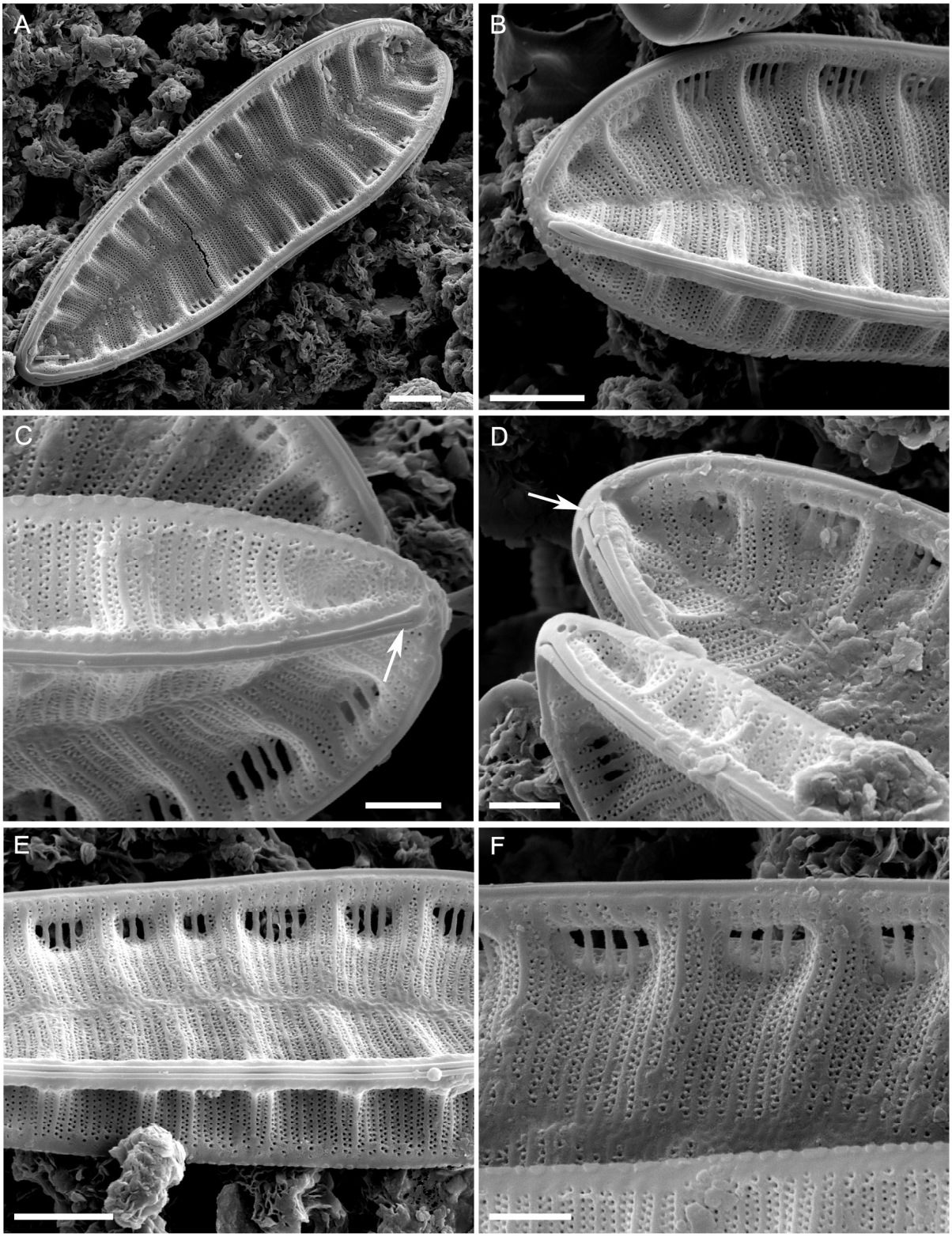


Fig. 8. *Surirella congolensis* sp. nov., type material from sample CCA 2071, Lomami River, DR Congo, SEM. External view. **A**. Overview. **B–C**. Detail of foot pole showing the straight not expanded raphe endings (arrow). **D**. Detail of the apical pole showing the slightly curved raphe endings (arrow). **E–F**. Detail of the biseriate striae and the open fenestrae with the fenestral bars. Scale bars: A–B = 2 µm; C–F = 1 µm.

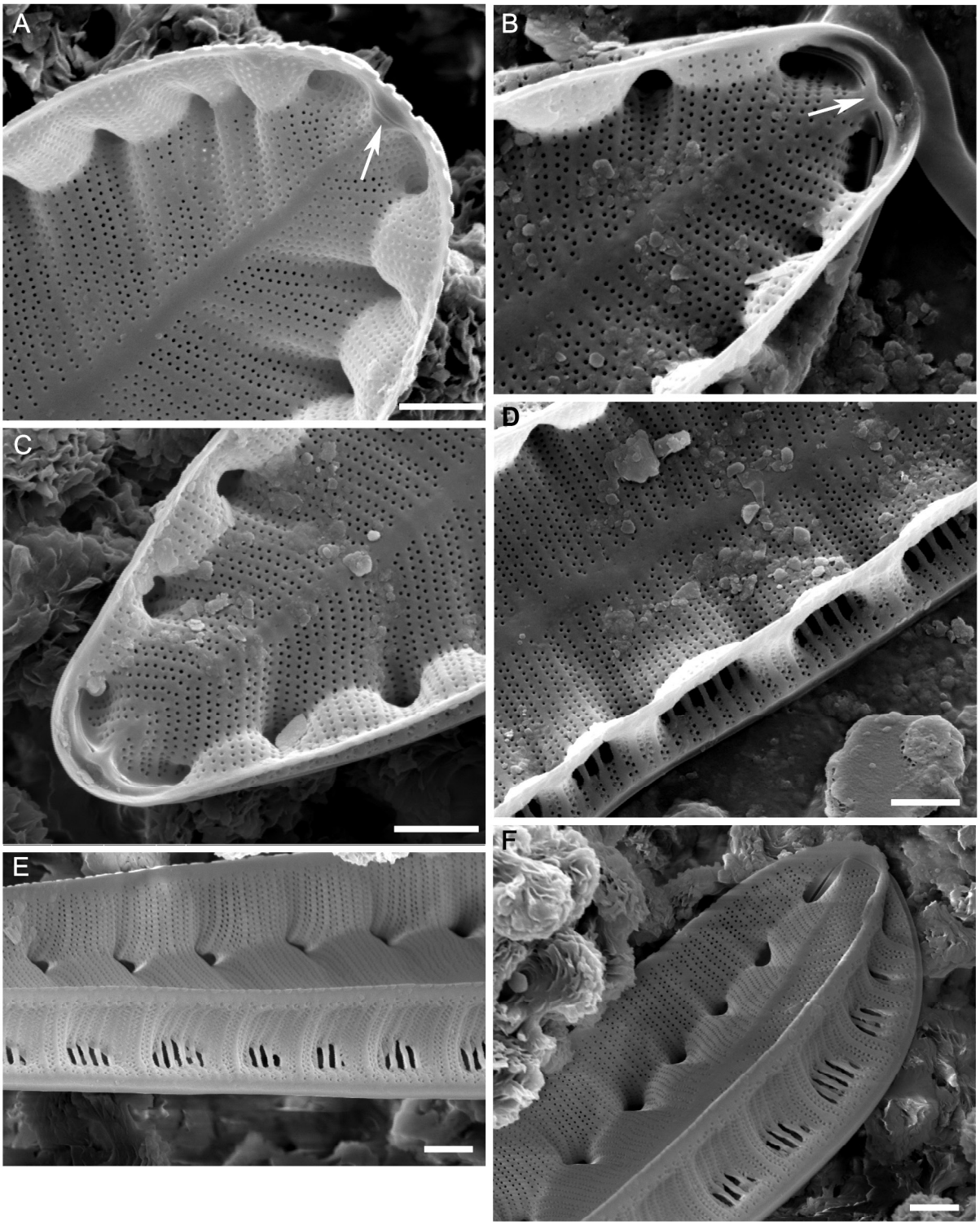


Fig. 9. *Surirella congolensis* sp. nov., type material from sample CCA 2071, Lomami River, DR Congo, SEM. Internal view. **A–B**. Head pole showing the continuous raphe (arrow). **C–D**. Foot pole showing the interruption of the raphe and the straight slightly expanded terminal raphe endings. **E–F**. Detail of the alar canals. Scale bars: B–C = 2 µm; A, D = 1 µm.

Morphological observations on scanning electron microscopy (Figs 8–9)

External view

Axial area narrow. Striae biseriate, continuing on the keel also above the fenestrae and on the mantle side. Raphe endings near the apical pole straight not enlarged (Fig. 8B–C), near the base pole straight and slightly bent towards the valve face (Fig. 8D). Striae composed of round areolae, in 10 µm, biseriate and continuing on the raphe sternum. Fenestrae open and divided by fenestral bars, openings between the fenestral bars located in the prolongation of the striae. No ornamentation such as spines or silica granules present on the valve face, except for silica plaques present at the edge of the valve mantle at the place where the valvocopula is attached to the valve (Fig. 8E).

Internal view

Raphe continuous on the apical pole (Fig. 9A) and interrupted near the foot pole with straight and slightly expanded raphe endings (Fig. 9B–C).

Girdle composed of several open, unornamented bands.

Ecology

Physical and chemical parameters measured at the type locality are as follows: temperature 25.7 °C, pH 6.06, conductivity 22.2 µS cm^{-1}, 0.060 mg l^{-1} NH_4, 0.005 mg l^{-1} NO_2, 0.446 mg l^{-1} NO_3, 0.086 mg l^{-1} soluble reactive phosphorous (SRP).

Surirella congolensis sp. nov. was observed in a diatom community dominated by *Navicula feuerbornii* var. *africana* Foged, *Gomphonema brasiliense* subsp. *pacificum* Gerd Moser, Lange-Bertalot & Metzeltin, *Orthoseira roeseana* (Rabenhorst) O'Meara, *Eolimna* spp. and *Eunotia* spp.

Discussion

Surirella ebalensis sp. nov. resembles *S. agonaensis* Foged described from Ghana in West Africa (Foged 1966; Cocquyt & Kusber 2010). The shapes of the valves differ slightly, but the valve width is distinctly narrower in *S. ebalensis* sp. nov. (16.1–19.4 µm) compared to the broader *S. agonaensis* (27.5–30.0 µm). The valve length also is smaller: 40.3–64.4 versus 78–80 µm, respectively. The alar canals are denser in *Surirella ebalensis* sp. nov. than in *S. agonaensis*: 2.2–3.0 compared to 1.4–1.6. Moreover, the wing projection is very distinct in the new species compared to the slightly distinct projection in *S. agonaensis*. Besides these differences observed in LM, the SEM study of *S. ebalensis* sp. nov. showed the presence of draped, often forked silica spine-like structures extending on the keel at the mantle side partially covering the fenestrae (Fig. 3C), these structures are absent in *S. agonaensis* (Cocquyt & Kusber 2010). The spines of variable length on the top of the porcae (Fig. 4A–B) as present in *S. ebalensis* sp. nov., have not until now been observed in *S. agonaensis*, where silica granules are the only ornamentation on the valve face.

The closely related *S. bonsaensis* Foged, also decribed from Ghana in West Africa (Foged 1966; Cocquyt & Kusber 2010) differs in valve shape, which is broadly linear and isopolar, and in larger valve dimensions (length 70–80 µm, width 24.5–26.0 µm). There exists a small overlap in number of alar canals in 10 µm: 2.2–3.0 in *S. ebalensis* sp. nov. and 2.0–2.4 in *S. bonsaensis*. As in *S. agonaensis* the wing projection in *S. bonsaensis* is also less pronounced than the distinct projection in *S. ebalensis* sp. nov.. Moreover the striation is coarser (25 in 10 µm in *S. bonsaensis*, 30 in 10 µm in *S. ebalensis* sp. nov.) and in LM no spines or other ornamentation of the valve face is visible.

A third taxon described from Ghana, *Surirella esamangensis* Foged (Foged 1966; Cocquyt & Kusber 2010), is also closely related to the new species described here. Some overlap exists in valve length (58.5–64.0 µm versus 40.3–64.5 µm), density of alar canals (2.0–2.4 in 10 µm versus 2.2–3.0 in 10 µm) and striae density (25–30 in 10 µm versus 30 in 10 µm) but not in valve width (22.5–24.0 µm versus

16.1–19.4 µm). Moreover, in *S. esamangensis* the valve shape is elliptic and slightly heteropolar with evenly rounded apical and base pole and a relatively indistinct wing projection.

Spines on the keel in other *Surirella* taxa were also observed in material from Sulawesi (Bramburger *et al.* 2006), e.g., *Surirella alata* Hustedt, *S. fimbriata* Hustedt and *S. tubicola* Bramburger & Hamilton. These spines are of different length than in the Congo material studied, but the spines in our material are often much larger. In the Sulawesi material spines were also observed on the valve face, for example in *S. tenacis* Bramburger & Hamilton. Both are hollow structures, different from the flat full spines found in for example *S. nervosa* (A.W.F. Schmidt) Mayer. Some of the spine-like structures present on the valve face resemble the four spines present on the valve face of *S. quadridentis* Bramburger & Hamilton. However, these four spines of *S. quadridentis* resemble more the spine-like structures of *Cymatopleura calcarata* Hustedt: also hollow structures (Cocquyt 2001) which are probably processes and not true spines. More studies, including internal valve views and cell wall ontogeny are needed to understand these spine-like structures.

Surirella congolensis sp. nov. is closely related to *S. takoradiensis* Foged, described from Ghana in West Africa (Foged 1966; Cocquyt & Kusber 2010). Although both taxa are heteropolar and about the same length (18.7–33.0 compared to 20.0–27.0 (38.0) µm for *S. takoradiensis*) the valve shape differs: lanceolate in *S. congolensis* sp. nov. while ovate to oval-elliptical in *S. takoradiensis*, slightly constricted in the larger valves compared to no constriction at all, subarcuate compared to rounded base pole. Valves are narrower in *S. congolensis* sp. nov. (5.5–6.9 µm, seldom up to 7.9 µm) than in *S. takoradiensis* (7.0–9.0 (10.5) µm), and the alar canals in 10 µm are mostly denser, 5.5–6.0 and 4.0–6.0, respectively. The fenestrae are lower in *S. congolensis* sp. nov. than in *S. takoradiensis* where the fenestrae are almost rectangular in girdle view.

Surirella congolensis sp. nov. is also related to *Surirella pseudotenuissima* Leclercq, described from Sumatra by Leclercq (1983) using material of the Friedrich Hustedt Diatom Collection (BRM X4/75, picture of the type in Hustedt 1938, tab. 43, fig. 8). Although *S. pseudotenuissima* is a tropical species it was reported from temperate regions in Europe (Belgium) by Denys (1985). It is a heteropolar species with a similar valve length of 25.0–57.0 µm, but much broader with a width of 9.5–13 µm. The alar canals (3.4–5.0 in 10 µm compared to 5.5–6.0 in 10 µm in *S. congolensis* sp. nov.) as well as the striae (23–26 striae in 10 µm compared to about 30 in 10 µm in *S. congolensis* sp. nov.) are coarser. The new taxon is also related to *Surirella tenuissima* Hustedt which has heteropolar valves with a length of 17.0–38.0 µm, a width of 6.0–11.0 µm and 4.0–7.0 alar canals in 10 µm, which are smaller than the fenestrae (Hustedt *in* Huber-Pestalozzi 1942). *Surirella tenuissima* is a littoral species reported from South America and Indo-Malaysia. In Sulawesi and the Philippines (Leyte) it was also found in the plankton (Hustedt *in* Huber-Pestalozzi 1942).

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Cavinula lilandae (Bacillariophyta), a new diatom species from the Congo Basin, Central Africa

Christine Cocquyt [a] , Myriam De Haan [a] & Jonathan C. Taylor [a b c]

[a] National Botanic Garden of Belgium, Meise, Belgium

[b] Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa

[c] South African Institute for Aquatic Biodiversity, Grahamstown, South Africa

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Cavinula lilandae (Bacillariophyta), a new diatom species from the Congo Basin, Central Africa

CHRISTINE COCQUYT[1]*, MYRIAM DE HAAN[1] & JONATHAN C. TAYLOR[1,2,3]

[1] *National Botanic Garden of Belgium, Meise, Belgium*
[2] *Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa*
[3] *South African Institute for Aquatic Biodiversity, Grahamstown, South Africa*

Cavinula lilandae, a small new diatom from the Congo Basin in Central Africa, was observed in acid riverine habitats in an almost pristine tropical rain forest. It occurred in a periphytic diatom community dominated by *Eunotia* spp. and *Fragilariforma strangulata* (Zanon) Williams & Round. Beside the type locality in the Baomba Stream, and the closely located Lilanda River, in the Democratic Republic of Congo, this taxon was also observed in a small river in Zambia, north of the Bangweulu swamps. *Cavinula lilandae* is distinguished from other taxa within the genus *Cavinula* D.G. Mann & A.J. Stickle by the following characteristics: uniseriate striae becoming irregularly biseriate near the poles; irregularly formed areolae; and irregular depressions in the valve face close to the axial area which are not perforating the valve face.

Keywords: *Africa, Congo, Zambia, diatoms,* Cavinula *taxonomy*

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Introduction

Algal reports from (sub-)tropical Africa are rather limited, especially for the Democratic Republic of Congo (DR Congo), formerly Belgian Congo and Zaire. Among the works that can be cited are Duvigneaud & Symoens (1949), Hustedt (1949), Kufferath (1956a, b), Compère & Symoens (1987, 1988), Compère et al. (1989), Golama Swana Kaketa (1992, 1996) and Compère (1995). Although some of these reports deal with the Congo Basin, nothing is known of the algal flora of the region covered by the Belgian-Congolese Boyekoli Ebale Congo 2010 Expedition. This expedition covered 250 km of the Congo River and some of its tributaries between Kisangani and Bumba (Fig. 1; see also Nagy et al. 2011, Virgilio et al. 2011, http://www.congobiodiv.org/nl/expeditie2010). Pristine habitats are rather scarce in this area where people from rural villages live predominantly from subsistence-based agriculture (closely associated with rivers) and fisheries. Oil palm and rubber plantations, as well as coffee, cacao, tea and cotton, are generally farmed by small landowners.

In terms of the diatom flora, the closest area that has been studied is near Kisangani, where Golama Swana Kaketa (1992, 1996) studied samples from 27 sites in rivers and pools. He reported 179 diatom taxa, 146 desmids and 46 euglenids. This work was followed by that of Compère (1995), who described a new diatom, *Gomphonema zairensis*, from the same region at the Tshopo waterfalls.

The diatom flora of the neighbouring country of Zambia was similarly under investigated, with the only in-depth study of the recent flora being that of Cholnoky (1970), in which he investigated the diatoms of Lake Bangweulu. A country-wide survey of the diatom flora of Zambia under the auspices of the Southern African River Assessment Scheme (SAFRASS project) was conducted from 2010 to 2012. Samples from the Lupososhi River in the far north-west of the country in a relatively unimpacted stream, with habitats comparable to the rather pristine sites sampled during the Boyekoli Ebale Congo 2010 Expedition, were investigated. The overall objective of the SAFRASS project is capacity building to improve safe water supply and protect riverine biodiversity in rivers of southern tropical Africa, as laid out under goals 2 and 3 of the United Nations Millennium Development Goal (http://www.safrass.com).

Material and methods

Material used for the present study was collected in May 2010 during the Boyekoli Ebale Congo 2010 Expedition near the village of Lilanda, in a small river (Lilanda) and a stream (Baomba) flowing through an almost pristine tropical lowland rainforest (Fig. 1). Epiphytic diatom samples were collected by scrapping material from plant stems or roots. The sampling localities are situated north-west of Yangambi, Oriental Province, DR Congo, at an altitude between 490 and 530 m above sea level (asl). In addition a single sample from Zambia was studied, collected from the Lupososhi River, in the north-west of Zambia at an altitude of 1250 m asl. This river drains through the Bangweulu

*Corresponding author. Email: christine.cocquyt@br.fgov.be

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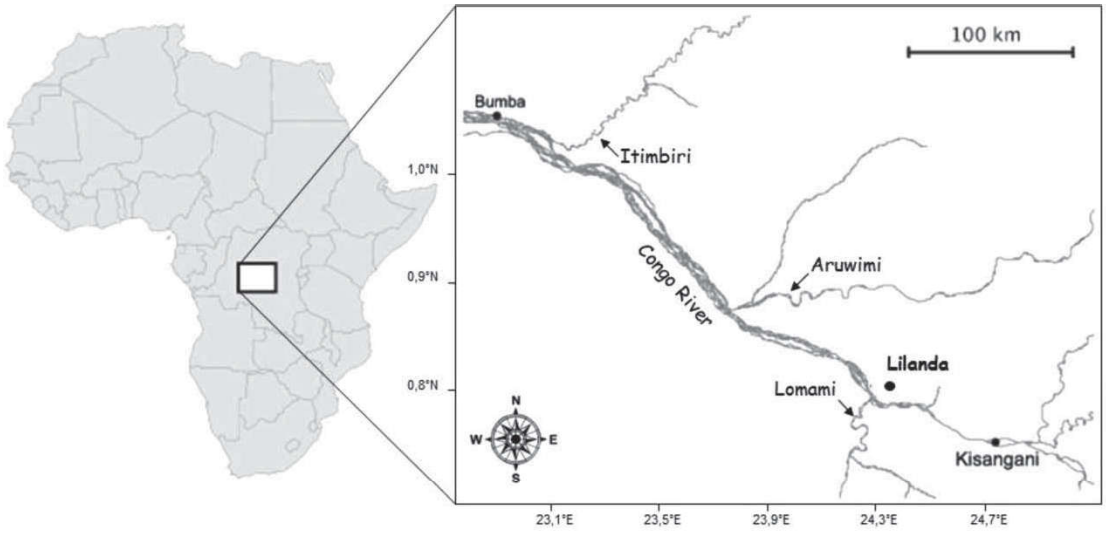


Fig. 1. Map showing the location of the Lilanda River and its tributary, the Baombo Stream. Modified from Virgilio et al. (2011).

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swamps and then via the Luapula River into Lake Mweru, and thus is part of the Congo Basin watershed.

The DR Congo sampling locations in the Lilanda River and Baombo Stream were all in the vicinity of 0.83128°N and 24.32931°E, and 0.84339°N and 24.34464°E, respectively. The only sample from Zambia was from the Lupososhi Stream at 10.35560°S and 29.48071°E. The following samples were examined:

CCA 1232: epiphyton on a liana from a stagnant water pool (5 m^2) at the edge of the Lilanda River; collected 8 May 2010;
CCA 1233: epiphyton on a submerged stem from the Lilanda River close to previous site; collected 8 May 2010;
CCA 1234: epiphyton on submerged roots from the Lilanda River, at the same site as the previous sample; collected 8 May 2010;
CCA 1235: epiphyton on a dead, partially submerged stem from the Lilanda River at 10 m downstream the confluence with the Baomba Stream; collected 8 May 2010;
CCA 1236: epiphyton between mosses on a partially submerged stem from the Lilanda River, at the same site as the previous samples; collected 8 May 2010;
CCA 1237: epiphyton on a submerged stem from the Lilanda River just after the confluence with the Baombo Stream; collected 8 May 2010;
CCA 1238: epipsammon from sandy and gravel substratum at the Baombo Stream; collected 8 May 2010; and
10-537 (35): epilithon from cobbles from the Lupososhi Stream; collected 18 June 2010.

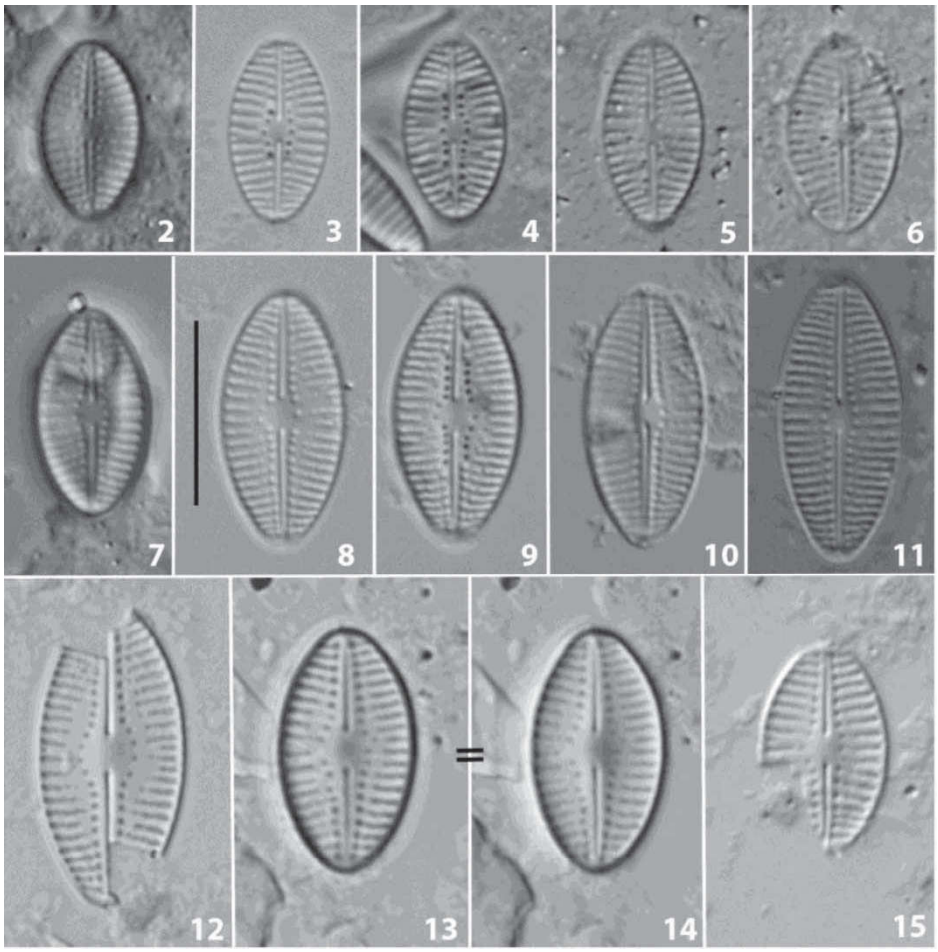
All samples, with the exception of the Zambian sample, were fixed *in situ* with 30% formalin and transported to Belgium at the end of the expedition. Material from these samples was oxidized with hydrogen peroxide (30%), rinsed five times with distilled water and embedded in Naphrax®. Light microscopy (LM) studies were carried out with an oil immersion objective (100×) using an Olympus BX 51 microscope, equipped with Nomarski differential interference contrast optics (DIC). Photographs were taken with an Olympus ColorView III digital camera. For scanning electron microscopy (SEM) studies, aliquots of the oxidized material was put on aluminium stubs and air-dried. The stubs were sputter-coated with 50 nm of gold and studied with a JEOL-5800LV operating at 25 kV at the National Botanic Garden of Belgium. The sample from Zambia was preserved with ethanol, cleaned using potassium permanganate and hot hydrochloric acid, mounted in Pleurax after rinsing with distilled water, and examined using a Nikon 80i microscope equipped with DIC. Photographs were taken with a Nikon DS Fi1 digital camera.

All raw and cleaned samples, and permanent slides from DR Congo, are housed at the herbarium of the National Botanic Garden of Belgium (BR). A copy of each permanent slide will be deposited at the Centre de Surveillance de la Biodiversité (CSB) and/or at the Herbarium of the University of Kisangani, DR Congo. The raw and cleaned Zambian materials as well as permanent diatom slides are housed at the North-West University, Potchefstroom, South Africa.

Water temperature, conductivity and pH were measured at every site in DR Congo using a YSI MPS 556 multiprobe field meter. Relative abundances of the diatom taxa were calculated from a count of 500 valves on each slide examined.

Results

Cavinula lilandae Cocquyt, M.de Haan & J.C. Taylor sp. nov. (Figs 2–11, 16–21)



Figs 2–15. *Cavinula lilandae* sp. nov. **Figs 2–11.** Valves from the holotype slide BR 4265, Baombo Stream, DR Congo, showing size range, valve view, LM. **Fig. 8.** Valve representing the holotype. **Figs 12–15.** Valves from material 10-537 (35) from the Lupososhi River in Zambia, LM. Scale bar = 5 μm.

Description. Valves elliptical with broadly rounded apices; length: 9.2–17.8 μm; width: 5.5–7.9 μm. Striae radiate, sometimes becoming parallel or slightly convergent, 15–20 in 10 μm, composed of 50–60 round areolae in 10 μm. Axial area elliptical, ornamented with a series of irregular depressions parallel to the raphe visible only externally.

Holotype. Slide BR 4265 from sample CCA 1238, National Botanic Garden of Belgium, Meise, Belgium (BR). The valve representing the holotype is here illustrated in Figure 8.

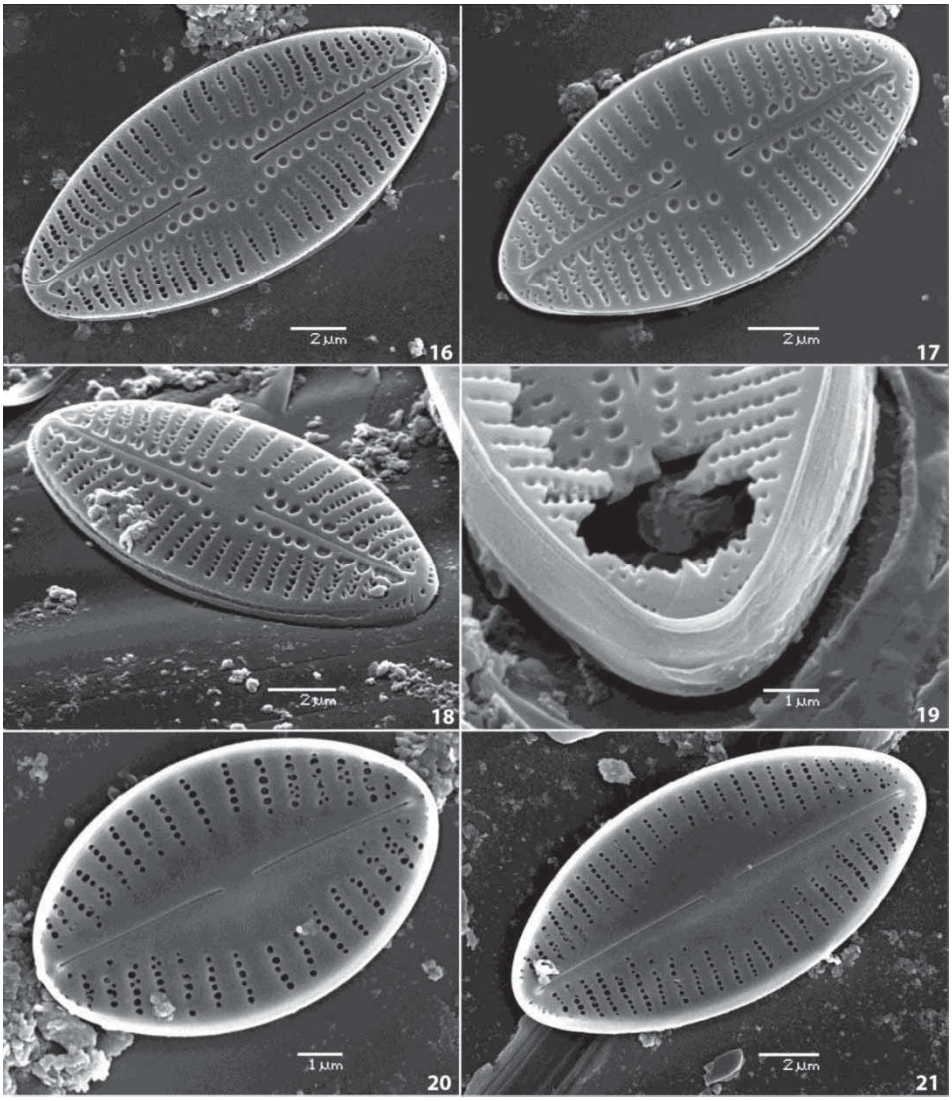
Isotype. Slide ZU8/48 from sample CCA 1238, the Friedrich Hustedt Diatom Collection, Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven, Germany (BRM).

Type locality. Boambo Stream, a small tributary of the Lilanda River, Oriental Province, DR Congo. 0.84339°N, 24.34464°E. An epipsammic taxon of acid running freshwater in a nearly pristine tropical rainforest: pH of 4.65 and conductivity of 12.5–15.0 μS cm^{-1}.

Etymology. The specific epithet refers to the Lilanda River, into which the Baombo Stream flows, where the species was also observed.

Morphological observations. Valves elliptical with broadly rounded apices; length: 9.2–17.8 μm; width: 5.5–7.9 μm; length:width ratio of 1.7–2.3 (n = 34) (Figs 2–11). Striae, 15–20 in 10 μm, composed of 50–60 round areolae in 10 μm, radiate sometimes becoming parallel or slightly convergent near the poles and not continuing over the valve face–mantle junction. Striae uniseriate becoming irregularly biseriate near the poles, with irregularly formed areolae. Axial area very elliptical, ornamented with series of irregular depressions parallel to the raphe not perforating the valve surface and only visible externally (Figs 16–18). Central area extending over half of the valve face with striae sometimes alternately shorter and longer (Fig. 19). A row of a few irregular elongated areolae present at the

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Figs 16–21. *Cavinula lilandae*, type material from sample CCA 1238, Baombo Stream, DR Congo, SEM. **Figs 16–19.** External view. **Figs 20–21.** Internal view. **Figs 16–17.** Valve face showing the irregular depressions in the axial area, the terminal raphe endings and the uniseriate striae becoming irregularly biseriate near the poles. **Fig. 18.** Whole valve with a shallow and indented mantle, and four to five irregular elongated areolae at the valve face–mantle junction on each side of the terminal raphe endings. **Fig. 19.** Valve broken at the apex showing the axial area depressions, not perforating the valve face, and part of a narrow advalvar girdle band and a much broader second band. **Figs 20–21.** Valves showing straight central raphe endings and small helictoglossae at the poles. Scale bars = 2 µm (Figs 16–18, 21); 1 µm (Figs 19–20).

junction of the valve face and mantle at the poles; the middle areola round and not elongated, and distinctly separated from the other four to five areolae on each side (Fig. 18). Valve face flat curving fairly abruptly into the valve mantle. Mantle shallow with a long depression that does not extend to the poles. Central raphe endings externally slightly expanded, internally unexpanded and straight (Figs 20–21). Terminal raphe endings abruptly curved in the same direction on the external valve face; a small helictoglossa present internally (Figs 20–21). Girdle bands without transverse rows of areolae or any other ornamentation; narrow advalvar girdle band, second band much broader (Fig. 19).

Remarks. The mean length and width of *C. lilandae* in the DR Congo material, including the type material, based on measurements of 34 valves, is 13.7 and 6.7 µm, respectively (Fig. 22). The valves observed in the Zambian material (Figs 12–15) fit within these dimensions, as well as conforming to the other characteristics of this newly described species. The single complete valve observed from Zambia (Figs 13–14) has a valve length of 13.7 µm, a width of 7.2 µm and 17 striae in 10 µm.

Ecology. Physical and chemical parameters are only available for the sampling localities in the DR Congo. At the time of sampling, the temperature of the Lilanda River

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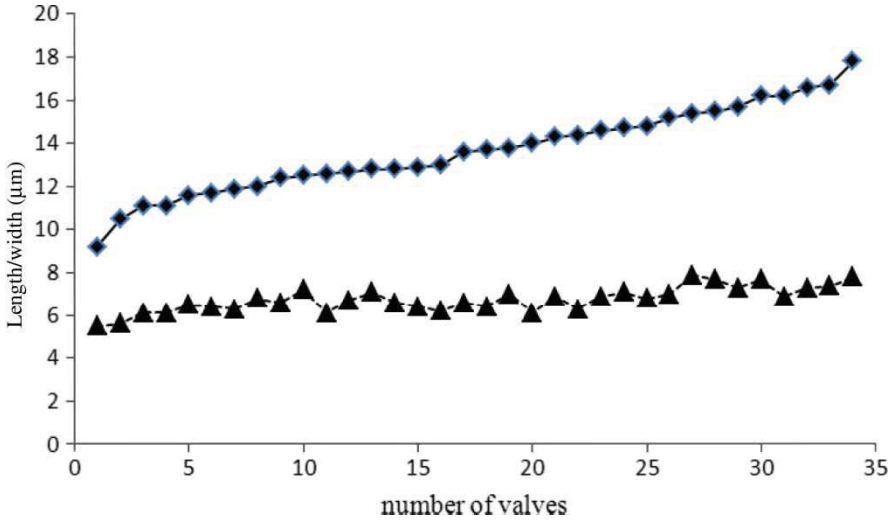


Fig. 22. Variation in the valve length (diamond) and width (triangle) for 34 valves of *Cavinula lilandae* measured in LM and SEM.

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varied between 24.3 and 24.4°C, measured between 9:45 am and 13:50 pm; the temperature of the Baombo Stream, the type locality, was 24.2°C at 12:00 am. The conductivity of the Baombo Stream (15.3 µS cm^{-1}) was slightly higher than that of the Lilanda River (12.3–12.5 µS cm^{-1}), while the pH value was somewhat lower in the Boambo Stream (4.65) compared to that of the Lilanda River (4.81–4.83).

Cavinula lilandae was observed in epiphytic and epipsammic diatom communities dominated by *Eunotia* spp., whereas *Fragilariforma strangulata* (Zanon) Williams & Round or *Sellaphora* cf. *seminulum* (Grunow) D.G. Mann may also be co-dominant (Table 1).

Discussion

The newly described *Cavinula* species was found at six of the seven sites near the village of Lilanda in the Baombo Stream, the type locality, and in the Lilanda River which flows through an almost pristine tropical lowland rainforest at an altitude between 490 and 530 m asl. In addition, this new species was found in the Lupososhi River in north-west Zambia. The taxon has not been recorded in other small acid rivers, which included effluents of the Aruwimi, Itimbiri and Lomami rivers sampled during the same expedition.

Cavinula lilandae was always present in low relative abundance (2.4%) in samples dominated by *Eunotia* Ehrenberg (Table 1) and was not observed in the sample CCA 1237 dominated by *Fragilariforma strangulata* (93.8%), in which *Eunotia* contributed only 2.3% of the species assemblage (Table 1). The Lupososhi River sample where this species was also observed was dominated by *Eunotia* spp. with *Fragilariforma strangulata* present, as in the DR Congo samples.

Cavinula lilandae forms part of both the epiphytic or epipsammic diatom assemblages in small tropical rivers and streams. The type habitat of *C. lilandae* is similar to that in which other representatives of the genus were found. According to Round et al. (1990), taxa belonging to the genus *Cavinula* D.G. Mann & A.J. Stickle are restricted to freshwater where they occur in the epipelon of oligotrophic lakes or in shady, moist subaerial habitats. Examples of species living in oligotrophic lakes are *C. lacustris* (Gregory) D.G. Mann & A.J. Stickle, which was described from Loch Leven in Scotland (Gregory 1856), and *C. jaernefeltii* (Hustedt) D.G. Mann & A.J. Stickle from a pond in Finland and from Lake Aokiko in Japan (Hustedt in Schmidt 1936). *Cavinula jaernefeltii* was also reported as abundant in an alkaline, oligotrophic pond from Prince Patrick Island, Nunavut, Canada comprising up to 54% of the total relative diatom abundance (Antoniades et al. 2009). Another taxon, *C. pseudoscutiformis* (Hustedt) D.G. Mann, has also been reported from the same Canadian High Arctic island,

Table 1. Relative abundance (%) of *Cavinula lilandae* and dominant diatom taxa in the epiphytic and epipsammic communities in the Lilanda River and Baomba Stream, Democratic Republic of Congo.

Sample	Community	*C. lilandae*	*Eunotia* spp.	*Fragilariforma strangulata*	*Sellaphora* cf. *seminulum*
CCA 1232	epiphyton	0.2	42.9	0	0
CCA 1233	epiphyton	0.5	23.3	35.1	0
CCA 1234	epiphyton	0.6	38.2	0	0
CCA 1235	epiphyton	2.0	30.1	0	0
CCA 1236	epiphyton	2.4	36.8	0	0
CCA 1237	epiphyton	0	2.3	93.8	0
CCA 1238	epipsammon	2.4	24.4	0	19.0

with a relative abundance up to 15% of the total diatom community in circumneutral (6.3–8.0) and low conductivity water (13–212 µm cm^{-1}) with moderate dissolved organic carbon concentrations (1.2–3.0 mg L^{-1}). Despite being thought by Round et al. (1990) that taxa belonging to *Cavinula* are restricted to freshwater, a new species, *C. kerguelensis* Riaux-Gobin & Compère, was described from a marine habitat at Observatory Bay, Kerguelen Island (Riaux-Gobin & Compère 2004).

The newly described taxon fits most of the morphological characteristics of the genus *Cavinula* as given in Round et al. (1990). The valve face is flat and curves fairly abruptly into a shallow mantle. The striae are uniseriate as is always the case in *Cavinula*, although they become irregularly biseriate near the poles. The raphe-sternum lies centrally and is only slightly thickened internally. The external central raphe endings are expanded and pore-like. However, the terminal raphe endings are deflected in the same direction, while in *Cavinula* they are deflected in opposite directions so that externally the raphe is slightly sigmoid. Internally the central raphe endings are not expanded in *C. lilandae*.

Cavinula lilandae also has characteristics of the genus *Placoneis* Mereschkowsky but differs from this genus. *Placoneis* has a valve face that is sharply differentiated from the valve mantle while in *Cavinula*, the mantle becomes much narrower near the poles. In *Placoneis*, the internal central raphe endings are hooked towards the primary side of the valve and the external terminal raphe endings are hooked in the same or in opposite directions.

Cavinula lilandae differs most markedly from other species of this genus by the presence of a single row of irregular depressions in the axial area running parallel to the raphe. Beside this most important distinguishing characteristic, *C. lilandae* differs from *C. jaernefeltii* (Antoniades et al. 2009), a species having a similar valve outline, length, width and number of striae (9.2–17.2 µm, 5.5–7.9 µm, 15–20 striae in 10 µm for *C. lilandae versus* 9.0–20.0 µm, 6–9 µm, 18–24 µm in 10 µm for *C. jaernefeltii*) in the greater number of areolae (50–60 *versus* 28–36) and the structure of the terminal raphe endings, which are abruptly curved in the same direction on the external valve face in contrast to the straight endings of *C. jaernefeltii.* Valve outline and radiate striae becoming parallel to slightly convergent near the poles differentiate *C. lilandae* from other *Cavinula* species such as *C. lacustris*, *C. scutelloides* (W. Smith) Lange-Bertalot, *C. pseudoscutiformis* and *C. vincentii* Antoniades & P.B. Hamilton.

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Stenopterobia cataractarum sp. nov. (Bacillariophyta), a new benthic diatom from a waterfall in Zambia, Africa

CHRISTINE COCQUYT [1], JONATHAN C. TAYLOR [1,2,3*] & CARLOS E. WETZEL[4]

[1]*National Botanic Garden of Belgium, Domein van Bouchout, 1860 Meise, Belgium*
[2] *School of Biological Sciences, North-West University, Potchefstroom, South Africa*
[3]*South African Institute for Aquatic Biodiversity, Grahamstown, South Africa*
[4] *Department of Environment and Agro-Biotechnologies (EVA), Public Research Centre -Gabriel Lippmann, 4422 Belvaux, Grand-duchy of Luxembourg.*
**Corresponding author: E-mail: Jonathan.Taylor@nwu.ac.za*

Abstract

Rivers, streams and associated habitats have been sampled for diatoms in the relatively understudied Congo and Zambezi sister basins. The primary purpose of the sampling effort was to collect samples for biological monitoring purposes and the establishment of a diatom-based monitoring programme. However, a large number of possibly endemic and novel taxa have been encountered in this region making the application of diatom-based indices at a species level difficult. In this paper we describe a new species of *Stenopterobia* from the Ntumbachushi Falls in the Luapula Province of Zambia. This taxon is closely related to *Stenopterobia delicatissima* but chiefly differentiated by a lower number of striae, a well-defined unornamented lanceolate axial area and shortened ribs which do not fuse with the fenestrae. This taxon also shows some similarity to *Stenopterobia delicatissima* var. *ghanaensis* but the valves are consistently narrower.

Key words: Africa, diatoms, freshwater, new species, Surirellaceae, taxonomy

Introduction

In recent decades diatoms have become a vital part of the suite of biological indicator organisms used to monitor changes and disturbances in water quality. The majority of this work has been done in Europe and North America with a number of diatom based water quality indices being developed. These indices and other diatom-based methods and metrics have proved invaluable for water quality monitoring (Smol & Stoermer & 2010). Recently these methods were applied in South Africa (e.g. Taylor et al. 2007) with some success. However Taylor et al. (2007) highlighted potential problems with the transfer of European based metrics when endemic African taxa are present as dominants in the samples. In this way, the EU ACP funded Southern African Rivers Assessment Scheme or SAFRASS (www.safrass.com) was undertaken in order to develop capacity building tools with the aim of introducing a suite of biomonitoring tools in Zambia; similarly the BELSPO funded Congo Zambezi Diatom Monitoring or COZADIMO project was initiated to determine the usefulness of diatoms as water quality indicators in the Congo and Zambezi sister basins.

Central Africa has a very large diversity of aquatics habitats and, given the size of this region, is remarkably understudied. Fortunately a large number of rivers and streams around Zambia and the Democratic Republic of the Congo were recently sampled and in addition a number of associated habitats such as waterfalls and seeps. On examination of some of the samples, collected under the auspices of the above mentioned projects, it has become clear that the majority of species encountered form part of the paleo- and neo-tropical flora and that a high percentage of these taxa are new to science and are possibly endemic to tropical central Africa. This makes the application of diatom indices rather difficult, especially at a species level with many taxa undescribed.

In this paper we examine a novel species from the genus *Stenopterobia* which forms a relatively small but heterogeneous group that comprises several taxa sometimes previous allocated to the genus *Surirella* Turpin (1828:

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362) and contains currently approximately 55 taxa (including species and varieties) widely distributed worldwide (Fourtanier & Kociolek, 2011). The genus *Stenopterobia* is characterized by cells that are solitary and sigmoid or straight and usually relatively narrow. The valve face is slightly undulate, with a fibulate raphe system around the whole circumference, the raphe system and the canal beneath it are raised above the general level of the valve. The striae are multiseriate containing tiny round poroids, which are not occluded by hymenes. The terminal raphe endings are simple or slightly curved externally and simple internally (Round et al. 1990). This genus is significant in that the member species usually indicate a specific suite of environmental conditions: oligotrophic acidic water with a low mineral content (Frankova *et al.* 2009, Lange-Bertalot & Metzeltin 1996, Sabater & Roca 1992). The new species can be loosely associated with the *Stenopterobia delicatissima* group but has several unique morphological characteristics which clearly separate it from *S. delicatissima* sensu stricto and other recently described taxa from this group (Siver & Hamilton 2005, Siver & Camfield 2007). In this paper we describe the epilithic diatom *Stenopterobia cataractarum sp. nov.* growing in the spray zone of the Ntumbachushi Falls, Zambia.

Material and methods

Epilithic diatom material for the present study (field sample number 12-349) was collected by scraping with a spoon from the surface of rocks covered with detrital material in a seep in the spray zone near to the main cataract of Ntumbachushi Falls (09.853736°S, 28.944683°E), Ngona River, Luapula Province, Zambia by J.C. Taylor. In total 8 samples were collected from this locality in June 2012 (winter, dry season), *Stenopterobia cataractarum sp. nov.* was found only in the sample mentioned above and sample 12-353 collected from damp wooden logs.

All samples were fixed in situ with ethanol (20% v.v). Material from these samples was oxidized with potassium permanganate (saturated solution), cleared with concentrated hydrochloric acid, rinsed 5 times with distilled water and embedded in Pleurax (Von Stosch 1974). Light microscopy (LM) studies were carried out at the National Botanic Garden of Belgium with an Olympus BX 51 microscope equipped with an oil immersion objective 1.4 N.A. (100×) and Nomarski differential interference contrast (DIC). Photographs were taken with an Olympus ColorView III digital camera. For scanning electron microscopy (SEM), a part of the oxidized suspension was filtered and rinsed with additional deionized water through a 3-µm Isopore™ polycarbonate membrane filter (Merck Millipore). Filters were mounted on aluminium stubs and coated with platinum using a BAL–TEC MED 020 (BAL–TEC AG, Balzers, Liechtenstein) for 30 s at 100 mA. An ultra-high-resolution analytical field emission (FE) scanning electron microscope Hitachi SU–70 (Hitachi High-Technologies Corporation, Tokyo, Japan) operated at 5 kV and 10 mm distance was used for the analysis, and SEM images were taken using the high (SE-H) detector signal at the Department of Environment and Agro-Biotechnologies (EVA), Public Research Centre-Gabriel Lippmann, Luxembourg. Supplementary SEM studies were carried out using a FEI QUANTA FSEM at the Laboratory for Electron microscopy North-West University, South Africa. Raw samples and permanent slides are kept at the herbarium of the South African National Diatom Collection housed at the North-West University, South Africa.

Morphological terminology used for the genus *Stenopterobia* is after Round et al. (1990) and Siver & Camfield (2007).

Physico-chemical variables were measured concurrent with the collection of the diatom sample with the following instruments: pH—Hanna HI 98129 portable meter, temperature, electrical conductivity and oxygen concentration using a YSI 556 MPS multi parameter meter.

Results

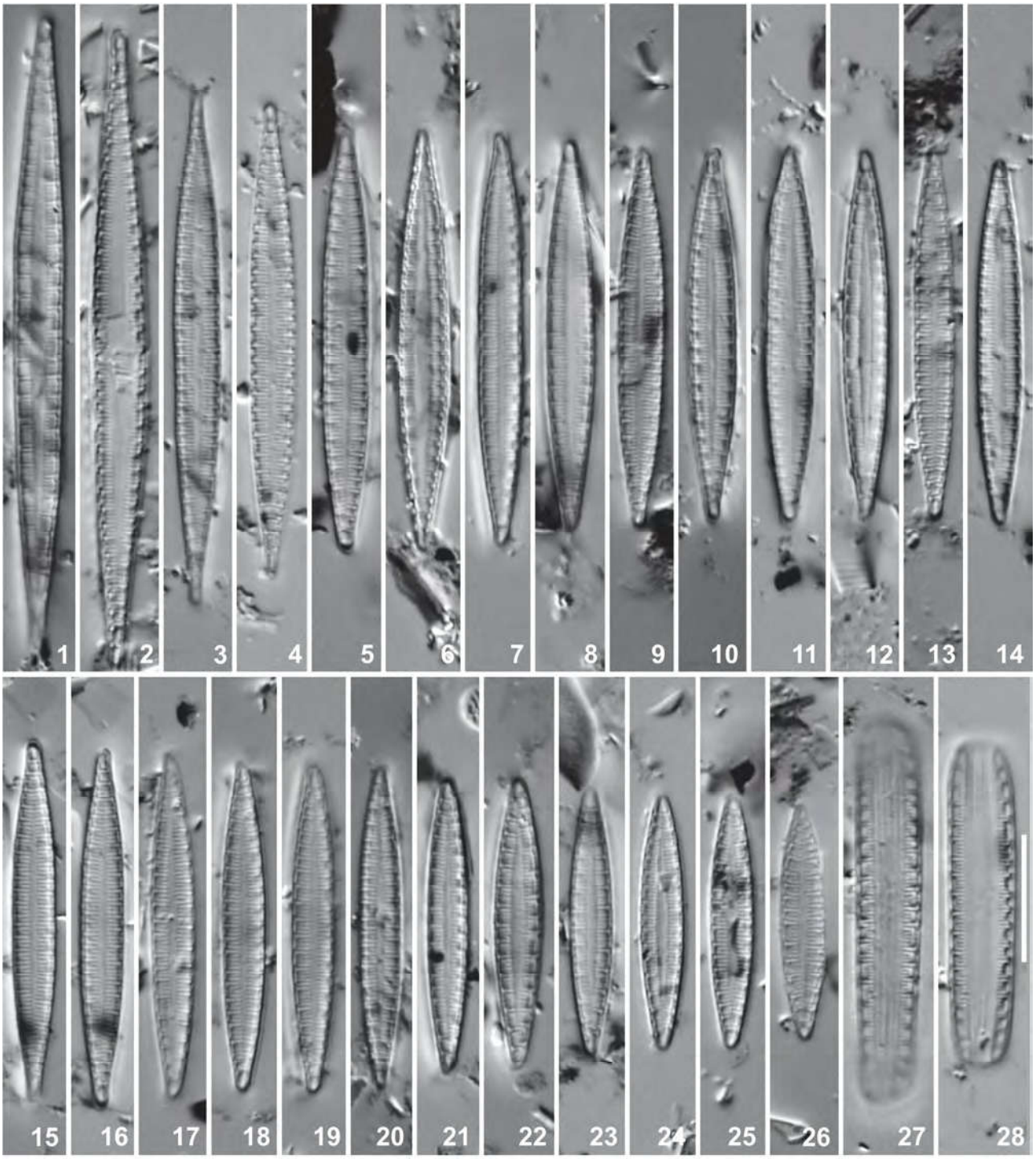
New Species Description

Division **Bacillariophyta**
Class **Bacillariophyceae** Haeckel 1878
Subclass **Bacillariophycidae** D.G. Mann in Round *et al.* 1990

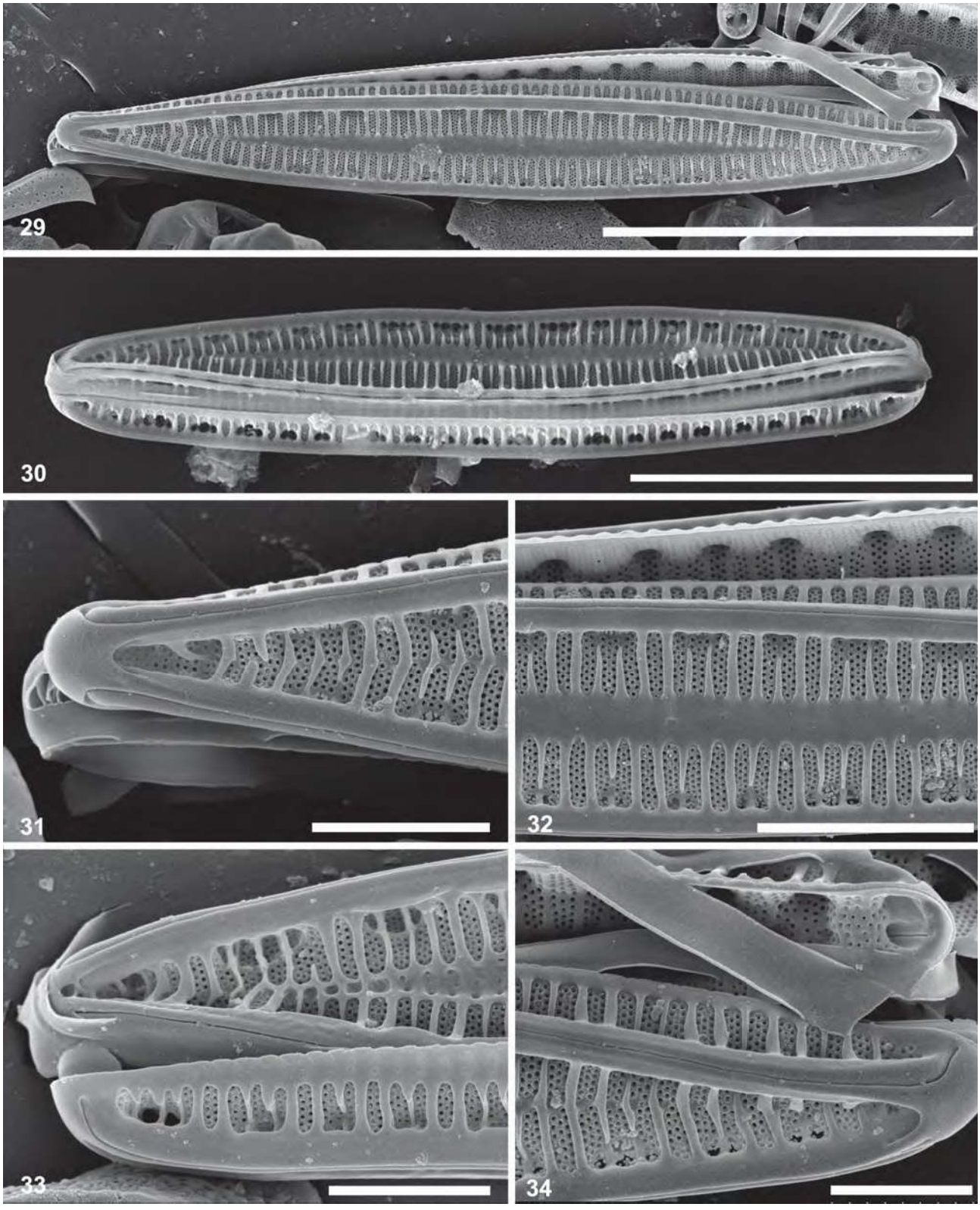
Order **Surirellales** D.G. Mann in Round *et al.* 1990
Family **Surirellaceae** Kützing 1844
Genus ***Stenopterobia*** Brébisson ex Van Heurck 1896

Stenopterobia cataractarum Cocquyt & J.C.Taylor *sp. nov.* (Figs. 1–38)

Valves isopolar, lanceolate, with slightly protracted rounded poles. Length: 18.5–54.6 µm, width: 3.5–4.6 (5.2) µm; length to width ratio: 4.9–12.6. Striae bi- to tri-seriate, 28–30 in 10 µm. Alar canals smaller than fenestrae, 6–8 in 10 µm, not reaching the middle of the valve face. Pairs of two to three ribs extending from the very broad axial area are connected with the keel and fusing with the alar canals. In between these pairs 1–3 shorter ribs, mostly 2, not connected to the keel.



FIGURES 1–28: *Stenopterobia cataractarum sp. nov.* LM (DIC). Figures 1–26. Valves from the holotype slide BR 4345, Ntumbachushi Falls, Luapula Province, Zambia, showing size range, valve view. Figures 27–28. Girdle views. Scale bar = 10 µm.

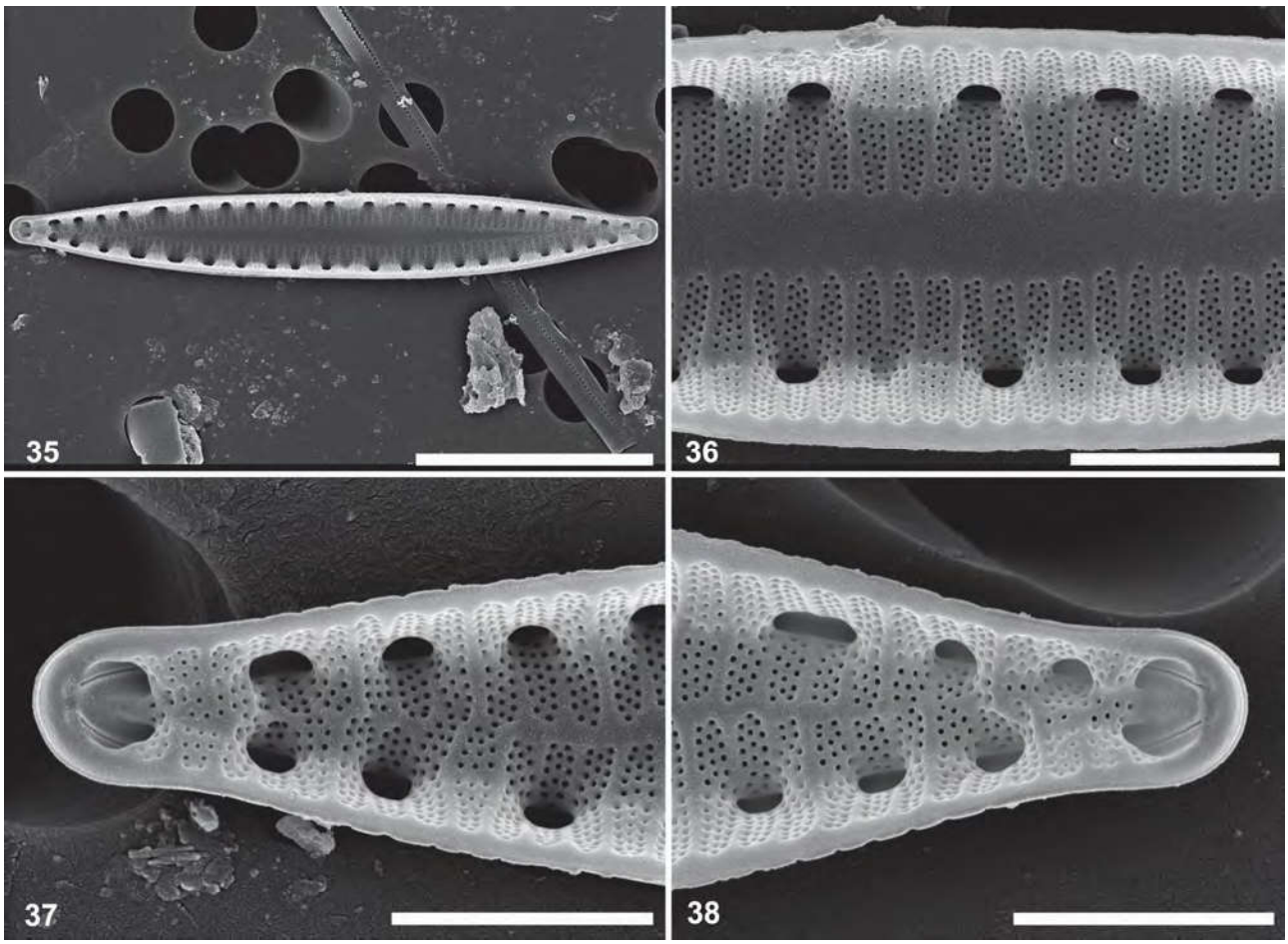


FIGURES 29–34: *Stenopterobia cataractarum sp. nov.*, type material from sample 12-349, Ntumbachushi Falls, Luapula Province, Zambia, external valve view. SEM. Figure 30. Entire valve showing the slightly elevated axial area on the valve face and the distinct fenestra on both valve face and valve mantle. Figures 31, 33–34. Detail of the terminal raphe fissures bent to the valve mantle. Figure 32. Valve face showing bi-to triseriate striae composed of round areolae, the broad axial area and one to two shortened transapical ribs in between the ribs connected to the alar wings. Figure 33. Mantle view showing the slightly comma-shaped terminal raphe fissures, the shortened ribs and one open girdle band near the apex. Figure 34. Junction of the valve face and mantle with the funnel-shaped thickenings near the apex. Scale bars: 10 μm (in Figs 29–30). , Scale bar: 3 μm (in Fig. 32). Scale bar: 2 μm (in Figs 31, 33–34) .

Holotype:—ZAMBIA. Luapula Province, Ntumbachushi Falls, 09.853736°S, 28.944683° E, epilithic sample, collected by J.C. Taylor (collector number 12-349), on slide *BR 4345, National Botanic Garden of Belgium sample number 12-349* (the valve representing the holotype is here illustrated as Figure 16), **holotype designated here**; slide *PUC 12-349, South African National Diatom Collection, North-West University, South Africa sample number 12-349,* **isotype designated here**.

Etymology:—The specific epithet refers to the waterfalls (cataracta), splashing on the wet steps where the species was found.

Description:—Valves isopolar, lanceolate, with slightly protracted rounded poles. Length: 18.5–54.6 µm, width: 3.5–4.6 (5.2) µm; Length to width ratio: 4.9–12.6. Striae around 28–30 in 10 µm, bi- to tri-seriate both on the valve face and mantle. They do not reach the middle of the valve but leave a broad lanceolate thickened axial area. Axial area does not extend up to the poles but is present on about two thirds of the valve length, and occupies less than one third of the valve width. No ornamentations or perforations are present in the axial area but occasionally unevenly distributed shallow depressions occur. The other parts of the external valve face are ornamented with regular transapically thickened ribs and striae. A single bi- to tri-seriate stria is present in between two transapical ribs. Striae composed of tiny round alreolae, about 100 in 10 µm. Externally the areolae are surrounded by a thickened ring which represents the remains of the caps; internally they are unoccluded.



FIGURES 35–38: *Stenopterobia cataractarum sp. nov.*, type material from sample 12-349, Ntumbachushi Falls, Luapula Province, Zambia, SEM. Internal valve view. Figure 35. Overview of the entire valve. Figure 36. Detail in the middle of the valve showing the simple portulae and the bi- to triseriate striae composed of unoccluded areolae. Figure 37. Apical pole with simple, slightly bent internal terminal raphe fissures. Figure 38. Base pole with simple, straighter terminal raphe fissures extending slightly onto the valve mantle. Scale bars: 10 µm (in Fig. 35). Scales bar: 2 µm (in Figs 36–38).

Alar canals about 8 in 10 µm, those in the middle are broader, almost double the size of those near the poles. The spaces between two alar canals form small fenestrae, divided by fenestral bars. A pair of two or three ribs extend from the axial area and connect with the keel and fuse with the alar canals, while between these pairs 1–3,

mostly 2, ribs are not connected to the keel. Their elongations form, together with the aligning ribs from the mantle, the fenestrae bars. The same rib structure is present on the slightly indented mantle. Internally the alar canals are composed of simple portulae, containing one to two striae, which are bi- to tri-seriate.

The raphe is located on the top of the keel which is smooth and without any ornamentation. The raphe ridge originates from the raphe ribs present near the apices and flares forming funnel-shaped thickenings. The funnel-shaped thickenings of both sides of the valve fuse near the apices forming a smooth area without any perforation. Near the apices, the distal raphe fissures bend down onto the mantle almost forming a right angle and are slightly comma-shaped; internally, these raphe fissures are not enlarged, slightly bent on the internal valve face near one pole and are straighter extending slightly onto the valve mantle at the other pole.

Girdle with several open, smooth bands, without any perforation or ornamentation.

Remarks:—The differences between the internal terminal raphe fissures of *Stenopterobia* points to a bipolarity in the valve as observed in *Surirella* (Cocquyt & Jahn 2007) where, even in isopolar valves, the internal terminal raphe fissures are continuous on one pole and interrupted near the other pole. The apex in *Surirella,* with the continuous internal raphe fissure, corresponds with the apical pole in the heteropolar species, while the apex with the interrupted fissure corresponds to the base pole. Externally no differences can be observed in the apices in isopolar valves in both *Surirella* as in *Stenopterobia*.

Ecology:—Freshwater, epilithic, acidophilic, oligotrophic taxon. The main stream of the falls had the following characteristics at the time of sampling (single measurement): pH 6.56, 18.13°C, 4 µS cm^{-1} electrical conductivity and an oxygen concentration of 9.70 mg l^{-1}.

Distribution:—ZAMBIA, Luapula Province, Ntumbachushi Falls.

Discussion and conclusion

Siver & Camfield (2007) studied material from the type locality of *Stenopterobia delicatissima* (F.W. Lewis) Brébisson ex Van Heurck (1896: 374) and provided an elaborated description and detailed SEM micrographs. According to their description, the valve shape is linear-lanceolate to linear in *S. delicatissima* while it is lanceolate in *S. cataractarum*. The apices also differ in the two species: triangular-shaped and pointed and protracted to needle-like, especially in longer valves of *S. delicatissima* in contrast to the acutely rounded apices in *S. cataractarum*. The valves of *S. cataractarum* are inclined to be shorter compared to those of *S. delicatissima*. However, different populations studied by Siver & Camfield (2007) showed that the minimal and maximal valve length ranged from 24–60µm to 38–72 µm. As a lower number of individuals and only one population of *S. cataractarum* was observed, the maximal valve length variability cannot be absolutely stated, however the shortest valves we measured of *S. cataractarum* were 18.5 µm as opposed to the 24 µm for *S. delicatissima*. In addition *S. delicatissima* has 30–35 striae per 10 µm while *S. cataractarum* has 28-30 per 10 µm. Other characteristics observed in SEM are different in the two taxa and are discussed below. Siver & Camfield (2007) stated that "at the apex of the valve, the raphe ridge closest to the valve face flares to form a funnel-shaped thickening, which fuses with its counterpart from the other side of the valve". The same structure can be observed in the novel *Stenopterobia* species but differs in the greater distance from the apices where the ribs fuse also taking into account that this distance varies with valve shape, being wider on valves with rounder ends and shorter on more protracted valves. Moreover, the typical shortened ribs not connected to the keel in *S. cataractarum* are not present in cells of *S. delicatissima* where all ribs are connected to the keel or run under the keel. The ribs running under the keel are fused with the aligning ribs from the mantle and form the fenestral bars. The fenestral bars in *S. cataractarum* are formed by the elongations of the ribs of the valve mantle.

Other closely related species are [*Stenopterobia*] *Surirella welshii* Cholnoky and *Stenopterobia krammeri* Metzeltin & Lange-Bertalot (1998: 220) (Table 1). Differences in morphological features can be observed in LM. The valve shape of *S. cataractarum* is lanceolate with rounded, sometimes slightly protracted poles while *S. welshii* is elliptical with acutely rounded poles. The width of the valve also differs, 3.6–4.4 µm in *S. cataractarum* compared to 5–7 µm for *S. welshii*, hence the length-to-width ratio is larger in *S. cataractarum* (4.9–12.6.) than in *S. welshii* (3.6–5). The striae density is slightly higher in *S. cataractarum* (28–30 in 10 µm) than in *S. welshii* (22–26). The density of the alar canals on the other hand lies in the same range, 6–8 compared to 5–7.5 in 10 µm. *Stenopterobia cataractarum* differs from *S. krammeri* which has cuneate, bluntly to acutely rounded poles, broader

valves (6–9 µm) and thus a lower length-to-width ratio (4–4.5). The density of the alar canals, 4–7 in 10 µm in *S. krammeri* overlaps partly tending to be coarser, as are the striae, 23–26 in 10 µm.

TABLE 1: Overview of the most important morphological features in *Stenopterobia cataractarum sp. nov.*, *S. delicatissima*, *S. welshii* and *S. krammeri*, according to this study, Siver and Camfield (2007), Cholnoky (1962) and Metzeltin & Lange-Bertalot (1998) respectively. Differentiating features indicated in bold.

	S. cataractarum	*S. delicatissima*	*S. welshii*	*S. krammeri*
Valve length (µm)	**18.5**–54.6	34–68	25–35 (41.7)	24–50
Valve width (µm)	3.5–4.6 (5.2)	3.5–5	5–7	6–9
Length/width ratio	**4.9–12.6**	–	3.6–5.0	4–4.5
Alar canals /10 µm	6–8	6.5–7.8	5–7.5	4–7
Alar canals	smaller than fenestrae	smaller than fenestrae	smaller than fenestrae	smaller than fenestrae
Striae/10 µm	**28–30** bi- to tri-seriate	30–35	22–26	23–26
Areolae /10 µm	100	–	–	–
Apical poles	isopolar	isopolar	isopolar	isopolar
	slightly protracted	protracted and needle-like, especially on longer valves	acutely rounded	cuneate, bluntly to acutely rounded
Valve shape	lanceolate	linear–lanceolate to linear	elliptical	linear-lanceolate
Valve margins	parallel mid-valve	parallel (can be slightly constricted centrally)	never parallel mid-valve (except in the initial and first cells)	
Axial area	lanceolate	narrow, linear–lanceolate (ca. 20% of the valve width)	broad, elliptical	irregular width
Wing projection	very indistinct	very indistinct	very indistinct	very indistinct

In LM the valve shape of *Stenopterobia delicatissima* var. *ghanaensis* (Foged) Cocquyt & Kusber described from Ghana (Foged 1966) looks almost identical to *S. cataractarum*. Plotting length against width data of the two taxa showed that Foged's taxon is somewhat broader and is distinctly separate from the new species (Fig. 39). Unfortunately no valves of *S. delicatissima* var. *ghanaensis* were observed in SEM but only valves belonging to *Surirella delicatissima* var. *africana* nom. inval. (Cocquyt & Kusber 2010), a taxon having biseriate striae. Thus no absolute confirmation can be given that *Stenopterobia delicatissima* var. *ghanaensis* and *S. cataractarum* do not belong to the same taxon, unless material from the type locality of *S. delicatissima* var. *ghanaensis* containing this taxon can be collected and studied thoroughly.

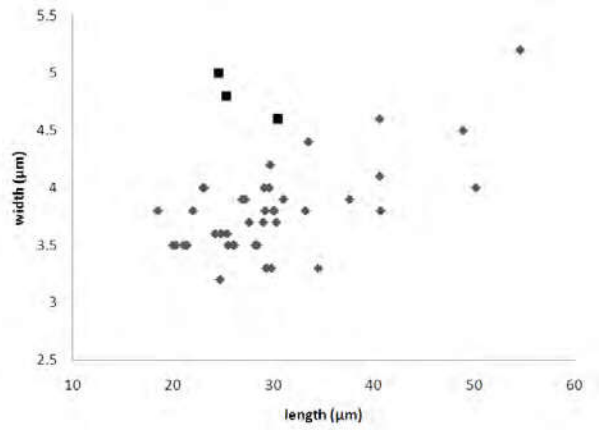


FIGURE 39: Length to width ratio of 141 valves of *Stenopterobia cataractarum* (diamonds) and 3 valves of *Stenopterobia delicatissima* var. *ghanaensis* (squares) from material of sample 12-349, Ntumbachushi Falls, Luapula Province, Zambia and Foged (Foged 1966, Cocquyt & Kusber 2010) respectively.

The ecology of *Stenopterobia cataractarum* fits well within the known ecology of the genus *Stenopterobia* according to Round et al. (1990): freshwater, (epipelic), apparently restricted to acid oligotrophic lakes and ombrogenous mires. It was found on periodically wetted stones (epilithic) but was also found in low numbers in other habitats including wooden logs in the spray zone at the type locality. It was not found in any of the samples from the Ngona River.

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Eunotia rudis sp. nov., a new diatom (Bacillariophyta) from the Man and Biosphere Reserve at Yangambi, Democratic Republic of the Congo

CHRISTINE COCQUYT[1,*], MYRIAM DE HAAN[1] & EDIT LOKELE NDJOMBO[2]
[1] *Botanic Garden Meise, 1860 Meise, Belgium*
[2] *Institut Facultaire des Sciences Agronomiques, Yangambi/Kisangani, Democratic Republic of the Congo*
** Corresponding author (E-mail: christine.cocquyt@botanicgardenmeise.be)*

Abstract

Eunotia rudis sp. nov. is described from material collected in acid rivers in an almost pristine tropical rain forest in the Congo Basin in Central Africa. The benthic diatom community was dominated by other *Eunotia* spp. and small naviculoid taxa. The morphological features of the new species are described and documented based on light and scanning electron microscopy investigations. *Eunotia rudis sp. nov*. can be distinguished from other taxa within the genus *Eunotia* by its typical slightly asymmetric valve shape with four dorsal undulations and the rough surface of the thick silica wall. In contrast to other *Eunotia* species, the number of dorsal undulations was constant in all observed populations. Differences between the new species and the related *Eunotia garucisa* and *E. garucisa* var. *polydentula* are discussed.

Key words: Africa, diatoms, freshwater, new species, taxonomy

Introduction

Algal reports from the Democratic Republic of the Congo (DRC), formerly Belgian Congo and Zaire, are rather limited. The studies conducted during the 20th century, *e.g*. Duvigneaud & Symoens (1949), Hustedt (1949), Kufferath (1956a, b), Cholnoky (1970), Compère & Symoens (1987, 1988), Compère *et al*. (1989), Golama Swana Kaketa (1992, 1996) and Compère (1995) do not deal with the region around Yangambi, even though it is an important agricultural research station along the Congo River, at about 80 km downstream from Kisangani (Tshopo Province, part of the formerly Orientale Provence).

Interest in the algal flora of this region started recently with the Belgian-Congolese Boyekoli Ebale Congo 2010 expedition, an expedition that covered 250 km of the Congo River and some of its tributaries between Kisangani and Bumba (Nagy *et al*. 2011, Virgilio *et al*. 2011, Cocquyt *et al*. 2013, www.congobiodiv.org/nl/expeditie2010). It is therefore not surprising that new species are to be discovered in the unexplored waters of the Yangambi region, which is nowadays a Man and Biosphere (MAB) Reserve (*e.g*. Cocquyt *et al*. 2013, Cocquyt & Taylor 2015, Karthick *et al*. 2016 and Taylor *et al*. 2016, in press). As observed during our investigations, members of the genus *Eunotia* Ehrenberg (1837: 44) are well represented in the waters of lower pH from DRC. Taylor *et al*. (in press) discussed in detail *Eunotia zygodon* Ehrenberg (1843: 415), based on material from the Lobaye River, a large tributary of the Lomami River, on the left bank of the Congo River (Fig. 1). They demonstrated the large morphological variability within *E. zygodon* with decreasing number of undulations of the dorsal margin during the diminishing of the valves in the cell cycle and they came to the conclusion that *E. tropica* Hustedt *sensu* Hustedt (1927: 159) and *E. monodon* var. *tropica* (Hustedt 1927: 159) Hustedt *sensu* Hustedt in Schmidt (1933: pl. 381) are synonyms. In the same paper, a new *Eunotia* taxon (Taylor *et al*. in press) is described from the Lobaye River. The present paper deals with the description of another new *Eunotia* species found in a stream in the MAB Reserve Yangambi on the right bank of the Congo River (Libongo, Fig. 1) and until now not observed in samples collected during the Congo 2010 expedition.

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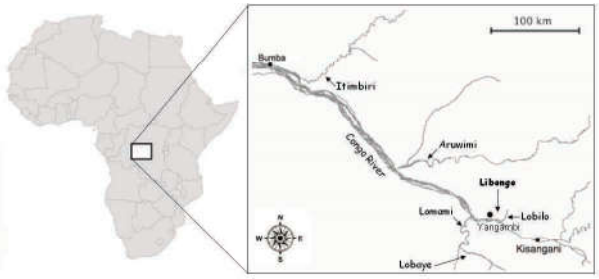


FIGURE 1. Map showing the location of the Libongo Stream on the right bank of the Congo River, D.R. Congo. Modified from Virgilio *et al*. (2011).

Materials and methods

Material used for the present study was collected by M. de Haan and D. Van den Broeck during a field trip in the frame of the COBIMBO (Congo basin integrated monitoring for forest carbon mitigation and biodiversity) project to the MAB Reserve Yangambi, Tshopo Province in October-November 2013. Samples were collected in three small streams/rivers, the Libongo (Fig. 1), the Loeo and the Moni, at an elevation between 400 and 460 meters above sea level. The following samples were studied:

CCA 3272: epipsammic sample from the River Moni, 0.825500° N and 24.521083° E, collected by D. Van den Broeck on 7 November 2013.

CCA 3273: epipsammic sample from the River Loeo, 0.820667° N and 24.529528° E, collected by D. Van den Broeck on 7 November 2013.

CCA 3279: benthos, scrapped from the bottom in the middle of the stream (muddy with some pebbles and small amounts of fine sand grains), from the River Libongo, 0.804057° N and 24.529932° E, collected by M. de Haan on 14 November 2013.

CCA 3282: benthos, scrapped from the bottom in the middle of the stream (muddy with some pebbles and small amounts of fine sand grains, from the River Libongo a couple of meters downstream CCA 3279, 0.804057° N and 24.529932° E, collected by M. de Haan on 14 November 2013.

CCA 3283: epiphyton on submerged roots and leafs at the River Libongo, 0.804057° N and 24.529932° E, collected by M. de Haan on 14 November 2013.

CCA 3284: phytoplankton and benthos at the spring of the River Libongo, 0.804057° N and 24.529982° E, collected by M. de Haan on 14 November 2013.

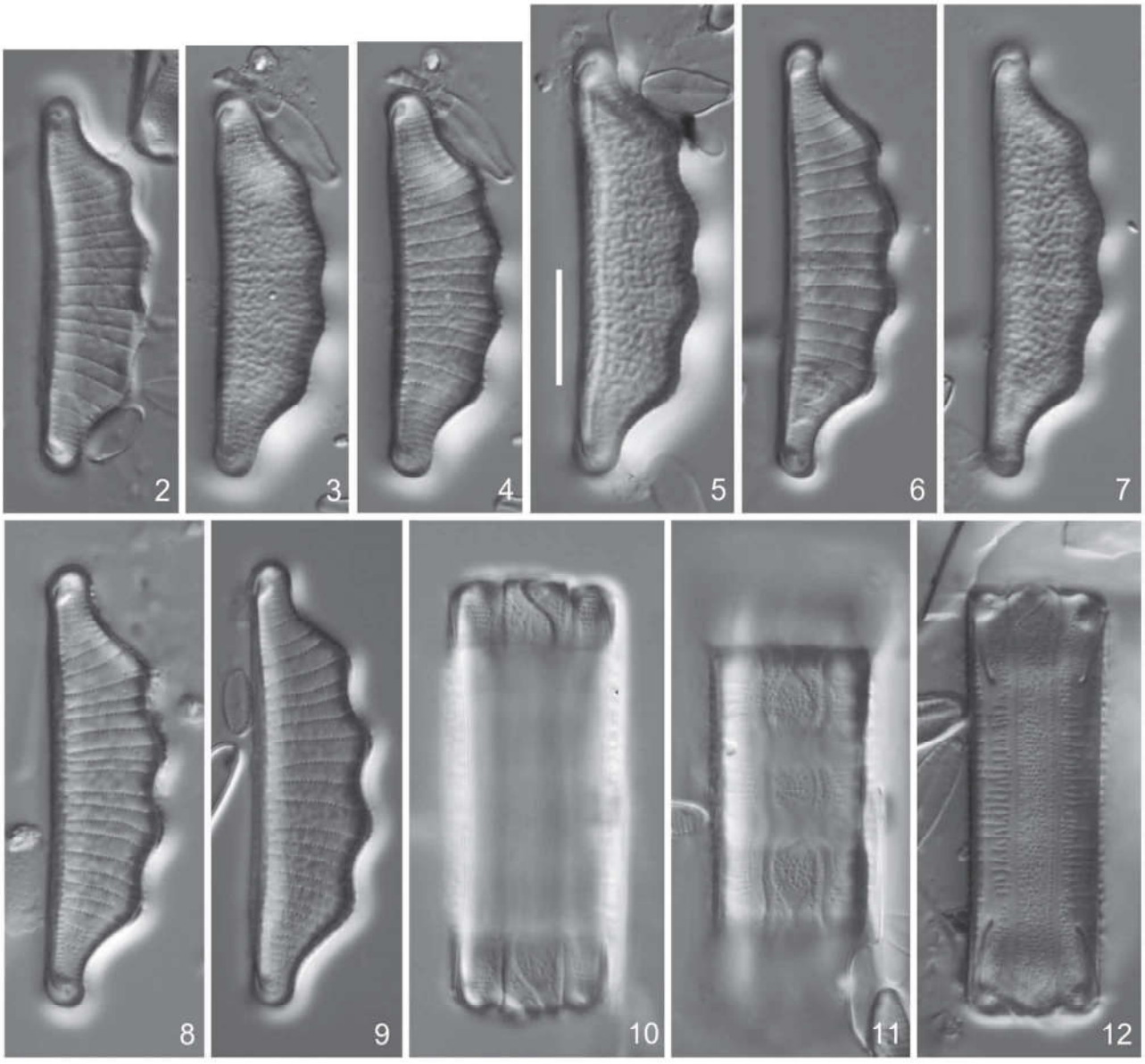
Epiphytic samples were collected by scrapping material from plant stems, roots or submerged leafs; benthic and epipsammic samples by scrapping off the sand respectively psammon from the bottom of the shallow water courses. Phytoplankton were collected in the middle of the river with a 10 µm mesh size net, without upwelling of bottom material. All samples were fixed *in situ* in a 20% v/v final concentration ethanol solution. Material from these samples were cleaned using hydroperoxide, rinsed three times with distilled water and mounted in Naphrax®. Light microscopy (LM) was carried out with an Olympus BX 51 microscope equipped with Nomarski differential interference contrast optics (DIC). Photographs were taken with an Olympus UC30 digital camera. For scanning electron microscopy (SEM), aliquots of the oxidized suspension were filtered and rinsed with additional distilled water through 1 µm isopore® polycarbonate membrane filters (Millipore). Filters were mounted on aluminum stubs, air-dried and sputter-coated with 50 nm of gold (Balzers Union SCD 020) and studied with a JEOL JSM-7100FLV Field Emission Scanning Electron Microscope (SEM) operating at 1 kV and 6.9 mm working distance. Relative abundances of the diatom taxa were calculated from counts of 500 valves examined on each slide, except for the diatom-poor sample CCA 3284 where only 100 valves were counted.

To study the species habitat preference and distribution in the Yangambi area, an extra sample, CCA 3583, was collected from epiphyton on *Vossia cuspidata* (Roxburgh 1814: 81) Griffith (1851: 153), known as 'hippo grass', at the River Loile, 0.585567° N and 24.929117° E, collected by E. Lokele and C. Cocquyt on 15 February 2015 during a field trip in the frame of the VLIR-UOS project. All raw materials, cleaned samples, permanent slides and SEM stubs are housed in the herbarium of the Botanic Garden Meise, Belgium (BR).

Results

Division **Bacillariophyta**
Class **Bacillariophyceae**
Subclass **Eunotiophycidae**
Order **Eunotiales**
Family **Eunotiaceae**
Genus ***Eunotia***

Eunotia rudis **Cocquyt & M. de Haan, *sp. nov*. (Figs 2–24)**

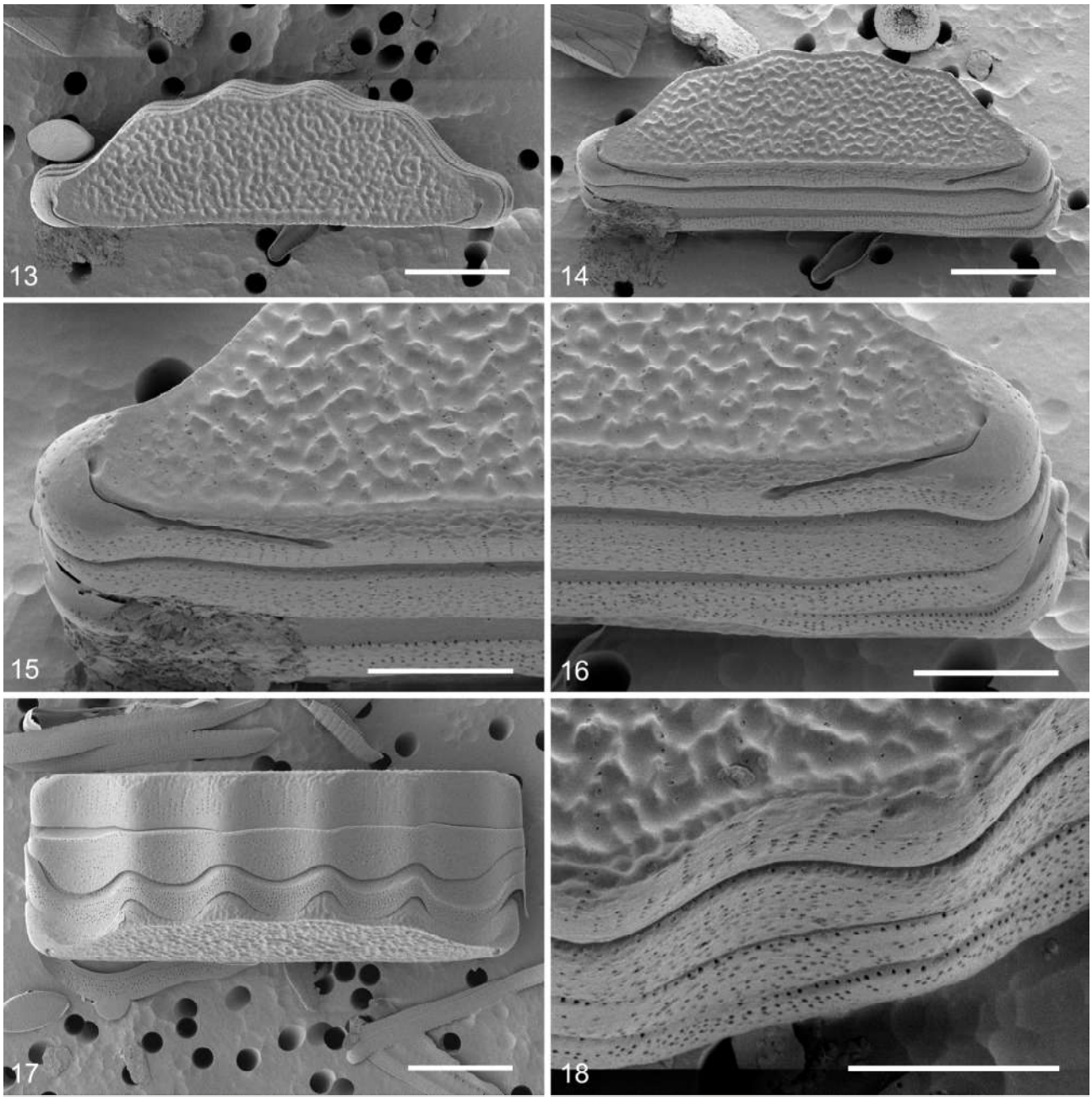


FIGURES 2–12. *Eunotia rudis*, LM. 2–9.Valve views showing size diminution series. 10–12. Girdle views. 3–4. Same valve taken at different foci. 6–7. Same valve taken at different foci. 10–11. Same frustule taken at different foci. Valves and frustules from the holotype slide BR 4447, Libongo River, Tshopo Province, Democratic Republic of the Congo. 6–7. Valve representing the holotype. Scale bar: 10 µm.

Valve with four undulations on the dorsal side and with straight ventral margin. Ends slightly heteropolar, with one pole more protracted than the other, and both broadly rounded. The slope between the dorsal valve undulation and the pole is steeper near the more protracted pole. Valve face with an irregular rough surface. Length (31.5) 36.0–48.0 µm, width 9.0–13.0 µm. Striae coarse and irregularly distributed on the valve face, 5.5–7.0, rarely up to 8.5 in 10 µm in the middle of the valve, becoming denser towards the

poles. Striae radiate almost throughout the valve, except for the ones in the middle of the valve. Short striae often present near the dorsal and the ventral side. Areolae very distinct, usually 24–26 in 10 µm, sporadically up to 28 in 10 µm, and often lacking on parts of the striae. Terminal nodules close to poles. Terminal raphe ends broadly curved on the valve face and clearly visible in LM. Large hyaline area is present around the raphe endings on the mantle side. Striae between the raphe and valve mantle margin denser than on the rest of the valve. Girdle bands with striae, around 25 in 10 µm, composed of large areolae and with clearly visible undulations on the dorsal side. In girdle view, the rough surface of the valve face is visible as a spiny unevenness. Girdle bands with distinct areolae arranged in rows but often irregularly distributed along these rows; around 25 rows in 10 µm.

Type:—DEMOCRATIC REPUBLIC OF THE CONGO. Tshopo Province: Libongo River, 0.804057° N, 24.529932° E, benthic sample CCA 3282, *M. de Haan, 14 November 2013* (holotype BR! 4447, Botanic Garden Meise, Belgium, the valve representing the holotype is here illustrated in Figs 6–7; isotype ZU! 10/69 BRM, Hustedt Diatom Collection, Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven, Germany).

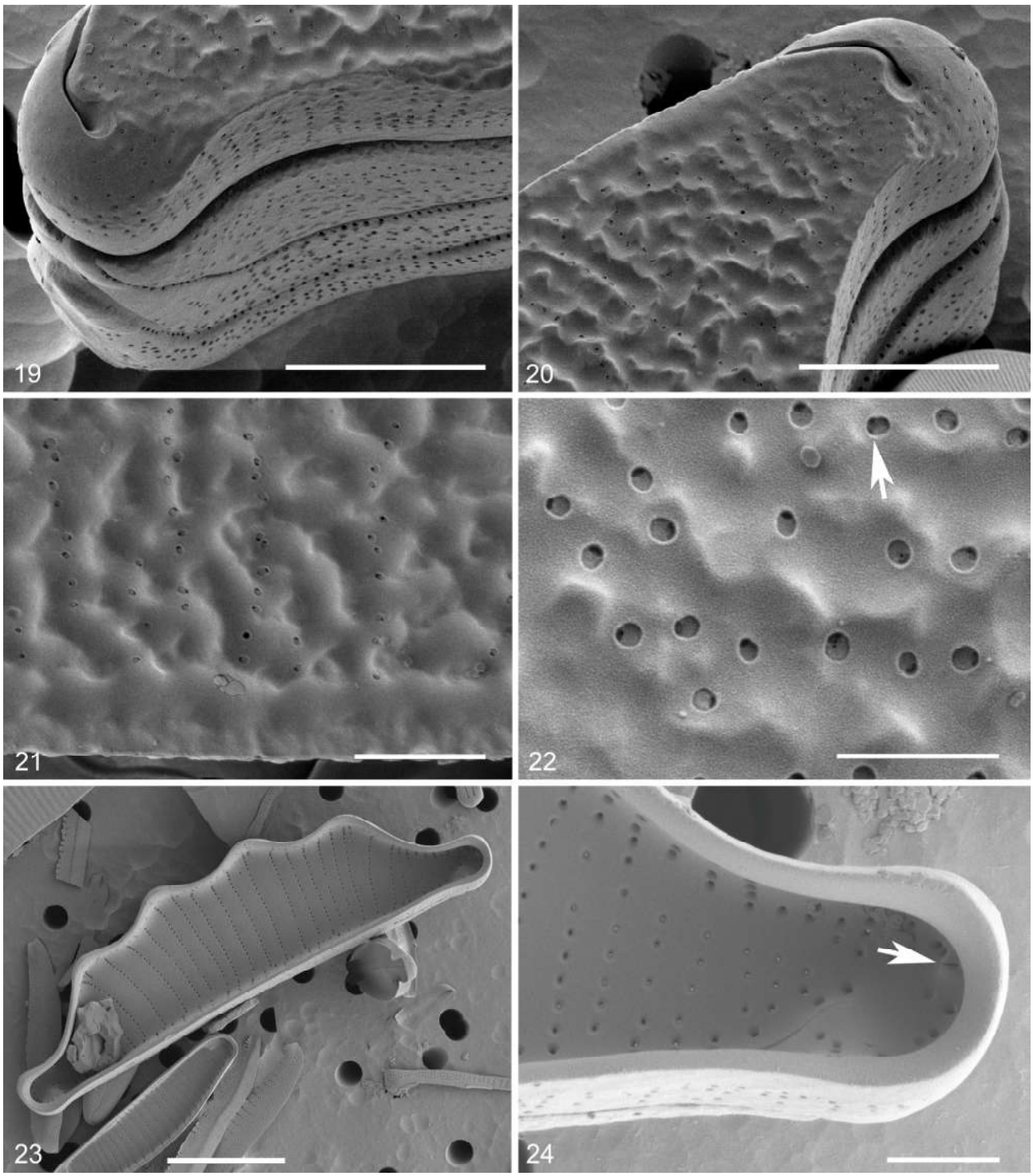


FIGURES 13–18. *Eunotia rudis* from type material CCA 3282, SEM external views. 13–14. Overview of an entire valve. 15–16. Detail of both poles of the same valve showing the enlarged terminal raphe endings. 17. Overview of an entire frustule in girdle view. 18. Detail of the girdle bands near the undulation on the dorsal side. Scale bar: 10 µm (Figs 13–14, 17), 5 µm (Figs 15–16, 18).

Etymology:—The specific epithet "*rudis*" refers to the rough look of the outside surface of the valve.

SEM observations (Figs 13–24):—Valve face with irregular shallow corrugations (Figs 13-21). Striae composed

of slightly elongated areolae oriented in different directions or rarely rounded (Fig. 21). External openings of the areolae obliquely perforating the thick silica wall; externally the areolae are covered by hymenes laying deep in the cell wall (Fig. 22 arrow). Internally the areolae are rounded (Fig. 23). Terminal nodules relatively close to the ends, expanded between the raphe endings and the poles (Figs 15–16, 19–20). Terminal raphe fissures short, straight on the mantle and extending onto the valve face with an angle of about 110° (Figs 15–16, 19–20). Terminal raphe endings enlarged at the valve surface, becoming narrower deeper in the thickened cell wall of the terminal nodule (Figs 15–16, 19–20). Internally the raphe terminates in a very small helictoglossa near the poles (Fig. 24). A single small rimoportula is present at one of the poles (Fig. 24 arrow). Spines are absent.



FIGURES 19–24. *Eunotia rudis* from type material CCA 3282. 19–22. SEM external views. 23–24. SEM internal views. 1–20. Detail of the girdle bands near both poles of the same frustule. 21–22. Details of the areolae, obliquely perforating the thick silica wall; hymen (arrow) laying deep in the cell wall. 23. Overview of an entire valve. 24. Detail of a terminal raphe ending with a small helictoglossa and rimoportula (arrow). Scale bar: 10 μm (Fig. 23), 5 μm (Figs 19–20), 2 μm (21, 24), 1 μm (22).

Girdle composed of at least three copulae (Fig. 17). Girdle bands almost smooth with rounded areolae irregular placed on striae (Figs 18–20).

Ecology and distribution:—Species of acid, running freshwater. Known from the Libongo River in the MAB Reserve of Yangambi and the Loile River located between Yangambi and Kisangani, Tshopo Province, D.R. Congo.

Discussion

The typical valve shape together with its rough surface makes this new *Eunotia* taxon easy to recognize. The rugose outside surface of the valve does not indicate this is a teratologic form. No evidence was found in other species from the studied material to point to deformations in valve formation. The accumulation curve of the valve length (n=45, Fig. 25) shows that our observations included the entire range in valve length, from the smallest (31.5 µm) to the largest (47.7 µm), but possibly not the initial cell. Morphologically most similar taxa are *Eunotia garusica* Cholnoky (1958: 113) and its var. *polydentula* Cholnoky (1958: 113). The main difference between the new described taxon and *E. garusica* and *E. garusica* var. *polydentula* is, besides the strongly rugose valve surface of *E. rudis*, the shape of the ventral margin (Table 1). The ventral margin is straight in the new taxon while is distinctly tumid in *E. garusica* and *E. garusica* var. *polydentula*, even in the smallest valves. Difference in the striation can also be observed: almost radiate throughout the valve in *E. rudis* while in *E. garusica* and *E. garusica* var. *polydentula* they are parallel throughout the largest part of the valve and becoming radiate near the poles. The undulations on the dorsal side of the valve were constant (four) in all valves of *E. rudis* observed in the studied samples (CCA 3272, CCA 3273, CCA 3279, CCA 3282, CCA 3283 and CCA 3583) but it cannot be excluded that this character varies in other populations of the newly described species. Although Cholnoky (1958) was convinced that larger valves of undulated *Eunotia* species have more undulations on their dorsal side than the smaller valves, he based his description of *Eunotia garusica* var. *polydentula* on the number of dorsal undulations (4 to 6, while the *Eunotia garusica* has only three undulations). He described the variety according to the accepted species concept at that time, but was aware that it wasn't a taxonomic entity as he remarks in the same publication that "forms with more undulations can't be differentiated genotypically". Reports of these two taxa are not limited to South Africa. *Eunotia garusica* was, among others, also reported from rivers in Burundi (Mpawenayo 1996), Ghana (Foged 1966, erroneously written as *E.* '*garussica*') and Sierra Leone (Molder 1962), and *E. garusica* var. *polydentula* from Sierra Leone (Molder 1962). However, up to now these two taxa have not been reported from D.R. Congo. The drawings given by Foged (1966), Mpawenayo (1996) and Molder (1962) are in agreement with Cholnoky's observation and the number of undulations on the dorsal side of the valve was 3 and 5 for the species and variety respectively. Taylor *et al*. (in press) demonstrated the statement of Cholnoky clearly by means of digital tracing of the valve outlines, including the raphe in a diminishing series of *Eunotia zygodon*. In the new *Eunotia rudis*, however, it seems that the number of undulations (4) stays constant. But we must remark that the range in the valve length in the 45 observed cells is not as large as in *E. zygodon*: 31.5–48.0 in the new taxon compared to 37.0–153.5 in *E. zygodon*.

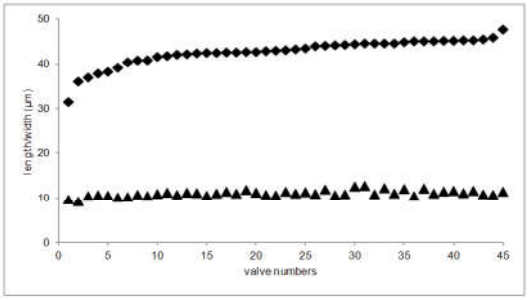


FIGURE 25. Variation in the valve length (diamond) and width (triangle) for 45 valves of *Eunotia rudis* measured in LM.

Eunotia rudis was observed in samples from the acidic Libongo River. Although no physical and chemical variables could be measured during the sampling, available information on other streams and rivers in the MAB reserve Yangambi (Cocquyt *et al*. 2013, Verheyen *et al*. in press) together with the diatom composition point to acidity. The new species was not uncommon in the benthic samples CCA 3282 and CCA 3279, and was sporadically observed in the epiphytic sample CCA 3283. However, the relative abundance was always low, less than 0.5%, but the unique shape of this rather large diatom makes it easy to detect even under low magnification. The diatom community of the

studied samples from the Libongo River was, besides *Eunotia* spp. with *E*. cf. *rhomboidea* Hustedt (1950: 435) as the relatively most important taxon good for 25% of the total relative abundance, dominated by small naviculoid taxa, such as *Nupela* Vyverman & Compère (1991: 175), *Eolimna* Lange-Bertalot & W. Schiller (1997: 166), and *Fallacia* Stickle & D.G. Mann in Round *et al*. (1990: 554, 667). These small diatoms accounted for 64.8 and 62.4% of the total relative abundances in the two benthic samples CCA 3282 and CCA 3279, and for 43.6% of the relative abundances in the epiphytic sample CCA 3283. The total relative abundances of *Eunotia* species in these three samples were 28.0, 30.8 and 37.6% respectively. In a fourth sample, CCA 3284, taken at the spring of the Libongo River and overall poor in diatoms, *Eunotia* was represented by 22.5% of the total diatom population but the new species was not observed. In the epipsammic samples from the Moni and Loeo Rivers the relative abundances of *Eunotia* reached 22.7 and 14.2% respectively. However, *Eunotia rudis* was not observed. Epipsammic environments are thus likely not the preferred habitats of the new taxon.

TABLE 1. Morphological comparisons between *Eunotia rudis*, *E. garusica* var. *garusica* and *E. garusica* var. *polydentula*.

	E. rudis	*E. garusica*	*E. garusica* var. *polydentula*
Valve length (µm)	(31.5) 36–48.0	23.0–42.0	40.0–60.0
Valve width (µm)	9.0–13.0	6.0–9.0	12.0–14.0
Striae in 10 µm	5.5–7.0 (8.5)	9.0–11.0	9.0–10.0
Areolae in 10 µm	24–26 (28)	18–19	22–24 *
Ventral side	straight	concave	concave
Dorsal undulations	4	3	4–6
Valve surface	rough	smooth	smooth
Reference	present study	Cholnoky (1952)	Cholnoky (1958)

*: measured on the drawings.

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Maurice Kottelat, Rue des Rauraques 6
CH–2800 Delémont, Switzerland
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Taxonomic evaluation of the *Hepsetus* from the Congo basin with the revalidation of *H. microlepis* (Teleostei: Hepsetidae)

Eva Decru*, Jos Snoeks* and Emmanuel Vreven*

A taxonomic evaluation was made of the Congolese *Hepsetus* based on 13 counts and 36 measurements on 158 specimens. Within the Congo basin, three species were found: *H. lineatus*, mainly a Lower Guinean species, is present in the Dja and other parts of the Sangha, but is also recorded in other parts of the Middle Congo (Lilande and Boende); *H. microlepis* occurs in large parts of the Congo basin, but is absent from the Dja and the southern part of the Kasai, and found also outside the Congo basin in the Gribingui (Chad basin); and *H. cuvieri*, which is common in the southern part of the Congo, is also present in the Quanza, Cunene, Zambezi and Okavango. *Hepsetus odoe microlepis* is elevated to species level. This species is characterised by its uniformly blackish colour. It is distinguished from *H. odoe*, *H. kingsleyae*, *H. lineatus* and *H. occidentalis* by its higher scale row numbers between the dorsal fin and the lateral line, and between the adipose fin and the lateral line; from *H. cuvieri*, *H. kingsleyae* and *H. occidentalis* by a broader head and snout; and from *H. lineatus* by a shorter dorsal-adipose distance and a higher number of gill rakers.

Introduction

For a long time, the family of the African pikes, Hepsetidae, was considered to be monospecific, until recent taxonomic studies of the *Hepsetus* from southern Africa, western Africa and the Lower Guinea ichthyofaunal province revealed an unexpected species richness with currently five valid species instead of one (Zengeya et al., 2011; Decru et al., 2012, 2013a–b). However, a review of the *Hepsetus* species within Congo basin taxa is still lacking. At present, the main *Hepsetus* species that is widespread in this basin is *H. odoe* (Bloch, 1794), until recently thought to be the only valid one within the genus. In the study of the southern African *Hepsetus* (Zengeya et al., 2011), specimens from the southernmost part of the Kasai and the Luapula River (Upper Congo) were found to be *H. cuvieri* (Castelnau, 1861). In addition, the revision of the species from Lower Guinea showed that *H. lineatus* (Pellegrin, 1926) occurs in the Dja (Decru et al., 2013a). This species was described as *Sarcodaces odoe* var. *lineata* and raised to species level but incorrectly named *H. lineata* by Decru et al. (2013a). Since the genus is masculine, the correct spelling is *H. lineatus*. Thus three species are presently known to occur in the Congo basin. However, Zengeya et al. (2011) already pointed

* KU Leuven, Laboratory of Biodiversity and Evolutionary Genomics, Charles Deberiotstraat 32, B–3000 Leuven, Belgium; and Royal Museum for Central Africa, Section Vertebrates, Ichthyology, Leuvensesteenweg 13, B–3080 Tervuren, Belgium. E-mail: eva.decru@africamuseum.be (corresponding author); jos.snoeks@africamuseum.be; emmanuel.vreven@africamuseum.be

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out that the specimens from the Lower and Middle Congo basins, except for the Kasai (their Congo basin North), were not conspecific with the holotype of *H. odoe*, thus indicating possible cryptic species diversity. Furthermore, *H. odoe microlepis* (Boulenger, 1901), was described as a variety from the Congo basin and Lake Ngami. However, the specimens mentioned by Boulenger from Lake Ngami could not be traced (Decru et al., 2012). The purpose of the present paper is to present the results of a morphometrical study of the *Hepsetus* taxa from the Congo ichthyofaunal province.

Material and methods

Abbreviations: DRC, Democratic Republic of the Congo; HL, head length; MWU test, Mann-Whitney U test; PCA, Principal Component Analysis; SL, standard length. Institutional abbreviations follow Sabaj Perez (2014); for collection numbers, the traditional abbreviations MRAC is used for the Royal Museum for Central Africa, Tervuren, and BMNH for the Natural History Museum, London. A total of 158 *Hepsetus* specimens from the Congo basin have been examined, including seven syntypes of *H. o. microlepis*. In addition, the types of *H. cuvieri*, *H. occidentalis* Decru, Vreven & Snoeks, 2013, *H. kingsleyae* Vreven, Decru & Snoeks 2013, *H. odoe*, and *H. lineatus* were included in the analyses. Data of additional specimens from the studies of other regions (Zengeya et al., 2011; Decru et al. 2012, 2013a, b), were used for the diagnoses and scatterplots. Since *Hepsetus akawo* was shown to be a synonym of *H. odoe* (Decru et al., 2013b), the types of this nominal species were not included in the analyses.

Counts and measurements follow Zengeya et al. (2011). Gill rakers of the holotype of *H. odoe* and the paralectotype of *H. lineatus* were not counted, as these specimens were not dissected. Principal Component Analyses (PCA) were used to explore the multivariate data matrix and to reduce the large number of variables into a few meaningful axes (Decru et al., 2012). Univariate comparisons between groups were made with non-parametric MWU tests using sequential Bonferroni correction (Zengeya et al., 2011). For the comparison of measurements, specimens of similar length classes were used to prevent interference of allometric growth effects. Hence, for the comparison of *H. lineatus* with *H. microlepis* only specimens larger than 145 mm SL were used. Significantly different variables were also evaluated individually with scatterplots to check the amount of overlap.

Results

A first PCA was carried out on 13 counts (n = 149). The highest loadings on PC I are for the three counts of the gill raker numbers on the first gill arch (Table 1). The highest loadings on PC II are for the number of scales between the dorsal and adipose fins, and for the number of scale rows between the adipose fin and the lateral line.

Five groups of specimens can be distinguished on this PCA (Fig. 1). A first group, situated en-

Table 1. PCA loadings for the first three PC axes of a PCA carried out on 13 counts of all examined specimens of *Hepsetus* (n = 149). The most important counts are in bold.

	PC I	PC II	PC III
Total number of lateral line scales	−0.085366	0.303875	−0.603620
Scale rows between dorsal fin and lateral line	−0.286573	−0.342257	−0.127234
Scale rows between adipose fin and lateral line	−0.281812	**−0.428288**	−0.082862
Scale rows between pelvic fin and lateral line	−0.324468	−0.101231	−0.114334
Scale rows between anal fin and lateral line	−0.281384	−0.347315	−0.129083
Pre-dorsal scales	−0.097467	0.065617	−0.442110
Dorsal-adipose scales	0.084293	**0.453609**	−0.283128
Adipose-caudal scales	−0.202687	−0.104299	−0.383731
Epibranchial gill rakers on first gill arch	**0.349447**	−0.308568	−0.080179
Epibranchial gill plates on first gill arch	−0.317824	0.162727	0.165000
Ceratobranchial gill rakers on first gill arch	**0.363688**	−0.168223	−0.207905
Ceratobranchial gill plates on first gill arch	−0.275072	0.150813	0.250299
Total number of gill rakers on first gill arch (UR/1/LR)	**0.397452**	−0.287283	−0.143017

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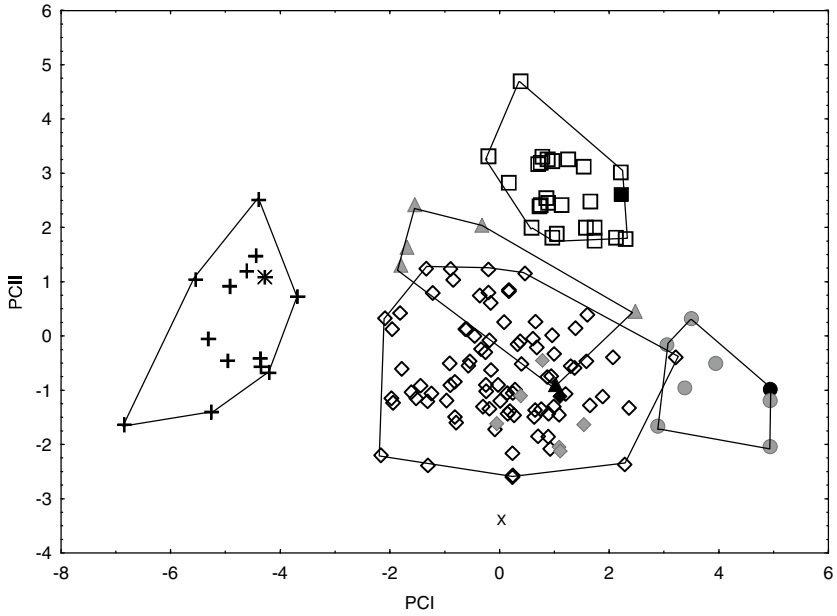


Fig. 1. Scatterplot of PCI against PCII for a PCA carried out on 13 meristics for all examined specimens (n = 149). *Hepsetus occidentalis*: holotype (▲) and paratypes (△); *H. kingsleyae*: holotype (●) and paratypes (○); *H. lineatus*: lectotype (■) and other specimens (□); *H. microlepis*: lectotype (◆), paralectotypes (◇) and other specimens (◇); and *H. cuvieri*: neotype (✳), other specimens (+), and *H.* cf. *cuvieri* (x).

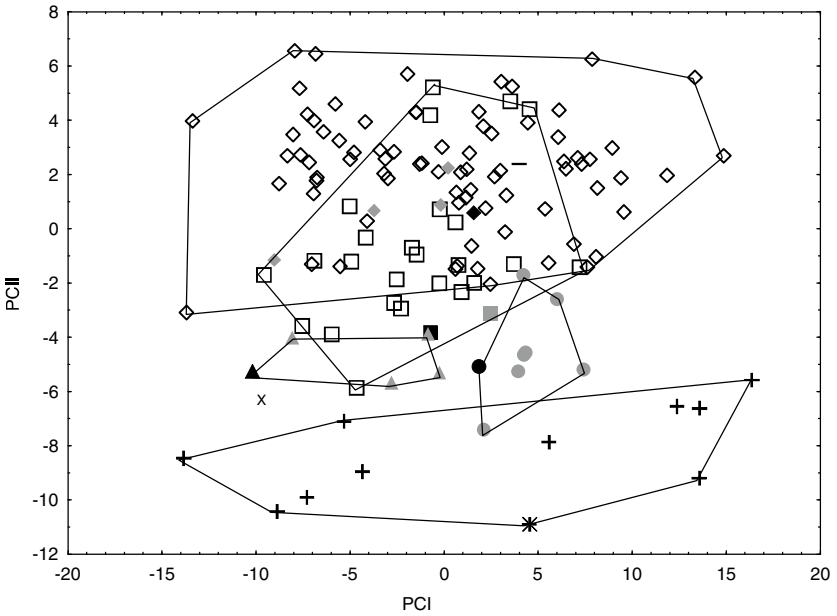


Fig. 2. Scatterplot of PCI against PCII for a PCA carried out on 34 measurements (expressed in percentages) for all examined specimens (n = 137). *Hepsetus occidentalis*: holotype (▲) and paratypes (△); *H. kingsleyae*: holotype (●) and paratypes (○); *H. lineatus*: lectotype (■), paralectotype (▣) and other specimens (□); *H. microlepis*: lectotype (◆), paralectotypes (◇) and other specimens (◇); and *H. cuvieri*: neotype (✳), other specimens (+) and *H.* cf. *cuvieri* (x); *H. odoe*: holotype (–).

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tirely on the negative part of PC I, corresponds to *H. cuvieri*, while the group situated entirely on the positive part of the same axis corresponds to *H. kingsleyae*. The group situated entirely on the positive part of PC II is conspecific with the lectotype of *H. lineatus*. Finally, two overlapping groups remain in the centre of the graph. The large group of specimens, centred around low absolute values on both axes, comprises all remaining Congo specimens, not belonging to one of the species already separated, but including the types of *H. o. microlepis* (further referred to as *H. microlepis*, see below). It overlaps considerably with the types of the West African *H. occidentalis*. One *H. microlepis* specimen overlaps with *H. kingsleyae* as it had more gill rakers on the epibranchiale of the first gill arch than the other *H. microlepis* specimens (13 vs. 4–9). There is also one Upper Congo specimen, identified as *H.* cf. *cuvieri*, with very low values on the second axis. This specimen has a higher number of gill rakers than the *H. cuvieri* specimens (20 vs. 8–13).

As the holotype of *H. odoe* and the paralectotype of *H. lineatus* lack data for the gill rakers, they were not included in the first PCA on the counts. Therefore, a second PCA on the counts excluding the gill raker counts, was performed including these two specimens (results not shown). For this analysis, the holotype of *H. odoe* falls just outside the group of *H. microlepis* specimens on PC I and the paralectotype of *H. lineatus* falls well within the group of *H. lineatus*.

Table 2. PCA loadings for the first three PC axes of a PCA carried out on 34 measurements in percentages of all examined specimens of *Hepsetus* (n = 137). The most important variables are in bold.

	PC I	PC II	PC III
Pre-dorsal length	0.057348	0.230422	0.286962
Dorsal-adipose length	0.006042	−0.105035	−0.303691
Adipose-caudal length	0.016881	0.001310	−0.064128
Pre-pelvic length	0.059979	0.056999	0.106827
Pelvic-pectoral length	−0.088456	−0.027787	−0.090842
Pectoral-anal length	−0.093416	−0.108831	0.067541
Anal-caudal length	−0.014442	−0.004854	−0.090730
Dorsal-fin base	−0.069798	−0.085126	−0.055304
Adipose-fin base	−0.016953	−0.022118	−0.033611
Pectoral-fin base	−0.041622	0.033409	−0.000070
Pelvic-fin base	−0.036437	0.017821	−0.004187
Anal-fin base	−0.028788	−0.044047	0.001844
Post-anal length	−0.064888	−0.012323	−0.044956
Dorsal-fin height	−0.078239	0.057390	0.001565
Adipose-fin height	0.033437	−0.060750	−0.303640
Pectoral-fin height	−0.106032	0.174443	0.017840
Pelvic-fin height	−0.024007	0.203881	0.128586
Anal-fin height	0.110955	0.170215	0.241866
Caudal-peduncle length	−0.014640	−0.014265	−0.009267
Caudal-peduncle depth	−0.058947	−0.022812	−0.054652
Body depth	−0.188187	−0.031288	−0.290942
Head length	0.048277	0.070884	0.031361
Nostril length	0.087433	0.026936	−0.044031
Nostril-eye length	0.009184	0.023831	0.002943
Snout length	0.058463	0.147072	0.205061
Eye diameter	0.241810	0.152882	0.072872
Head depth	−0.311919	−0.160214	0.092381
Eye-lower jaw length	**−0.432968**	**−0.338693**	0.051474
Nostril-lower jaw length	−0.316423	−0.234733	0.132789
Lower jaw length	−0.179786	−0.105518	0.398504
Upper jaw length	−0.171067	−0.170738	0.444921
Head width	**−0.458802**	0.325880	−0.295690
Interorbital distance	**−0.345471**	**0.469929**	0.063638
Internasal distance	−0.209849	**0.430052**	0.001080

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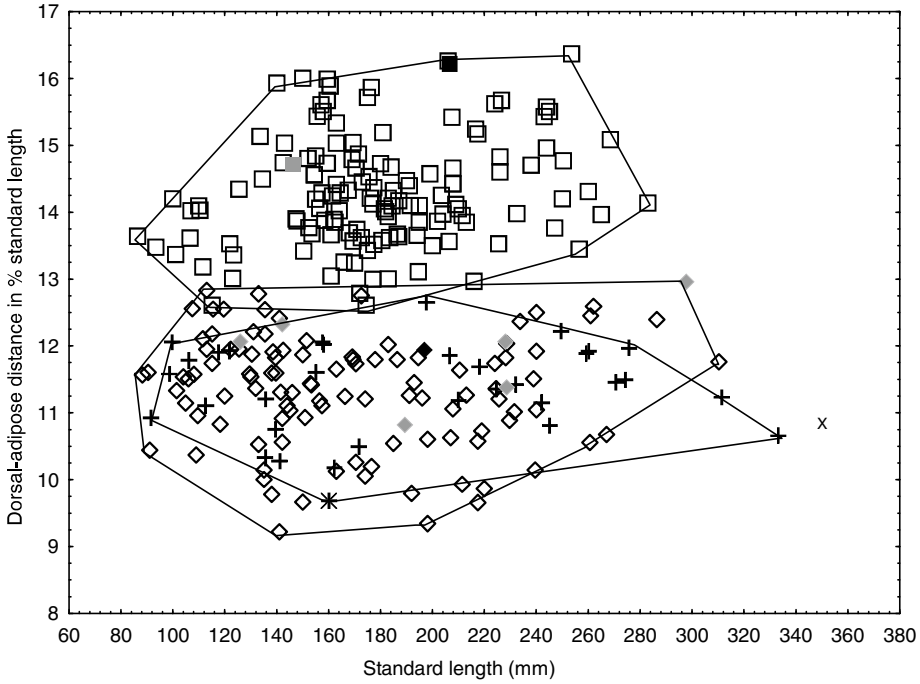


Fig. 3. Scatterplot of dorsal-adipose distance (in % SL) against standard length (in mm). *Hepsetus lineatus*: lectotype (■), paralectotype (■) and other specimens (□); *H. microlepis*: lectotype (◆), paralectotypes (◆) and other specimens (◇); and *H. cuvieri*: neotype (✳), other specimens (+), and *H.* cf. *cuvieri* (×).

A PCA was carried out on 36 measurements expressed as percentages (n = 137) (Fig. 2). The highest loadings on PCI are for the head width, the distance between eye and lower jaw and interorbital distance (Table 2). The highest loadings on PCII are for the interorbital and internasal distances and the distance between eye and lower jaw. Specimens of *H. cuvieri* (and *H.* cf. *cuvieri*) are entirely situated on the negative part of PCII and overlap slightly with *H. kingsleyae*. There is also

Table 3. Results (p-values) of Mann-Whitney U tests for the 13 counts of *Hepsetus* species included in the PCA. Significant values ($p < 0.05$ after Bonferroni correction) are in bold; highly significant values ($p < 0.001$ after Bonferroni correction) are in bold and underlined.

	H. microlepis × *H. cuvieri*	*H. microlepis* × *H. lineatus*	*H. cuvieri* × *H. lineatus*
Total number of lateral line scales	0.635203	**0.000328**	0.030686
Scale rows between dorsal fin and lateral line	**0.000005**	**0.000000**	**0.000000**
Scale rows between adipose fin and lateral line	**0.000009**	**0.000000**	**0.000000**
Scale rows between pelvic fin and lateral line	**0.000004**	0.011041	**0.000000**
Scale rows between anal fin and lateral line	0.000851	**0.000000**	**0.000000**
Pre-dorsal scales	0.130542	0.065549	0.976341
Dorsal-adipose scales	0.794714	**0.000000**	**0.000038**
Adipose-caudal scales	0.236085	0.014547	0.008412
Epibranchial gill rakers on first gill arch	**0.000000**	**0.000000**	**0.000001**
Epibranchial gill plates on first gill arch	**0.000000**	0.012453	**0.000004**
Ceratobranchial gill rakers on first gill arch	**0.000000**	0.261922	**0.000013**
Ceratobranchial gill plates on first gill arch	**0.000000**	0.201324	**0.000003**
Total number of gill rakers on first gill arch (UR/1/LR)	**0.000000**	**0.000006**	**0.000001**

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an overlap between specimens of *H. microlepis* and *H. lineatus*, and the holotype of *H. odoe* falls within both these groups. However, based on the PCAs of counts and measurements together, it can be concluded that *H. microlepis* is not conspecific with any of the other valid *Hepsetus* species.

An additional PCA (not illustrated) was carried out on the measurements expressed as percentages, excluding the specimens of *H. cuvieri* (and *H.* cf. *cuvieri*), which were already well separated in the previous analysis. In this PCA, specimens of *H. microlepis* were almost completely separated from all other species.

Since the PCAs showed that the Congo specimens are not conspecific with either *H. odoe*, *H. kingsleyae* or *H. occidentalis*, MWU tests (tables 3 and 4) were carried out on the specimens of the Congo ichthyofaunal province only. The sole specimen of *Hepsetus* cf. *cuvieri* was also excluded from the MWU tests. Many counts were found to be significantly different between all three species from the Congo province (Table 3). Between *H. cuvieri* and *H. microlepis*, 8 out of 13 counts are highly significantly different, between *H. cuvieri* and *H. lineatus*, 10 out of 13 counts are significantly different, 8 of which are highly significant. Gener-

Table 4. Results (p-values) of Mann-Whitney U tests for the 36 measurements (expressed in percentages) of *Hepsetus* species included in the PCA. Significant values ($p < 0.05$ after Bonferroni correction) are in bold; highly significant values ($p < 0.001$ after Bonferroni correction) are in bold and underlined. For the comparison of *H. microlepis* with *H. lineatus* a size class of SL > 145 mm is used.

	H. microlepis × *H. cuvieri*	*H. microlepis* × *H. lineatus*	*H. cuvieri* × *H. lineatus*
Standard length	0.552235	0.929147	0.783353
Pre-dorsal length	0.001274	**0.000000**	0.000620
Dorsal-adipose length	0.353635	**0.000000**	**0.000000**
Adipose-caudal length	0.874605	**0.000026**	0.003412
Pre-pelvic length	0.016835	0.616190	0.287575
Pelvic-pectoral length	0.489659	0.014092	0.048710
Pectoral-anal length	0.722366	0.046669	0.989032
Anal-caudal length	0.300481	**0.000083**	**0.000270**
Dorsal-fin base	**0.000027**	0.010867	0.088435
Adipose-fin base	0.001208	**0.000219**	0.901539
Pectoral-fin base	**0.000210**	0.001058	0.001421
Pelvic-fin base	0.020272	0.441159	0.032275
Anal-fin base	0.189327	0.974510	0.449578
Pre-anal length	0.062634	0.550611	0.179840
Post-anal length	0.061887	0.085630	0.650030
Dorsal-fin height	0.737465	0.325746	0.664893
Adipose-fin height	**0.000227**	0.003458	0.016141
Pectoral-fin height	0.003847	0.001973	0.021853
Pelvic-fin height	**0.000083**	**0.000491**	0.050387
Anal-fin height	0.078069	0.172324	0.664907
Caudal-peduncle length	0.269292	0.310918	0.113916
Caudal-peduncle depth	0.476419	**0.000334**	0.283600
Body depth	0.826341	**0.000023**	0.006766
Head length	0.007118	0.490721	0.025048
Nostril length	0.357625	0.034570	0.242607
Nostril-eye length	0.425587	0.499328	0.815203
Snout length	**0.000000**	**0.000000**	0.005478
Eye diameter	0.197812	0.787695	0.690154
Head depth	0.104304	0.408713	0.309021
Eye-lower jaw length	0.022917	0.476426	0.371580
Nostril-lower jaw length	0.032463	0.862297	0.289819
Lower jaw length	0.584671	**0.000119**	0.364205
Upper jaw length	0.823334	**0.000041**	0.253869
Head width	0.001574	0.367675	**0.000022**
Interorbital distance	**0.000001**	**0.000000**	0.000756
Internasal distance	**0.000000**	**0.000001**	**0.000009**

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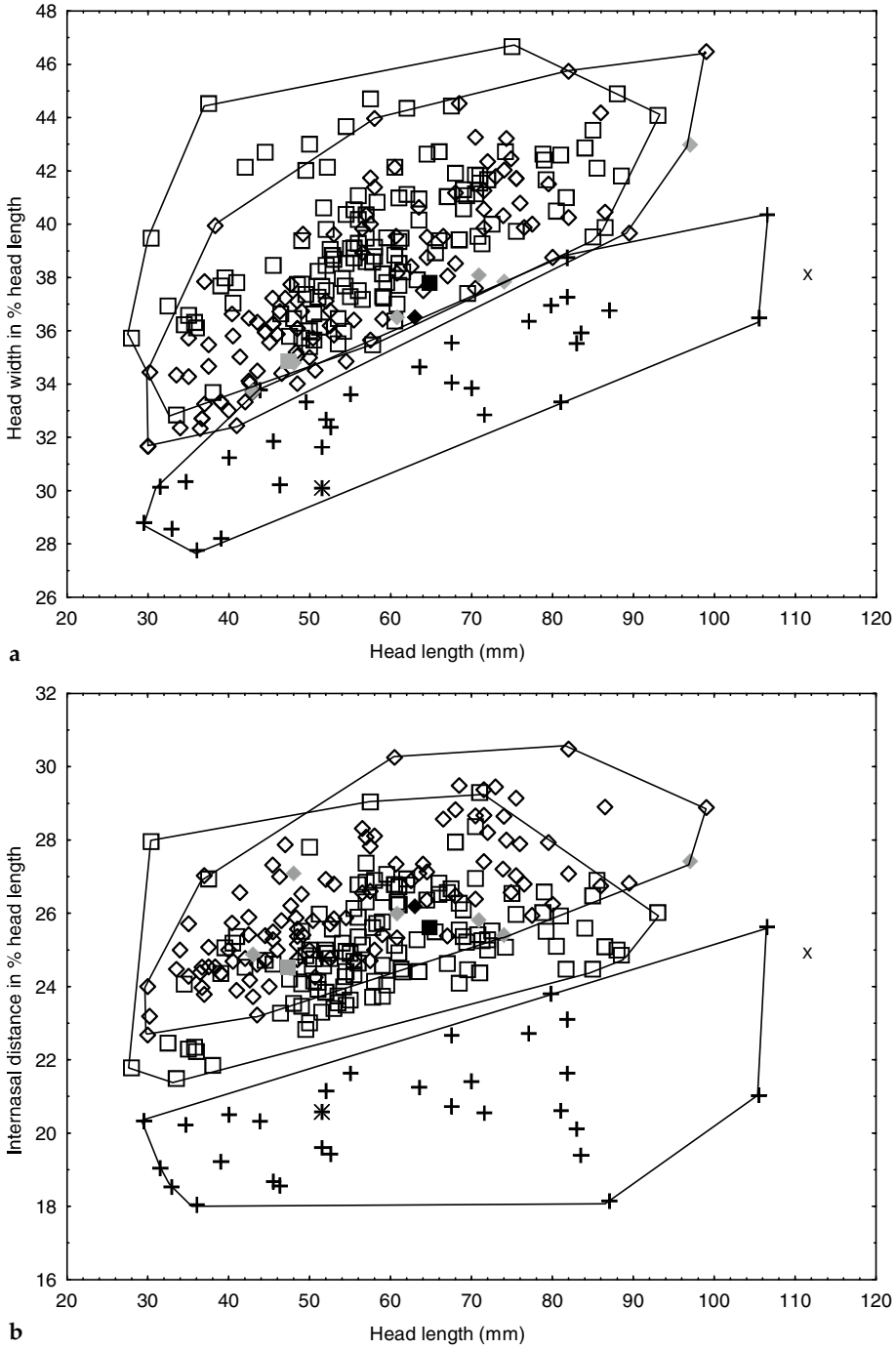


Fig. 4. Scatterplot of: (**a**) head width (in % HL) against head length (HL) (in mm) and (**b**) internasal distance (in % HL) against head length (HL) (in mm). *Hepsetus lineatus*: lectotype (■), paralectotype (▩) and other specimens (□); *H. microlepis*: lectotype (◆), paralectotypes (◈) and other specimens (◇); and *H. cuvieri*: neotype (✳), other specimens (+), and *H.* cf. *cuvieri* (x).

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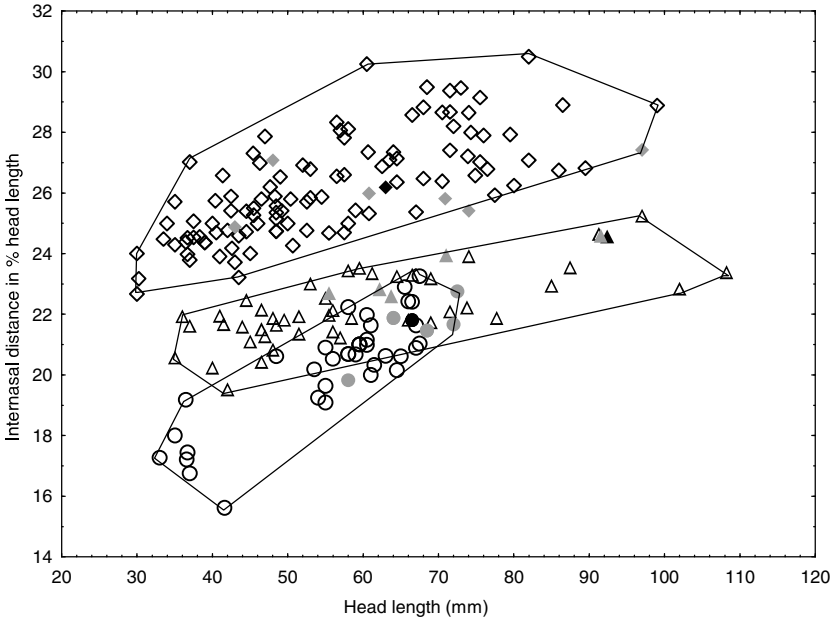


Fig. 5. Scatterplot of internasal distance (in % HL) against head length (HL) (in mm). *Hepsetus occidentalis*: holotype (▲), paratypes (▲) and other specimens (△); *H. kingsleyae*: holotype (●), paratypes (●) and other specimens (○); *H. microlepis*: lectotype (◆), paralectotypes (◆) and other specimens (◇).

ally, differences between *H. cuvieri* and these two species are similar, with *H. cuvieri* having higher values in scale row counts between the dorsal fin and the lateral line (10.5–11.5 vs. 9.5–10.5 in *H. microlepis* and 7.5–9.5 in *H. lineatus*), and the adipose fin and the lateral line (6.5–7.5 vs. respectively 5.5–6.5 and 4.5–5.5); and a lower number of gill rakers (8–13 vs. respectively 13–24 and 12–20). The results of the comparison of *H. cuvieri* and *H. microlepis* are in accordance with the findings of Zengeya et al. (2011). Between *H. microlepis* and *H. lineatus*, 7 out of 13 counts are significantly different, 6 of which are highly significant. These include scale row counts, with *H. microlepis* having a higher number of scale rows between the dorsal fin and lateral line (9.5–10.5 vs. 7.5–9.5) and the adipose fin and the lateral line (5.6–6.5 vs. 4.5–5.5); gill raker counts, with *H. microlepis* having generally higher values (13–24 vs. 12–20); and scales on the lateral line between dorsal and adipose fin, with *H. microlepis* generally having lower values (16–23 vs. 15–28).

For the MWU tests on the measurements (Table 4), 7 out of 35 measurements are significantly different between *H. microlepis* and *H. cuvieri*, 4 of which are highly significant. Between *H. microlepis* and *H. lineatus*, 13 out of 35 measurements are significantly different, 5 of which are highly significant. Scatterplots of these variables show that especially the distance between dorsal fin and adipose fin, which is longer in *H. lineatus* [12.6–16.4 % SL (mean 14.4) vs. 9.2–12.8 (11.3)] (Fig. 3), and the snout length, which is shorter in *H. lineatus* [35–40 % HL (mean 37.9) vs. 37–43 (40.2)] and is negatively allometric (not shown), separate the two species best.

Finally, between *H. cuvieri* and *H. lineatus* 4 out of 35 measurements are significantly different, 2 of which highly significant. Examination of these variables shows that head width (Fig. 4a), which is smaller in *H. cuvieri* [29–40 % HL (mean 33.6) vs. 33–47 (39.4)]; internasal distance (Fig. 4b), which is smaller in *H. cuvieri* [18–26 % HL (mean 20.7) vs. 22–29 (25.1)] and distance between dorsal and adipose fin, which is smaller in *H. cuvieri* [9.0–12.7 % SL (mean 12.2) vs. 12.6–16.4 (14.3)] (Fig. 3) are important discriminating characters.

The different analyses clearly show that, within the Congo ichthyofaunal province, three *Hepsetus* species can be recognised. The first one is *H. cuvieri*, which also occurs in southern Africa (Zengeya et al., 2011). The second one is *H. lineatus*, which also occurs in Lower Guinea (Decru et al., 2013a), whereas the third species

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is conspecific with the seven examined syntypes of *H. microlepis* and occurs mainly in the Congo basin. This subspecies is here elevated to species level and a redescription is given below.

Hepsetus microlepis (Boulenger, 1901)
(Fig. 6, Tables 5 and 6)

Material examined. Ordered by river system: Lower Congo: MRAC 15386, 1, 141.0 mm SL; DRC: Zambi; 5°51'S 12°50'E; Lang & Chapin, 1 Jun 1915. – MRAC 119120, 1, 239.0 mm SL; DRC: Luki, Mayumbe; 5°39'S 13°04'E; Wagemans; 01 Jan 1957. – MRAC A6-007-P-1529-1531, 3, 141.0-217.5 mm SL; DRC: Luibi River, little coastal basin near. Kingalasa, Lower Congo; 5°56'11"S 12°29'51"E; Exp. Bas-Congo, 2 Oct 2005.

Middle Congo: BMNH 1897.9.30.15, 1, paralectotype, 228.5 mm SL; DRC: Stanley Falls; 0°30'N 25°12'E; W. H. Bentley, no date. – BMNH 1899.9.26.78–79, 2, paralectotypes; 189.5–126.0 mm SL; DRC: Ibali, Lake Leopold II (Lake Mai-Ndombe); 2°03'00"S 18°11'00"E; M.P. Delhez, no date – BMNH 2011.7.21.1, lectotype, 196.8 mm SL; DRC: Ibali, Lake Leopold II (Lake Mai-Ndombe); 2°03'00"S 18°11'00"E; M.P. Delhez, no date MNHN 1925-0246, 1, 109.5 mm SL; DRC: Ikengo; H. Schouteden, no date. – MRAC 709–710, 2, paralectotypes, 142.0–228.0 mm SL; DRC: Ibali, Lake Leopold II (Lake Mai-Ndombe); 2°03'S 18°11'E; M. P. Delhez, no date. – MRAC 711, 1, paralectotype, 297.8 mm SL; DRC: M'Bali; 1°49'S 18°07'E; M. P. Delhez, no date. – MRAC 1235, 1, 211.5 mm SL; DRC: Banzyville, Ubangi River, 4°18'N 21°10'E; Royaux, 1 Jan 1901. – MRAC 1352, 1, 286.5 mm SL; DRC: Eala; 0°04'N 18°20'E; M. Laurent, 1 Jan 1906. – MRAC 2251, 1,172.5 mm SL; DRC: Ilando, 1°01'N 23°58'E; C. Christy, 8 Jun 1912. – MRAC 2304, 1, 129.5 mmSL; DRC: Stanley-Falls; 0°30'N 25°12'E; C. Christy, 22 Jun 2012. – MRAC 3370, 1, 101.5 mm SL; DRC: Kondue, Sankuru; 4°58'S 23°16'E; E. Luja, no date. – MRAC 7995, 1, 135.0 mm SL; DRC: Poko; 3°09'N 26°53'E; C. Christy, 1 Jan 2012. – MRAC 19591, 1, 119.5 mm SL; DRC: Bikoro; 0°40'S 18°02'E; H. Schouteden, no date. – MRAC 19777, 1, 133.0 mm SL; DRC: Dika, Upper Uélé; 4°16'N 27°42'E; H. Schouteden, 1 Jan 1925. – MRAC 23337, 1, 91.0 mm SL; DRC: Buta; 2°47'N 24°50'E; H. Schouteden, no date. – MRAC 38015-38022, 3, 111.5–151.5 mm SL; DRC: Kunungu; 2°06'S 16°26'E; H. Schouteden, 1 Jan 1932. – MRAC 46691, 1, 224.0 mm SL; DRC: Eala, Ruki River; 0°04'N 18°20'E; J. Ghesquière, 4 Aug 1936. – MRAC 46752, 1, 108.0 mm SL; DRC: Karawa; 3°20'N 20°18'E; Wallin, 1 Jan 1936. – MRAC 47669, 1, 210.5 mm SL; DRC: Eala, Ruki River; 0°04'N 18°20'E; J. Ghesquiére, 23 Oct 1936. – MRAC 47981-47982, 2, 132.0–146.0 mm SL; DRC: Flandria; 0°20'S 19°05'E; G. Hulstaert, 1 Jan 1936. – MRAC 57592, 1, 261.0 mm SL; DRC: Kunungu; 2°06'S 16°26'E; H. Schouteden, 3 May 1938. – MRAC 57760, 1, 267.0 mm SL; DRC: Lake Tumba; 0°37'S 17°49'E / 1°00'S 18°09'E; G. de Loneux, 1 Jan 1938. – MRAC 58408-58410, 2, 151.0-156.5 mm SL; DRC: Yakoma, Ubangi River; 4°06'N 22°23'E; M. Rosy, 1 Jan 1938. – MRAC 67081, 1, 240.0 mm SL; DRC: Mushie region, Kasai River; 3°02'S 16°55'E; C. Vleeschouwers, 1 Oct 1945. – MRAC 68941, 1, 133.0 mm SL; DRC: Bomili region, Nepoko River; 1°40'N 27°01'E; A. Henrion, 1 Jan 1946. – MRAC 74072, 1, 139.5 mm SL; DRC: Bokalakala; 2°08'S 16°22'E; J. D. Viccars, 1 Jan 1950. – MRAC 75619-621,75629, 4, 130.0-183.0 mm SL; DRC: Bokungu, Tshuapa; 0°41'S 22°19'E; L. Dupuis, 1 Jan 1950. – MRAC 78012-78017, 3, 104.0-120.0 mm SL; DRC: Dungu River, km 999 royal road Congo-Nile; 3°47'N; 28°34'E; C. Vleeschouwers, 1 Jan 1950. – MRAC 78117, 1, 135.5 mm SL; DRC: Kinshasa; 4°18'S 15°18'E; M. H. Pierret, 1 Jan 1951. – MRAC 78565, 1, 157.5 mm SL; Bokuma, Mbandaka; 0°00'N 18°20'E; P. Lootens, 7 Jul 1951 – MRAC 78858–859, 2, 106.0–115.0 mm SL; DRC: Kwamatelsa River at Limeté, near Kinshasa; 4°28'S 15°21'E; Richard, 1 Jan 1952. – MRAC 79471, 1, 105.0 mm SL; DRC: Botanankasa, Ebale River; 2°16'S 16°15'E; N'Kele, 1 Jan 1952. – MRAC 80797, 1, 198.0 mm SL; DRC: Bamania; 0°00'S 18°20'E; G. Hulstaert, 1 Jan 1952. – MRAC 87669, 1, 90.5 mm SL; DRC: Boangi, Momboyo River; 1°51'S 20°56'E; P. Verbuyt, 1 Jan 1953. – MRAC 96062, 1, 196.0 mm SL; DRC: Sassilez-Bambesa; 3°35'N 25°42'E; P. Depasse, 1 Jan 1951. – MRAC 101162, 1, 113.0 mm SL; DRC: Wamba; 0°16'N 21°54'E; G. Vandebroek, 1 Feb 1955. – MRAC 102424–425, 2, 186.5–194.5 mm SL; DRC: Bungumu pond, near Bambesa, Uélé; 3°22'N 25°44'E; P. Charlier, 28 Jul 1955. – MRAC 102479, 1, 198.0 mm SL; DRC: Mokpete, near Bambesa, Uélé; 3°22'N; 25°44'E; P. Charlier, 27 Jul 1955. – MRAC 116788, 1, 219.0 mm SL; DRC: Stanley-Pool; 4°06'S 15°15'E / 4°20'S 15°23'E; Brien et al., 1 Sep 1957. – MRAC 131853, 131855, 2, 158.5–217.5 mm SL; DRC: Yangole, Lilanda River; 0°48'N 24°17'E; J. P. Gosse, 22 Feb 1955. – MRAC 131856, 1, 135.5 mm SL; DRC: Bohonde River; 0°46'N 24°23'E; J. P. Gosse 15 Sep 1954. – MRAC 131857, 1, 169.0 mm SL; DRC: Yaekama, Biaka marsh; 0°47'N 24°18'E; J. P. Gosse, 12 Nov 1953. – MRAC 131859, 1, 131.0 mm SL; DRC: Yaekama, Ikonga River; 0°48'N 24°23'E; J. P. Gosse, 31 Aug 1954. – MRAC 175180, 1, 150.0 mm SL; DRC: Bolobo; 2°09'S 16°14'E; N'Kele, 1 Jan 1957. – MRAC 175319, 1, 93.5 mm SL; DRC: Boende, creek; 0°14'S 20°50'E; M. Erkens, 10 Feb 1960. – MRAC 67647–648, 2, 113.0–115.0 mm SL; DRC: Keseki village, near Mistandunga, Mambuti River; 2°02'S 16°24'E; H. Schouteden, no date. – MRAC 73-23-P-102–104, 3, 50.0–228.3 mm SL; DRC: Boende, Tshuapa River; 0°14'S 20°50'E; R. Philippe, 1 Aug 1956. – MRAC 73-23-P-119–141, 15, 135.0–260.5 mm SL; DRC: Lienard, Buta; 3°04'N 25°31'E; R. Philippe, 1 Aug 1958. – MRAC 82-21-P-120–123, 4, 138.5–170.0 mm SL; DRC: Liwa River (5 km), affluent of River Ouaka; 5°39'N 20°41'E; J. P. Marquet, 2 May 1982. – MRAC 88-25-P-827, 1, 88.2 mm SL; DRC: Bumba, affluent of Congo River; 2°11'N 22°32'E; L. De Vos, 29 Jan 1988. – MRAC 89-43-P-1455-456, 1, 115.5 mm SL; DRC: Lobaye River, left bank near Yaeti, road Yaongama-Yaeti; 0°15'N 24°46'E;

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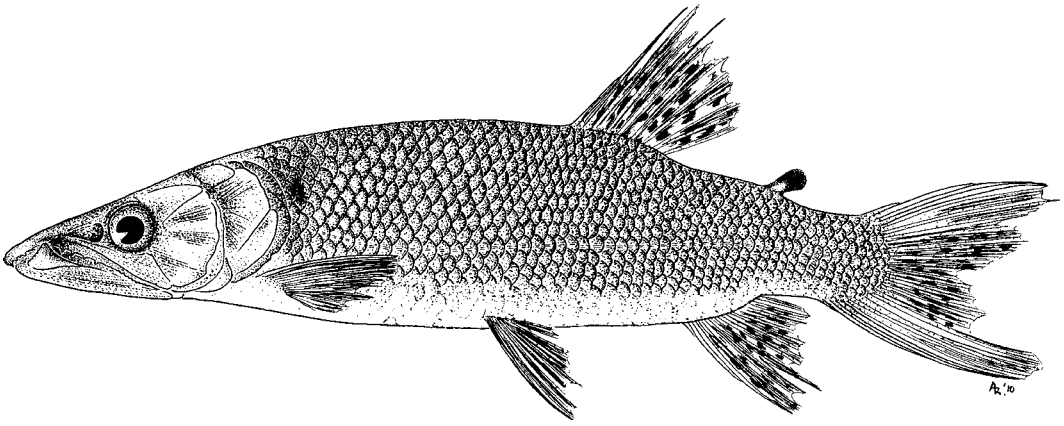


Fig. 6. *Hepsetus microlepis*, BMNH 1899.9.26-78, lectotype, 196.8 mm SL; DRC: Ibali, Lake Leopold II (Mai-Ndombe).

De Vos and Vandelanoote, 20 Feb 1989. – MRAC A4-046-P-0784, 1, 262.0 mm SL; DRC: Léfini River, 3 km upstream of confluence with the Louna. Natural Reserve Lésio-Louna, Congo basin; 2°99'S 15°50'E; Mamonekene et al., 17 Sep 2004. – MRAC A4-046-P-0788–0789, 2, 130.5–174.0 mm SL; DRC: Komo River, affluent of Alima River, near RN2 bridge, Congo basin; 1°11'S 15°57'E; Mamonekene et al., 23 Apr 2004. – MRAC A5-036-P-0514, 1, 220.0 mm SL; DRC: River "Likouala-aux-Herbes" at Itanga, Community Reserve of Lake Télé, Congo basin; 1°13'N 17°25'E; V. Mamonekene, 27 Jul 2005. – MRAC A7-031-P-0273, 1, 240.0 mm SL; DRC: Blue Lake, Léfini basin; 3°19'S 15°29'E; Ibala Zamba et al., 20 Sep 2007. – MRAC A7-031-P-0274–0275, 2, 163.0–145.0 mm SL; DRC: Léfini River, at confluence Léfini-Nambouli, left bank; 2°54'S 15°07'E; Ibala Zamba et al., 28 Sep 2007. – MRAC A7-031-P-0277–0279, 3, 109.0–142.5 mm SL; DRC: Ntiene, affluent of Lésio River, Léfini basin; 3°16'S 15°29'E; Ibala Zamba et al., 20 Sep 2007. – MRAC A9-024-P-0001, 1, 193.0 mm SL; DRC: River Mundamba, across Lisala, Congo basin; 2°06'31"N 21°32'29"E; Danadu and Snoeks, 17 Jun 2009 – MRAC A9-024-P-0002, 1, 231.5 mm SL; DRC: Langalange, Langalanga River, ±5 km downstream of Lisala, affluent of Congo basin; 2°08'23"N 21°27'42"E; Danadu and Snoeks 19 Jun 2009. – MRAC B1-09-P-31, 1, 174.1 mm SL; DRC: Lomami, Djabir River, right bank (0°30'07"N 24°10'39"E; Moelants and Danadu, 28 Mar 2010. – MRAC B2-036-P-0001, 1, 118.2 mm SL; DRC: Lake 1, national park Dzangha-Sangha in the middle of the park; 2°28'56"N 16°13'05"E; M. Sévilla, 2 Feb 2012. – CU 87457, 89447, 89468, 89591, 92974, 5, 122.2–310.4 mm SL; DRC: Lékoli River, Odzala National Park; 0°37'16"N 14°55'05"E; no collector, 7 Aug 2002. – CU 87576, 1,138.7 mm SL; DRC: small channel around island in Lékoli River, Odzala National Park; 0°37'12"N 14°55'06"E; collector unknown, 16 Aug 2002.

Diagnosis. *Hepsetus microlepis* is distinguished from *H. occidentalis* by a larger internasal distance [23–31 % HL (mean 26.1) vs. 20–25 (22.3)] (Fig. 5) and smaller nostril–lower jaw distance [35–44 % HL (mean 40.0) vs. 40–48 (41.8)] (not illustrated), both positively allometric characters. *Hepsetus microlepis* is distinguished from *H. odoe* by a generally higher number of lateral line scales [47–60 (median: 52–53) vs. 43–51 (48)] and by a uniform body colour vs. a vague stripe pattern from the adipose fin to the caudal fin. *Hepsetus microlepis* is distinguished from *H. kingsleyae* by a greater internasal distance [23–31 % HL (mean 26.1) vs. 16–23 (20.6)] (Fig. 5), and a greater head width [32–46 % HL (mean 37.3) vs. 26–36 (33.1)] (not illustrated), which are both positively allometric. *Hepsetus microlepis* is distinguished from *H. lineatus* by a combination of characters: a higher number of epibranchial gill rakers on the first gill arch [4–9, exceptionally 13 (median 6) vs. 0–6 (3)] and a smaller distance between dorsal and adipose fin [9.2–12.8 % SL (mean 11.3) vs. 12.6–16.4 (14.4)] (Fig. 3). *Hepsetus microlepis* also has a uniform body colour, whereas *H. lineatus* has a prominent stripe on its flanks. *Hepsetus microlepis* is distinguished from *H. cuvieri* by a higher number of gill rakers on the first arch [13–24 (median 17) vs. 8–13 (10)], and by a greater head width [32–46 (mean 37.3) % HL vs. 29–40 (33.7)] (Fig. 4a), and also a greater internasal distance [23–31 (mean 26.1) % HL vs. 19–26 (21.6)] (Fig. 4b), with the latter two being positively allometric.

Description. The lectotype is illustrated in Figure 6. Maximum observed size: 267.0 mm SL. Meristic and morphometric data are given in Tables 5–6.

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Table 5. Counts of *Hepsetus microlepis*.

	lectotype	min.	max.	n	median
Total number of lateral line scales	53	47	60	111	53
Scales between dorsal fin and lateral line	10½	9½	10½	114	9½
Scales between adipose fin and lateral line	6½	5½	6½	114	6½
Scales between pelvic fin and lateral line	4	4	6	111	4
Scales between anal fin and lateral line	6	5	7	112	6
Pre-dorsal scales	17	15	22	110	18
Dorsal-adipose scales	21	16	23	111	19
Adipose-caudal scales	15	13	18	112	15
Epibranchial gill rakers on first gill arch	6	4	13	104	6
Epibranchial gill plates on first gill arch	2	0	5	104	2
Ceratobranchial gill rakers on first gill arch	11	8	12	105	10
Ceratobranchial gill plates on first gill arch	0	0	7	105	1
Total number of gill rakers on first gill arch (UR/1/LR)	18	13	24	104	17

Table 6. Measurements of *Hepsetus microlepis*.

	lectotype	min.	max.	n	mean
Body measurements in percents of standard length					
Pre-dorsal length	67.8	65.1	72.4	116	68.0
Dorsal-adipose length	11.9	9.2	12.8	116	11.3
Adipose-caudal length	8.0	6.1	10.8	114	7.9
Pre-pelvic length	30.3	28.7	33.5	114	31.4
Pelvic-pectoral length	21.8	20.4	28.4	111	24.4
Pectoral-anal length	20.7	17.6	24.5	111	21.2
Anal-caudal length	9.1	6.1	12.6	114	9.9
Dorsal-fin base	11.2	8.2	13.3	114	10.4
Adipose-fin base	1.2	1.0	2.5	112	1.6
Pectoral-fin base	3.5	2.3	4.4	113	3.6
Pelvic-fin base	3.6	2.1	4.2	111	3.3
Anal-fin base	9.3	8.4	11.7	114	9.9
Pre-anal length	73.8	70.9	82.0	96	77.6
Post-anal length	22.4	13.3	26.4	112	23.7
Dorsal-fin height	20.8	15.6	28.1	106	21.8
Adipose-fin height	15.9	12.8	29.8	113	18.6
Pectoral-fin height	15.9	13.2	21.7	107	18.3
Pelvic-fin height	16.8	14.2	23.3	103	17.5
Anal-fin height	18.4	14.2	27.6	106	21.1
Caudal-peduncle length	12.9	9.9	14.2	114	12.0
Caudal-peduncle depth	8.6	7.1	10.2	114	8.6
Body depth	20.1	13.7	24.3	114	19.9
Head measurements in percents of head length					
Head length	32	30	36	115	33.1
Nostril length	6	5	9	113	6.7
Nostril-eye length	3	2	4	114	2.5
Snout length	40	37	43	114	40.2
Eye diameter	19	15	25	112	19.5
Head depth	43	38	48	114	42.5
Eye-lower jaw length	25	18	33	114	24.4
Nostril-lower jaw length	40	35	44	113	40.0
Lower jaw length	62	50	68	114	63.4
Upper jaw length	67	64	73	114	68.6
Head width	37	32	46	115	37.3
Interorbital distance	33	28	39	112	33.3
Internasal distance	26	23	31	115	26.1

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Body elongate, greatest depth at or just anterior of dorsal fin origin. Dorsal profile from vertical through first series of scales to dorsal-fin origin mostly straight or slightly convex, straight along dorsal-fin base up to adipose fin. Dorsal and ventral profile of caudal peduncle slightly concave. Caudal peduncle somewhat longer than deep. Head large and broad [head width 32–46 % HL (mean 37.3)] with convex to straight profile. Snout large and elongated. Nostrils positioned just in front of eye, anterior one close to posterior, inter-nasal distance large [23–31 % HL (mean 26.1)]. Mouth terminal with upper jaw slightly prognathous. Maxillary bone fully toothed and reaching posterior margin of orbit. Teeth in both jaws conical. Premaxillary teeth in a single row. Maxillary and dentary with two teeth rows, outer row with large, sharp teeth of variable size, inner row with small teeth of uniform size lying internal to a shallow trench. A pair of triangular dermic folds on lower jaw and small and rounded folds on upper jaw, variable in size. Variation does not seem to be size or sex related.

Dorsal and anal fins set well back on body, insertion of dorsal fin posterior to insertion of pelvic fin. Dorsal fin margin slightly convex, margin of anal fin straight. Caudal fin forked, with lobes of equal size. Adipose fin inserted above posterior part of anal fin.

Body covered with well-developed cycloid scales. Lateral line completely pored, with a large range of lateral line scales (47–60). Large number of scale rows between dorsal fin and lateral line (9.5–10.5); scale rows between lateral line and pelvic fin origin 4–6.

Between 13–24 gill rakers on first gill arch, with 4–13 on epibranchial and 8–12 on ceratobranchial. Gill rakers long and slender. Vertebrae 39–40 excluding the Weberian apparatus (counted in 12 specimens based on X-ray photographs).

Coloration. Preserved specimens: Overall body colour varying from light brown to blackish in most specimens; some specimens with light coloured ventral parts. Fins brown to blackish, yellowish in light brown specimens; generally translucent distally. Caudal fin light greyish to yellowish, translucent in mid-centre and central parts of upper and lower lobes. Dorsal, anal and caudal fins with prominent small, rounded, dark-brown spots, more obvious in larger individuals (more than about 155 mm SL). Adipose fin creamy basally, black distally. Three well-marked, dark-brown bands radiate from infraorbitals behind eye over pre-operculum to operculum. A black dorso-ventrally elongated spot behind head above lateral line. In a few specimens, a series of 6–10 small greyish elongated vertical bars on body, first one situated just behind operculum and last one just anterior to caudal-fin base.

Etymology. The species name *microlepis* is derived from the Greek mikros meaning small, and lepis meaning scale.

Distribution (Fig. 7)**.** *Hepsetus microlepis* occurs in most parts of the Lower and Middle Congo but has not been found in the southern part of the Kasai and the Dja, and is virtually absent from the Upper Congo, except for one recently collected specimen from just upstream of the Wagenia rapids in Kisangani (MRAC B0-009-P-3). In addition, *H. microlepis* is also found in the Gribingui River, Chad basin (Central African Republic).

Discussion

Three species of *Hepsetus* (*H. microlepis*, *H. lineatus* and *H. cuvieri*) occur within the Congo ichthyofaunal province. *Hepsetus microlepis* was originally described as a subspecies of *H. odoe* by Boulenger (1901). The name *H. o. microlepis* has never been used after the description and it was listed under *H. odoe* by Roberts (1984), and is here elevated to species level. Some characters were found to differ notably with the data given by Boulenger (1901) in the original description. For example Boulenger (1901) counted 53–58 (8 specimens) lateral line scales and considered the character as diagnostic. In this study the number of lateral line scales was found to vary between 47–60 (104 specimens) making this character unsuitable as a diagnostic feature, as it overlaps greatly with the range in *H. lineatus* (50–64) and *H. cuvieri* (48–55). One of the syntypes mentioned by Boulenger (1901) is from Lake N'gami (Botswana), which is also the type locality of *H. cuvieri*. This syntype was not found in the BMNH collection, but most probably is a specimen of *H. cuvieri* and not of *H. microlepis* (see also Zengeya et al., 2011).

Hepsetus lineatus occurs in the Dja and other upper reaches of the Sangha basin. In addition, two Middle Congo basin specimens have been

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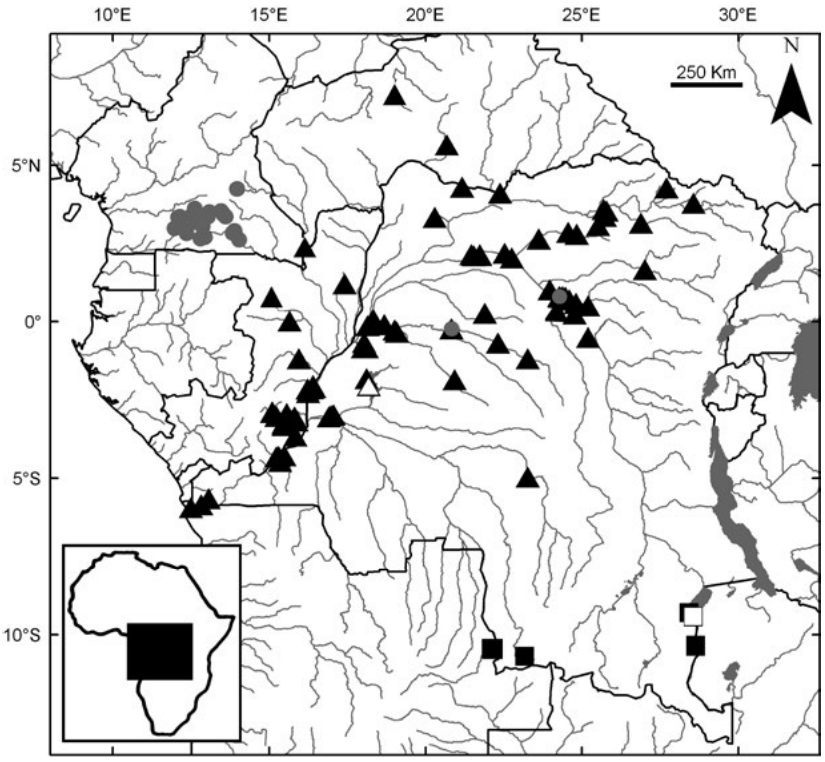


Fig. 7. Distribution of *Hepsetus microlepis*, *H. lineatus* and *H. cuvieri* within the Congo ichthyofaunal province. *Hepsetus microlepis*: lectotype (△) and other specimens (▲); *H. lineatus* (●); and *H.* cf. *cuvieri* (□) and other specimens (■).

identified as *H. lineatus* (MRAC 131855, 175319). Both specimens fall well within the distribution area of *H. microlepis* and it is remarkable that *H. lineatus* occurs in this part of the basin. The unexpected occurrence does probably not result from mislabelling, and at present there is no evidence that another type of mistake, such as putting specimens back in the wrong jars, has occurred. Therefore, these two occurrences indicate an important range extension within the basin for *H. lineatus* and at the same time indicates the rarity of the species in this part of the basin.

One specimen from the Upper Congo is here identified as *H.* cf. *cuvieri* (MRAC 81286), as it has a much higher number of gill rakers on the first gill arch than *H. cuvieri* (20 vs. 8–13). This is remarkable since the number of gill rakers is an important diagnostic character for *H. cuvieri*. For all other diagnostic counts, i. e. the number of scales between the dorsal fin and the lateral line (10½) and between the adipose fin and the lateral line (6½), and measurements, i. e. the narrow head and snout, this specimen falls within the range of *H. cuvieri*. The specimen does deviate from *H. cuvieri* in its colour pattern and lacks the typical brown blotches on the sides.

Another case of deviating gill raker count was found in one *H. microlepis* specimen (MRAC 1352) with an exceptionally high number of gill rakers on the epibranchial (13 vs. 4–9). In all other diagnostic meristic and morphometric characters this specimen matches *H. microlepis*.

Hepsetus lineatus has a limited distribution within the Congo basin (Fig. 7). It is likely that its presence results from past changes in the course of the Dja River. At present, the upper part of the Dja flows westwards, then suddenly changes direction, and flows eastwards to finally reach the Sangha, a right bank affluent of the Middle Congo (Olivry, 1986). At an unknown time in the past, however, the Dja River formed the upper course of the Nyong River, a coastal basin in Cameroon and part of the Lower Guinea ichthyofaunal province (Olivry, 1986). Through capture of the Dja

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by the Sangha, specimens of *H. lineatus* (mainly a Lower Guinea species), seem to have entered the Sangha River. Other fish species with similar distribution patterns are *Parauchenoglanis guttatus* (Lönnberg, 1985) (Claroteidae) (Geerinckx et al., 2007), *Nannocharax intermedium* (Boulenger, 1903) (Distichodontidae) (Vari, 2007), and *Sarotherodon mvogoi* (Thys van den Audenaerde, 1965) (Cichlidae) (Stiassny et al., 2007), all of which occur in the upper parts of the Dja and in the Nyong. Another species, *Mastacembelus marchei* Sauvage, 1879, mainly occurs in the coastal basins of Lower Guinea, but a few specimens have been found in the Dja, but also in the lower Congo and Pool Malebo (Vreven, 2007; Vreven & Stiassny, 2009). *Hepsetus lineatus* occurs in the upper reaches of the Sangha River, even upstream from the mouth of the Dja, while *H. microlepis* has been found in the Sangha more towards the confluence with the Congo main channel (Fig. 7). Whether or not the presence of *H. lineatus* there is a result of upstream dispersal or another river capture event is not known.

Hepsetus microlepis has also been found outside the Congo basin in the Gribingui River, which is part of the Chad basin in the Central African Republic. There is no permanent river connection between the Gribingui and the Congo basin, but during flood periods, *H. microlepis* may have invaded the Gribingui from the Congo basin. Pellegrin (1919) already stated that the Gribingui is rich in fish species because it is located close to the Congo basin, implying an exchange of fishes between the two river systems. Similarly, *Mastacembelus congicus* Boulenger 1896 is mainly a Congo species and it has been reported from the Upper Chad basin (Vreven, 2003).

Within the Congo ichthyofaunal province, *H. cuvieri* is reported from the Upper Congo and the southern part of the Kasai River. Outside the Congo basin, *H. cuvieri* is also known from the Quanza, Cunene, Okavango, upper Zambezi and Kafue. Possible connections and dispersal routes between these rivers of southern Africa (which used to form one single basin) and the Upper Congo and Kasai have been discussed in Zengeya et al. (2011).

Although the genus is widespread within the Congo basin, there is no record from large parts of the Kasai, the Lomami and the Lualaba basins (Fig. 7). This region is visible as an apparent distribution gap between the southern edge of the distribution of *H. microlepis* and the northern one of *H. cuvieri*. Only a single *H. microlepis* specimen has been collected in this area, at Kondue on the Sankuru River, a main tributary of the Kasai River (DRC). It seems that *H. microlepis*, in contrast to *H. cuvieri*, does not occupy the more southern parts of the Congo basin. However, as the distribution gap is situated in an area where not much sampling has been undertaken in the past, it is still possible that the absence of *Hepsetus* specimens from this area is partially due to the limited sampling. On the other hand, as shown above, the distribution pattern of the three *Hepsetus* species in the Congo basin are rather complex and could reflect the complicated history of the basin itself (e. g. Stankiewicz & de Wit, 2005).

Additional specimens examined and analysed (*Hepsetus cuvieri*), not in Zengeya et al. (2011). Kasai: MRAC B2-30-P-1386–1388, 3, 98.4–259.1 mm SL; DRC: Lualaba Province, Kasai river below and above Saipako Falls ± 15 km from airline district, NNW of Dilolo at border to Angola; 10°33'10"S 22°18'36"E.

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Geophagus diamantinensis (photograph by José L. O. Mattos)
José L. O. Mattos, Wilson J. E. M. Costa and Alexandre C. A. Santos
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Taxonomic challenges in freshwater fishes: a mismatch between morphology and DNA barcoding in fish of the north-eastern part of the Congo basin

EVA DECRU,*† TUUR MOELANTS,*† KOEN DE GELAS,‡ EMMANUEL VREVEN,*† ERIK VERHEYEN‡§ and JOS SNOEKS*†

*Department of Biology, Laboratory of Biodiversity and Evolutionary Genomics, KU Leuven, Charles Deberiotstraat 32, 3000 Leuven, Belgium, †Section Vertebrates, Ichthyology, Royal Museum for Central Africa, Leuvensesteenweg 13, 3080 Tervuren, Belgium, ‡OD Taxonomy and Phylogeny, Royal Belgian Institute for Natural Sciences, Vautierstraat 29, 1000 Brussels, Belgium, §Biology Department, Evolutionary Ecology Group, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerpen, Belgium

Abstract

This study evaluates the utility of DNA barcoding to traditional morphology-based species identifications for the fish fauna of the north-eastern Congo basin. We compared DNA sequences (COI) of 821 samples from 206 morphologically identified species. Best match, best close match and all species barcoding analyses resulted in a rather low identification success of 87.5%, 84.5% and 64.1%, respectively. The ratio 'nearest-neighbour distance/maximum intraspecific divergence' was lower than 1 for 26.1% of the samples, indicating possible taxonomic problems. In ten genera, belonging to six families, the number of species inferred from mtDNA data exceeded the number of species identified using morphological features; and in four cases indications of possible synonymy were detected. Finally, the DNA barcodes confirmed previously known identification problems within certain genera of the Clariidae, Cyprinidae and Mormyridae. Our results underscore the large number of taxonomic problems lingering in the taxonomy of the fish fauna of the Congo basin and illustrate why DNA barcodes will contribute to future efforts to compile a reliable taxonomic inventory of the Congo basin fish fauna. Therefore, the obtained barcodes were deposited in the reference barcode library of the Barcode of Life Initiative.

Keywords: cytochrome *c* oxidase subunit 1, Congo Basin, taxonomic diversity detection, biodiversity

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Introduction

The Congo basin is the most species rich basin of Africa with over 1250 valid fish species described and is worldwide second in species richness, after the Amazon Basin (Runge 2007; Snoeks *et al*. 2011). Traditionally, the Congo basin is divided into the Upper Congo (Lualaba) from its origin in Zambia to the Wagenia or Boyoma falls near Kisangani, the Middle Congo downstream from these rapids to Pool Malebo near Kinshasa, and the Lower Congo downstream of Pool Malebo to the mouth at the Atlantic Ocean. Each of these main divisions harbours a characteristic fauna with a large number of endemics (Snoeks *et al*. 2011). Despite the status of diversity hotspot, the fish fauna remains poorly documented (Snoeks *et al*. 2011). Recent surveys in affluents of the Middle Congo, that is the Léfini River (Ibala Zamba 2010), the N'Sele River (Monsembula Iyaba *et al*. 2013), the rivers in Salonga National Park (Monsembula Iyaba & Stiassny 2013), and the Kwilu River (Mbimbi Mayi Munene & Stiassny 2011); and in the Lower Congo (Lowenstein *et al*. 2011), and one of its affluents, the Inkisi River (Wamuini Lunkayilakio *et al*. 2010), have greatly improved knowledge on the taxonomic diversity in these watersheds. However, the fish faunas of many regions remain poorly or unexplored, such as the north-eastern tributaries in the vicinity of Kisangani. Several fish families within the Congo basin, such as in the Clariidae (e.g. Devaere *et al*. 2007; Hanssens 2009) and Cyprinidae (e.g. Lowenstein *et al*. 2011) are difficult to identify using morphological features alone. In such cases, molecular techniques have been proposed to facilitate species identifications (Schindel & Miller 2008). It has been suggested that this approach can speed up the rate at which taxonomists can revise and describe the estimated 5–100 million species that await discovery (Meier *et al*. 2006). Hebert *et al*. (2003) proposed a DNA barcoding system for animals based on

Correspondence: Eva Decru, Fax: +32 2 769 56 42;
E-mail: eva.decru@africamuseum.be

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the sequence diversity in the mitochondrial gene for the cytochrome *c* oxidase subunit 1 (COI). The DNA barcoding approach is based on the premise that the interspecific variation of a gene exceeds the intraspecific variation. The successful identification of species using the barcoding approach has been demonstrated to be high in fish species, varying between 80% and 100% for both marine and freshwater taxa (Becker *et al.* 2011; Pereira *et al.* 2013). In many taxonomic groups, DNA barcoding can be a powerful tool to detect cryptic species diversity or to reveal taxonomic inconsistencies (Goodier *et al.* 2011; Collins & Cruickshank 2012). In this context, this study tests (i) whether DNA barcoding also provides complementary information that may speed up the species inventory of the fish fauna in the region around Kisangani and (ii) provides data that will highlight which species groups need a taxonomic revision.

Furthermore, the DNA barcoding approach can also be used to reveal intraspecific genetic diversity in fish species (Pereira *et al.* 2013). The fragmented nature of freshwater ecosystems caused by (eventually temporary) physical and chemical barriers among watersheds, can restrict gene flow, and thereby cause genetic divergence due to geographical isolation. In this study region, the Congo River passes the Wagenia or Boyoma falls, a series of rapids of about 60 km just upstream of Kisangani and further referred to as the Wagenia rapids to avoid confusion. They are traditionally seen as an ichthyological barrier between the Middle and Upper Congo (Poll 1963). Another barrier, the ±15 m high Ngoy waterfall, is situated in the Upper Aruwimi and separates the Ituri River from its tributary the Epulu River. Our study will use DNA barcodes to test the hypothesis that the Wagenia rapids and Ngoy falls represent a physical barrier that restricts gene flow in species occurring on both sides of these falls.

Materials and methods

Samples

Fishes were collected during five expeditions between 2009 and 2011 in the Kisangani area downstream (Lobaye, Lomami and main channel of the Middle Congo River) and upstream (Loboya, Maiko and main channel of the Upper Congo River) of the Wagenia rapids and on the north-eastern affluents of the Congo Basin (the lower reaches of the Itimbiri and Aruwimi, and the upstream tributaries of the latter, that is the Ituri and Epulu rivers) (Fig. 1). In all these instances, the collected specimens were fin-clipped for DNA barcoding.

All specimens were identified up to species level by the authors, in collaboration with other experts within the Ichthyology unit of the Royal Museum for Central Africa (RMCA, Tervuren). The most recent information was used to identify the specimens (see Table S1, Supporting Information), but identification keys were often lacking. In these cases, characteristics mentioned in the original species description were used for identification, which sometimes comprised only minimal morphological features to recognize a certain species. For difficult identifications, specimens were compared with type material when available.

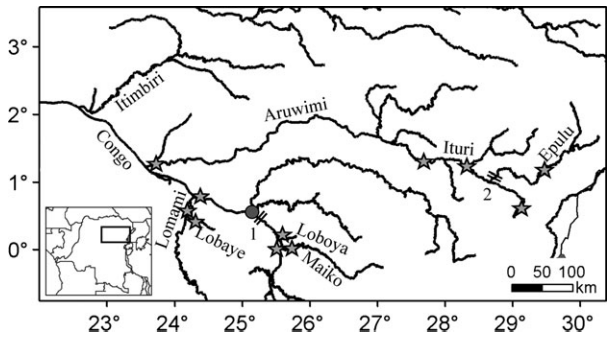


Fig. 1 Map of the north-eastern part of the Congo basin with the main rivers and rapids and falls of the study. Sampling locations are indicated as stars, the city of Kisangani as a circle. 1: Wagenia rapids; 2: Ngoy fall. Insert map shows the positioning of the sampling region in the Democratic Republic of the Congo.

From each river, two samples of every species that could be identified on the basis of its morphological features were sequenced. Additional samples of *Enteromius miolepis*, *Bryconaethiops macrops*, *Bryconaethiops microstoma* and *Brycinus imberi* were included to test whether DNA barcoding could confirm the observed morphological differences. A total of 821 sequences were successfully obtained: 160 from the Itimbiri, 374 from the Aruwimi and 287 from the Kisangani region. The latter selection consisted of 57 sequences from the Middle Congo, 46 from the Lomami, 29 from the Lobaye, 54 from the Upper Congo, 52 from the Maiko and 49 from the Loboya (Fig. 1). For 818 of the 821 sequences, voucher specimens are available and deposited at the RMCA.

DNA extraction, PCR amplification and sequencing

DNA was extracted using either a DNeasy Blood and Tissue kit (Qiagen Ltd., UK) or a NucleoSpin® Tissue kit (Macherey-Nagel, Germany) following the instructions of the manufacturer. Fragments of ~562 up to 652 bp of the mitochondrial cytochrome c oxidase subunit I (COI) gene were amplified by polymerase chain reactions (PCRs) using three different primer combinations: the majority of the samples was amplified with an M13 tailed primer cocktail containing the primers VF2_t1, FishF2_t1, FishR2_t1 and VR1d_t1 (Ivanova *et al.* 2007); for samples of the genus *Bryconaethiops* and *Synodontis*,

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new primer pairs were developed, respectively, Bryco_F_d (ACC CTC YAC CTT GTA TTT GGT G), Bryco_R_d (GCA GGG TCA AAR AAG GTG GT) and SYNF (GTG CCT GAG CCG GAA TAG), SYNR_d (CTC CTG CRG GGT CRA AGA AAG). PCR was carried out using a Qiagen PCR kit in a total volume of 25 μL, containing 2.5 μL PCR buffer (10×), 2.5 μL dNTP (2 mM), 1.25 μL primer cocktail (2 μM), 0.2 μL Taq DNA polymerase (5 units per μL), 1.0 μL of extracted DNA and 17.75 μL of mQ-H_2O. For the specific primers, 2.5 μL of each primer (2 μM) was used. The PCR profile was 3 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 40 s at annealing T (see further), 1 min 30 s at 72 °C and a final extension of 10 min at 72 °C. Annealing temperature for the three different primer combinations were 51, 55 and 55 °C, respectively. Amplified products were verified on 1.2% agarose gels. The PCR products were purified using a NucleoFast® 96 PCR kit (Macherey-Nagel). PCR products were sequenced bidirectionally using BIGDYE TERMINATOR v.3.1 and an ABI 3130 capillary sequencer. The DNA sequences were assembled and visually checked in CodonCode Aligner (CodonCode Corporation) and aligned in MEGA 6.06 (Tamura *et al.* 2013) using Muscle Alignment (Edgar 2004).

Distance analyses and identification techniques

Pairwise distances for all intraspecific and interspecific samples were calculated with SPECIESIDENTIFIER v1.7.8 (Meier *et al.* 2006). As this study concerns a noncomprehensive and very diverse group of samples, intraspecific variation can differ between species (Collins & Cruickshank 2012). Therefore, the constructed histogram of these distances was not informative to test for overlap between intraspecific and interspecific genetic variability. Scatterplots were made instead, in which for each sample the distance to the furthest conspecific is plotted against the nearest-neighbour distance (nearest nonconspecific, congeneric distance) (Robinson *et al.* 2009). For obvious reasons, singletons were not included in this analysis. A Model test was performed in MEGA 6.06, and the model GTR + G + I was revealed to be the most suitable for the data using the Akaike information criterium. Neighbour joining (NJ) and maximum-likelihood (ML) trees with 100 bootstrap replications were also constructed in MEGA 6.06.

To examine identification success, the three criteria as proposed by Meier *et al.* (2006) were applied: best match (BM), best close match (BCM) and all species barcodes (ASB). Under the best match criterion, each sequence is assigned to the species name of its closest match in the alignment regardless of similarity. Identification is successful if the sequence and the match are conspecific, fails if they are allospecific, and is ambiguous if there are equally good matches among sequences from two or more species. The best close match criterion is similar, except that here a species name is only assigned if the sequence and the match are sufficiently similar. A threshold value is determined below which 95% of all intraspecific distances are found. Under the all species barcode criterion, a list of barcodes is compiled for each query sequence, sorted by their similarity with the query sequence under the same threshold as under the BCM. Identifications are considered to be successful when the query sequence is followed by all (at least two) conspecific barcodes and ambiguous when they are followed by only one conspecific barcode or only some of the conspecific barcodes. Queries are considered misidentified when the query sequence is more similar to all sequences from another species (Meier *et al.* 2006; Mattio & Payri 2010). These success rates for the data set, threshold values and pairwise distances were calculated with SpeciesIdentifier v1.7.8 (Meier *et al.* 2006). Species that are only represented by a single sample in the data set (singletons) will automatically be incorrectly identified when using the BM and BCM criteria as there are no possible conspecific sequences to match with. Therefore, results for all analyses will be represented with and without singletons (46 sequences).

Results

We obtained sequences of 821 specimens belonging to 27 families and 206 species as identified morphologically. This included 31 taxa with working names or uncertain identifications, referred to as 'cf.'.

Intra- and interspecific variability

Of the 602 samples (excluding singletons and samples without congeneric sequences) analysed, 445 (73.9%) have a nearest-neighbour value higher than the maximum intraspecific distance, pointing to the presence of a barcoding gap (Fig. 2). The remaining 157 samples (26.1%) have a nearest-neighbour value lower than the maximum intraspecific distance, indicating possible taxonomic problems. They concern mainly samples of the families Cyprinidae and Mormyridae. More than one-fourth of the samples (26.1%) belonged to *Enteromius miolepis* (25) and *Brycinus imberi* (16).

Tree-based identification techniques

As both NJ and ML trees gave similar results, only the NJ tree is illustrated (Fig. S1, Supporting Information). In the collapsed tree, the statistical node support of both the NJ and ML bootstrap methods is shown. In the expanded tree, only NJ bootstrap values are shown (see

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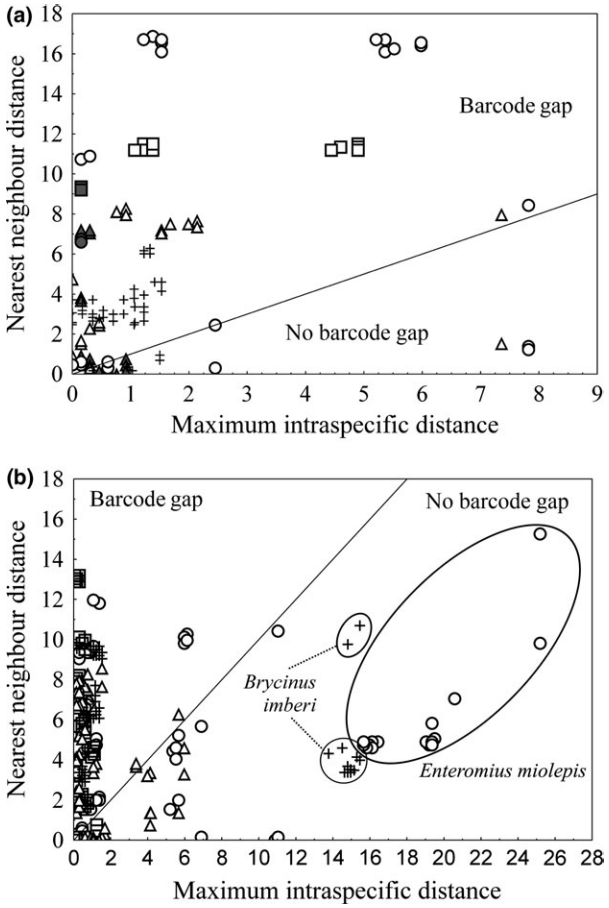


Fig. 2 Maximum intraspecific divergence compared with nearest-neighbour distance for congeners. a. (+) Mochokidae, (○) Cichlidae, (□) Schilbeidae, (Δ) Clariidae, (■) Channidae, (●) Nothobranchiidae. b. (+) Alestidae; (○) Cyprinidae, (□) Distichodontidae, (Δ) Mormyridae.

Data Accessibility). Many sequences failed to form species-specific clusters. Indeed, some species are scattered over different genetic clusters, possibly representing cases of undetected taxonomic diversity. Conversely, in some instances, different species (mostly congeneric) clustered in the same lineage.

The proportion of fish that could be identified correctly depended on the criterion used. It was highest when using the BM and BCM criteria, both with (87.45% and 84.53%) and without (93.93% and 90.58%) singletons (Table 1). It decreased to 64.06% and 69.54%, respectively, under the ASB criterion, mainly due to the higher number of ambiguous identifications (26.55% and 25.80%, respectively).

Discussion

DNA barcoding was evaluated as a complementary tool for the identification of fish species of the north-eastern Congo basin. In comparison with a similar study on

Table 1 Results of best match, best close match and all species barcodes analyses, including (n = 821) and excluding (n = 775) singletons

	Best match (%)	Best close match (%)	All species barcodes (%)
With singletons			
Correct ID	718 (87.45)	694 (84.53)	526 (64.06)
Ambiguous ID	38 (4.62)	34 (4.14)	218 (26.55)
Incorrect ID	65 (7.91)	25 (3.04)	9 (1.09)
Sequences without any match closer than threshold	NA	68 (8.28)	68 (8.28)
Without singletons			
Correct ID	728 (93.93)	702 (90.58)	539 (69.54)
Ambiguous ID	24 (3.09)	23 (2.96)	200 (25.8)
Incorrect ID	23 (2.96)	17 (2.19)	3 (0.38)
Sequences without any match closer than threshold	NA	33 (4.25)	33 (4.25)

Neotropical fishes (Pereira *et al.* 2013), our findings suggest that the identification success rates using the DNA barcode approach is relatively low (95% using the BM and BMC criteria, and 65% for the ASB criterion see Table 1). In Pereira *et al.* (2013), 252 out of 254 species (99.2%) of the Upper Parana River basin were correctly identified. However, the fish fauna of the Upper Parana is well studied, and may even be considered to be the best known fish fauna in the Neotropical region (Pereira *et al.* 2013). This probably explains the high identification success found in the study. The ichthyofauna of the Congo basin on the other hand is still underexplored. Identification keys and detailed information to distinguish the different species are often lacking, and the distribution areas are often not sufficiently known. Some families, genera or species are also in need of revisions. Thus, in this study of the Congolese fish fauna, barcoding is rather used as an attempt to refine the present knowledge concerning species richness and distributions instead of an identification system where sequences are compared with a reliable DNA database. Moreover, this study contributes to the construction of a DNA database of the Congolese fish fauna. Obviously, the ID success is higher when excluding singletons (Table 1), as the sequence with the smallest distance from the query (the singleton) will always be allospecific, which leads to ambiguous or incorrect identifications and thus a lower identification success.

One side of the coin: confirmation and detection of undescribed diversity

Two or more distinct genetic groups were found in *Hydrocynus vittatus*, *Brycinus imberi*, *Brachypetersius altus*,

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Pareutropius debauwi, *Hemichromis lifalili*, *Oreochromis niloticus*, *Leptocypris lujae*, *Petrocephalus christyi*, *Enteromius miolepis* and *Enteromius brazzai* (Fig. S1, Supporting Information).

Three different genetic groups were found within *H. vittatus* (Fig. S1, Supporting Information), each containing samples from different rivers: one group from the Aruwimi and Itimbiri, one from the Ituri and Maiko and one with a single sample from Upper Congo. A further inspection of the specimens (no detailed morphometric study done) did not reveal any morphological differences between these groups. Indications of possible new species have already been reported in a molecular (cytb, mtDNA) study of *Hydrocynus* (Goodier *et al.* 2011). Five unknown lineages, three of which from the Congo basin, were found in addition to the five valid species already known. This was also an indication that the diversity within *H. vittatus* from the Congo basin might be larger than could be detected by morphology. However, also in this study, a thorough morphometric revision was not included.

The presence of two genetic groups within *H. lifalili* (one with samples from the Ituri and Aruwimi, the other from the Loboya and Lomami) is also not surprising. Several potential new species within the genus have been mentioned (Lamboj 2004). An example is *Hemichromis* sp. 'Bangui', which occurs in the northern parts of the Congo and which represents either a new species or a geographic variety of *H. lifalili* or *H. stellifer* (Loiselle 1979). The two different genetic groups within *H. lifalili* in our data set confirm the findings of Lamboj (2004) that a thorough examination of the group is necessary.

The cryptic diversity found in *B. imberi* and *E. miolepis* (genus recently revalidated by Yang *et al.* 2015) was not unexpected as both have a very wide distribution area (Paugy 1984, 2003; Skelton 2001; Van Steenberge *et al.* 2014), and taxonomic problems are known to exist within these taxa (Musschoot, pers. comm.; Moelants & Tweddle 2010). Moreover, both taxa are often used as a 'wastebasket taxon', to which any specimen with a comparable morphology is assigned to, but without further consideration. This raises the question whether *B. imberi* and *E. miolepis* are indeed single widespread species with a high intraspecific genetic variability or actually multispecific taxa. Such poorly diagnosed, supposedly species are often revealed to represent species complexes when examined more closely after broad geographic sampling (Snoeks *et al.* 2011).

In the NJ tree (Fig. S1, Supporting Information), *B. imberi* appears to be paraphyletic and two lineages can be recognized herein. Both groups have a striking genetic divergence (about 12% for phylogroups sampled syntopically in the Ituri); one group is more closely related to *B. bimaculatus* and *B. kingsleyae*, and the other group to *B.* sp. 'Epulu', a new species to be described soon. This species can be distinguished from *B. imberi* by fewer premaxillary teeth and a horizontal black band on the body (E. Decru, E. Vreven, O. Sadio and J. Snoeks, in prep.). The *B. imberi* group closest to *B. bimaculatus* contains relatively small specimens with a more silvery colour pattern (in preserved specimens). This could be a first indication that a more thorough morphological examination might reveal the existence of several species within what is currently considered to be *B. imberi*. Based on our results spanning a relatively small geographic region and in view of the wide distribution of *B. imberi*, we expect to discover additional cases of genetic differentiation in allopatric populations of *B. imberi*.

Enteromius miolepis also splits into two groups, and a third lineage containing a single sample (Fig. 3). The two groups contain samples from the Ituri River, but from different locations. At first sight, no morphological differences between specimens from the two lineages could be detected. *Enteromius brazzai* as well, splits into two groups of specimens between which no morphological differences were observed at first sight. This again underscores the need for a thorough taxonomic revision of the genus *Enteromius* from the Congo, many of which are morphologically very similar. Therefore, a more extensive study on *E. miolepis* and some related species of the Congo basin is in progress.

There is a clear difference between the issues in *B. imberi* and *E. miolepis*, and those in the other discussed cases (*H. vittatus* and *H. lifalili*). In the latter, the different lineages are found in samples from different rivers, which would point to phylogeographical variation rather than undetected taxonomic diversity. In the former cases, the different lineages occur in the same rivers. This observation and the fact that the genetic divergence is supported by morphological differences in *B. imberi*, supports the hypothesis of multiple species within what is currently considered as one species.

The fact that two different lineages of *O. niloticus* were found in the NJ tree (Fig. S1, Supporting Information) can be attributed to cryptic diversity, but more likely to introduction. Indeed, the species is widely introduced for aquaculture (Trewavas 1983). As a consequence, many different 'domesticated' genetic strains exist within this species. The two lineages, one containing sequences from the Middle Congo and the Aruwimi, the other also from the Middle Congo as well as the Upper Congo, thus probably point to the presence of different introduced strains in our sampling material.

Also in *P. christyi*, two genetic lineages were found in the NJ tree (Fig. S1, Supporting Information). Recently, several new species have been described within the genus (Lavoué *et al.* 2010; Lavoué & Sullivan 2014), some of which morphologically very similar to *P. christyi*. Yet,

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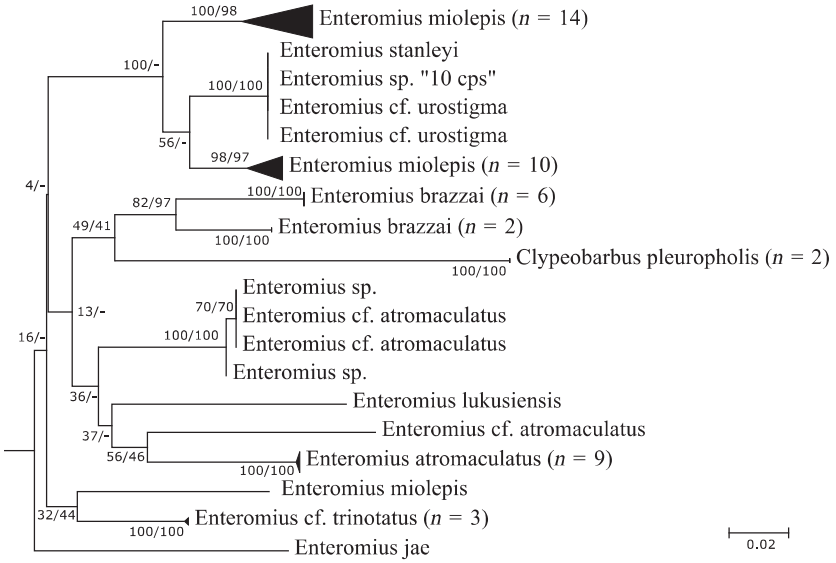


Fig. 3 Phylogram constructed from the mitochondrial COI sequences of *Enteromius*. Statistical node support is shown as NJ bootstrap/ML bootstrap. Branch lengths indicate the expected number of substitutions per site under NJ inference.

all specimens from both genetic lineages keyed out as *P. christyi*. Additional taxonomic research is necessary to determine whether one of the two lineages represents a new species.

Multiple genetic groups were found also within *B. altus*, *P. debauwi* and *L. lujae* (Fig. S1, Supporting Information). These were rather unexpected observations as taxonomic problems were not known to occur within these taxa, and identification of the specimens was considered to be straightforward. The fact that different genetic lineages occur, underscores the need of a taxonomic revision of these taxa to examine whether this genetic diversity is the result of cryptic diversity or intraspecific genetic structure.

The other side of the coin: species clustering

In several instances, morphologically different species clustered in the same genetic lineage. This was the case for the following species pairs: *Chrysichthys longipinnis* and *C. congicus*; *Chrysichthys habereri* and *C. cranchii*; *Marcusenius monteiri* and *M. stanleyanus*; and *Synodontis iturii* and *S. acanthomias*.

While *C. congicus* could not be separated from *C. longipinnis* based on their COI gene (Fig. 4), their morphology is clearly distinct. *Chrysichthys congicus* can be distinguished from *C. longipinnis* by its shorter dorsal fin, that is 22.3–26.8%SL (vs. 25.8–36.9), longer distance between the anal and caudal fins, that is 17.0–22.7%SL (vs. 12–17.9), reduced nasal barbels, that is 2.0–6.4%HL (vs. 8.9–19.5) and narrower premaxillary toothplate, that is 16.2–24.6% HL (vs. 24.5–32.6) (Risch 1986). *Chrysichthys habereri* and *C. cranchii* are also relatively easily distinguished from each other. The mouth of *C. cranchii* is inferior, unlike in *C. habereri*, where the mouth is terminal, often with a prognathous lower jaw. In addition, the adipose fin in *C. habereri* is slightly shorter than in *C. cranchii* (9.6–14.2 vs 10.1–17.9% SL). Both mitochondrial introgression and incomplete lineage sorting could explain the similarity in mtDNA haplotypes between two species that are clearly morphologically different. Introgression following hybridization could have caused a transfer of mtDNA from one species to another. On the other hand, the speciation events between these closely related species could have been so recent that the mtDNA lineage sorting among species is incomplete (Moran & Kornfield 1993), and the mitochondrial genomes of the descendent species have not yet accumulated species-specific mutations. However, incomplete lineage sorting would become a very unlikely hypothesis in two instances: (i) if other populations of the same species would be found with very divergent mitochondrial haplotypes and (ii) if alleles would be geographically concentrated near species boundaries or known hybrid zones of mainly allopatric species. Indeed, if alleles from a common ancestor have been retained in descendent species, these alleles are expected to be randomly distributed across descendant populations, and not concentrated geographically near species boundaries or known hybrid zones (McGuire *et al*. 2007). However, in the present study, both species within each species pair of *Chrysichthys* occur syntopically (caught in the same gill net); the sympatric distribution of both taxa of each species pair is also reported in the literature (Risch 1986). Therefore, introgression through hybridization seems the most valid hypothesis for the similarity in mtDNA in these clearly morphological different species.

Incomplete lineage sorting or introgressive hybridization can also explain the clustering of *M. monteiri* and *M. stanleyanus* (Fig. 5). However, this case is slightly dif-

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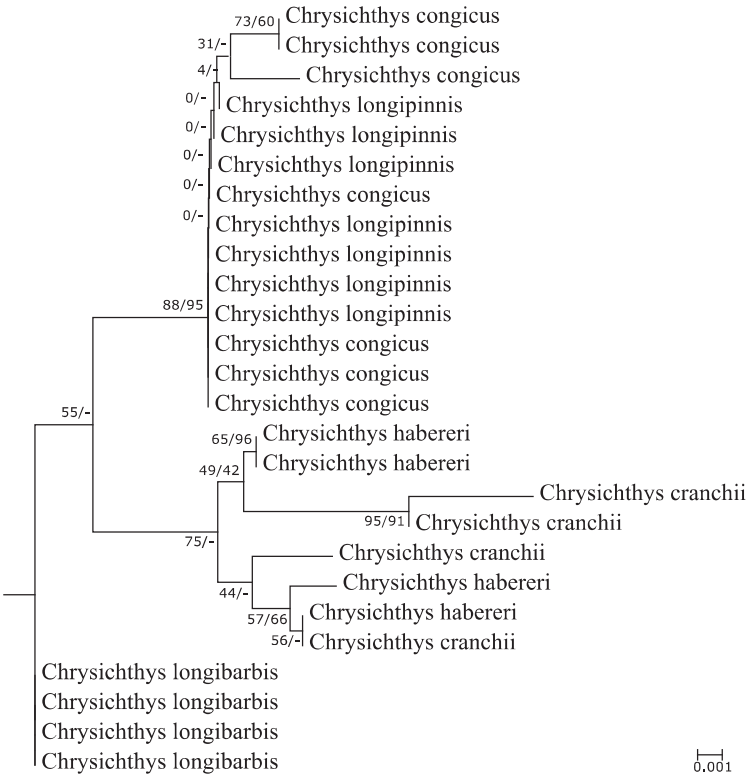


Fig. 4 Phylogram constructed from the mitochondrial COI sequences of the *Chrysichtyhys* complex. Statistical node support is shown as NJ bootstrap/ML bootstrap. Branch lengths indicate the expected number of substitutions per site under NJ inference.

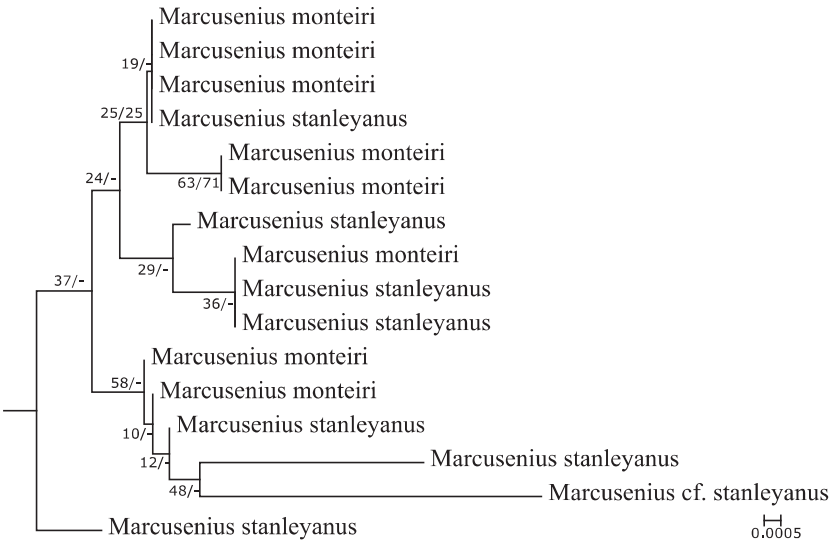


Fig. 5 Phylogram constructed from the mitochondrial COI sequences of *Marcusenius monteiri* and *Marcusenius stanleyanus*. Statistical node support is shown as NJ bootstrap/ML bootstrap. Branch lengths indicate the expected number of substitutions per site under NJ inference.

ferent as the morphological distinction between both taxa is not as straightforward as in *Chrysichthys*. The 38 species of *Marcusenius* Gill 1862 are generally split in two groups: one with eight scales around the caudal peduncle (large-scaled *Marcusenius* species) and a second with 12–16 scales (small-scaled *Marcusenius* species). The first group has already been revised (Boden *et al.* 1997), while a thorough study of the small-scaled *Marcusenius* species is still lacking. According to the original descriptions and an ongoing morphometric study of the small-scaled *Marcusenius* species of the Congo, the only clear characteristic to distinguish *M. monteiri* from *M. stanleyanus* is tooth morphology; teeth are notched in *M. monteiri* and conical or truncated in *M. stanleyanus*. However, in some cases, both tooth shapes were encountered in the same specimens. This could indicate that tooth morphology may

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not be stable within one species or that hybridization has occurred.

The last example where two species cluster involves *S. iturii* and *S. acanthomias* (Fig. S1, Supporting Information). *Synodontis iturii* is endemic to the Ituri River (affluent of the Aruwimi) in the Congo basin. According to Poll (1971), it can mainly be distinguished from *S. acanthomias*, which is described from Boma (Lower Congo) and occurs throughout the Congo basin, by its smooth humeral process (vs. serrated). As further differences between both species are small, a thorough morphological comparison has been executed at the RMCA (unpublished). This revealed that *S. iturii* and *S. acanthomias* indeed are two different species that can be distinguished based on the humeral process length, head width and depth, dorsal-adipose distance and the number of teeth. Again, their clustering can be explained by incomplete lineage sorting or introgressive hybridization.

Identification problems within certain genera

In addition to the above-discussed cases of undetected taxonomic diversity and species clustering, intrageneric problems were observed within *Clarias*, *Enteromius*, *Labeo* and to a lesser extent in *Petrocephalus*. These problems were already observed during the morphology-based identification of the specimens. Therefore, working names were given when specimens could not be unambiguously assigned to one of the valid species.

In 1986, Teugels made a pan-African revision of the catfish genus *Clarias* (Teugels 1986). Taxonomic difficulties remain though, some of which were addressed by Hanssens (2009) for species of the Lower Congo and Pool Malebo. Also in the current study, several groups of specimens of *Clarias* could not be assigned to a valid species based on their morphology alone. Working names were given to such specimens. In addition, their sequences did not fall within any of the valid species clusters (Fig. S1, Supporting Information), which confirms the difficulty experienced during the identification process; these include *Clarias* sp. 'angolensis sole-shaped', *Clarias* sp. 'pachynema long barbels' and *Clarias* sp. 'pachynema long barbels/pectorals'.

In addition to the taxonomic problems within the genus *Clarias*, the results point to problems on the genus level and indicate that this genus is paraphyletic (Fig. S1, Supporting Information). The family contains an estimated 15 genera and 114 species (Eschmeyer 2014), but delineation of genera and subgenera is rather difficult (Hanssens 2009). This confusion clearly complicates identifications efforts and a revision of certain complexes is necessary. Our molecular results are comparable with those of a study on the phylogeny of African Clariidae that included 32 different species (Agnèse & Teugels 2005). In our NJ tree, two distinct lineages are observed (Fig. S1, Supporting Information). The first lineage (A) is composed of *Clarias gariepinus* and *Heterobranchus* sp. (not identified up to species level as the specimens were not preserved). All other species from the genera *Clarias*, *Clariallabes* and *Channalabes* belong to the second lineage (B). Agnèse & Teugels (2005) had a similar outcome (but with more species in each lineage) and stated that the name *Clarias* should be restricted to the monophyletic group formed by *C. ngamensis*, *C. gariepinus*, *C. anguillaris* and *Bathyclarias* species (our lineage A), and that all other species presently arranged in *Clarias* should be placed in other genera. Our results clearly indicate that rearrangements are necessary and a more thorough molecular study with multiple markers is needed.

The small African barbs also represent numerous taxonomic problems (e.g. Lévêque 1989a,b; Berrebi *et al.* 1996; Roberts 2010; Bamba *et al.* 2011; Banyankimbona *et al.* 2012). Recently, the species referred to as '*Barbus*' following the convention of Berrebi *et al.* (1996), have been revalidated as *Enteromius* (Yang *et al.* 2015). In addition to the genetic diversity found in *E. miolepis* and *E. brazzai* discussed above, many other specimens have been given a working name (Fig. 3). In the genetic tree, we found different taxa that clustered together. For example, cluster *Enteromius* complex 1 consisted of *E.* cf. *urostigma*, *E. stanleyi* and *E.* sp. '10 cps'. On the other hand, supposedly conspecific specimens were found in different parts of the tree (e.g. *E.* cf. *atromaculatus*). A revision of the *Enteromius* species of the Congo basin is clearly needed based on a combined morphological and molecular approach.

Several specimens of the genus *Labeo* belonged to the '*L. forskahlii* group' as defined by Reid (1985) and to the group with plicate lips and concave or falciform fin of Tshibwabwa (1997a,b). However, they were difficult to identify and were mostly given working names. Similar to the situation within *Enteromius*, not all specimens from the same species (*L. lukulae*, *L. parvus*) clustered together in the genetic tree (Fig. 6). These results clearly illustrate that, despite of two revisions of Reid (1985) and Tshibwabwa (1997a,b), taxonomic problems are still abound within the genus, a view shared by Lowenstein *et al.* (2011).

Confirmation of provisional results of morphological studies

DNA barcoding is not only a useful tool to uncover potential taxonomical problems, but can also evoke solutions to pending taxonomic issues in African fish (e.g. Sullivan *et al.* 2000; Lowenstein *et al.* 2011). In the Congo basin, *Bryconaethiops macrops* can only be unambiguously

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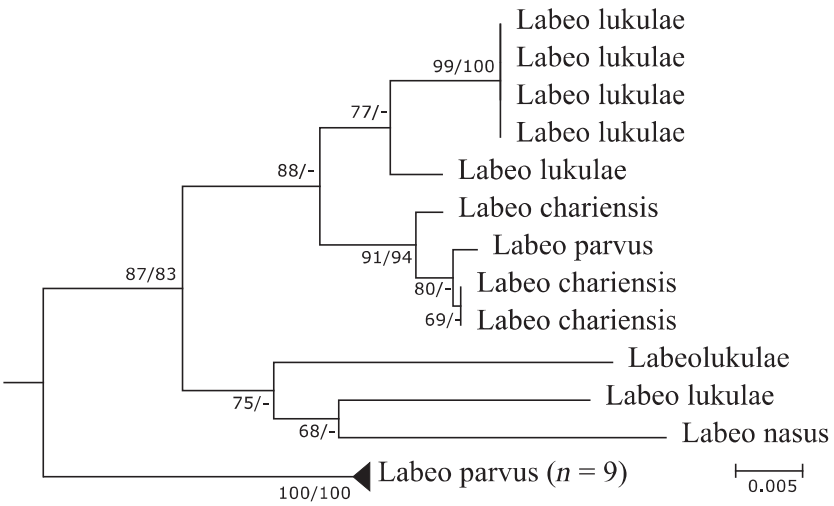


Fig. 6 Phylogram constructed from the mitochondrial COI sequences of the *Labeo 'forskahlii'* complex. Statistical node support is shown as NJ bootstrap/ML bootstrap. Branch lengths indicate the expected number of substitutions per site under NJ inference.

separated from *Bryconaethiops microstoma* by the number of scales around the caudal peduncle, that is 10 vs. 12 (Ibala Zamba *et al.* 2007). Because of their striking overall morphological resemblance, we reluctantly accepted this criterion to separate specimens of both taxa in the present study. Surprisingly, the NJ tree shows a large interspecific divergence (10.5%) between the two taxa, which confirms their validity (Fig. S1, Supporting Information).

Another case of a questionable species delineation concerns *Distichodus* cf. *teugelsi*. The specimens of this taxon are very similar to *D. teugelsi* in terms of their basic colour and the number of scales around the caudal peduncle (16) (Mamonekene & Vreven 2008). However, they had dark spots on the flanks along the longitudinal line, a melanin pattern not found in *D. teugelsi*, but present in *D. decemmaculatus* Pellegrin 1926. The latter species had 20 caudal peduncle scales though. The barcoding results indicate that the specimens of *D.* cf. *teugelsi* indeed appear to be conspecific with *D. teugelsi* (Fig. S1, Supporting Information) and that this species appears to display a much more variable colour pattern than previously reported.

Geographical divergence

The Wagenia rapids in the Kisangani region are traditionally regarded as an ichthyo-geographical barrier between the Upper and Middle Congo (Poll 1963). In the present study, differences were found between COI haplotypes of localities upstream (Upper Congo, Maiko and Loboya) and downstream (Middle Congo, Lomami, Aruwimi, Itimbiri and Lobaye) in some taxa occurring up and downstream of these rapids. For *P. debauwi*, a minimal distance of 5.7% was found between clusters of sequences from up- and downstream the falls, 5.0% for *Schilbe intermedius*, 2.5% for *Bathyaethiops caudomaculatus*, 1.5% for *Mesoborus crocodilus*, 1.0% for *Lamprologus mocquardi*, 0.3% for *Auchenoglanis occidentalis* and 0.2% for *Synodontis congicus*, *S. decorus*, *S. notatus*, *Phago intermedius* and *Campylomormyrus alces*. There are also recurrent patterns of genetic divergence within the Aruwimi basin between samples originating from the Ituri and the Epulu, separated by the Ngoy falls, for seven of 14 species that occur in both systems. This is the case for *Opsaridium ubangiense* (1.6% divergence), *Microctenopoma uelense* (1.1%), *Hypsopanchax* (1.1%), *Raiamas salmolucius* (0.4%), *Myomyrus macrops* (0.2%), *Euchilichthys royauxi* (0.2%) and *Enteromius* cf. *trinotatus* (0.2%). We are aware that the number of samples per location might be insufficient to make strong inferences on geographic divergence within species, as sampling only a few specimens per location could lead to the detection of geographic patterns by chance, or could, on the other hand, leave patterns undetected. Yet, the deep intraspecific divergence found in some species, illustrates that the Wagenia rapids and the Ngoy falls could have acted as a geographical barrier. Moreover, the possible impact of the Ngoy falls is confirmed by the impoverished ichthyofauna of the Epulu that includes only 37 valid species. The level of intraspecific divergence differed strongly between species found up- and downstream the Wagenia rapids. This could either indicate that not one single event was responsible for the isolation of these populations, or that the amount of gene flow is taxon specific.

This study indicates that DNA barcoding has great value as an explorative technique in taxonomy, can reveal cryptic diversity and highlight taxa that need morphological revisions. In four cases, mtDNA of closely related, but morphologically different species was identical, most likely due to introgression through hybridization. If DNA barcoding is applied on collections that have not been identified yet based on morphology,

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species richness could thus be underestimated. The combination of morphological and molecular data will allow a better delimitation of species, and speed up the exploration the Congo basin ichthyodiversity.

The usefulness of the DNA barcoding approach as a reliable species identification method will increase with the number of reliable species for which DNA barcodes become available. Therefore, the DNA barcodes from the poorly known fish fauna from the north-eastern part of the Congo basin generated in our study will contribute to document the online databases and facilitate species identifications.

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T.M., J.S. and E.Vr. sampled the fish; the molecular work was supervised by K.D. and executed by E.D. and T.M; analyses were executed by E.D and T.M. Morphological identifications were principally executed by E.D. and T.M. The study was mainly written by E.D. and T.M. and revised by E.Ve. and J.S. All authors contributed to the design of the study.

Data accessibility

COI sequences: GenBank accessions KT192741 – KT193561

Final DNA sequence assembly: Dryad entry doi:10.5061/dryad.n571 m

Phylogenetic data: Dryad entry doi:10.5061/dryad.n571m

BOLD project DCF, accessions DCF001-15 – DCF821-15

Applied references for morphological identifications: online Supporting Information

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 References used for morphological identification of the specimens.

Fig. S1 Phylogram constructed from the mitochondrial COI sequences.

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Managing Editor
Maurice Kottelat, Rue des Rauraques 6
CH–2800 Delémont, Switzerland
Tel. +41 32 4623175 · Fax +41 32 4622259 · E-mail mkottelat@dplanet.ch

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Brycinus epuluensis, a new species from the Epulu River (Congo basin), Africa (Teleostei: Alestidae)

Eva Decru*, Emmanuel Vreven*, Oumar Sadio and Jos Snoeks***

Brycinus epuluensis, new species, is described from the Epulu River, an affluent of the Ituri River (Aruwimi basin, Middle Congo basin, DR Congo). It most closely resembles the widespread species *B. imberi* from the nurse group, although it has a broad black mid-lateral band on the body, which is absent in *B. imberi*. This black band is, however, also present in specimens from the neighbouring population in the Ituri River itself. *Brycinus epuluensis* differs from the types of *B. imberi* and its five junior synonyms, as well as from the neighbouring Ituri population, mainly in having only 6 instead of 8 premaxillary teeth, arranged in a single row. This low number of premaxillary teeth is characteristic for species of the longipinnis group. However, *B. epuluensis* differs from all members of the longipinnis group by the absence of a fronto-parietal fontanelle in adults and the following combination of characteristics: 18–22 lateral line scales and 12–15 branched anal-fin rays. The Ituri population could not be separated from *B. imberi* and most of its synonyms on morphometrics, but does differ by the presence of the black mid-lateral band.

Brycinus epuluensis, nouvelle espèce, est décrite de la rivière Epulu, un affluent de la rivière Ituri (bassin Aruwimi, bassin du Congo Moyen, RD Congo). Elle ressemble plus à l'espèce largement distribuée qu'est *Brycinus imberi* du groupe nurse, mais présente une large bande noire horizontale mi-latérale alors que chez *B. imberi* cette bande est absente. Toutefois, cette bande noire est aussi présente dans des spécimens de la population voisine de la rivière Ituri. *Brycinus epuluensis* diffère des types de *B. imberi* et de ses cinq synonymes juniors, ainsi que des spécimens de la population voisine de la rivière Ituri, principalement en ayant seulement 6 au lieu de 8 dents prémaxillaires, disposées en une seule rangée. Ce nombre de dents prémaxillaires est typique des espèces du groupe longipinnis. Néanmoins, *B. epuluensis* diffère de tous les membres du groupe longipinnis par l'absence de la fontanelle fronto-pariétale chez les adultes, ainsi que par la combinaison des caractères suivants: 18–22 écailles en ligne latérale et 12–15 rayons branchus à la nageoire anale. La population de la rivière Ituri n'a pas pu être séparée de *B. imberi* et la plupart de ses synonymes par la morphométrie, mais diffère toutefois par la présence de la bande noire mi-latérale.

* Royal Museum for Central Africa, Section Vertebrates, Ichthyology, Leuvensesteenweg 13, B–3080 Tervuren, Belgium; and KU Leuven, Laboratory of Biodiversity and Evolutionary Genomics, Charles Deberiotstraat 32, B–3000 Leuven, Belgium. E-mail: eva.decru@africamuseum.be (corresponding author); jos.snoeks@africamuseum.be; emmanuel.vreven@africamuseum.be

** Institut de Recherche pour le Développement (IRD), B.P. 1386 – CP 18524, Dakar, Sénégal. E-mail: oumar.sadio@ird.fr

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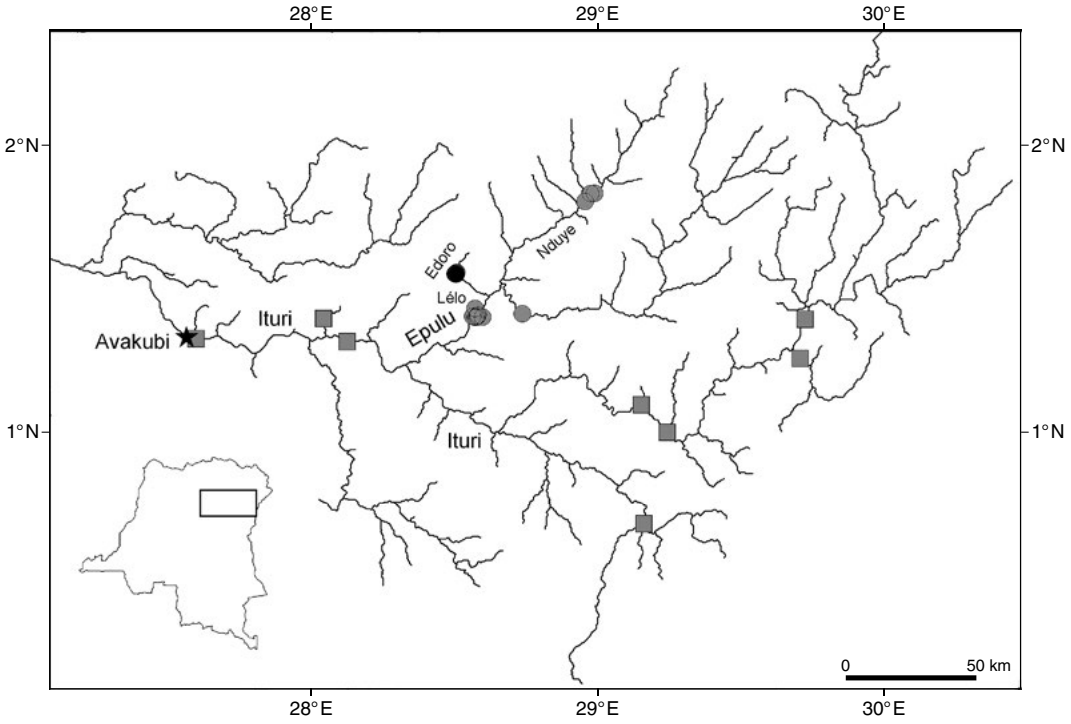


Fig. 1. Distribution of *Brycinus epuluensis*: type locality (●) and other localities (●), indicating also the sampling sites of *B.* cf. *imberi* included in the analyses (■), and the type locality of *B. curtus* and *B. bequaerti* (★).

Introduction

With about 120 species sharing a large range of sizes, shapes and ecological niches, Alestidae is the most speciose of the African characiform families (Paugy & Schaefer, 2007; Arroyave & Stiassny, 2011). Although many morphological (Vari, 1979; Buckup, 1998; Zanata & Vari, 2005) and molecular (Ortì & Meyer, 1998; Murray & Stewart, 2002; Hubert et al., 2005; Calcagnotto et al., 2005) studies proved the family to be monophyletic, a recent study on the phylogenetic relationships of the Alestidae rejected this monophyly (Arroyave & Stiassny, 2011). For pragmatic reasons, the genera of the Alestidae are artificially classified into three groups, mainly based on tooth morphology: the genus *Hydrocynus*, with a single row of caniniform teeth; the tribe Alestiini with more modest, pluricuspid teeth and molariform teeth in the inner row of the premaxilla; and the Petersiini or dwarf Alestidae, with small pluricuspid teeth and non-molariform teeth in the inner premaxillary row (Paugy & Schaefer, 2007). The genus *Brycinus* Valenciennes, 1849 belongs to the Alestiini and is characterized by the presence of a rudimentary adipose eyelid, and by having two rows of pluricuspid teeth on the upper jaw (Paugy, 2003). The genus (together with *Alestes* Müller & Troschel, 1844) was revised by Paugy (1986), who divided the species of *Brycinus* into three groups: the *B. macrolepidotus* group with large-sized species (maximum 530 mm SL) in which the fronto-parietal fontanelle is always lacking; the *B. nurse* group with medium-sized species (maximum 270 mm SL), in which the fronto-parietal fontanelle is present in juveniles but closes in adults; and the *B. longipinnis* group with small-sized species (maximum 140 mm SL), in which the fronto-parietal fontanelle is always present. A detailed study on osteological and morphological characters (Zanata & Vari, 2005) did not confirm the monophyly of *Brycinus* sensu lato as defined by Paugy (1986), and revealed that the *B. longipinnis* group within *Brycinus* is not most closely related to the other members of that genus. As such they propose to allocate members of the *B. longipinnis* group to the genus *Bryconalestes* Hoedeman, 1951. Also according to Hubert et

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Fig. 2. *Brycinus imberi*, SAIAB 97226, 113.5 mm SL; Mozambique: lodge on Zambezi-harbour.

al. (2005), a study combining morphological and mitochondrial characters, the three groups as defined by Paugy (1986) are not monophyletic, and the *B. longipinnis* group should be excluded from the genus, resulting in *Brycinus* sensu stricto including only the *B. macrolepidotus* group and the *B. nurse* group.

While identifying the *Brycinus* specimens collected by one of us (EV) during the 'Okapi Wildlife Reserve' 2009 expedition, the specimens from the Epulu River (Fig. 1) could not be attributed with certainty to one of the presently known *Brycinus* species. When using the identification key of Paugy (1986), the specimens keyed out as *B. imberi* (Peters, 1852) (Fig. 2), a species belonging to the *B. nurse* group. However, the preserved specimens from the Epulu had a broad mid-lateral band, while *B. imberi* lacks this band and only has a humeral and a caudal spot. *Brycinus imberi* is a very widespread species (Paugy, 1986) that occurs in West Africa in the coastal basins of the Gulf of Guinea (Paugy, 2003), in the Congo River Basin, in East Africa in tributaries of the Great Lakes, and in southern Africa in the Middle and Lower Zambezi (Balon, 1971; Bell-Cross, 1976) and Phongolo systems (Skelton, 2001). Five nominal species (all from the Congo basin) were synonymized with *B. imberi* by Paugy (1984): *Alestes bequaerti* Boulenger, 1920 and *Alestes curtus* Boulenger, 1920, from Avakubi on the Ituri River; *Alestes fuchsii* Boulenger, 1899, from Boma, Kutu and Stanley-Pool (now Pool Malebo); *Alestes fuchsii taeniata* Pellegrin, 1903, from the Uélé River; and *Alestes lemairii* Boulenger, 1899, from Lake Mweru. *Brycinus imberi* itself was described from the Zambezi River.

To assess the status of the Epulu population, a morphometric study has been done, including the Epulu specimens and type specimens of *B. imberi* and the above-mentioned nominal species. Equally included were specimens of the neighbouring *B.* cf. *imberi* population from the Ituri River (of which the Epulu is the most important right bank affluent), which share the presence of a black mid-lateral band with the Epulu population.

Material and methods

Methods for counts and measurements are based on Lunkayilakio & Vreven (2008) with the addition of the following: number of scales between the dorsal and adipose fins; number of premaxillary tooth rows; number of pectoral- and pelvic-fin rays; pelvic–adipose fin distance (from anterior point of pelvic-fin base to origin of adipose fin); pectoral–adipose fins distance (from anterior point of pectoral-fin base to origin of adipose fin); body depth at anus, measured just anterior to the anal-fin base and perpendicular to the standard length; 'dorsal-adipose distance 1' (from anterior point of dorsal fin base to origin of adipose fin; while 'dorsal–adipose distance 2' is measured from the posterior edge of the dorsal-fin base); pectoral and pelvic fin length (from insertion point of the first ray to the most distal point of respectively pectoral and pelvic fin); pectoral–pelvic fin distance (from anterior point of pectoral-fin base to anterior point of pelvic-fin base); pectoral–dorsal fin distance (from anterior point of pectoral-fin base to anterior point of dorsal-fin

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base); pelvic–anal distance (from anterior point of pelvic-fin base to anterior point of anal-fin base); snout-occiput distance (from the anterior border of snout to occiput); occiput-dorsal fin distance (from occiput to anterior point of dorsal-fin base); and occiput-adipose fin distance (from occiput to origin of adipose fin).

Fourteen counts and 31 measurements were taken on 53 specimens from the Epulu, 42 from the Ituri and 10 type specimens of *B. imberi* and its five synonyms (see comparative material). Principal Component Analyses (PCA) were used to explore the multivariate data matrix and to reduce the large number of variables into a few meaningful axes (Snoeks, 2004). Univariate comparisons between the two groups were executed with non-parametric Mann-Whitney U (MWU) tests, with Bonferroni corrections according to Rice (1989). Counts with invariable values were omitted from the analyses, i.e. the number of branched and unbranched dorsal-fin rays, the number of unbranched anal-fin rays, the number of scales between dorsal fin and lateral line, and the number of scales between pelvic fin and lateral line. Abbreviations: BMNH, British Museum of Natural History, London; MNHN, Muséum national d'Histoire naturelle, Paris; MRAC, Musée Royal de l'Afrique Centrale; RMCA, Royal Museum for Central Africa, Tervuren; MWU, Mann-Whitney U; PCA, Principal Component Analysis; SL, standard length.

Table 1. Variable loadings for first three principal axes of a principal component analysis carried out on 31 log-transformed measurements for all examined specimens (n = 105); most important measurements for second and third axis in bold; results (p-values) of MWU tests on measurements (in %) between *B. epuluensis* (n = 53) and *B.* cf. *imberi* from the Ituri (n = 42), significant values ($p < 0.05$ after Bonferroni correction) in bold; highly significant values ($p < 0.001$ after Bonferroni correction) in bold and underlined.

log-transformed measurements	PC I	PC II	PC III	MWU
Standard length	−0.169951	−0.105665	−0.004719	0.033816
Head length	−0.150174	0.013572	0.004472	0.040413
Snout length	−0.171541	0.080604	0.173644	0.003600
Eye diameter	−0.121079	0.102223	0.042550	0.183480
Pelvic-adipose distance	−0.197545	−0.049724	−0.037432	**0.000002**
Pectoral-adipose distance	−0.189998	−0.072627	−0.082432	**0.000025**
Body depth at pelvic fins	−0.205518	0.166426	0.019119	**0.000000**
Body depth at anus	−0.208418	**0.283467**	0.111552	**0.000000**
Caudal peduncle depth	−0.192290	0.180794	0.029192	**0.000000**
Caudal peduncle length	−0.139553	**−0.731436**	**0.514903**	**0.000000**
Pre-anal distance	−0.178377	−0.070607	−0.099667	**0.000116**
Pre-pelvic distance	−0.168105	−0.033304	−0.073307	**0.000045**
Pre-pectoral distance	−0.152799	−0.000988	−0.011664	0.100001
Dorsal-adipose distance 1	−0.178635	−0.150162	−0.130952	0.144976
Dorsal-adipose distance 2	−0.176456	−0.188416	−0.213031	0.008439
Dorsal fin base length	−0.189348	−0.035647	0.104911	0.009214
Pectoral fin length	−0.166693	0.035752	−0.008901	**0.000001**
Pectoral-pelvic distance	−0.190664	−0.048731	−0.180336	**0.000820**
Pelvic fin length	−0.164175	0.031350	−0.049554	**0.000055**
Pectoral-dorsal distance	−0.198002	0.091692	−0.020056	**0.000000**
Pectoral-anal distance	−0.198885	−0.118916	−0.161319	0.006599
Pelvic-anal distance	−0.206208	−0.127865	−0.201547	0.056505
Anal fin base length	−0.180867	0.179600	**0.638220**	**0.000077**
Inter-orbital distance	−0.206182	**0.292035**	0.111603	**0.000000**
Snout-occiput distance	−0.137703	0.015018	0.019577	0.985053
Occiput-dorsal distance	−0.210228	0.053000	−0.073559	**0.000000**
Occiput-adipose distance	−0.190009	−0.072998	−0.090466	**0.000028**
Head width	−0.189982	0.122040	0.090569	**0.000000**
Predorsal length	−0.180354	0.011843	−0.039132	**0.000000**
Dorsal fin length	−0.163257	0.076175	−0.054962	**0.000000**
Anal fin length	−0.150900	−0.180366	−0.208362	0.041023

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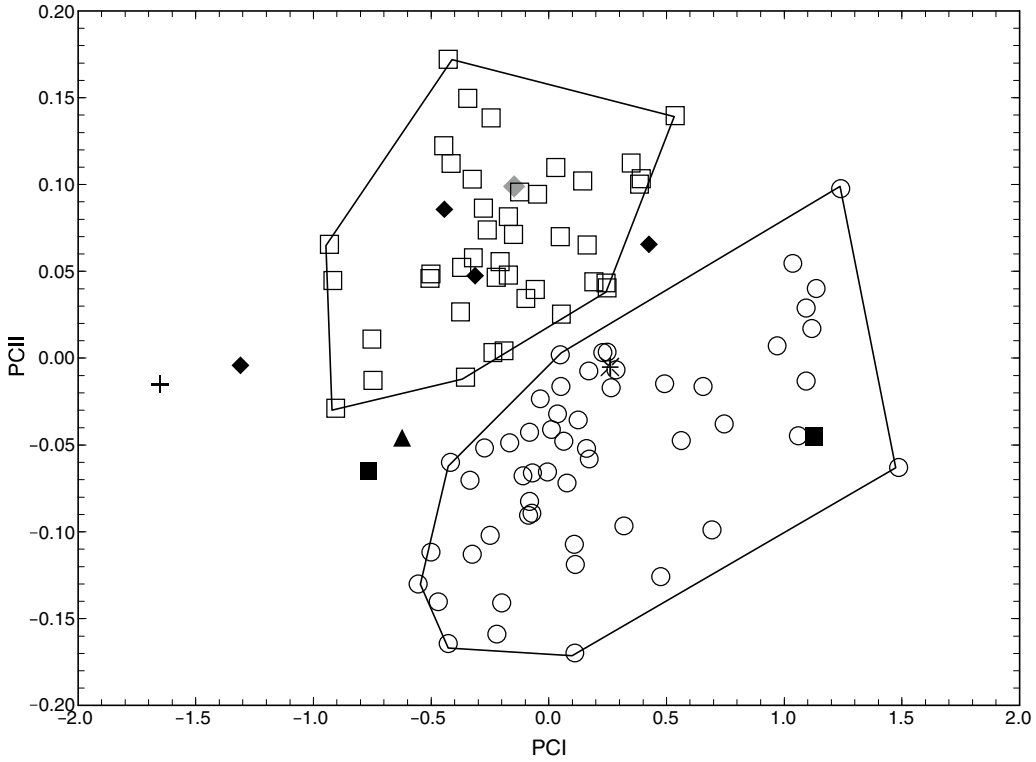


Fig. 3. Scatterplot of PCII against PCI for a principal component analysis on 31 log-transformed measurements for all examined specimens (n = 105) of *Brycinus* species. ✳ holotype of *B. bequaerti*; + holotype of *B. curtus*; ◆ syntypes of *B. fuchsii*; ◆ holotype of *B. fuchsii taeniata*; ▲ syntype of *B. lemairii*; ■ paralectotypes of *B. imberi*; ○ *B. epuluensis*; □ *B.* cf. *imberi* from the Ituri.

Results

A PCA was performed on 9 meristic characters (n = 105) (not shown). On this PCA, the specimens from the Epulu River fall completely apart from the type specimens and the Ituri population (not shown), due to their lower number of lateral line scales (18–22 vs. 21–28), premaxillary teeth (6, exceptionally 7, vs. 8, exceptionally 7), premaxillary tooth rows (1 vs. 1–2), and pectoral-fin rays (12–14 vs. 14–16). The Ituri population, on the other hand, cannot be separated from most of the type specimens. MWU tests between the Epulu and Ituri specimens on the meristic characters (not shown) revealed that 4 out of 9 meristic characters were highly significantly different after Bonferroni correction. These are the number of premaxillary teeth (6, exceptionally 7 in the Epulu population vs. 8 exceptionally 7 in the Ituri population), the number of premaxillary tooth rows (1 vs. 1–2); the number of lateral line scales (18–22 vs. 21–25) and the number of pectoral-fin rays (12–14 vs. 14–16).

A subsequent PCA was performed on 31 log-transformed measurements (n = 105) (Fig. 3). The most important loadings on PC II are for the caudal peduncle length, the interorbital distance and the body depth at anus (Table 1). PC I is a proxy for size, with the smallest specimens located on the positive part of PC I (since the loadings are negative). PC II appears to be allometric, with the Epulu specimens having generally lower values than the other specimens on this axis for similar size classes. The specimens from the Epulu are fully separated from the Ituri specimens, but the holotype of *B. bequaerti* and the smallest paralectotype of *B. imberi* fall within the group of Epulu specimens. The largest paralectotype of *B. imberi* and the syntype of *B. lemairii* are located close to the Epulu specimens. The specimens from the Ituri fall together with most of the type specimens. The MWU tests on the measurements (Table 1) revealed that 19 out of 30 measurements were

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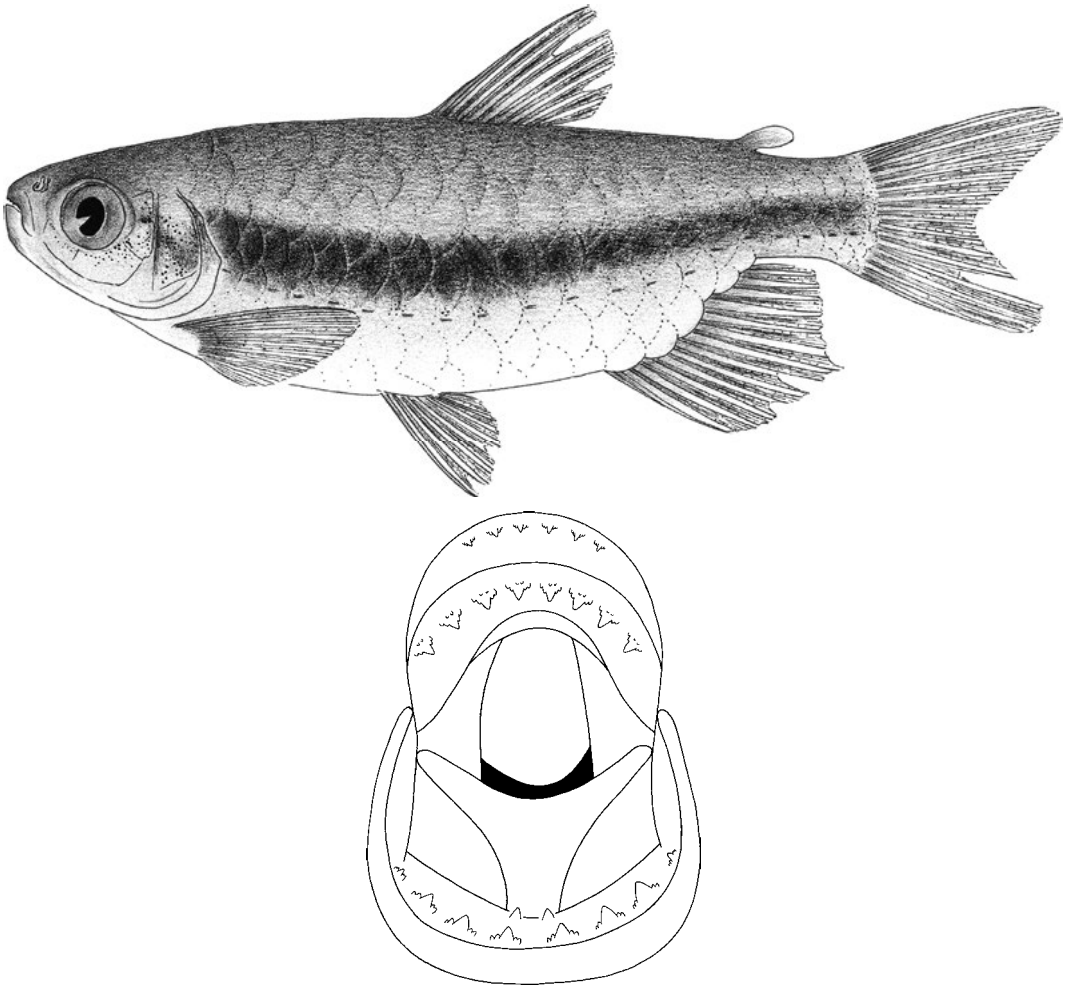


Fig. 4. *Brycinus epuluensis*, MRAC A9-029-P-0612, holotype, 85.1 mm SL; DRC: Edoro River, affluent of Epulu River, Ituri basin; above: lateral view; below: open mouth in lingual view.

significantly different between the Epulu and Ituri populations after Bonferroni correction, 15 of which highly significant. Body depth at anus and interorbital distance revealed to be the most diagnostic characteristics, with specimens from the Epulu generally having lower values for both measurements (respectively 20.9–26.2 % SL vs. 26.0–32.1 and 10–11 % SL vs. 11–13). The smallest paralectotype of *B. imberi* falls within the range of the Epulu specimens for the body depth at anus (22.5 % SL), but has a smaller interorbital distance (9 % SL), while the largest paralectotype falls within their range for interorbital distance (11 % SL). However both specimens were in a very bad shape (orbits broken and body very soft) and measurements could often not be taken accurately. The holotype of *B. bequaerti* also falls within the range of the Epulu specimens for interorbital distance (10 % SL), but can be distinguished based on the body depth at anus (29.9 % SL) and on the meristic characters.

The combination of counts and measurements demonstrates that the specimens from the Epulu are different from the examined types of *B. imberi* and its five junior synonyms, and from the population from the Ituri. As such, the specimens from the Epulu are considered a different species: *B. epuluensis*. A description is given below. While the Ituri population corresponds to *B. imberi* as currently defined, it differs by the presence of

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Fig. 5. *Brycinus epuluensis*, MRAC A9-029-P-0612, holotype, male, 85.1 mm SL; DRC: Edoro River, affluent of Epulu River, Ituri basin.

a mid-lateral black band; therefore it is named *B.* cf. *imberi*.

Brycinus epuluensis, new species
(Figs. 4, 5)

Holotype. MRAC A9-029-P-0612, 1, 85.1 mm SL; DRC: Ituri basin: Edoro River, upstream of the bridge, near the research camp at Edoro-Afarama, affluent of Epulu River, 1°33'01"N 28°30'41"E; Exp. Réserve Okapis, 6 Jul 2009.

Paratypes. MRAC A9-29-P-0613, 1, 83.7 mm SL; same data as holotype. – MRAC A9-029-P-0573–0575, 3, 90.2–95.2 mm SL; DRC: Ituri basin; Epulu River at Epulu, ca. 250 m upstream of the bridge, before the GIC building, 1°24'08"N 28°34'44"E; Exp. Réserve Okapis, 24 Jun 2009.

Additional material. MRAC A9-029-P-0567–0570, 4, 48.1–68.7 mm SL; DRC: Ituri basin: Epulu River at Epulu, right bank, downstream of the bridge, 1°24'01"N 28°34'32"E; Exp. Réserve Okapis, 1 Jun 2009. – MRAC A9-029-P-0571–0572, 0576–0578, 5, 50.8–94.2 mm SL; DRC: Ituri basin: Epulu River at Epulu, right bank, upstream of the bridge, across the chimpanzee island, 1°24'35"N 28°35'07"E; Exp. Réserve Okapis, 4 Jun 2009. – MRAC A9-029-P-0579–0580, 1024–1026, 5, 81.3–90.9 mm SL; DRC: Ituri basin, Lelo River, agricultural area of the Epulu centre, affluent of Epulu River, in primary forest, 1°25'53"N 28°34'27"E; Exp. Réserve Okapis, 29 Jun 2009. – MRAC A9-029-P-0581–0582, 2, 78.8–104.4 mm SL; DRC: Ituri basin, Epulu River at Bandisende, 30 km from the RFO station, 1°24'47"N 28°44'2"E; Exp. Réserve Okapis, 30 Jun 2009. – MRAC A9-029-P-0583–0611, 8, 81.7–105.6 mm SL; DRC: Ituri basin: Edoro River, upstream of the bridge, near the research camp at Edoro-Afarama, affluent of Epulu River, 1°33'01"N 28°30'41"E; Exp. Réserve Okapis, 8 Jul 2009. – MRAC A9-029-P-0614–0615, 2, 100.2–103.8 mm SL; DRC: Ituri basin: Afarama River, affluent of Edoro River, affluent of Epulu River, 1°33'06"N 28°30'17"E; Exp. Réserve Okapis, 12 Jul 2009. – MRAC A9-029-P-0616–0619, -0621, 1028–1031, 9, 54.2–99.8 mm SL; DRC: Ituri basin: Nduye River at Nduye, downstream of bridge, affluent of Epulu River, 1°50'01"N 28°59'31"E; Exp. Réserve Okapis, 23 Jul 2009. – MRAC A9-029-P-0622–0631, 10, 54.8–109.8 mm SL; DRC: Ituri basin, Nduye River at Nduye, upstream of bridge, behind police camp, aflluent of Epulu River, 1°49'56"N 28°58'40"E; Exp. Réserve Okapis, 25 Jul 2009. – MRAC A9-029-P-1021–1023, 3, 75.2–88.3 mm SL; DRC: Ituri basin; Epulu River at Epulu, ca. 250 m upstream of the bridge, before GIC building, 1°24'08"N 28°34'44"E; Exp. Réserve Okapis, 24 Jun 2009.

Diagnosis. *Brycinus epuluensis* is distinguished from all species belonging to the macrolepidotus group by having a short snout (more than 3 times in head length vs. less than 3 times), and fewer premaxillary teeth (6–7, vs. 8–11). It is distinguished from all species belonging to the longipinnis group by the absence of a fronto-parietal fontanelle in adults (larger than ca. 75 mm SL) (vs. fronto-parietal fontanelle always present), fewer lateral line scales (18–22 vs. 25–36), and generally fewer anal-fin rays (iii12–15 vs. iii14–25). It is distinguished from all species of the nurse group by having 6 (exceptionally 7) premaxillary teeth (vs. 8–10, exceptionally 7). The species is most similar to *B. imberi*, from which it is mainly distinguished by fewer premaxillary teeth (6, exceptionally 7 vs. 8, exceptionally 7), fewer lateral line scales (18–22 vs. 21–31), and the presence of a black mid-lateral band.

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Description. Morphometric counts and measurements given in Table 2. Medium-sized species with maximum observed size 109.8 mm SL. Body relatively slender (body depth at pelvic fins 27.9–34.0 % SL, mean 30.9), with greatest depth at vertical through dorsal-fin origin. Dorsal profile straight along head, straight to slightly convex from occipital process to adipose fin, and slightly

Table 2. Morphometric and meristic data for *Brycinus epuluensis* (n = 53).

	holotype	all specimens examined		
		min	max	mean
Standard length (mm)	85.1	48.1	109.8	80.9
In percentage of standard length				
Pelvic-adipose distance	49.9	39.9	51.4	45.6
Pectoral-adipose distance	68.6	60.5	71.3	65.8
Body depth at pelvic fin	32.2	27.9	34.0	30.9
Body depth at anus	23.1	20.9	26.2	24.2
Caudal peduncle depth	10.8	8.8	11.9	10.8
Caudal peduncle length	10.0	7.8	13.2	10.3
Pre-anal distance	76.0	72.4	81.5	76.7
Pre-pelvic distance	51.4	48.6	56.2	52.5
Pre-pectoral distance	27.9	25.9	32.0	28.5
Dorsal-adipose distance 1	40.0	34.4	40.7	38.1
Dorsal-adipose distance 2	27.3	23.0	29.5	26.7
Dorsal fin base length	11.8	9.5	13.2	11.6
Pectoral fin length	20.8	19.9	23.9	21.8
Pectoral-pelvic distance	25.5	21.6	28.5	25.3
Pelvic fin length	17.3	16.4	21.8	18.7
Pectoral-dorsal distance	37.1	30.1	38.8	35.3
Pectoral-anal distance	51.5	45.6	56.9	50.3
Pelvic-anal distance	27.6	22.0	30.3	26.2
Anal fin base length	19.4	13.4	22.7	16.3
Occiput-dorsal distance	32.5	25.8	34.4	31.5
Occiput-adipose distance	70.3	62.5	73.3	68.9
Predorsal length	50.6	45.6	54.1	50.6
Dorsal fin length	23.3	22.7	28.3	24.8
Anal fin length	14.2	12.7	18.9	15.9
Head length	26.8	24.4	29.5	26.9
Snout length	8.3	7.4	9.7	8.5
Eye diameter	9.1	6.4	10.2	8.2
Inter-orbital distance	10.5	9.5	11.0	10.3
Head width	14.5	12.5	15.2	14.1
Snout-occiput distance	20.7	16.6	22.9	19.7
Counts				median
Unbranched dorsal-fin rays	2	2	2	2
Branched dorsal-fin rays	8	8	8	8
Unbranched anal-fin rays	3	3	3	3
Branched anal-fin rays	14	12	15	14
Lateral line scales	20	18	22	20
Scales between dorsal and lateral line	4.5	4.5	4.5	4.5
Scales between lateral line and pelvic	2	2	2	2
Caudal peduncle scales	10	8	10	10
Predorsal scales	9	9	10	10
Scales between dorsal and adipose fin	6	6	8	7
Premaxillary teeth	6	6	7	6
Tooth rows on premaxilla	1	1	2	1
Pectoral-fin rays	13	12	14	13
Pelvic-fin rays	9	8	10	9

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concave between adipose and caudal fins. Ventral body profile convex between isthmus and anal-fin base, straight to convex along anal-fin base, and slightly concave between anal and caudal fins. Caudal peduncle slightly longer than deep. Head narrow (width 13–15 % SL, mean 14.1); fronto-parietal fontanelle absent in adults (closes at ca. 75 mm SL). Snout rounded and relatively short (7–10 % SL, mean 8.5), jaws isognathous, mouth terminal. Orbit relatively large, length larger than snout length.

Upper jaw with two tooth rows. Six, exceptionally seven pluricuspid teeth in outer row of premaxilla, aligned in a single row, with a crenelated appearance and an enlarged median cusp flanked by one or two smaller cusps. Inner row of premaxilla with eight stout teeth, anteriorly with two blunt broad-based cusps, and with a large primary cusp flanked by several secondary cusps on posterior edge. Eight outer row teeth on lower jaw, each with a large central cusp and one to three secondary cusps on either side. Two outermost teeth much smaller than median six. A single pair of small conical teeth on inner row of lower jaw.

Dorsal fin located at midbody, origin of pelvic fin at same level or slightly anterior to dorsal-fin origin. Dorsal-fin margin straight to slightly convex. Caudal fin forked, with rounded lobes of equal size. Adipose fin inserted above posterior border of anal fin.

Sexual dimorphism in anal-fin shape with a concave distal border in males and a straight border in females.

Body covered with well-developed, relatively large cycloid scales. Lateral line completely pored, with a low number of scales (18–22).

Gill rakers long and slender, 10–13 on upper branch, 13–17 on lower of first gill arch; total number of gill rakers 25–30 (counted on 20 specimens). Vertebrae 33–34 excluding Weberian apparatus (counted on 20 specimens based on X-ray photographs).

Colour pattern. Preserved specimens. Dorsal and dorso-lateral parts of head and body olive-brownish, ventral parts of body light brown to yellowish; head yellow to silvery below eye. A broad blackish mid-lateral band, spanning one to two scale rows, from operculum to caudal-fin base. In males, band more pronounced than in females; in small specimens (smaller than ca. 75 mm SL), band vague or even absent. Humeral and caudal spots always present. All fins greyish, except pelvic fin yellowish.

Colour pattern in vivo. Dorsal parts of body olive-yellowish, ventro-lateral and ventral parts grey to silvery. Dorsal, caudal and anal fins olive-yellowish; pectoral and pelvic fins grey to silvery; adipose fin orange. Black mid-lateral band on flank in males, apparently absent in females. Humeral and caudal spots present in both sexes.

Etymology. The Latin adjective *epuluensis* refers to the type locality of the species, the Epulu River.

Distribution. *Brycinus epuluensis* is endemic to the Epulu River and its affluents. The Epulu River is the most important right bank affluent of the Ituri River, from which it is separated by the Arabia waterfall. Within the Epulu basin, *B. epuluensis* is captured in the main stream and its affluents, the Lélo, Edoro and Nduye rivers (Fig. 1).

Discussion

Following the identification key of Paugy (1986), the *Brycinus* specimens from the Epulu keyed out as *B. imberi*. However, the presence of the mid-lateral black band raised some doubts concerning their identification. Multivariate and univariate analyses of counts and measurements demonstrated that the Epulu specimens are different from the types of *B. imberi* and its synonyms, and are therefore considered a new species, *B. epuluensis*. *Brycinus epuluensis* also differs from the specimens from the Ituri River, which have a mid-lateral black band as well. The latter cannot be separated from *B. imberi* based on the morphological analyses and are therefore named *Brycinus* cf. *imberi*.

Species of the genus that are known to have some form of a lateral band are *B. opisthotaenia* (Boulenger, 1903), *B. peringueyi* (Boulenger, 1923) and *B. kingsleyae* (Günther, 1896). In *B. peringueyi*, the black band starts only at the level between the pelvic and anal fins (Paugy, 1986). In *B. opisthotaenia* and *B. kingsleyae* a complete band can be present, but the part anterior to the dorsal fin is always vaguer or even absent (Toham & Teugels, 1997). Besides the shape of the band, *B. epuluensis* is clearly different from these species in, among others, a lower number of premaxillary teeth (6–7 vs. 8), and a lower number of lateral line scales (18–22 vs. respectively 27–32, 29–31 and 26–28).

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A COI barcoding study (Decru et al., 2016) already indicated that the *Brycinus* samples from the Epulu differed from a cluster of *B. imberi* from the Ituri River and the Maiko River (a second level affluent of the Congo basin near Kisangani) by 3 %. Our morphological study, which includes the specimens from the Epulu and Ituri that were sequenced for that barcoding study, indeed confirms that the Epulu population represents a separate species.

Brycinus epuluensis cannot really be attributed to one of the three groups within the genus delineated in the revision of Paugy (1986). Because of its medium adult size and short snout, it corresponds most with the nurse group, to which *B. imberi* also belongs. However, due to the presence of only 6 (exceptionally 7) premaxillary teeth, it does not fit the range (8–10) of this character found in the nurse group. Similarly it does not fit within the macrolepidotus group with 8–12 premaxillary teeth. In fact, the occurrence of only 6 premaxillary teeth is characteristic for the longipinnis group. However, species of this group only reach a small adult size and always have a fronto-parietal fontanelle (also in adults), while the fontanelle is closed in adult specimens of *B. epuluensis*. *Brycinus epuluensis* thus seems to be an exceptional species that cannot be allocated to one of the three groups within the genus, unless their definitions are modified. Problems in the delineation of the groups were already mentioned by Zanata & Vari (2005) and Hubert et al. (2005), who demonstrated that these groups are not monophyletic and that the *B. longipinnis* group should be excluded from the genus.

Only one specimen of *B. epuluensis* had seven premaxillary teeth; three arranged in a single row on the left half, and four arranged in two rows on the right half. Since it was only one specimen from the 53 examined, this configuration is most probably just an aberrancy, meaning that typically, *B. epuluensis* is characterised by 6 premaxillary teeth. The holotype of *B. fuchsii taeniata* (synonym of *B. imberi*), described from the Uélé River, does not have any premaxillary teeth, but there seemed to be also 7 dental cavities on the premaxilla. *Brycinus fuchsii taeniata* also has a broad mid-lateral black band, like *B. epuluensis*. However, based on other characters [e. g. 12–14 pectoral-fin rays vs. 16; a smaller body depth at anus (20.9–26.2 % SL vs. 29.0), and a smaller interorbital distance (10–11 % SL vs. 13)], *B. epuluensis* is clearly different from *B. fuchsii taeniata*.

Up to now, *B. epuluensis* has only been found in the Epulu River. The comparison with the neighbouring population in the Ituri River has shown that the two populations are not conspecific. For meristics, *B. epuluensis* differs mainly from *B.* cf. *imberi* from the Ituri in having 6 (exceptionally 7) premaxillary teeth arranged in a single row (vs. 8, exceptionally 7, arranged in a single or double row), a lower number of lateral line scales (18–22 vs. 21–25), and a lower number of pectoral-fin rays (12–14 vs. 14–16). For measurements, *B. epuluensis* has a smaller body depth at anus [20.9–26.2 (mean 24.2) % SL vs. 26.0–30.9 (mean 28.4)] and smaller interorbital distance [10–11 (mean 10.3) % SL vs. 10–13 (mean 12.1)]. The Epulu is separated from the Ituri by the Arabia waterfall. By forming a barrier to the dispersal of certain fish species, the fall may have isolated the fauna of the Epulu, eventually leading to allopatric speciation. However, up to now, only one other valid species is known to be endemic to the Epulu River, *Labeobarbus macroceps* (Fowler, 1936) (Cyprinidae: Cypriniformes).

It is puzzling that *B. epuluensis* and *B.* cf. *imberi* from the Ituri both display the same melanin pattern, which is sex related. In preserved specimens, males have a well-marked black mid-lateral band, which consists of densely grouped melanine dots, while the band is vaguer in females where the melanine dots are lighter and more sparsely distributed. Smaller specimens (smaller than about 75 mm SL) only have a very vague band, made up by some scattered vague melanin dots on the midlateral scales. The band seems to become better visible after preservation, as in vivo, the band appears to be completely lacking in adult females. Thus, although *B.* cf. *imberi* and *B. epuluensis* can easily be distinguished from each other, they share the same characteristic melanin pattern. Whether this similarity has a geographic basis, as both species occur in the upper parts of the Ituri basin, is difficult to assess at the moment. From preliminary genetical (COI) results (Decru et al., 2016), it is clear that the Ituri and the Epulu populations do not constitute a monophyletic clade. Indeed, specimens from the Ituri clustered in the same lineage as specimens from the Maiko River, which lack a mid-lateral band.

Brycinus imberi is a very widespread species that displays a large amount of intraspecific variation compared to other *Brycinus* species (Paugy, 1986), for example in the number of lateral line scales, which range from 21 to 31. Therefore, many

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populations can be and are indeed allocated in the literature to *B. imberi*, which as currently defined may represent a polyspecific complex rather than a highly variable species. Indeed, the population of the Ituri is for now still called *B.* cf. *imberi*, as no clear morphometric differences with the type specimens were found, except for the melanin pattern. A complete revision of *B. imberi* is planned to assess the intraspecific variation within *B. imberi* as currently defined.

Comparative material. *Brycinus bequaerti*: MRAC 14538, holotype, 77.0 mm SL; DRC: Avakubi.

B. curtus: MRAC 7584, holotype, 163.5 mm SL; DRC: Avakubi.

B. fuchsii: MRAC 796–797, 2 syntypes, 71.3–94.5 mm SL; DRC: Kutu; Delhez, 1899. – MRAC 798, 1 syntype, 99.6 mm SL; DRC: Stanley Pool; Delhez, 1899. – MRAC 799, 1 syntype, 142.5 mm SL; DRC: Boma; Delhez, 1899.

B. fuchsii taeniata: MNHN 1903-029, holotype, 88.6 mm SL; DRC: Uélé River.

B. lemairii: MRAC 221, 1 syntype, 109.8 mm SL; DRC: Pweto: Lake Moero.

B. imberi: BMNH 1861.3.10.1–2, 2 paralectotypes, 57.0–113.5 mm SL; Mozambique: Zambezi River.

B. cf. *imberi*: MRAC A9-029-P-1020, 1, 91.6 mm SL; DRC: Ituri River: Bango village, just upstream of bridge, 1°05'45" N 29°09'19" E; Exp. Réserve Okapis, 10 Jun 2009. – MRAC A9-029-P-1027, 1, 102.0 mm SL; DRC: Angolomu River at Babeke, confluence with Isaye River, affluent of Ituri River, 1°23'52" N 28°02'45" E; Exp. Réserve Okapis, 28 May 2009. – MRAC A9-029-P- 1032–1033, 1043–1058, 10, 59.4–100.6 mm SL; DRC: Ituri River at Avakubi, just downstream of bridge, 1°19'37" N 27°33'08" E; Exp. Réserve Okapis, 10 Jun 2009. – MRAC A9-029-P-1034–1042, 1051–1053, 12, 85.7–122.5 mm SL; DRC: Ituri River, across Penge village, just downstream of Penge rapids, 1°19'03" N 28°07'40" E; Exp. Réserve Okapis, 17 Aug 2009. – MRAC B1-011-P-0201–0207, 7, 65.7–93.2 mm SL; DRC: Ituri basin: Loya River, near bridge on Komanda-Beni road, Loya village, 1°15.89' N 29°41.82' E; Vreven et al., 25 May 2011. – MRAC B1-011-P-0208, 1, 119.5 mm SL; DRC: Ituri basin: Loya River, upstream of bridge on Komanda-Beni road, Loya village, 1°25.48' N 29°42.68' E; Vreven et al., 27 May 2011. – MRAC B1-011-P-0209–0217, 9, 70.1–86.7 mm SL; DRC: Asisa River, left bank affluent of Ituri River, upstream of rapids and bridge, Vonkoutou village, 1°23.68' N 29°43.67' E; Vreven et al., 4 Jun 2011. – MRAC B1-011-P-0218–0221, 4, 89.9–120.2 mm SL; DRC: Ebiena River, left bank affluent of Ituri River, quarry of camp Kisangani III, upstream of Ebiena-Luhulo confluence, 0°40.76' N 29°09.88' E; Vreven et al., 16 Jun 2011. – MRAC B1-011-P-0222, 1, 88.5 mm SL; DRC: Ituri River, Elake village, area of confluence with Elake River, 1°00.27' N 29°14.80' E; Vreven et al., 2 Jul 2011.

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Phytoplankton dynamics in the Congo River

JEAN-PIERRE DESCY*,†, FRANÇOIS DARCHAMBEAU†, THIBAULT LAMBERT†, MAYA P. STOYNEVA-GAERTNER‡, STEVEN BOUILLON§ AND ALBERTO V. BORGES†

*Research Unit in Organismal Biology (URBE), University of Namur, Namur, Belgium

†Unité d'Océanographie Chimique, Université de Liège, Liège, Belgium

‡Department of Botany, University of Sofia St. Kl. Ohridski, Sofia, Bulgaria

§Department of Earth & Environmental Sciences, KU Leuven, Leuven, Belgium

SUMMARY

1. We report a dataset of phytoplankton in the Congo River, acquired along a 1700-km stretch in the mainstem during high water (HW, December 2013) and falling water (FW, June 2014). Samples for phytoplankton analysis were collected in the main river, in tributaries and one lake, and various relevant environmental variables were measured. Phytoplankton biomass and composition were determined by high-performance liquid chromatography analysis of chlorophyll *a* (Chl *a*) and marker pigments and by microscopy. Primary production measurements were made using the ^{13}C incubation technique. In addition, data are also reported from a 19-month regular sampling (bi-monthly) at a fixed station in the mainstem of the upper Congo (at the city of Kisangani).

2. Chl *a* concentrations differed between the two periods studied: in the mainstem, they varied between 0.07 and 1.77 μg L^{-1} in HW conditions and between 1.13 and 7.68 μg L^{-1} in FW conditions. The relative contribution to phytoplankton biomass from tributaries (mostly black waters) and from a few permanent lakes was low, and the main confluences resulted in phytoplankton dilution. Based on marker pigment concentration, green algae (both chlorophytes and streptophytes) dominated in the mainstem in HW, whereas diatoms dominated in FW; cryptophytes and cyanobacteria were more abundant but still relatively low in the FW period, both in the tributaries and in the main channel.

3. Daily integrated production measured in the mainstem (n = 15) varied between 64.3 and 434.1 mg C m^{-2} day^{-1} in FW conditions and between 51.5 and 247.6 mg C m^{-2} day^{-1} in HW. Phytoplankton biomass in the Congo River mainstem was likely constrained by hydrological factors (accumulation due to increased retention time during FW, dilution by increased discharge during HW), even though increased nutrient availability in the FW period might have also stimulated phytoplankton production.

4. In contrast to other tropical river systems where connectivity with the floodplain and the presence of natural lakes and man-made reservoirs play a prominent role in the recruitment of phytoplankton to the main river, our results show that phytoplankton growth in the Congo River can take place in the main channel, with hydrological processes allowing maintenance of phytoplankton biomass even during HW.

Keywords: diatoms, green algae, large tropical river, physical control, potamoplankton

Introduction

Most studies on potamoplankton, i.e. the assemblage of suspended algae in flowing water, have been conducted in temperate lowland rivers, starting at the end of the 19th century with the first comprehensive reports on the presence and often substantial amounts of suspended algae in river waters (review by Welch, 1952). These early reports highlighted the role of physical factors in controlling growth and losses of algae during downstream transport, a view which was largely confirmed afterwards (e.g. Reynolds, 1988; Reynolds & Descy,

Correspondence: Jean-Pierre Descy, Professor emeritus, University of Namur, Belgium. E-mail: jpdescy@unamur.be

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1996). The origin of this plankton was discussed by several authors, but even though riverine phytoplankton always contains a variable contribution of benthic forms brought into suspension (tychoplankton), inocula of true planktonic taxa from tributaries and retention/accumulation zones (so-called hydrological 'dead zones' by Reynolds, Carling & Beven, 1991 and Reynolds, 2006) are needed for the development of cell populations in the main channel. These hydrological dead zones (or retention zones) may be generated by the heterogeneity of flow across a river channel (Reynolds, 1988) or constituted by backwaters with a sufficient hydrological connection with the main channel to provide inocula of cells (Reynolds *et al.*, 1991). In retention zones, phytoplankton photosynthesis may result in net production and growth of phytoplankton, as long as the ratio euphotic depth:river channel depth allows gross primary production to exceed autotrophic respiration losses. Once in the mainstem, cell populations are transported downstream and their survival depends on their capacity to offset advective losses (Reynolds, 1988) and dilution by inputs from the catchment (Descy *et al.*, 1987). Therefore, discharge is a key factor determining transport time and suspended matter load, which influences light penetration in the water column, with mean light exposure depending on channel depth. In other words, it is the physical setting, constrained by hydrology and river morphology, that controls phytoplankton development, and algal populations are submitted to a strong selection by light and temperature: traits such as the ability to achieve net photosynthesis at low light and high specific growth rates are essential for survival and for population increase (Reynolds, 1994). Therefore, in temperate rivers, diatoms, which are well adapted to low temperature and low light, have been generally reported as dominant by biomass in rivers at all times from spring to autumn, with coccal green algae being most species rich and developing whenever conditions allow for greater light availability (Rojo, Alvarez-Cobelas & Arauzo, 1994; Reynolds & Descy, 1996). Besides, evidence for nutrient limitation of phytoplankton growth in rivers has been scarce (Basu & Pick, 1996), even though measures for reducing the nutrient loading to major rivers may have resulted in effective control of phytoplankton biomass (Minaudo *et al.*, 2015).

While it has been stated that 'tropical rivers are poorly understood compared with their temperate counterparts' (Davies, Bunn & Hamilton, 2008), the studies conducted in the tropical and subtropical regions suggest that phytoplankton dynamics in tropical rivers obey the same rules as in temperate rivers given above, even though specific features of tropical rivers further constrain development of algal populations within the river channel. The best studied tropical rivers are South American rivers such as the Amazon (Fisher & Parsley, 1979; Wissmar *et al.*, 1981; Forsberg *et al.*, 1993), but also the Uruguay (O'Farrell & Izaguirre, 2014), Paraná (Bonetto, 1983; Garcia de Emiliani, 1990) and Paraguay (Zalocar De Domitrovic, 2002; Zalocar de Domitrovic, Devercelli & Garcia de Emiliani, 2007; Devercelli *et al.*, 2014). The emerging general view is that, in lowland high-order rivers, phytoplankton production in river channels is 'exceedingly low' (Lewis, Hamilton & Saunders, 1995) due to extreme light limitation, combined in black water rivers with low nutrient concentrations (Wissmar *et al.*, 1981). In fact, *in situ* phytoplankton growth seems extremely low in lowland large South American rivers and appears to be actually imported from adjacent floodplain lakes, where high phytoplankton production occurs (Vasquez & Wilbert, 1992; Melack & Forsberg, 2001). Phytoplankton production in floodplain lakes is fuelled by nutrient input from the rising river waters at the onset of the wet season, when settling of suspended matter allows light penetration into the water column. This stresses the major role of the exchange of nutrients and organic matter between the rivers and the floodplain lakes that takes place during the wet season in most tropical catchments (Junk, Bayley & Sparks, 1989; Neiff, 1996).

Regarding river phytoplankton dynamics, the best studied river in Africa is the Nile, where high phytoplankton biomass and production have been reported (Prowse & Talling, 1958). However, the Nile River is a river–lake system, allowing increased retention times and recruitment from the lakes and relatively high abundance of planktonic species in the main river channel. As such, it behaves very differently from the above-mentioned South American rivers. Sub-Saharan rivers are poorly characterised with regard to phytoplankton dynamics: data have been obtained in small river networks such as rivers in Ivory Coast (Iltis, 1982; Koné *et al.*, 2009; Ouffoue *et al.*, 2013) and the Tana (Tamooh *et al.*, 2012) and only in one large river, the Niger (Imevbore & Visser, 1969; Imevbore, 1970).

Regarding the general taxonomic composition of phytoplankton assemblages, selection by turbulence and light availability, rather than by nutrient availability or grazing, is the key process shaping phytoplankton assemblages in most rivers (Reynolds, 1994). According to the review by Rojo *et al.* (1994), diatoms comprise the majority of species numbers in temperate rivers but are substituted in tropical rivers by desmids and coccal

chlorophytes (when benthic species are not taken into account). In terms of biomass and abundance, diatoms often dominate in tropical rivers, with *Aulacoseira granulata* and other *Aulacoseira* taxa often reported (e.g. Garcia de Emiliani, 1990; Hötzel & Croome, 1996). These diatoms are typical *r* strategists (Reynolds, 1984), able to withstand the variability associated with variations of flow and able to achieve net growth within the time frame imposed by downstream transport and low retention time. Coccal green algae, unicellular and colonial, are also present. They may even be the most important group in terms of abundance and diversity when water transparency and depth allow greater light availability (e.g. in the upper section of the Paraguay River; Zalocar de Domitrovic *et al.* 2014) than in river sections with high turbidity, where low light adaptation is an absolute requirement for growth and survival (Kilham, Kilham & Hecky, 1986). Other taxonomic groups are also reported, but they become significant only in particular conditions, for instance where substantial interactions take place between the river and the floodplain and backwaters, or with reservoirs.

This study presents data from the Congo River, the second most important river in the world in terms of catchment size and freshwater discharge, covering a 1700-km transect from Kisangani to Kinshasa (Fig. 1). The objectives of this article are to examine phytoplankton longitudinal dynamics in the river mainstem during two contrasting hydrographic conditions (high and falling water) and to identify the factors controlling phytoplankton development. We used marker pigment analyses coupled with identification of the dominant algal species by microscopy and primary production measurements. Phytoplankton primary production is a marginal component of overall carbon flows in tropical rivers that are dominated by inputs from land and from wetlands (macrophytes and flooded forest), as shown in the Amazon (Abril *et al.*, 2014). However, phytoplankton provides organic matter with a high nutritional value compared with more refractory and N and P poor organic matter of terrestrial origin. As such, phytoplankton has been shown to be important in sustaining pelagic food webs in tropical rivers including fish (Araújo-Lima *et al.*, 1986; Forsberg *et al.*, 1993; Benedito-Cecilio *et al.*, 2000) that can sustain an important economic activity and a source of animal protein for the local populations.

Methods

Study site and sampling

The Congo River (Fig. 1) is the second largest river in the world in terms of discharge (1457 km^3 $year^{-1}$) and

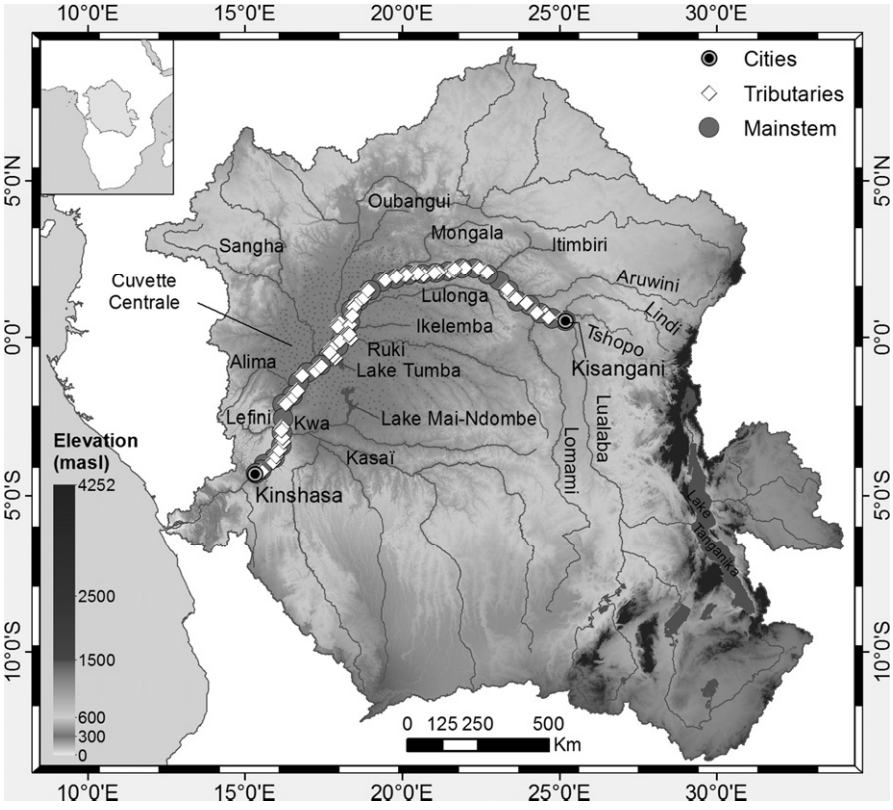


Fig. 1 Location of sampling stations during two cruises (December 2013 and June 2014) in the Congo River at the scale of the whole basin overlain on elevation. Inset: Location of the Congo River basin in Africa. Map was generated with ArcGIS® using publically available spatial datasets.

drainage basin (3.75 million km^2). Flooded areas in the Congo basin largely consist of flooded forest (Bwangoy *et al.*, 2010), and these are mostly permanently flooded unlike the Amazon floodplains that are seasonally flooded. In addition, there are no temporary floodplain lakes but only a handful of relatively large permanent lakes (Lake Mai-Ndombe, 2300 km^2; Lake Tumba, 765 km^2). Floating macrophytes (mainly hippo grass, *Vossia cuspidata*) commonly occur along channel edges and within channels and form large meadows in streams, rivers and the mainstem, in all types of waters (white and black). The water height variations in the Congo mainstem are small (3–4 m), because the Congo basin straddles on the equator: the dry season on the northern part of the basin is compensated by the rainy season on the southern part of the basin, and *vice versa*, leading to a regulation of seasonal water height variations (Runge, 2008).

Two sampling campaigns were carried out, in the high water (HW) period, in December 2013 (75 sites), and in the falling water (FW) period in June 2014 (89 sites). The sampling was carried out in the Congo River mainstem and in tributaries, as well as in the outlet of one of the two main lakes (Lake Tumba). A few additional samples were collected on the lower stretch of the mainstem downstream of the confluence with the Kasai River in May 2015 (FW period). Despite large difference in discharge, the water height in the Congo River mainstem did not differ much between the two main campaigns (Fig. 2 and Table 1). In addition, a temporal survey, with fortnightly sampling of the Congo River, was carried out at Kisangani from November 2012 to May 2014.

Water temperature, conductivity, O_2 and pH were measured *in situ* with a YSI ProPlus CTD calibrated using standard protocols. As cross-section CTD casts at several sites did not show any variation of pH, O_2, temperature and conductivity with depth, vertical homogeneity of the water column was assumed. Secchi depth was measured with a Secchi disk and water column depth was measured with a portable probe. Water was collected just below the surface in 5-L plastic bottles kept in the dark until further processing within 30 min (at most) of collection. Phytoplankton samples were collected with a 28-μm mesh plankton net and preserved in 20-mL plastic bottles with Lugol's iodine.

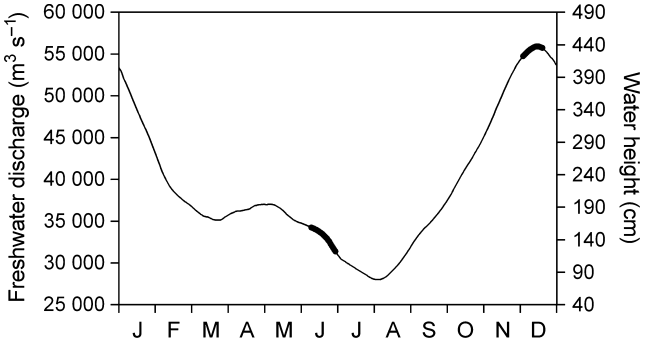


Fig. 2 Average freshwater discharge (Q in m^3 s^{-1}) of the Congo River at Kinshasa and corresponding water height at the gauging station, for the period 2003–13. Timing of the two cruises is indicated by thicker lines.

The vertical light attenuation coefficient (Ke in m^{-1}) was calculated from simultaneous measurements of surface irradiance with a Li-Cor LI-190 Quantum Sensor and underwater PAR measurements with a submersible Li-Cor LI-193SA Spherical Quantum Sensor (Kirk, 1994). The average light to which phytoplankton was exposed was calculated according to Riley (1957) taking into account river depth and vertical light extinction at the site of primary production measurement.

Particulate and dissolved matter analyses

Samples for total suspended matter (TSM) were obtained by filtering a known volume of water on pre-combusted (4 h at 500 °C) and pre-weighed glass fibre filters (47 mm GF/F, 0.7 μm nominal pore size) and air-dried. These were later oven-dried prior to weighing to determine TSM load. Samples for the determination of particulate organic carbon (POC), particulate nitrogen (PN) and particulate phosphorus (PartP) were collected from a known volume of water on pre-combusted 25 mm GF/F filters (0.7 μm nominal pore size). The filtrate from the TSM filtrations was further filtered on 0.2-μm polyethersulfone syringe filters (16532-Q; Sartorius, Goettingen) for dissolved organic carbon (DOC) (40-mL glass vials with polytetrafluoroethylene-coated septa, poisoned with H_3PO_4), coloured dissolved organic matter (CDOM) (20-mL amber glass vials with PTFE-coated septa) and inorganic nutrients (NO_3^-, NH_4^+, dissolved Si and soluble reactive phosphorus – SRP) (20-mL PET vials, frozen at −20 °C).

The 25-mm filters for POC and PN were decarbonated with HCl fumes for 4 h, re-dried and packed in Ag cups. POC and PN were determined on an elemental analyser – isotope ratio mass spectrometer (EA-IRMS; ThermoFinnigan DeltaV Advantage, ThermoFischer Scientific, Waltham) using the thermal conductivity detector signal as described by Bouillon *et al.* (2012). PartP was analysed by spectrophotometric determination of phosphate after digestion with potassium persulfate and boric acid (Valderrama, 1981). DOC was analysed on an Aurora1030 TOC analyser (OI Analytical, College Station) coupled to a Delta V Advantage

Table 1 Average values and range of the variables relevant to phytoplankton ecology, for the mainstem Congo River and tributaries during high water and low water conditions. POC, particulate organic carbon; DOC, dissolved organic carbon; TSM, total suspended matter; SRP, soluble reactive phosphorus; PartP, particulate phosphorus.

	Congo River main stem (34 sites)						Tributaries/lakes					
	High water			Falling water			High water (41 sites)			Falling water (55 sites)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Temperature (°C)	27.4	26.0	28.2	28.0	27.0	29.0	26.1	23.4	27.8	26.1	23.6	29.1
DO (% saturation)	60.6	48.4	89.2	85.7	76.1	93.4	37.6	4.2	111.3	44.5	0.3	103.0
Specific conductivity (μS cm^{-1})	38.2	26.6	48.3	60.3	32.0	79.3	27.5	7.3	55.1	31.8	6.7	87.6
pH	6.49	6.07	6.92	6.84	6.20	7.38	5.48	3.91	6.87	4.89	3.60	7.05
Channel depth (m)	10.2	3.3	38.6	8.7	2.0	37.3	4.9	1.0	10.8	4.1	0.5	18.9
Secchi depth (cm)	52	3	80	47	35	62	82	15	250	73	1	205
Light attenuation coefficient (m^{-1})	1.54	1.06	2.83	3.93	2.60	4.65	1.52	0.44	2.79	3.35	1.48	5.16
Euphotic depth (Zeu) (m)	3.13	1.63	4.35	1.19	0.99	1.77	3.43	1.65	10.36	1.48	0.89	3.11
NH_4^+ (μM)	2.27	0.58	6.45	1.33	0.53	7.09	2.82	0.65	5.68	2.10	0.38	20.01
NO_3^- (μM)	11.31	0.00	19.60	36.68	28.33	47.08	4.02	0.00	16.29	15.96	0.00	89.94
Si (μM)	195.7	169.2	248.8	218.5	186.8	255.8	162.4	61.1	283.0	157.6	25.2	295.6
SRP (μM)	0.36	0.11	1.00	2.38	0.67	4.42	0.31	0.05	0.94	0.73	0.10	1.73
TSM (mg L^{-1})	29.8	14.0	99.7	31.9	4.0	45.4	10.0	0.7	71.4	8.4	0.5	43.0
POC (mg L^{-1})	1.81	0.93	4.58	2.26	1.16	3.46	1.04	0.30	3.56	1.11	0.23	3.67
DOC (mg L^{-1})	8.0	5.4	13.9	5.9	4.2	9.7	15.9	1.8	47.8	17.9	1.8	67.8
a_{350} (m^{-1})	29.18	19.67	45.39	23.15	16.08	45.32	60.5	3.6	180.5	69.2	6.5	249.4
PartP (μM)	1.46	0.86	3.01	2.21	0.37	3.47	0.95	0.42	2.92	1.09	0.08	2.81
POC/PN (molar ratio)	12.8	10.9	15.5	12.9	11.6	14.7	13.0	8.8	16.4	13.5	8.4	20.9
POC/PartP (molar ratio)	100.8	73.5	149.4	77.7	55.7	117.7	92	41	186	132	10	767
Chlorophyll *a* (μg L^{-1})	0.84	0.10	1.77	3.98	1.13	7.68	0.44	0.00	3.58	0.85	0.01	6.39
Green algae (μg Chl *a* L^{-1})	0.75	0.10	1.58	0.97	0.28	1.68	0.38	0.00	3.14	0.22	0.00	1.24
Cryptophytes (μg Chl *a* L^{-1})	0.01	0.00	0.06	0.31	0.08	0.64	0.01	0.00	0.20	0.10	0.00	0.67
Cyanobacteria (μg Chl *a* L^{-1})	0.00	0.00	0.00	0.34	0.00	1.15	0.00	0.00	0.06	0.01	0.00	0.20
Diatoms (μg Chl *a* L^{-1})	0.09	0.00	0.22	2.38	0.67	4.42	0.04	0.00	0.39	0.71	0.00	4.90
Mean light in the water column (μE m^{-2} s^{-1})	71	12	195	35	5	108	–	–	–	–	–	–
Phytoplankton production (mg C m^{-2} day^{-1})	109	51	248	229	64	434	285	35	581	125	3	262

IRMS, with a precision better than ±5%. Absorbance was recorded on a UV/Vis 650S spectrophotometer (Perkin-Elmer, Waltham, MA) using a 1-cm quartz cuvette. Absorbance spectra were used to report CDOM as the absorption coefficient at 350 nm (a_{350}). Details on data acquisition and corrections can be found in Lambert *et al.* (2015). NO_3^- and NH_4^+ concentrations were estimated by spectrophotometry, using the dichloroisocyanurate-salicylate-nitroprussiate colorimetric method for NH_4^+ (Westwood, 1981), the sulfanilamide colorimetric after vanadium reduction method for NO_3^- (American Public Health Association, 1998). SRP was determined by spectrophotometry using the ammonium molybdate-potassium antimonyl tartrate method (American Public Health Association, 1998). Si was measured with inductively coupled plasma mass spectrometry (Agilent 7700x, Santa Clara). The detection limits were 0.30, 0.15, 0.03 and 8.0 μmol L^{-1} for NH_4^+, NO_3^-, SRP and Si, respectively.

Phytoplankton analyses

Pigments were analysed by high-performance liquid chromatography (HPLC) after acetone extraction (90%) of the material filtered on Macherey–Nagel 47 mm diameter GF-5 filters. Filters were frozen (−20 °C), and within a few days, the extractions were carried out in 5-mL acetone (90%) and a 1-mL extracts subsamples were kept in a portable deep-freezer until transport to Belgium. Chl *a* and marker pigments were determined according to Descy *et al.* (2005), using a HPLC system (Waters, Milton) equipped with a Waters 996 PDA detector and a Water 470 fluorescence detector. The contribution of phytoplankton classes was calculated using CHEMTAX (Mackey *et al.*, 1996), with input ratio matrices defined from microscopic examination of net samples (see below), allowing to verify the presence of diatoms, green algae (chlorophytes and streptophytes), cyanobacteria, cryptophytes, chrysophytes, dinoflag-

ellates and euglenophytes. Owing to the low concentrations of pigment in the HW samples, only a few markers were used for assessing the biomass of the main phytoplankton classes: fucoxanthin (diatoms and chrysophytes), lutein and chlorophyll *b* (green algae), alloxanthin (cryptophytes) and zeaxanthin (cyanobacteria and green algae). For the FW samples, more marker pigments were used, including neoxanthin and violaxanthin (green algae), myxoxanthophyll (cyanobacteria type 2; Wright & Jeffrey, 2006), diadinoxanthin and diatoxanthin (diatoms), and chlorophyll *c*1–*c*2 (diatoms and cryptophytes).

Phytoplankton samples were examined at the microscope (Leitz Laborlux S, Leitz, Oberkochen and Motic BA 400, Motic, Wetzlar) for identification to species level whenever possible. We are aware that these samples may be biased towards the largest phytoplankton taxa, which were retained by the net. Therefore, the microscope data were used here for assessing taxa frequency of occurrence, and for all quantitative phytoplankton assessments, we used biomass estimates at the class level derived from processing the marker pigments concentrations with CHEMTAX.

Primary production

The primary production rate was determined from photosynthesis-irradiance incubations using $H^{13}CO_3^-$ as a tracer for incorporation of dissolved inorganic carbon (DIC) into biomass. A solution was prepared with 500 mL of surface water spiked with $NaH^{13}CO_3$. Subsamples of the spiked solution were preserved in triplicate in 12 mL Exetainer vials (Labco, Lampeter) and poisoned with a saturated solution of $HgCl_2$, for the determination of the δ^{13}C-DIC value. Eight 50-mL polycarbonate flasks were filled with the spiked solution and were placed into a floating incubator providing a range from 0 to 90% of natural light energy and incubated *in situ* around mid-day for *c.* 2 h just below the surface. Incident light was monitored by a LI-190SB quantum sensor (Li-Cor, Lincoln) during daytime (5-min interval acquisition) for the entire duration of the cruises. The incubation was stopped by adding neutral formaldehyde and water was filtered on pre-combusted Macherey–Nägel GF5 filters (25 mm diameter). Filters were rinsed with HCl 0.1 N and preserved dry. For the analysis of δ^{13}C-DIC, a 2-mL helium (He) headspace was created, and H_3PO_4 was added to convert all DIC species to CO_2. After overnight equilibration, part of the headspace was injected into the He stream on the above-mentioned EA-IRMS. Given the high ^{13}C-enrichments, the obtained δ^{13}C data were not corrected for isotopic equilibration between gaseous and dissolved CO_2. The δ^{13}C-POC was determined on the above-mentioned EA-IRMS using an internally calibrated acetanilide and sucrose (IAEA-C6) to calibrate the δ^{13}C-POC (for the natural abundance data).

The specific photosynthetic rate in an individual bottle *i*, P_i (in μg C L^{-1} h^{-1}) was calculated following Dauchez, Legendre & Fortier (1995) based on the initial and final δ^{13}C-POC values and δ^{13}C-DIC of the spiked incubated solution, and considering that isotopic discrimination is negligible (Legendre & Gosselin, 1996), at most 2.5% according to Hama *et al.* (1983). For each experiment, the maximum specific photosynthetic rate P_m (in μg C L^{-1} h^{-1}) and the irradiance at the onset of light saturation I_k (μE m^{-2} s^{-1}) were determined by fitting P_i to the irradiance gradient provided by the incubator I_i (μE m^{-2} s^{-1}), using the following equation:

$$P_i = 2P_m \left[\frac{I_i/2I_k}{1 + (I_i/2I_k)^2} \right] \quad (1)$$

which is the Vollenweider's equation with $a = 1$ and $n = 1$, allowing for photoinhibition (Vollenweider, 1965), with P_i the photosynthetic rate in bottle *i* during the incubation, and with I_i the corresponding mean light during the incubation. Fitting was performed using the Gauss–Newton algorithm for nonlinear least squares regression with TATISTICA software (StaSoft, Tulsa).

Daily depth-integrated primary production (mg C m^{-2} day^{-1}) was calculated according to Kirk (1994) using the following equation:

$$P(z,t) = 2P_m \left[\frac{I(z,t)/2I_k}{1 + (I(z,t)/2I_k)^2} \right] \quad (2)$$

where $P(z, t)$ is the photosynthesis at depth z and time t, and $I(z, t)$ is the underwater light determined from Ke and surface irradiance recorded every 5 min, and assuming a vertically homogenous Chl *a* profile. Assuming that short-term incubations provide an estimate which is close to gross primary production (GPP), we calculated water column daily respiration (R, mg C m^{-2} day^{-1}) as in Reynolds (2006), considering a respiration rate of 0.16 mg C mg Chl *a* h^{-1} at 18 °C (based on Lopez-Sandoval *et al.*, 2014), a Q_{10} of 2 for adjusting for river temperature, a constant respiration rate over 24 h, and the whole river depth at the study sites. Subtracting R from GPP provided net primary production estimates, which in turn provided estimates of phytoplankton growth rate. Community growth rate was calculated using a C:Chl *a* ratio of 40, according to

Harris (1986). This is an estimate of the maximal growth rate, not taking into account mortality, sedimentation and grazing losses, nor possible nutrient limitation.

Statistical and multivariate analyses

A principal component analysis (PCA) was performed on scaled variable using the *prcomp* function in the R software (R Development Core Team, 2008). Physical and chemical data from the mainstem were used as the variables for the PCA, with the objective of determining their correlation to chlorophyll *a* and to explore the biogeochemical and ecological gradients during the two main sampling series. The following variables (see Table 1 for acronyms) were included: Rkm (River km, or distance downstream of Kisangani), Secchi depth, total suspended solids, POC, PN, PartP, a_{350}, SRP, nitrate, ammonia, dissolved oxygen, conductivity and pH. A similar analysis was run for all sites, including the tributaries. Given the different units of the variables used in the PCA, data were scaled to zero-mean and unit-variance as recommended (Borcard, Gillet & Legendre, 2011).

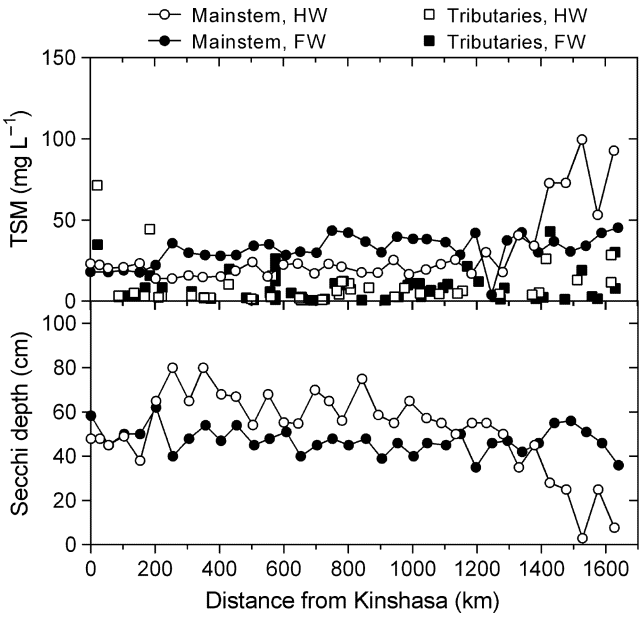


Fig. 3 Longitudinal variation of total suspended matter (TSM, mg L^{-1}) and of Secchi depth (cm) in the Congo River mainstem and in the tributaries during the sampling campaigns of December 2013 (high water, HW) and June 2014 (falling water, FW).

Results

The data are summarised in Table 1, presenting the mean and the range of the environmental data, of phytoplankton biomass and production, in the sampled tributaries and lakes, and in 34 sites along the mainstem, under both hydrological conditions (HW; FW).

Physical and chemical data

Below we focus on the variables which could influence phytoplankton development in the Congo River mainstem. The Congo River main channel is relatively shallow and its depth varies little between FW and HW (Fig. 2 and Table 1): in most sites, the depth did not exceed 10 m and was often lower, except in the lower part (between the mouth of the Kasai and the entrance of the Malebo pool), where the river channel is narrower and deeper (22–37 m). TSM concentrations (Fig. 3) decreased longitudinally and were higher in FW conditions, resulting in lower Secchi depth (Fig. 3) and depth of the euphotic zone (Zeu), compared to HW conditions (mean Zeu 3.1 m in HW versus 1.2 m in FW). Generally, TSM was lower in the tributaries than in the mainstem, with the exception of the Kasai River (confluence 30-km upstream of Kinshasa), and no distinct seasonal variation in the TSM of tributaries was apparent. SRP and DIN concentrations were higher in the mainstem at FW than at HW (Fig. 4). This pattern was also observed in the tributaries. The POC:PN and POC:PartP ratios (Table 1) did not vary much among sites in both hydrological conditions: the molar POC:PN averaged 12.8 (FW) and 12.9 (HW), and the mean molar POC:PartP was lower in FW (78) than in HW (101). Extremely low pH values (<4) and O_2 concentrations close to zero were measured in some of the small and slow-moving tributaries draining the Cuvette Centrale (Table 1), in accordance with extremely high DOC content, and CO_2 and CH_4 concentrations (Borges *et al.*, 2015a,b; Lambert *et al.*, 2016).

Phytoplankton abundance and composition

Chlorophyll *a* concentrations differed between the two periods studied: in the mainstem, they varied between 0.07 and 1.77 μg L^{-1} in HW conditions and between 1.13 and 7.68 μg L^{-1} in FW conditions (Table 1). The range in the tributaries was even larger: 0–3.58 μg L^{-1} in the HW period, with maxima in the Oubangui River (3.58 μg L^{-1}), in the outlet of Lake Tumba (1.85 μg L^{-1}) and in the Lefini River (1.71 μg L^{-1}) downstream of the Imboulou dam. In the FW period, Chl *a* in the tributaries varied between 0.02 and 6.39 μg L^{-1}, with maxima recorded in the Oubangui River (6.39 μg L^{-1}) and lake

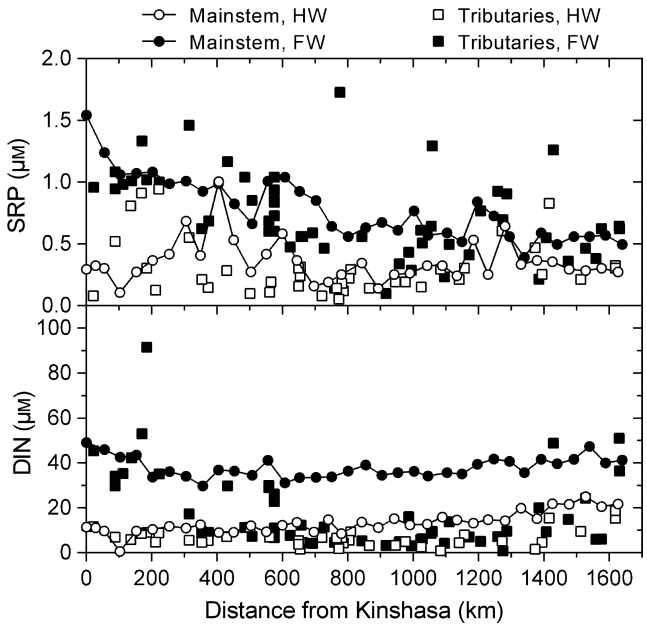


Fig. 4 Longitudinal variation of soluble reactive phosphorus (SRP, µM) and of inorganic nitrogen (DIN, µM) in the Congo River mainstem and in the tributaries during the sampling campaigns of December 2013 (high water, HW) and June 2014 (falling water, FW).

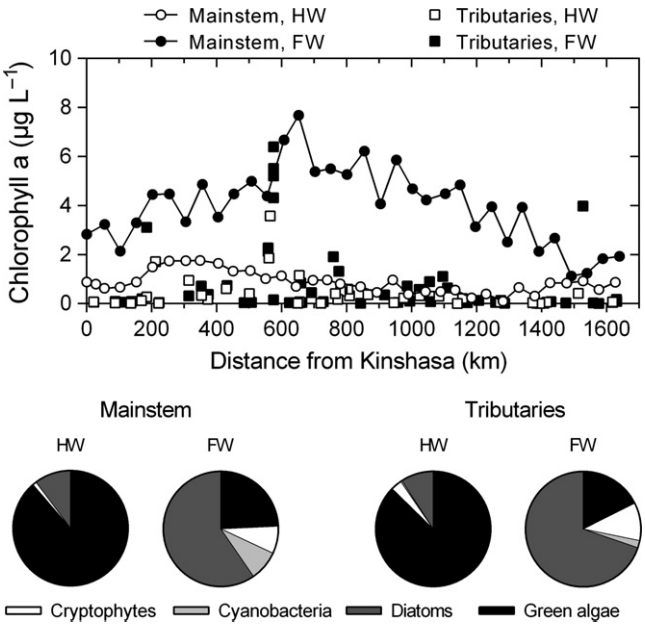


Fig. 5 Longitudinal variation of chlorophyll *a* (µg L^{-1}) in the Congo River mainstem and in the tributaries during the sampling campaigns of December 2013 (high water, HW) and June 2014 (falling water, FW). The average relative contribution to chlorophyll *a* of the main phytoplankton groups is shown below.

Tumba (2.28 µg L^{-1}), but with higher concentrations than in HW in many tributaries, suggesting that phytoplankton development occurred throughout the Congo catchment. Substantial amounts of Chl *a* degradation products (chlorophyllide a, phaeophytins a and phaeophorbides) were detected in most samples (on average 23% of total phorbins-a at FW), indicating the transport of dead and senescent cells among living, healthy cells and possibly of plant detritus. The longitudinal variation of Chl *a* and the contribution of the main phytoplankton groups are shown in Fig. 5. In the mainstem, Chl *a* exhibited a hump-shaped pattern, with a maximum between 200 and 600 km from Kinshasa in the HW period, and a maximum around 700 km from Kinshasa in the FW period. Chl *a* in most tributaries (mostly black waters) was generally lower than in the mainstem, with few exceptions (e.g. Oubangui River and outlet of Lake Tumba in the HW period).

The difference in phytoplankton composition in the two periods is striking: both pigment data and microscopic examination of samples showed that green algae dominated in the mainstem in HW, whereas diatoms dominated in FW (Fig. 6). Green algae dominance in HW was also observed in the tributaries, where they represented on average 88% of Chl *a* (Table 1); a sample taken in a *Vossia* sp. patch in HW contained only green algae, with 98% of contribution to total Chl *a*. Cryptophytes and cyanobacteria were more abundant in the FW period, both in the tributaries and in the main channel. Overall, taxonomic diversity was greater in the FW period. The most frequent phytoplankton taxa in the Congo mainstem were *Aphanocapsa* spp., *Aphanothece* spp., *Limnococcus limneticus*, *Planktolyngbya* spp. (cyanobacteria/cyanoprokaryotes), *Actinastrum rhaphidioides*, *Coelastrum* spp., *Coenochloris* spp., *Desmodesmus magnus*, *D. perforatus*, *Scenedesmus communis*, *S. oahuensis*, *Planktosphaeria gelatinosa* (chlorophytes), *Closterium* spp., *Hyalotheca* cf. *mucosa* (streptophytes), *Euglena* spp., *Strombomonas* spp. (euglenophytes), *Salpingoeca* spp. (chrysophytes), *A. granulata* (including var. *angustissima*), *A. agassizii*, *Cyclostephanos invisitatus*, *Cyclotella* spp., *Discotella pseudostelligera*, *Staurosirella* spp. and *Thalassiosira rudolfii* (diatoms).

The PCA (Fig. 7) performed on the physical and chemical variables and Chl *a* from the main channel (70.9% of cumulated variance associated with the two first principal components) showed a clear discrimination between the sampling series (sites from the FW campaign in the upper part of the diagram), resulting from higher inorganic nutrient concentrations (except NH_4^+), dissolved oxygen, pH, particulate phosphorus and Chl *a* in the FW conditions, and from higher a_{350} in

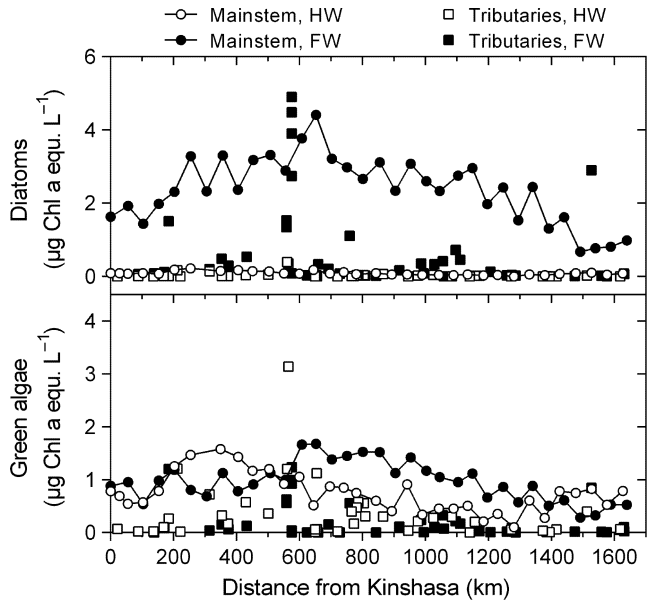


Fig. 6 Longitudinal variation of diatoms and green algae (expressed as chlorophyll *a*, µg L^{-1}) in the Congo River mainstem and in the tributaries during the sampling campaigns of December 2013 (high water, HW) and June 2014 (falling water, FW).

HW conditions. Within each sampling series, the sites are distributed along the first axis of the PCA, which represents a longitudinal gradient, well-marked for the HW series, depending mainly on TSM, POC, PN and Secchi depth. The PCA (Figure S1) performed on all sites, including the tributaries, essentially discriminated the tributaries from the mainstem, based on higher DOM concentration (measured by a_{350} and DOC) and lower TSM, pH, POC and PN.

The temporal survey at Kisangani provided additional data, allowing to observe a strong variation of Chl *a* (Fig. 8) over time. Phytoplankton biomass was low (0.49–1.43 µg Chl *a* L^{-1}) during the HW period and increased up to 5.72 µg Chl *a* L^{-1} when discharge and TSM decreased.

Phytoplankton photosynthesis and production

The average light to which phytoplankton was exposed in the mainstem varied between 5 and 108 µE m^{-2} s^{-1} in FW and between 12 and 195 µE m^{-2} s^{-1} in HW (Table 1). Phytoplankton photosynthesis parameters were similar in the two sampling series: the mean maximal photosynthetic rate was 10 µg C L^{-1} h^{-1} in HW and 11.5 µg C L^{-1} h^{-1} in FW, with mean Ik (light at the onset of saturation of photosynthesis) of 311 µE m^{-2} s^{-1}. Daily integrated GPP measured in the mainstem varied between 64.3 and 434.1 mg C m^{-2} day^{-1} in FW conditions and between 51.5 and 247.6 mg C m^{-2} day^{-1} in HW. Phytoplankton production was close to that of the mainstem in the main tributaries, i.e. in the rivers Tshopo (FW: 261.6 mg C m^{-2} day^{-1}), Lomami (FW: 245.6 mg C m^{-2} day^{-1}), Oubangui (HW: 417.6 mg C m^{-2} day^{-1}; FW: 236 mg C m^{-2} day^{-1}) and Kasai (HW: 34.7 mg C m^{-2} day^{-1}; FW: 168 mg C m^{-2} day^{-1}). In both sampling series, primary production increased regularly in the Congo River (Fig. 9) between 1200 and 800 km (from Kinshasa) and dropped around the confluence with the river Oubangui, at *c.* 500 km. This was related to a decrease of Chl *a* (Fig. 5) likely resulting from dilution and TSM input by this tributary, despite that it carried significant phytoplankton biomass during FW. During HW, the decrease of Chl *a* occurred further downstream after the confluence of the Kasai River that brought high TSM to the mainstem.

Net primary production estimated for the Congo River sites ($n = 30$) varied between −442.6 and 148.4 mg C m^{-2} day^{-1} in FW conditions and between −64.7 and 204.0 mg C m^{-2} day^{-1} in HW. Community

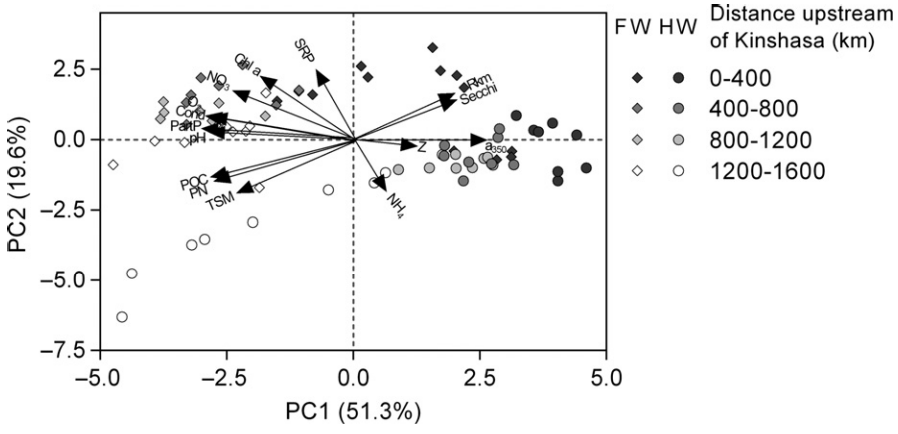


Fig. 7 Results of a principal component analysis carried out on the physical and chemical data in the Congo River mainstem, from the sampling campaigns of December 2013 (high water, HW) and June 2014 (falling water, FW). The dots are the sampling sites; their shading depends on the distance from Kinshasa. See Table 1 for acronyms.

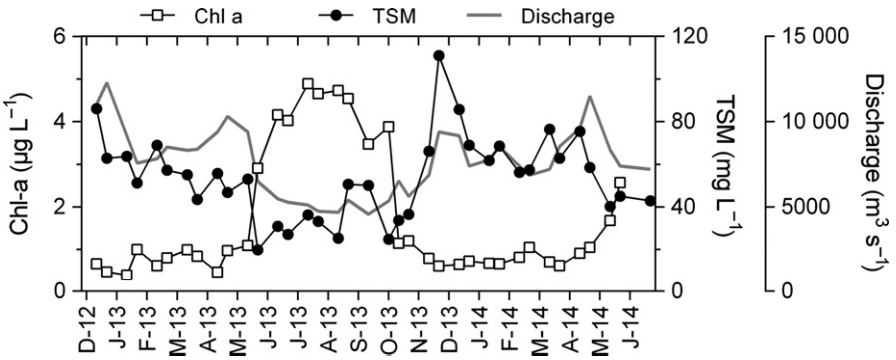


Fig. 8 Variation of discharge (m^3 s^{-1}), total suspended matter (TSM, mg L^{-1}) and chlorophyll *a* (µg L^{-1}) in the Congo River at Kisangani between December 2012 and June 2014.

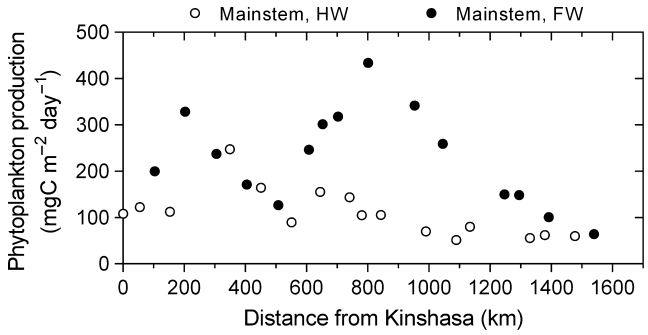


Fig. 9 Longitudinal variation of phytoplankton production (mg C m^{-2} day^{-1}) in the Congo River mainstem during the sampling campaigns of December 2013 (high water, HW) and June 2014 (falling water, FW).

growth rates were comprised between −0.13 and 0.43 day^{-1} in FW and −0.07 and 0.63 day^{-1} in HW.

Discussion

The results of this study provide evidence of significant phytoplankton production in the main channel in the second largest river of the world, the Congo River. Phytoplankton biomass developed mainly in the period of low water and FW, when the lower discharge (resulting in higher residence time and lower dilution by tributaries) and the lower TSM load allowed sufficient light penetration for substantial primary production to occur. Chl *a* reached a maximal concentration (up to *c.* 8 µg L^{-1}) after a gradual increase along a *c.* 1000-km transect in the FW period, resulting from net phytoplankton increase, most probably allowed by lower dilution by tributaries than in the HW period. In the Congo River, phytoplankton biomass may be controlled by dilution rather than by residence time. Indeed, given the relatively high production rates of the phytoplankton, the river is long enough to allow sufficient residence time for biomass increase, irrespective of the discharge: based on a simple calculation, the water residence time from Kisangani to Kinshasa was *c.* 25 days in HW, and it did not increase much (*c.* 30 days) in FW. By contrast, the discharge of all tributaries, estimated from the few data available, was about twice as high in HW than in FW, and the main tributaries contributed a significant proportion of the main river flow. For instance, it could be estimated that the Kasai River, the Oubangui River and the Ruki River contribute, respectively, to 21, 8 and 12% of the discharge recorded in Kinshasa, whereas the river network located upstream of Kisangani contributes to 20% of the total river flow. The temporal survey at Kisangani provided further evidence that phytoplankton development was controlled by hydrological factors and turbidity.

In the most downstream section of the Congo River, phytoplankton biomass declined, as a result from combined effects of dilution, TSM inputs by major tributaries (Oubangui and Kasai rivers) and greater channel depth. The region after the confluence of the Kasai also corresponds to a narrowing and deepening of the Congo upstream of Pool Malebo (Runge, 2008; O'Loughlin *et al.*, 2013). Schematically, the longitudinal profile of phytoplankton biomass in the Congo River is, therefore, similar to that described in the early publications on phytoplankton in large temperate rivers (e.g. Welch, 1952): an absence or small amount of true plankton in an upstream section, followed by a downstream increase up to a maximum in a middle section, and decline in the lower section. This similarity clearly stems from the same controlling factor, i.e. hydrology, influencing residence time, dilution during downstream transport and suspended matter loading, which determines the availability of light.

Our ^{13}C uptake measurements and estimates of daily primary production provide evidence for significant phytoplankton production in the mainstem of the Congo River, particularly in shallow sections of the river between 400 and 1400 km from Kinshasa, where organic C losses from respiration were reduced, allowing positive net production to take place. The substantial

phytoplankton production occurring in the main channel may be a major difference between the mainstem of the Congo River and that of most other large tropical rivers. The general pattern in the rivers of South America (e.g. Amazon, Orinoco, Paraná) is that phytoplankton growth is not possible in their mainstem, due to a very low euphotic depth:river depth ratio (e.g. Fisher & Parsley, 1979), resulting from high TSM loading in a deep river channel. Instead, in the South American floodplains, the bulk of planktonic primary production takes place in lakes that receive nutrients from the river during the 'flood pulse' (Junk *et al*., 1989) in the rising water period, and most phytoplankton present in the rivers is imported from the lakes. In a similar way, in catchments without extensive seasonally inundated floodplains, natural lakes and man-made reservoirs in the catchment act as reactors where phytoplankton is produced, resulting from improved light penetration from sedimentation of suspended matter, and their overflow provides planktonic algae and cyanobacteria to the downstream river section. As described by Talling & Prowse (2010), these inocula can be substantial and are transported far downstream, but the maintenance of populations in the river depends on ability to grow in a turbulent, usually turbid, environment with short retention time. However, transport from floodplain lakes and reservoirs is not the only process allowing phytoplankton presence in the main channel of tropical rivers. Studies conducted in tropical rivers have shown that a substantial part of the phytoplankton production can take place in slow-flowing areas of the main channel, as in the Orinoco River (Lewis, 1988) or in the Daly River, tropical Australia (Townsend, Przybylska & Miloshis, 2012). Moreover, the success of *Aulacoseira* species, which are abundant in the turbulent and turbid waters of the Paraná river, while being absent from the floodplain lakes (O'Farrell, Izaguirre & Vinocur, 1996; Devercelli *et al*., 2014), suggests that they originate from the river environment rather than being imported from lakes.

In contrast to the Nile and to its South American counterparts, the Congo River has only a few lakes in its catchment and lacks an extensive seasonal floodplain (Borges *et al*., 2015a). the floodplain consists of permanently flooded forest, with little variation in water depth between the extremes of the hydrograph. A factor explaining the possibility of net phytoplankton growth in the river main channel is the relatively low TSM load over most of the studied transect, compared for instance with the Amazon. Indeed, the average TSM in the Amazon mainstem at Obidos of 230 mg L^{-1} (range 93–385 mg L^{-1}, Richey *et al*., 1986) is distinctly higher than the average TSM in the Congo mainstem at Kinshasa of 26 mg L^{-1} (range 15–40 mg L^{-1}, Coynel *et al*., 2005) and at Kisangani of 53 mg L^{-1} (range 13–111 mg L^{-1}, A.V. Borges, own unpubl. data). Another factor is the low mean depth in most of the Congo River mainstem that allowed a euphotic depth:channel depth ratio favourable for sufficient net photosynthesis to sustain algal growth.

Two different parts can be distinguished in the Kisangani–Kinshasa transect: the upper sector of about 200 km in length where TSM is higher during the HW period and the whole river downstream of this upper sector, where TSM is actually lower in HW conditions than in FW. This could be explained by deposition of sediments in the 'Cuvette Centrale Congolaise' region during HWs, whereas sediments and nutrients were transported back to the rivers during FWs. Several results of our study point towards this process: higher TSM in most of the river transect and increased inorganic macro-nutrients (NO_3^- and SRP) both in the main river and the tributaries during FW. This nutrient increase might have favoured phytoplankton growth, although there was no clear evidence for a change in phytoplankton nutrient status from elemental ratios. Indeed, POC:PN and POC:PartP ratios cannot be relied on as indicators of the phytoplankton nutrient status, as POC:Chl *a* ratios were quite high, showing that phytoplankton carbon was a small fraction of the POC (2–8%, using a Chl *a*:C ratio of 40; Harris, 1986).

Phytoplankton composition was quite different between HW and at FW. In FW conditions in the mainstem, diatoms contributed on average to 60% of Chl *a* and were the dominant group at all sites, in agreement to what was reported in most studies on tropical river phytoplankton. Accordingly, Hughes *et al.* (2011) found that the largest fraction of biogenic silica (BSi) in the Congo River was composed of diatoms, with significant uptake of dissolved silicon, affecting its concentration in the river, as well as silicon isotopic fractionation. This confirms that significant diatom production does occur in the Congo River mainstem at falling and probably low waters.

The most abundant taxa were those adapted to low light conditions, such as diverse taxa of *Aulacoseira*, described as the most competitive at high Si:light ratio (Kilham *et al*., 1986). The second most important phytoplankton taxa in the FW period were the green algae, which accounted for 10% of Chl *a*, while the other groups (cyanobacteria and cryptophytes) accounted for 8% of Chl *a* on average. In contrast, during HW, green algae contributed to 88% of Chl *a* in the Congo River mainstem conditions, and 87% in the tributaries,

whereas diatoms barely reached 10% of total phytoplankton biomass. This is quite different from most reports on phytoplankton composition in large tropical rivers, particularly of South America (e.g. Garcia de Emiliani, 1990; Lewis *et al.*, 1995; Davies *et al.*, 2008; Devercelli *et al.*, 2014; O'Farrell & Izaguirre, 2014) but also from Africa (Prowse & Talling, 1958; Talling & Rzóska, 1967): one would have expected dominance of diatoms during both discharge conditions, owing to light limitation resulting from the TSM concentration in the mainstem and large tributaries, and from high DOC in the blackwater tributaries (Mayora, Devercelli & Frau, 2016). Indeed, considering a mean daily surface irradiance of 750 μE m^{-2} s^{-1}, calculated from continuous recordings during the cruises, the mean light exposure of phytoplankton would have been on average 35 μE m^{-2} s^{-1} in FW versus 71 μE m^{-2} s^{-1} in HW (Table 1). Under such conditions, diatoms, which are highly efficient at photosynthesis at low light (Kilham *et al.*, 1986; Kirk, 1994), would have been able to grow, so that the most likely explanation for their lack of development at HW is dilution by water inputs and lack of time to build populations in the fast-flowing mainstem. In contrast, green algae typically need high light for growth: Dauta (1982) measured optimal light for photosynthesis (I_{opt}) at different temperatures for some coccal green taxa and found I_{opt} values between 180 and 281 μE m^{-2} s^{-1} in the 25–30 °C range. The question thus arises how did green algae manage to maintain significant populations during HW. The most likely mechanism by which they can access these light levels and achieve high growth rates may be that they grew in the well-developed littoral/wetland aquatic vegetation of the main river, thereby providing inocula to the mainstem. This hypothesis, suggested by the presence and high abundance of green algae in a sample taken at HW in the fringing aquatic meadow, should be further substantiated by specific observations. The general descriptions of the ecology and distribution of key coccal and filamentous conjugate genera found in the Congo River show their belonging to the metaphyton, that is, algae loosely attached to the submerged macrophytes, from which they may be dislodged by the waves and current and then occur as tychoplankters in open waters (e.g. Behre, 1956; John *et al.* 2011). In these shallow environments, green algae may have benefited from adequate light conditions for growth, and water exchanges with the main channel may have supplied cells to the mainstem, according to the process described by Reynolds *et al.* (1991) involving the hydrological 'dead zones'. Similarly, the observations of Stoyneva (1994) suggest the role of macrophytes growing in the shallow river margin for sustaining the planktonic green algae in the Danube River. Once in the mainstem, green algae may have been able to maintain populations, provided that they were able to sustain a high photosynthetic rate. More research would be needed to assess whether plankton retention in the fringing aquatic vegetation actually plays a role in the maintenance of phytoplankton production in the mainstem of the Congo River during the different hydrological periods.

The fact that phytoplankton production occurs in the Congo River mainstem is of some biogeochemical significance, such as the effect of diatom silicon uptake on the Si cycle (Hughes *et al.*, 2011), but this production remains marginal in terms of the overall river network carbon budget. For instance, the emission of CO_2 to the atmosphere from the Congo River fuelled by processing of organic matter from land and wetland (13 800–18 250 mg C m^{-2} day^{-1}, Borges *et al.*, 2015b) is two orders of magnitude higher than primary production (<500 mg C m^{-2} day^{-1}). The major contribution of phytoplankton primary production and biomass may lie in its role in sustaining the food web. Indeed, as for the Amazon River (Forsberg *et al.*, 1993) where a key role of autochthonous C production by phytoplankton in fish diet was evidenced, there are indications that phytoplankton is an important component of the diet of some fish species in the Congo River. This is the case, based on gut content analysis, in two cyprinid species (Pwema *et al.*, 2015) and in a catfish (Tembeni Makiadi *et al.*, 2013) in the Malebo pool. This stresses the importance of understanding food-web processes, and in particular, the role of allochthonous versus autochthonous C, in large tropical rivers, where the fisheries contribute to the local economies and provide food for the human populations.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Results of a principal component analysis carried out on the physical and chemical data in the Congo River mainstem and its tributaries, from the sampling campaigns of December 2013 (high water, HW) and June 2014 (falling water, FW). The dots are the sampling sites; their shading depends on the distances from Kinshasa. See Table 1 for acronyms.

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ORIGINAL PAPER

Invasive South American floating plants are a successful substrate for native Central African pleuston

Janet Higuti · Koen Martens

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Abstract *Eichhornia crassipes*, commonly known as water hyacinth, is a free-floating perennial aquatic plant native to South America, which has been widely introduced on different continents, including Africa. *E. crassipes* is abundant in both the Congo (Africa) and Amazon (South America) River catchments. We performed a comparative analysis of the ostracod communities (Crustacea, Ostracoda) in the *E. crassipes* pleuston in the Amazon (South America) and Congo (Africa) River catchments. We also compared the ostracod communities from the invasive *E. crassipes* with those associated with stands of the native African macrophyte *Vossia cuspidata*. We recorded 25 species of ostracods associated with *E. crassipes* in the Amazon and 40 in the Congo River catchments, distributed over 31 ostracod species in *E. crassipes* and 27 in *V. cuspidata*. No South American invasive ostracod species were found in the Congolese pleuston. Diversity and richness of Congolese ostracod communities was higher in the invasive (*Eichhornia*) than in a native plant (*Vossia*). The highest diversity and abundance of ostracod communities were recorded in the Congo River. The result of principal coordinates analysis, used to evaluate the (dis)similarity between different catchments, showed significant differences in species composition of the communities. However, a dispersion homogeneity test (PERMDISP) showed no significant differences in the variability of the composition of species of ostracods (beta diversity) within Congo and Amazon River catchments. It appears that local ostracod faunas have adapted to exploit the opportunities presented by the floating invasive *Eichhornia*, which did not act as "Noah's Ark" by introducing South American ostracods in the Congo River.

Keywords Ostracoda · Aquatic macrophytes · Beta diversity · Amazon · Congo

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J. Higuti
Nupelia, PEA, Universidade Estadual de Maringá, Av. Colombo 5790, Maringá, PR CEP 87020-900, Brazil
e-mail: janethiguti@gmail.com

K. Martens (✉)
Freshwater Biology, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1000 Brussels, Belgium
e-mail: darwinula@gmail.com; martens@naturalsciences.be

K. Martens
Department of Biology, University of Ghent, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

Introduction

Several aquatic plants native to South America have successfully invaded other continents. *E. crassipes* (Mart.) Solms, commonly known as the water hyacinth,

has invaded freshwater systems in over 50 countries on five continents (Villamagna and Murphy 2010). *Eichhornia crassipes* is a free-floating perennial aquatic plant and is especially pervasive throughout Southeast Asia, the southeastern United States, central and western Africa and Central America (see overview in Villamagna and Murphy 2010). The water hyacinth is a pest species in many waterbodies, this in spite of many efforts to eliminate or at least control its populations. Introduction of this plant species in different parts of the world might have been accidental and/or intentional. Indeed, intentional introductions to ponds have been common, as the water hyacinth is an ornamental plant that also reduces nutrient concentrations and algae blooms (Villamagna and Murphy 2010).

Floating vegetation (i.e. not rooted in the sediment) is a typical feature of South American floodplains, where its submerged system of mostly roots hosts rich communities of invertebrate (and fish) taxa; these communities are called pleuston.

Ostracods are small bivalved crustaceans inhabiting sediments (Pieri et al. 2007; Van der Meeren et al. 2010; Szlauer-Lukaszewska 2014) and aquatic macrophytes (Higuti et al. 2010; Liberto et al. 2012; Mazzini et al. 2014; Matsuda et al. 2015), which are common in inland waters. There are about 2000 species of non-marine Ostracoda described in approximately 200 genera in various non- marine environments in the world (Martens et al. 2008), of which about 275 species in 55 genera are known from South and Central America (Martens and Behen 1994; Martens et al. 2008), while nearly 500 species in 84 genera of ostracods have been described from African inland waters (Martens 1984, 1998; Martens et al. 2008).

Currently, 117 species represented by 37 genera have been reported from Brazil (Martens and Behen 1994; Martens et al. 2008; Würdig and Pinto 2001; Pinto et al. 2003, 2004, 2005a, b, 2008; Higuti et al. 2009, 2013; Higuti and Martens 2012a, b, 2014). In both Africa and South America, more than 80 % of all species are endemic to their continent and zoogeographical region.

Eichhornia crassipes, native to South America and invasive in Africa, is abundant in both the Congo (Democratic Republic of Congo) and Amazon (Brazil) River catchments. Many studies reported on the negative impact of invasive aquatic plants on their own communities and/or on the ecosystems in general. However, there is little information about the introduction of aquatic organisms (periphyton, invertebrates) together with these invasive plants. Some biological features of ostracods could facilitate their long-distance dispersal, such as the presence of drought-resistant eggs (Horne and Martens 1998) so that they might colonize a new habitat and successfully establish a new population. Furthermore, the reproduction of many species of ostracods happens by parthenogenesis (Martens et al. 2008), so that a new population can be formed by a single founder specimen or egg.

The objectives of the present study were to determine (1) if Congolese *Eichhornia* comprises exclusive Congolese faunas or if also invasive (South American) species occur; (2) if ostracods communities in native (*Vossia cuspidata* (Roxb.) Griff.) and invasive (*E. crassipes*) macrophyte stands in the Congo River catchment differ and (3) if ostracod biodiversity within and between the Amazon and Congo River catchments are significantly different.

Materials and methods

Study area

The Amazon River in South America is the second longest in the world and by far the largest by water flow with an average discharge of approximately 214 million liters per second. During the rainy season, the water discharged in the Atlantic Ocean travels approximately 160 km out into sea. The Amazon has the largest river basin in the world, occupying more than 6.8 million km^2; rainfall is evenly distributed spatially and temporally, ranging from 1500 to 2500 mm annually, for about 6 months a year (Goulding et al. 2003). The Amazon River and its tributaries are accompanied along their middle and lower courses by large fringing floodplains that cover an area of about 300,000 km^2, the flooded forest is known as *várzea* (Irion et al. 1997).

The Congo River has the second highest discharge (1325 km^3/year or 42 million liters/s) of any river in the world after the Amazon (Coynel et al. 2005). It drains 3.7 million km^2 of the African continent (Campbell 2005). It covers about 25 % of the total wet tropical zone and is responsible for almost 3.4 % of the freshwater inputs to the Atlantic Ocean (Probst and Tardy 1987). In the wet season, most of the forests

are flooded, while in the dry season they dry out. The Congo Basin has a humid tropical climate: the mean annual precipitation, calculated between 1980 and 1990 is close to 1550 mm (Mahé 1993).

The Amazon and Congo systems, situated across the equator, are characterized by tropical rain forest and by extensive floodplains. However, there are major differences in catchment characteristics. For example, the runoff in the Amazon River is higher (Filizola 2003) because the headwaters of the Amazon catchment are located in high mountains in contrast with the plateaus or hills of the Congo catchment (Meybeck et al. 2001).

Collections and measurements

The ostracod collection of the Amazon floodplain used in the present study results from a project in the SISBIOTA program (National System of Biodiversity Research) funded by CNPq/Fundação Araucária. The project covered four Brazilian floodplains (in addition to the Amazon: Paraná, Araguaia and Pantanal). Two sampling campaigns were conducted in the Amazon floodplain, the first one was done in October 2011 (dry season) and the second one in May 2012 (rainy season). More than 20 lakes or lake-like habitats were sampled, mostly for their pleuston communities in *Eichhornia* stands.

The ostracod collection from the Congo River was effected during the large Congo-expedition in May and June 2010 (Boyekoli Ebala Congo 2010—http://www.congobiodiv.org/en). More than 50 samples were collected, either in the pleuston of the invasive species *E. crassipes* or amongst stands of the native *V. cuspidata.*

We used data from ostracods associated with *E. crassipes* and *V. cuspidata* of the same sampling period in both river catchments to compare the ostracod fauna. The localities sampled in the Amazon and Congo River catchments are generally located close to cities or village. Thirteen localities were selected for each of the river catchments (Fig. 1). In each locality, two replicate samples of each aquatic macrophyte (*E. crassipes* in Amazon, *E. crassipes* and *V. cuspidata* in Congo) were taken.

Eichhornia crassipes individuals of similar size were hand collected and were placed in plastic buckets to remove the ostracods; roots were separated from the leaves and were washed in the bucket. The residuals were filtered in a hand net (mesh size c 160 µm). Ostracods in *Vossia* stands were collected directly by moving the handnet through the rooted plants for c 5 min. Ostracods were killed by adding 97 % ethanol to the wet residual; all samples were washed again in the lab and were transferred to fresh ethanol (70 %). Samples were sorted under a microscope stereoscopic. Specimens were identified using valves (Scanning Electron Microscopy) and appendages (soft parts dissected in slides with light microscopy).

The environmental variables measured in the field included water temperature (°C) and dissolved oxygen (mg L^{-1}) (oxymeter-YSI), pH and electrical conductivity (µS cm^{-1}) (YSI 63). Some geographical, physical, and chemical features of the waterbodies of Amazon and Congo River catchments are listed in Table 1.

Data analyses

Richness, abundance, Shannon–Wiener diversity and evenness were calculated to assess differences in the ostracods communities in Amazon and Congo River catchments. A parametric analysis of variance (ANOVA) was performed to test for significance of differences in species richness, abundance, diversity and evenness of ostracods between plants species and between catchments. When the homogeneity assumption required for ANOVA was not fulfilled, a non-parametric Kruskal–Wallis test was used. Analyses of variance were performed in Statistica 7.1 (StatSoft 2005).

Rarefaction analysis was performed, because of differences in abundance amongst the samples, in order to compare species richness between the sampling units at comparable levels of density in both catchments. Rarefaction curves were computed using the BioDiversity Pro Programme (McAleece et al. 1997).

Principal coordinates analysis (PCoA) was used to evaluate the (dis)similarity of species composition between different catchments. Differences between the two catchments were tested by ANOVA applied to the scores of PCoA axes. The dispersion homogeneity test (PERMDISP) was performed to test the variability in the ostracods species composition (beta diversity) within Amazon and Congo River catchments. This analysis calculates a centroid for each catchment, and determines the Bray–Curtis distance of each sampling

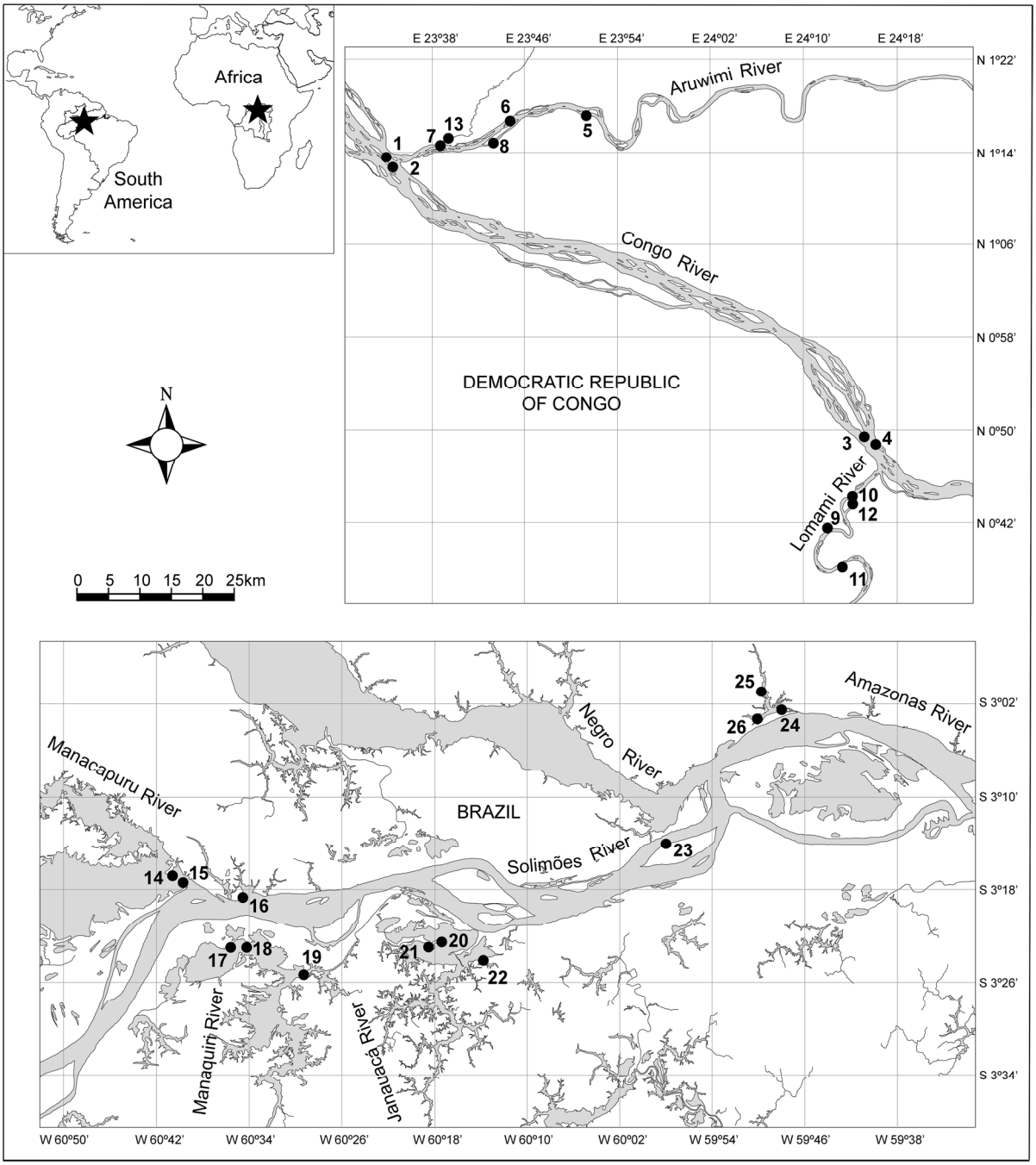


Fig. 1 Location of the sampling stations of Congo River catchment (localities numbers *1–13*) and Amazon River catchment (localities numbers *14–26*). *Source* Original (see acknowledgments)

locality to the centroid. The higher average of Bray–Curtis distances to the centroid means greater dissimilarity in species composition, suggesting an increase in beta diversity. The significance ($p < 0.05$) in beta diversity between catchments was analyzed by a permutation test of least squares residuals, using 999 permutations. The analyses was performed using the R 3.0.1 software (R Development Core Team 2013)

Table 1 Geographical coordinates and some abiotic characteristics of the localities of Congo and Amazon River catchments

Locality name	Collection number	Date	Coordinates	Catchment	Substrate	WT	DO	pH	EC
1. Congo	DRC KM 10.020	21.05.2010	1°13′24.5″N 23°34′31.2″E	Congo	*E. crassipes*	28.5	–	7.1	57.3
2. Congo	DRC KM 10.017	21.05.2010	1°12′59.1″N 23°34′15.2″E	Congo	*V. cuspidata*	28.7	–	7.1	67.2
3. Congo	DRC KM 10.152	02.06.2010	0°49′31.8″N 24°15′14.8″E	Congo	*E. crassipes*	28.9	3	7.4	77.1
4. Congo	DRC KM 10.154	02.06.2010	0°49′10.6″N 24°16′9.2″E	Congo	*V. cuspidata*	28.7	3.1	7.3	65.7
5. Aruwimi	DRC KM 10.008	20.05.2010	1°17′8.8″N 23°51′22.5″E	Congo	*E. crassipes*	29.6	–	6.8	44.1
6. Aruwimi	DRC KM 10.016	20.05.2010	1°16′32.2″N 23°44′47.9″E	Congo	*E. crassipes*	30.5	–	7.1	45.7
7. Aruwimi	DRC KM 10.029	21.05.2010	1°14′33″N 23°39′5.8″E	Congo	*E. crassipes*	25.9	–	6.7	44.2
8. Aruwimi	DRC KM 10.051	24.05.2010	1°14′48.4″N 23°43′16.2″E	Congo	*E. crassipes*	28.6	3	6.8	44.8
9. Lomami	DRC KM 10.086	28.05.2010	0°41′30.5″N 24°11′59.9″E	Congo	*V. cuspidata*	28	2.6	6.5	24.5
10. Lomami	DRC KM 10.097	28.05.2010	0°44′10.5″N 24°14′6.7″E	Congo	*V. cuspidata*	28.5	3.3	6.4	24.8
11. Lomami	DRC KM 10.120	30.05.2010	0°38′14.3″N 24°13′19.3″E	Congo	*E. crassipes*	28.7	3.7	6.7	25.4
12. Lomami	DRC KM 10.141	01.06.2010	0°44′23.5″N 24°14′22.1″E	Congo	*E. crassipes*	28.1	–	6.5	23.1
12. Lomami	DRC KM 10.139	01.06.2010	0°44′34″N 24°14′15.6″E	Congo	*V. cuspidata*	28.3	–	6.4	25.8
13. Lulu	DRC KM 10.026	26.05.2010	1°14′50.5″N 23°39′2.7″E	Congo	*V. cuspidata*	25.8	–	4.5	16.5
14. Monte Cristo	AMA 42/AMA 44	15.05.2012	3°16′43.4″S 60°40′28″W	Amazon	*E. crassipes*	31.7	0.4	6.5	67.1
15. Piranha	AMA 46/AMA 47	15.05.2012	3°17′7.5″S 60°39′50.4″W	Amazon	*E. crassipes*	31.7	0.6	6.6	70.1
16. Calado	AMA 51/AMA 52	15.05.2012	3°18′32″S 60°34′28″W	Amazon	*E. crassipes*	34.3	3.1	6.8	67.8
17. Grande	AMA 55/AMA 56	16.05.2012	3°22′33.9″S 60°35′20.9″W	Amazon	*E. crassipes*	31.5	1.2	6.6	54.1
18. Curuça	AMA 58/AMA 59	16.05.2012	3°23′0″S 60°34′9.4″W	Amazon	*E. crassipes*	31.5	1.4	6.6	51.8
19. Fuxico	AMA 66/AMA 68	16.05.2012	3°25′19″S 60°29′18.4″W	Amazon	*E. crassipes*	32.9	1.3	6.5	41.5
20. Jamauacá	AMA 70/AMA 71	17.05.2012	3°22′29.1″S 60°17′34.3″W	Amazon	*E. crassipes*	32.3	3	6.5	42.5
21. Jutaí	AMA 74/AMA 75	17.05.2012	3°22′51.1″S 60°18′22.1″W	Amazon	*E. crassipes*	32.7	2.2	6.5	42.9
22. Castanho	AMA 78/AMA 80	17.05.2012	3°24′6.5″S 60°13′43.4″W	Amazon	*E. crassipes*	32.5	0.9	6.4	43.5
23. Comprida	AMA 82/AMA 83	17.05.2012	3°14′0.3″S 59°5.7′53.8″W	Amazon	*E. crassipes*	31.5	1.8	6.7	65.1
24. Poraquequara IV	AMA 86/AMA 87	18.05.2012	3°2′22.7″S 59°48′3.3″W	Amazon	*E. crassipes*	31.5	2.1	9.7	51.5
25. Poraquequara II	AMA 94/AMA 95	18.05.2012	3°0′46.7″S 59°49′40.2″W	Amazon	*E. crassipes*	32.3	3.9	9.3	53.4
26. Poraquequara I	AMA 98/AMA 99	18.05.2012	3°3′12″S 59°50′1.1″W	Amazon	*E. crassipes*	31.9	2.6	9.1	50.8

WT water temperature (°C), *DO* dissolved oxygen (mg L^{-1}), *EC* electrical conductivity (μS cm^{-1})

through vegan and permute packages (Oksanen et al. 2013).

Results

We recorded 25 species of ostracods associated to *E. crassipes* in the Amazon River catchment and 40 species in the Congo River catchment. Of these 40 Congolese species, 31 were found in the invasive *E. crassipes* and 27 ostracod species occurred in the native *V. cuspidata* (See appendix 1 and 2 in Electronic supplementary material). The most speciose ostracod subfamily was Cypricercinae (8 species in *E. crassipes*) in the Amazon; and Cypridopsinae (9 species in both *E. crassipes* and *V. cuspidata*) and Herpetocypridinae (7 species in *E. crassipes* and 6 species in *V. cuspidata*) in the Congo River catchment (Table 2). The only ostracod species present in both catchments was *Stenocypris major*, a circumtropical species. Several ostracod species in the Amazon and nearly all species in the Congo River catchment are new to science and remain to be described.

The highest diversity and abundance of ostracods were recorded in the Congo River catchment in both aquatic plants species, where richness and Shannon diversity values were higher in the invasive *E. crassipes* (Fig. 2a–c). However, no significant difference was observed regarding these attributes between *E. crassipes* and *V. cuspidata*. When comparing diversity of ostracods associated with *E. crassipes* between both catchments, significantly higher values of species richness ($p < 0.01$) and diversity ($p < 0.05$) were found in the invasive plant from the Congo River catchment. The evenness values were similar between Amazon and Congo River catchments (Fig. 2d).

Rarefaction curves indicated higher number of ostracods species in the Congo River catchment, especially on the invasive *E. crassipes*, for similar numbers of individuals. The richness reached an asymptote in the *E. crassipes* samples from the Amazon River catchment, while it is still increasing in the *E. crassipes* and *V. cuspidata* samples of the Congo River catchment (Fig. 3), meaning that with more sampling most likely still more species could be found there.

The result of the PCoA, used to evaluate the (dis)similarity between different catchments, showed significant differences in species composition, assessed for the scores of PCoA, axis 1 ($F = 170.98$; $p < 0.00$) and axis 2 ($F = 5.39$; $p < 0.05$). The dispersion homogeneity test (PERMDISP) showed no significant differences ($F = 0.31$; $p = 0.59$) in the variability of ostracod species composition (beta diversity) within Amazon and Congo River catchments. The highest average distance to the centroid was recorded in the Amazon River catchment (0.58) compared to the Congo River catchment (0.56) (Fig. 4).

Discussion

Composition of ostracod communities

Ostracods are amongst the most abundant invertebrates associated with macrophytes and are themselves a group that have a high diversity of species (Thomaz et al. 2008; Higuti et al. 2007, 2010; Liberto et al. 2012; Mazzini et al. 2014; Matsuda et al. 2015).

The Cyprididae are the most species ostracod family in the Amazon and Congo River catchments. This is in line with the fact that this family comprises c 50 % of all known Recent non-marine ostracod species and more than 80 % of all species in most tropical regions (Martens 1998). The patterns of species composition are different between both catchments: Cypricercinae (32 % of species) and Cypridopsinae (20 %) are the most dominant subfamilies in the Amazon River catchment, Cypridopsinae (30 %) and Herpetocypridinae (20 %) in the Congo River catchment. These findings are in agreement with those of Martens (1998), who compared the diversity of non-marine Ostracoda from Africa and South America.

The comparison of the ostracod communities between the two catchments clearly shows that the pleuston in the Amazon River catchment only contained South American species, while those in the Congo River catchment only contained Congolese ostracod species, or in any case did not have any identifiable South American (or other non-African) ostracod species, with *Stenocypris major* being the only shared, circumtropical, ostracod species. The invasive *E. crassipes* therefore did not act as a "Noah's Arc" by transporting also invasive ostracod species into that part of the Congo River catchment.

Table 2 Comparisons between ostracods fauna from Amazon (South America) and Congo River catchments (Africa)

Family	Subfamily	AMAZON		CONGO			
		E. crassipes		*V. cuspidata*		*E. crassipes*	
		Genera	Species	Genera	Species	Genera	Species
Cyprididae	Cypricercinae	3	8	1	5	1	4
	Cypridinae	1	2	–	–	–	–
	Cypridopsinae	4	5	2	9	2	9
	Cyprettinae	1	3	–	–	–	–
	Herpetocypridinae	1	1	3	6	3	7
Candonidae	Candoninae	2	3	1	2	1	2
	Cyclocypridinae	1	2	–	–	2	2
Limnocytheridae	Timiriaseviinae	1	1	2	3	3	4
Darwinulidae		–	–	2	2	3	3
		14	25	11	27	15	31

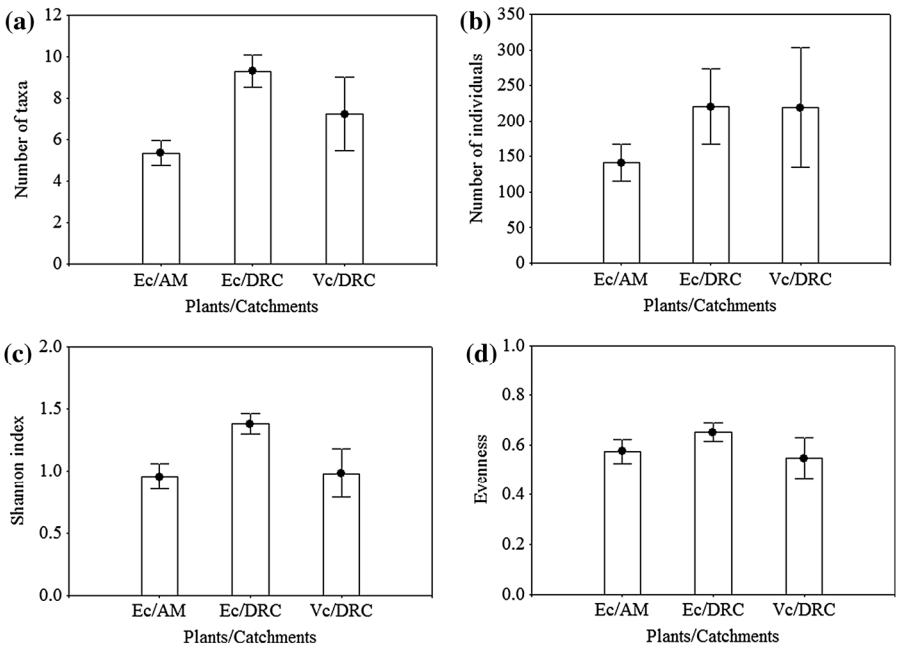


Fig. 2 Mean values and standard error of **a** richness, **b** abundance, **c** Shannon diversity and **d** evenness of ostracods community associated with *E. crassipes* (Ec) and *V. cuspidata* (Vc) in Amazon (AM) and Congo River (DRC) catchments

This can be either because the invasive plant was introduced as seeds or propagules, in which case no pleuston was associated with it yet, or because the invasive pleuston in adult plants failed to establish viable communities in the new environment.

However, several ostracod species do have the capacity to invade new areas, and have already done so in the past (see Table 3).

Comparison between ostracod communities in native (Amazon) and invasive (Congo) *E. crassipes*

Our results showed higher ostracod diversity and abundance in the invasive *E. crassipes* in the Congo River catchment than in the native *E. crassipes* specimens from the Amazon River catchment. This

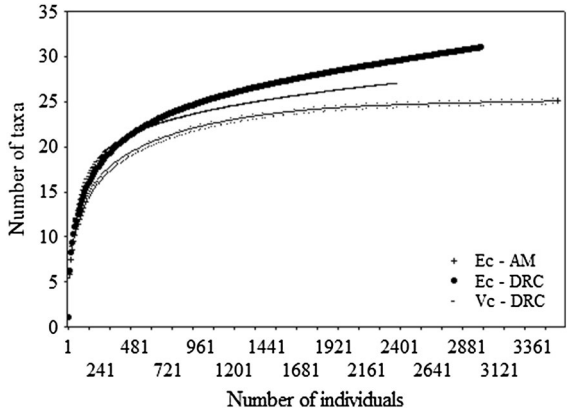


Fig. 3 Rarefaction curves of ostracods species associated with different aquatic plants in Amazon (AM) and Congo River catchments (DRC). Ec = *E. crassipes* and Vc = *V. cuspidata*. +: native *E. crassipes* of the Amazon River catchment, ●: invasive *E. crassipes* of the Congo River catchment, -: native *V. cuspidata* of the Congo River catchment

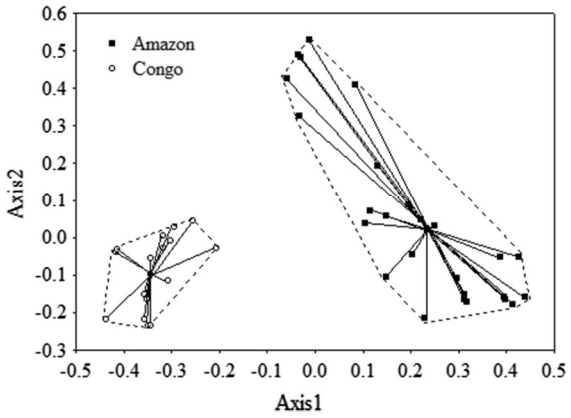


Fig. 4 Multivariate permutation analysis diagram showing the variability in ostracods species composition associated with *E. crassipes* between different catchments. *Full square* Amazon River catchment, circle: Congo River catchment

is a rather unexpected result, as one could predict to have a greater diversity of ostracod species in *E. crassipes* of the Amazon River catchment, where this plant is native and where ostracod species are expected to be better adapted to life in the root system of this (and other) floating plants. The fact that the collections were sampled in a small part of the Amazon floodplain relatively near to the city of Manaus might be part of the reason. However, the rarefaction analyses showed that the cumulative curve of ostracod species in *E. crassipes* from the Amazon River catchment had reached the maximum plateau with our collections. The results are thus representative for at least that part of the Amazon River catchment. Moreover, size of root systems in this macrophyte species can vary considerably and is known to depend at least in part on water chemistry (Kobayashi et al. 2008). Observations showed that the root systems of this plant in the Congo River catchment samples were much smaller than those from the Amazon River catchment, down to less than half the volume (results not shown). This makes the higher values of richness, diversity and abundance of the ostracod communities in the Congolese samples even more surprising.

The present results indicate that invasive species such as *E. crassipes* do not necessarily always have a fully negative effect on the invaded communities. Invasive *E. crassipes* root systems obviously offer novel substrates for native animals, such as ostracods, that can adapt to life in pleuston. However, this is clearly only one side of a broader picture, where other native communities such as zooplankton or whole community levels might be negatively impacted.

Comparison between ostracod communities in native (*V. cuspidata*) and invasive (*E. crassipes*) plant species in the Congo River catchment

We observed that the ostracod communities associated with an invasive plant species, *E. crassipes*, have a higher richness, diversity and abundance than those associated to a native plant species, *V. cuspidata*, in the Congo River catchment. This is a clear example of an invasive species physically facilitating communities of native species [see review in Rodriguez (2006)].

Also Mormul et al. (2010) observed higher values of these attributes of ostracod communities in an invasive plant, *Hydrilla verticillata* when compared to a native macrophyte, *Egeria najas*, in the Paraná River floodplain, although these differences were not significant. However, cumulative curves did indicate higher diversity of ostracod species on the invasive *H. verticillata*. The invasive plants might thus provide favorable habitats for native ostracod communities. The experiment by Mormul et al. (2010) was performed to test the effect of an invasive plant on the ostracod communities, using two plants with similar architecture (as measured in fractal heterogeneity). In the present case, *E. crassipes* and *V. cuspidata* do not have similar architecture: *Eichhornia* is a floating plant with normally dense root systems with high heterogeneity, whereas *Vossia* is a grass-like rooted

Table 3 Examples of invasive ostracod species from or to South America and Africa

Species	Origin	Destination	Dispersal	Reference
Chlamydotheca incisa	South America	Europe	Tobacco, tomato and sunflowers	McKenzie and Moroni (1986)
C. arcuata	South America	Europe	Aquatic plants	Matzke-Karasz et al. (2014)
Chrissia sp.	Africa	Europe	Rice	Rossi et al. (2003)
Cypris latissima		Africa	–	Bruton and Merron (1985)
Hemicypris sp.		Africa	–	Bruton and Merron (1985)
Stenocypris sp.		Africa	–	Bruton and Merron (1985)
Strandesia bicuspis	South America	Europe	Aquatic plants	Matzke-Karasz et al. (2014)
S. caudata	Africa	Europe	Rice, hemp and saffron	McKenzie and Moroni (1986)
S. spinulosa	Africa	Europe	Rice, hemp and saffron	McKenzie and Moroni (1986)
S. tonolli	Africa	Europe	Rice, hemp and saffron	McKenzie and Moroni (1986)

plant, with low individual heterogeneity but occurring in dense stands. This density of the macrophyte stands will to a certain degree compensate for the lower individual heterogeneity.

Figueiredo et al. (2015) conducted an experiment using a native (*E. najas*) and an invasive (*H. verticillata)* macrophyte species to test the preference of a native fish prey species for either macrophyte as a refuge in the presence of an invasive voracious predator. It appeared that the prey fish did not show preference for one plant over the other, and they concluded that habitat structure affects choice more than evolutionary history between fish and macrophytes and this is in line with our results.

The effects of invasive plants on aquatic communities, however, is more complex and depends on more factors than on heterogeneity of the habitat only. Villamagna and Murphy (2010) showed that abundance and diversity of aquatic invertebrates generally increase in response to increased habitat heterogeneity and structural complexity provided by *E. crassipes*, but can also decrease due to decreased planktonic algae (food) availability. Stiers et al. (2011) compared macrophytes and macroinvertebrates communities of 32 Belgium ponds, of which 22 included invasive plants. Negative impact on native plants and macroinvertebrates was recorded for three invasive plants, reducing the richnees of both communities. Schultz and Dibble (2012) summarized the role of invasive plants on freshwater fish and macroinvertebrate communities. They found that positive effects of invasive macrophytes on fish and macroinvertebrate communities were associated with characteristics held in common with native macrophytes such as photosynthesis, increasing habitat heterogeneity, and stabilizing substrate. The authors found that three other traits of invasive plants can largely be responsible for negative effects on fish and macroinvertebrate communities: increased growth rate, allelopathic chemical production, and phenotypic plasticity that allow for greater adaptation to environmental conditions and resource utilization than native species.

Conclusions

Ostracod communities in Congo and Amazon River catchments are highly dissimilar, indicating that the invasive *E. crassipes* root systems in the Congo River catchment were colonized by African ostracods, and that the arrival of invasive *E. crassipes* did not bring along successful invasions of South American ostracod species, nor species from other parts of the world from where the plants may have been introduced into the Congo basin. Also, richness, diversity and abundance of ostracod communities in the invasive Congolese plants are higher than in the native Amazonian plants. Finally, richness, diversity and abundance is also higher in the invasive *E. crassipes* than in the native *V. cuspidata* in the Congo River catchment, but this could in part be owing to lower complexity of the native plant. It appears that local African ostracod faunas have adapted to exploit the habitat opportunities presented by the floating invasive *E. crassipes*.

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Gomphonema grande sp. nov., a new diatom (Bacillariophyta) from the Democratic Republic of the Congo, Tropical Africa

BALASUBRAMANIAN KARTHICK[1, 2], J. PATRICK KOCIOLEK[3], JONATHAN C. TAYLOR[1, 4, 5 *] & CHRISTINE COCQUYT[5]

[1]*School of Biological Sciences, North-West University, Potchefstroom, South Africa*
[2]*Present Address: Biodiversity & Paleobiology Group, Agharkar Research Institute, Pune – 411004, India*
[3]*Museum of Natural History and Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado, 80309 USA*
[4]*South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown, South Africa*
[5]*Botanic Garden Meise, 1860 Meise, Belgium*
** Corresponding author (E-mail: Jonathan.Taylor@nwu.ac.za)*

Abstract

Gomphonema grande sp. nov. is described from a single sample collected from the Congo River Basin, Tropical Africa. The morphological features of the new species are described and documented through light microscopy and scanning electron microscopy analyses. *Gomphonema grande* differs from most species of the genus by its relatively large size, robust valves, unique shape, and distinctive suite of valve features such as a labium-shaped internal stigma opening. Further discussion on the diverse nature of certain characters in the genus *Gomphonema* is presented.

Keywords: Gomphonemoid, doubly-punctate, robust valves, stigma, checklist, new species

Introduction

The freshwater diatom flora of Africa is rather poorly known given the size of the continent and the multitude of habitat types present. Perhaps the best-studied area in Africa in terms of the taxonomy and diversity of the group is South Africa, where Cholnoky published 38 papers over a 20 years period, describing many hundreds of new taxa. His work was followed up by Schoeman and Archibald who worked together for a number of years in close collaboration (*e.g*. Schoeman & Archibald 1986), and more recently Taylor (*e.g*. Taylor & Lange-Bertalot 2006). Another fairly well-documented region on the African continent is the East African Great Lakes, where endemic genera and species have been described (Müller 1904a, b, 1905, 1910, Cocquyt 1998, 2000, 2003, Cocquyt & Schram 2000). West African freshwater diatoms have been documented by Carter & Denny (1982, 1987, 1992), Foged (1966) and Woodhead and Tweed (1958a, 1960), and diatoms from North Africa have been addressed by Maillard (1977) as well as others. New species of diatoms have been described from Africa intermittently (*e.g*. Dickie 1880, Zanon 1941, Klee & Casper 1992, Cocquyt & Jahn 2007a, b, Cocquyt *et al*. 2012, 2013). Perhaps the least well-known elements of the African freshwater diatom flora are in the central part of the continent, with work from this region coming from Hustedt (1949) and Kufferath (1956a) among others, and with the body of this work being focused on the phytoplankton.

Species of the diatom genus *Gomphonema* Ehrenberg (1832: 87) have been described from southern Africa (Cholnoky 1954a, b, 1959, Passy *et al*. 1997), Democratic Republic of the Congo—formerly Belgian Congo and Zaire—by Compère (1995) and Hustedt (1949), Rwanda in central Africa by Kufferath (1957), and the East African Great Lakes (Fricke in Schmidt 1902, West 1907, Kociolek & Stoermer 1991). A total of 56 *Gomphonema* species have been described from the African continent (Table 1). Of these, the morphology of only a few species has been studied in detail with scanning electron microscopy, which has shown that some of the African species have double rows of areolae per stria (*e.g*. Compère 1995) and elongated internal stigma openings (*e.g*. Kociolek & Stoermer 1991, Compère 1995), similar to species described from Madagascar (Spaulding & Kociolek 1998) and India (Karthick *et al*. 2011, Karthick & Kociolek 2012).

In the present paper, we describe a new species, *Gomphonema grande*, from the Democratic Republic of the

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Congo, central Africa, and document its valve morphology with light and scanning electron microscopy. We compare this species with others from the African continent, and other taxa within the genus.

Materials and Methods

The studied material was collected from the Congo basin downstream of Kisangani in the Democratic Republic of the Congo, by François Darchambeau and Ernest Tambwe in November 2012 during an expedition in the frame of the Belgium COBAFISH project. For the present paper following sample was studied: CCA 2071, epiphyton on dead submerged wood at Lomami River, 0.49339° N, 24.16960° E.

The sample was cleaned using hydrogen peroxide (30%) and centrifuged, then rinsed five times with distilled water. The cleaned material was used for preparation of permanent slides, mounted in Naphrax®. Light microscope (LM) images were obtained using an Olympus BX51 microscope equipped with DIC optics and a 100× 1.4 n.a. oil immersion objective and an Olympus UC30 digital camera at the Botanic Garden Meise, Belgium. For scanning electron microscopy (SEM) studies, aliquots of the cleaned material were air dried on coverslips and fixed to aluminium stubs using double sided carbon tape, sputter-coated with gold palladium and studied using a FEI Quanta 2000 F-SEM (10 kV, WD 10 mm) at the Laboratory for Electron Microscopy, North-West University, Potchefstroom, South Africa. Raw and cleaned samples and permanent slides used in this study are housed at the Botanic Garden Meise, Belgium, and at the South African National Diatom Collection at the North-West University, Potchefstroom, South Africa. A copy of the permanent slide will be deposited at the Centre de Surveillance de la Biodiversité (CSB) and/or at the Herbarium of the University of Kisangani, DR Congo.

Concurrent with the collection of the algae samples, physical and chemical parameters were measured. Water temperature, conductivity and pH were measured with an YSI ProPlus multiprobe field meter equipped with a pH sensor 1001 ProSeries. Samples for nutrient analyses (NH_4, NO_3, NO_2 and soluble reactive phosphorus SRP) were filtered and acidified in situ, and kept frozen until spectrophotometric analysis in the laboratory of the Unité d'Océanographie chimique, Département d'Astrophysique, Géophysique et Océanographie, University of Liège, Belgium.

Results

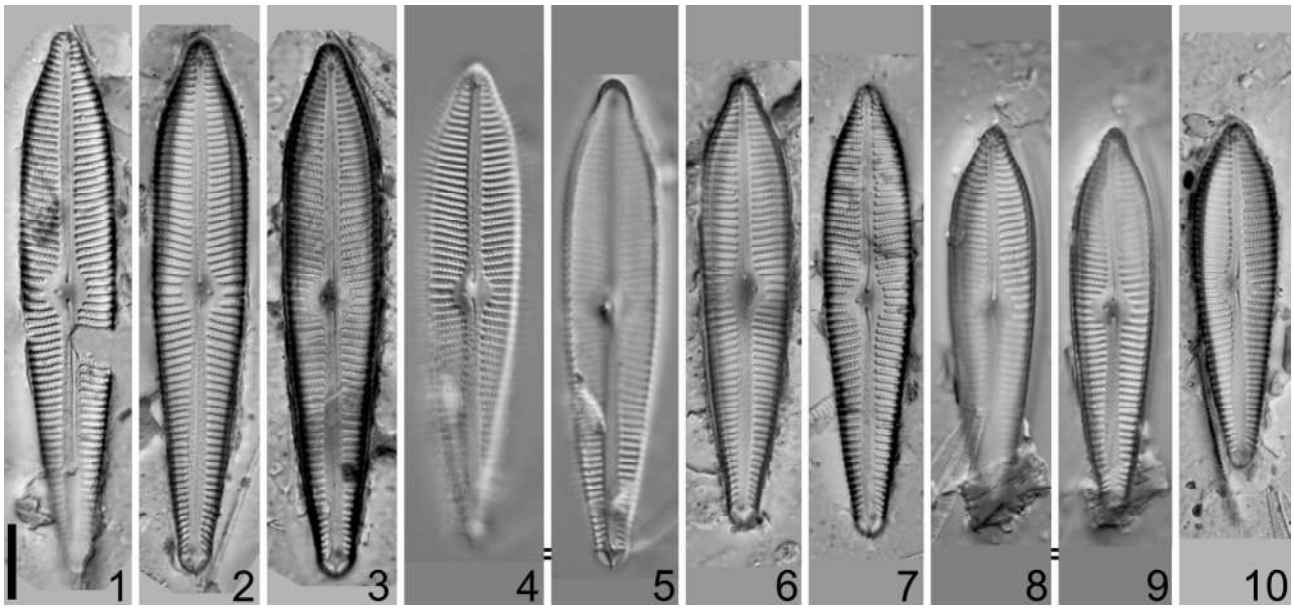
Division **Bacillariophyta**
Class **Bacillariophyceae**
Subclass **Bacillariophycidae**
Order **Cymbellales**
Family **Gomphonemataceae**
Genus ***Gomphonema*** **Ehrenberg 1832**

Gomphonema grande **B. Karthick, Kociolek, J.C. Taylor & Cocquyt, *sp. nov*. (Figs 1–24)**

Valves lanceolate-clavate, head pole broadly apiculate, foot pole rounded. Length 46–72 µm, breadth 10–12 µm. Axial area straight, usually with a mottled appearance, expanded to form an asymmetrical, elliptical central area. A prominent stigma present in the central area. Raphe broadly lateral, straight. External proximal raphe ends dilated, distinct. External distal raphe ends deflected towards the direction opposite the stigma opening. Striae radiate around the central area, becoming parallel towards the poles, convergent at the head pole, strongly radiate at the foot pole. Striae broad, comprised of two rows of areolae. Striae number 10–12/10 µm. Bilobed apical pore field prominent at the footpole. Septa and pseudosepta present at both poles.

Type:—DEMOCRATIC REPUBLIC OF THE CONGO. Oriental Province: Lomami River, 0.49339° N, 24.16960° E, epiphytic sample on dead submerged wood sample CCA 2071 (holotype BR 4426, valve representing the holotype here illustrated as Fig. 2, isotype PUC 13-572)

Etymology:—The specific epithet '*grande'* refers to the generally large size of the cell and distinct nature of the ornamentation.



FIGURES 1–10. *Gomphonema grande*, LM (DIC). Valve views showing size diminution series. Valves from the type slide BR 4426, Lomami River, Oriental Province, Democratic Republic of the Congo. 2. Valve representing the holotype. Scale bar: 10 µm.

Description:—In the SEM, the valve exterior appears rugose with a network of silica present along the striae, a raised area of silica is present along the margin (Figs 11–17). A single, small spine is present at the head pole at the junction of the valve face and the valve mantle tapering off in a shallow silica ridge on the valve mantle (Figs 11–17). Areolae appear to be sunken within elevated outlines of silica (Figs 11, 12, 15). The axial area has many depressions along its entire length (Figs 11, 13). Distal raphe ends are deflected far onto the valve mantle (Figs 11, 12, 14). Proximal raphe ends are tear-drop shaped, and the stigma opening is elliptical in outline (Figs 11, 13). Areolar openings are occluded by siliceous flaps that form C-shaped openings (Figs 13, 15, 16). Apical pore fields are bisected by deflected distal raphe ends (Fig. 14). Porelli are small, and physically differentiated from the areolae (Figs 11, 14, 17) and extend from the valve face onto the mantle. Areolae extend on to the mantle, and also occur in depressions surrounded by raised silica (Figs 15–17). The valve interior is dominated by a prominent central nodule, helictoglossae and pseudosepta (Figs 18–24). At the poles, pseudosepta are evident, but not extensive (Fig. 18). Helictoglossae are positioned near the valve terminus, and offset from the main raphe branches (Figs 18, 19, 21, 24). The virgae appear slightly undulate (arrow in Figs 20, 21), with small silica extensions present (Figs 18–23). The central nodule is raised internally, and the internal proximal raphe ends are recurved against the edges of the nodule. The internal stigma opening appears similar to the helictoglossae, bordered by thickened extensions and giving a lip-like appearance (Figs 18, 20, 23). At the foot pole, porelli are distinct, the lobes of the apical pore fields are separated by the extension of the axial area (Figs 21, 24). Striae extend from the valve face to the mantle (Fig. 22). Girdle composed of several open, smooth bands. At least one band with a row of small poroids near the edge away from the valve (Fig. 15).

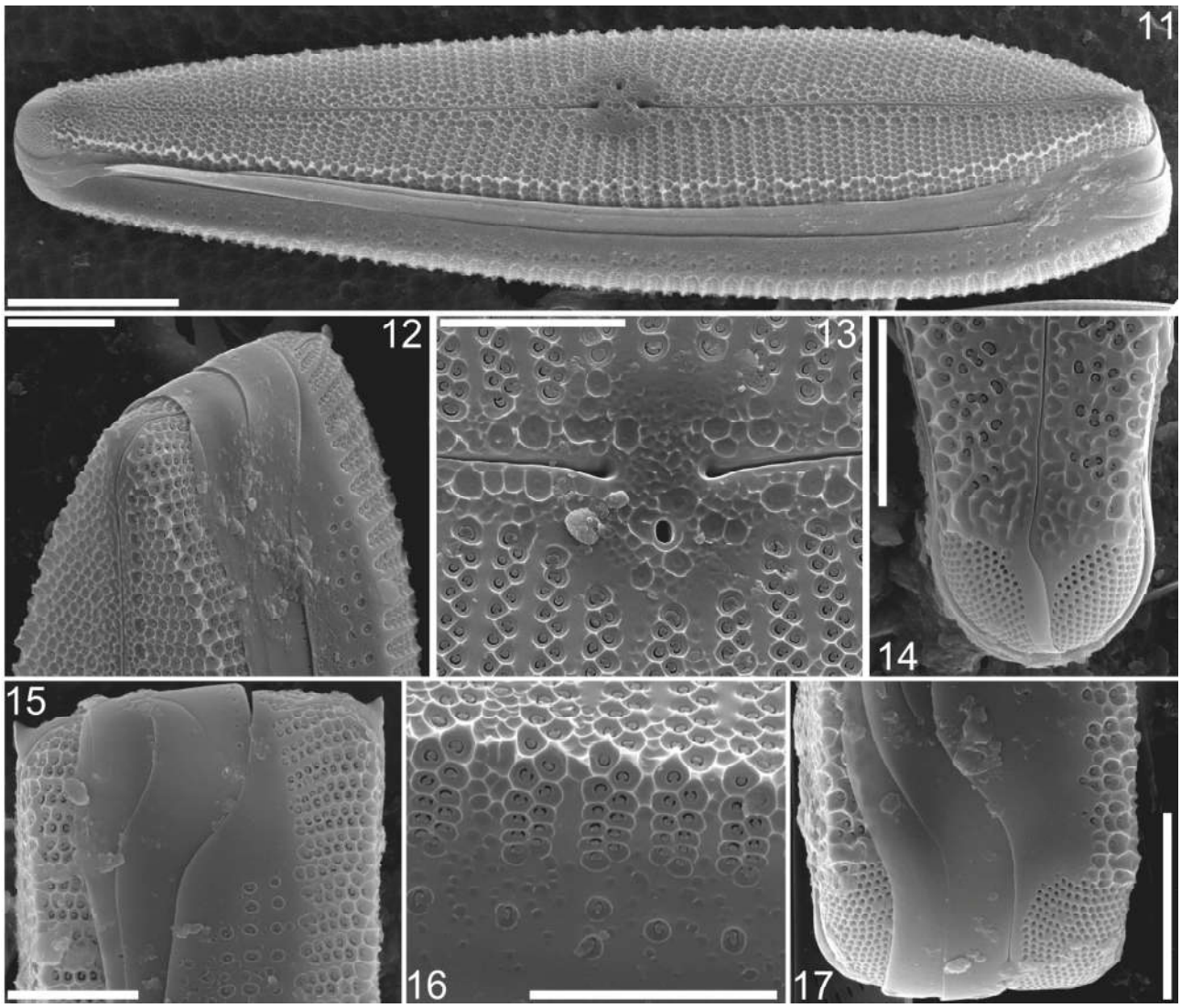
Ecology:—Freshwater, epiphytic, acidophilic taxon. At the sampling site in the Lomami River the following characteristics were measured on 24 November 2012 at the time of sampling: water temperature 25.7 °C, pH 6.06, conductivity 22.2 µS cm^{-1}, 0.060 mg l^{-1} NH_4, 0.005 mg l^{-1} NO_2, 0.446 mg l^{-1} NO_3, and 0.086 mg l^{-1} SRP.

Distribution:—only known from the type locality.

Discussion

Gomphonema grande differs from most species of the genus by its relatively large size, robust valves, unique shape, and distinctive suite of valve features. While there have been larger species described from African previously (West 1907, Kufferath 1956a, Kociolek & Stoermer 1991), none of these taxa, including *G. africanum* f. *turris* Kufferath (1956b: 37) which has a similarly-shaped headpole, have the distinctive set of features found in *G. grande*. For example, the presence of doubly-punctate striae is found in *G. grande* and several other species described from the African continent (Compère 1995, Passy *et al*. 1997). This feature, seen across many species of gomphonemoid diatoms (Dawson 1974,

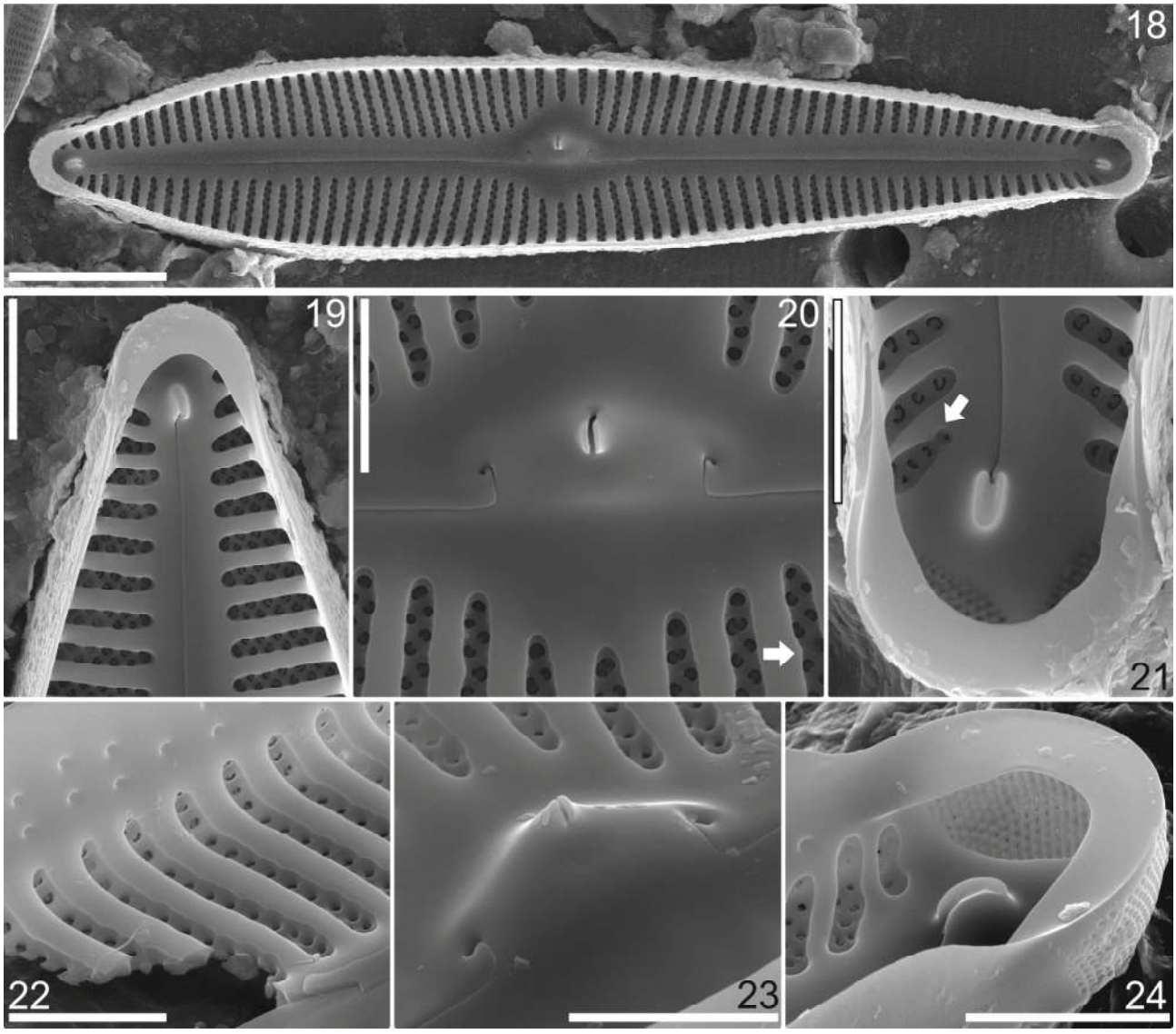
Hustedt 1942, Reichardt 2007, Kociolek & Stoermer 1988) is quite evident in *G. grande*. The large, round external stigma opening seen in *G. grande* is also present in *G. zairense* Compère (1995: 32) and *G. latistigmatum* Kociolek, Passy & Lowe in Passy *et al*. (1997: 463–464). At the ultrastructural level, the lip-like internal stigma opening is distinctive for *G. grande*. The only species we know of with doubly-punctate striae and a labium-shaped stigma opening is *G. zairense*, described from a waterfall of a tributary of the Congo River, also in Oriental Province in the Democratic Republic of the Congo (Compère 1995). The two species differ in size (*G. zairense* is smaller, reported as 34–54 µm long compared to 46–72 µm for *G. grande*), shape of the headpole (*G. zairense* has a rounded headpole, not protracted to the degree seen in *G. grande*) and the lack of a high degree of silicification in *G. zairense*.



FIGURES 11–17. *Gomphonema grande* from type material CCA 2071, SEM external views. 11. Whole valve exterior showing raised area of silica along the margin and sunken areolae within elevated outlines of silica. 12. Head pole showing single, small spine tapering in a shallow silica ridge on the valve mantle, and distal raphe curves onto valve mantle. 13. Central area showing tear drop shaped proximal raphe ends, stigmal opening and depressions. 14. Foot pole, with apical pore field composed of round porelli, physically and morphologically distinct from the areolae. 15. Girdle view of head pole with one spine on each valve. 16. View of valve showing the "C" shaped areolae extend on to the mantle. 17. Girdle view of the foot pole showing porelli extend on to the mantel and girdle band. Scale bar: 10 µm (Fig. 11), 4 µm (Figs 12–17).

The genus *Gomphonema* is diverse in terms of the number of character states seen for many of its features. For example, stigmata can be absent (*e.g*. many examples from South America, Metzeltin & Lange-Bertalot 1998) or present. If present, the internal stigma opening may be a simple round pore (Kociolek *et al*. in press), a slit (*e.g*. Dawson 1973), an elongated slit (Passy *et al*. 1997, Kociolek & Stoermer 1993, Karthick & Kociolek 2012), an opening covered with delicate siliceous coverings (Reichardt 1999) and, in the case of *G. grande*, an inwardly-projecting, labiate-like opening. Striae may be short, giving the impression of wide axial area (Reichardt 2005), or the striae may

extend towards the center to form a narrow axial area (Dawson 1972). Septa and pseudosepta can be present (Kociolek & Stoermer 1993) or lacking (Dawson 1973). Helictoglossae are found to be aligned with the raphe slit, or offset to one side (Karthick & Kociolek 2012, Liu *et al*. 2013, Kociolek *et al*. in press). Striae can be uniseriate or biseriate, and the openings can be slit-like (Kociolek *et al*. in press), C- or S-shaped or round with siliceous 'flaps' (Dawson 1973). While some have suggested that doubly-punctate striae are restricted to the closely related genus *Gomphoneis* Cleve (1894: 73) (Dawson 1974), others have shown the presence of doubly-punctate striae in numerous species of *Gomphonema* (Hustedt 1942, Lange-Bertalot 1980, Reichardt 2007, Kociolek *et al*. in press) and, conversely, there are species of *Gomphoneis* that have uniseriate striae (*e.g*. *G*. *cantalicum* Brun & Héribaud in Héribaud (1893: 219), Kociolek & Stoermer (1986)).



FIGURES 18–24. *Gomphonema grande* from type material CCA 2071, SEM internal views. 18. Whole valve internal view showing prominent central nodule, pseudosepta and helictoglossae. 19. Head pole showing helictoglossa offset from the main raphe branches and pseudoseptum. 20. Raised central nodule showing raphe ends recurved against the edge of the nodule and "lip-like" internal stigma opening. 21. Foot pole showing porelli, lobes of the apical pore field and helictoglossa. 22. Internal valve showing the extension of striae to the mantle. 23. Tilted view of raised central nodule with lip-like internal stigma opening. 24. Foot pool showing the helictoglossa and pseudoseptum. Scale bar: 10 µm (Fig. 18), 3 µm (Figs 12–17).

The thickened valves of *G*. *grande*, with a marginal ridge of silica along the outline and a small, thick spine at the headpole, are features shared with large, heavily-silicified species of gomphonemoid diatoms such as *Gomphoneis mammilla* (Ehrenberg 1854: 37) Cleve (1894: 73) (Kociolek & Rosen 1984), *Gomphonema imprime* Kociolek *et al*. (2004: 427–444) and *G*. *trulliforme* Kociolek *et al*. (2004: 427–444), and cymbelloid diatoms such as *Didymopshenia geminata* (Lyngbye 1819: 219) M. Schmidt (1899: 214) (Dawson 1974), as well as some centric diatoms (*Stephanodiscus*

superiorensis Stoermer & Theriot in Theriot & Stoermer (1984: 48). A spine at the head pole has also been described for *G. gomphopleuroides* Amossé (1969: 332) (Kociolek *et al*. 2015). These heavily-silicified valves appear to be the ways some species exist in high-silica environments (Theriot & Stoermer 1984).

At this point in time, large parts of the African continent, especially the Congo Basin, remain one of the least explored areas in terms of the diversity of its diatom flora. The continent has habitats ranging from tropical rain forest to dessert and even contains a unique biome, the Fynbos of the Western Cape, South Africa. In particular, concerning benthic diatoms, the study region covered in the present paper has been largely under-sampled when considering the relatively greater body of work on planktonic and lacustrine species. However, there is a growing interest in the diatoms of this region and we hope the research of which this paper forms a part will continue to find support and grow.

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TABLE 1. *Gomphonema* taxa described from Africa (including Madagascar, indicated with *).
G. angustissimum var. *africanum* Cholnoky (1959: 28)
G. acidoclinatiforme Metzeltin & Lange-Bertalot (2002: 31)*
G. aequatoriale Hustedt (1949: 119)
G. africanum G.S. West (1907:159)
G. africanum f. *turris* Kufferath (1956b: 37–38)
G. apicetrapezon Kufferath (1956b: 38)
G. brachyneura O. Müller (1905: 145)
G. cholnokyi Passy, Kociolek & Lowe (1997: 466–468)
G. clarkii Gasse (1980: 72)
G. clevei Fricke in Schmidt (1902: 234)
G. constrictum [var. *capitata*] f. *bipunctata* Kufferath (1957: 30)
G. crassestriatum Fritsch & Rich (1924: 389)
G. crocodilei Kociolek, Passy & Lowe (1997: 455)
G. dintara J.R. Carter & Denny (1982: 298)
G. eurycephalum Spaulding & Kociolek (1998: 365–366)*
G. evanescens Spaulding & Kociolek (1998: 363–365)*
G. hasta Metzeltin & Lange-Bertalot (2002: 33)*
G. invidia J.R. Carter & Denny (1982: 298)
G. frickei O. Müller (1905: 145)
G. kilhamii Kociolek & Stoermer (1991: 277)
G. latistigmata Kociolek, Passy & Lowe (1997: 463–464)
G. leemanniae Cholnoky (1954a: 415)
G. madagascarense Spaulding & Kociolek (1998: 363)*
G. migmatitum Woodhead & Tweed (1958b:201)
G. naviculla O. Müller (1905: 142)
G. oceanicum Cholnoky (1968: 36)
G. paddockii Kociolek & Stoermer (1991: 279)
G. parvuloides Cholnoky (1966: 23)
G. peracutum Spaulding & Kociolek (1998: 367–368)*
G. perinsignis Spaulding & Kociolek (1998: 362)*
G. perminutum Cholnoky (1957: 56)
G. pfannkucheae Cholnoky (1966: 25)
G. pulvillum Spaulding & Kociolek (1998: 366)*
G. quadratarea Spaulding & Kociolek (1998: 368)*
G. quasicrocodilei Passy, Kociolek & Lowe (1997: 458–459)
G. rautenbachiae Cholnoky (1959: 29)
G. resendei A.R. Moura (1963: 149)
G. schweickerdtii Cholnoky (1954: 143)
G. sonfonense Woodhead & Tweed (1958b: 201)
G. sparsistriatum O. Müller (1905: 144)
G. spauldingiae Metzeltin & Lange-Bertalot (2002: 34)
G. sphaerophorum var. *parvum* Cholnoky (1954a: 416)
G. stigmatellum Spaulding & Kociolek (1998: 367)*
G. streetii Gasse (1978: 110)
G. subapitatum Fritsch & Rich (1929: 109)
G. subapitatum f. *curtum* Fritsch & Rich (1929: 109)
G. subula J.R. Carter & Denny (1982: 299)
G. suhmii Foged (1966: 145)
*G. taiaensi*s J.R. Carter & Denny (1982: 299)
G. venustum Passy, Kociolek & Lowe (1997: 460–461)
G. westii Spaulding & Kociolek (1998: 367)*
G. wulasiense Foged (1966: 145)
G. wulasiense var. *nunguaensis* Foged (1966: 146)
G. wulasiense var. *voltaensis* Foged (1966: 146)
G. zairense Compère (1995: 32)
G. zambezicum Cholnoky (1954b: 416)

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Managing Editor
Maurice Kottelat, Rue des Rauraques 6
CH–2800 Delémont, Switzerland
Tel. +41 32 4623175 · Fax +41 32 4622259 · E-mail mkottelat@dplanet.ch

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Marcusenius kaninginii, a new species of elephantfish from the Lowa River basin, Democratic Republic of the Congo (Osteoglossiformes: Mormyridae)

Tchalondawa Kisekelwa*,, Gert Boden**, Jos Snoeks*,** and Emmanuel Vreven*,****

Marcusenius kaninginii, a new *Marcusenius* species with eight large circumpeduncular scales, is described from the Lowa River, a right bank tributary of the Lualaba (Upper Congo basin). It is distinguished from all other *Marcusenius* species with eight circumpeduncular scales by the following unique combination of characters: 43–47 lateral line scales; 18–23 dorsal-fin rays; 27–30 anal-fin rays; 42–44 vertebrae; a relatively shallow body at the anterior base of the anal fin (depth 25.0–28.6 % SL); a deep caudal peduncle (39–57 % of caudal peduncle length); and a short pectoral fin (15.5–19.1 % SL). Additionally, it has a typical dark-brown body coloration with a black transversal bar between the anterior parts of the dorsal and anal fins, and a black horizontal band along the lateral line from just behind the opercle up to the transversal bar.

Marcusenius kaninginii, une nouvelle espèce de *Marcusenius* à huit écailles autour du pédoncule caudal, est décrite de la rivière Lowa, un affluent de la rive droite du Lualaba (bassin du Congo Supérieur). Il se distingue des autres *Marcusenius* à huit écailles autour du pédoncule caudal par la combinaison unique des caractères suivants: 43–47 écailles sur la ligne latérale; 18–23 rayons à la dorsale; 27–30 rayons à l'anale; 42–44 vertèbres; corps relativement moins élevé à la base antérieure de la nageoire anale (hauteur 25,0–28,6 % LS); un pédoncule caudal haut, (39–57 % de la longueur du pédoncule caudal); et une nageoire pectorale courte (15,5–19,1 % LS). De plus, il présente une coloration corporelle typique noire-brunâtre avec une bande transversale noire entre les parties antérieures des nageoires dorsale et anale ainsi qu'une bande noire horizontale le long de la ligne latérale allant de l'opercule jusqu'à la bande transversale.

Introduction

The Mormyridae, or elephantfishes, are by far the largest and the most diverse family within the order of the Osteoglossiformes. Species of the family generally have an elongated to deep and laterally compressed body covered by small cycloid scales. The mouth is small and non-protrusible, the snout variable in form and sometimes tubular (Bigorne, 2003; Hopkins et al., 2007), and the chin

* KU Leuven, Laboratory of Biodiversity and Evolutionary Genomics, Charles Deberiotstraat 32, B–3000 Leuven, Belgium. E-mail: kisekelwa.tchalondawa@student.kuleuven.be; jos.snoeks@bio.kuleuven.be

** Royal Museum for Central Africa, Section Vertebrates, Ichthyology, Leuvensesteenweg 13, B–3080, Tervuren, Belgium. E-mail: kisengoja@yahoo.fr; Kisekelwa.Kise@africamuseum.be; gert.boden@africamuseum.be; jos.snoeks@africamuseum.be; emmanuel.vreven@africamuseum.be (corresponding author)

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occasionally bears a mental lobe. Mormyridae are widespread in Africa and are often a dominant group in riverine habitats in sub-Saharan Africa (Hopkins et al., 2007). Mormyrids have received considerable attention due to their remarkable ability to emit and detect electric signals for communication and object location (Boden et al., 1997). Their weak electric discharges are repeated pulses, which are often species- and even sex-specific (Hopkins et al., 2007).

Currently, 216 valid species of Mormyridae are reported belonging to 21 genera (Gosse, 1984; Poll & Gosse, 1995; Van der Bank & Kramer, 1996; Hopkins et al., 2007). Recently, multidisciplinary approaches including morphology, genetics and/or the study of the electric organ discharges (EODs) have considerably improved our systematic knowledge of many genera from western and southern Africa (Sullivan et al., 2000; Lavoué et al., 2004; Hopkins et al., 2007; Kramer et al., 2007; Kramer & Van der Bank, 2011; Kramer, 2013; Kramer & Wink, 2013; Maake et al., 2014). However, particularly in central Africa, many of the genera and species are poorly diagnosed (Boden et al., 1997; Teugels & Hopkins, 1998; Hopkins et al., 2007).

The genus *Marcusenius* Gill, 1862 is one of the many lesser known genera in Central Africa. The genus is diagnosed by the following unique combination of characters (Taverne, 1971): body moderately elongated; snout poorly developed and shorter than postorbital length; a fleshy submental swelling; lower jaw slightly longer than upper; nostrils situated at about mid-distance between eye and snout tip; caudal peduncle depth two to five times in its length; dorsal fin with 19–36 rays and shorter than anal fin with 25–43 rays; pectoral fin with 10–12 rays; pelvic fin with 6 rays; distance between pectoral and pelvic fins shorter than distance between pelvic and anal fins; caudal fin with 36–46 rays; 38–98 lateral line scales; 8–26/12–28 transversal line scales; 7–21/7–21 transversal line scales between dorsal and anal fins; 8–18 caudal-peduncle scales; and 3–8/3–10 conical or bicuspid oral jaw teeth.

With 40 valid species (Boden et al., 1997; Teugels et al., 2001; Kramer, 2013; Kramer & Wink, 2013; Maake et al., 2014), the genus *Marcusenius* is one of the largest genera within the mormyrids and forms a polyphyletic group (Lavoué et al., 2000). Based on the number of circumpeduncular scales, the *Marcusenius* species can be divided into two species groups (Boden et al., 1997): a first one containing the species with eight large scales, referred to as the large-scaled *Marcusenius* by Boden et al. (1997), and a second one composed of species with 12–18 smaller scales. Within the former group, Boden et al. (1997) recognized eight valid species: *M. dundoensis* (Poll, 1967), *M. fuscus* (Pellegrin, 1901), *M. ghesquierei* (Poll, 1945), *M. intermedius* Pellegrin, 1924, *M. kutuensis* (Boulenger, 1899), *M. moorii* (Günther, 1867); *M. sanagaensis* Boden, Teugels & Hopkins, 1997 and *M. schilthuisiae* (Boulenger, 1899). Most of these species are endemic to a few rivers of the Central Congo basin except for *M. moorii*, which is widespread in Lower Guinea and the Congo basin. The only other species of Lower Guinea, *M. sanagaensis*, is only known from the Sanaga River (Cameroon) (Boden et al., 1997).

Between 2009 and 2013, several expeditions were carried out by TK in the Lowa River, a right bank affluent of the Upper Congo River in the eastern part of the Democratic Republic of the Congo. During these expeditions, *Marcusenius* specimens with eight large circumpeduncular scales were collected, which at first sight were somewhat similar to *M. intermedius* and *M. moorii*. A detailed comparison with these species however revealed the Lowa specimens to be different. Therefore, the Lowa species is described below as a new species.

Materials and methods

A total of 57 *Marcusenius* specimens were examined, including 31 specimens from the Lowa River and 26 other specimens for comparative purposes. The latter group included the holotype and two additional specimens of *M. intermedius*, and 23 specimens of *M. moorii*, including its neotype, 11 specimens from the Ogowe basin (Gabon) from which *M. moorii* has been described, two from the Nyong basin (Cameroon) and nine from the cuvette central of the Congo basin (DRC) (see list of material examined).

A total of 13 counts and 28 point to point measurements were taken on each specimen. Meristics and measurements follow Boden et al. (1997), but with one additional measurement: the body depth at the anterior base of the anal fin.

Principal Component Analysis (PCA) was performed on the raw meristics using the correlation matrix while the log-transformed measurements were analysed using the covariance matrix

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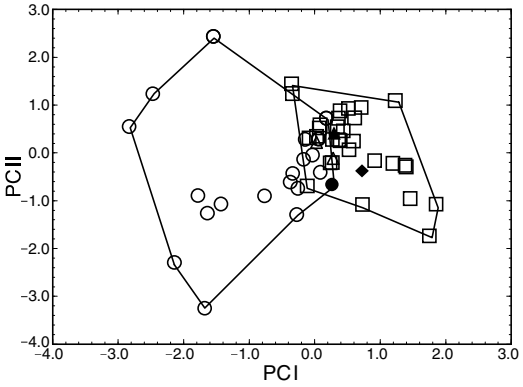


Fig. 1. Scatterplot of PCI against PCII for a PCA on 10 counts for all examined specimens (n = 57). *Marcusenius kaninginii*: ◆, holotype; □, other specimens; *M. intermedius*: ▲, holotype; △, other specimens; *M. moorii*: ●, neotype; ○, other specimens.

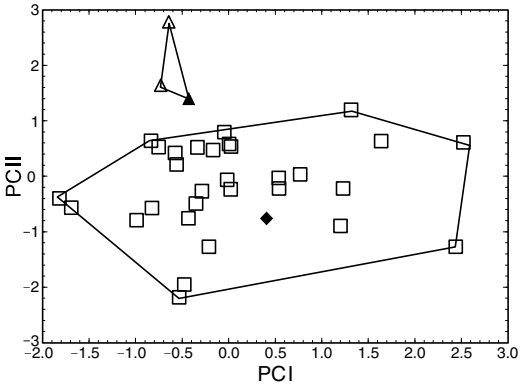


Fig. 2. Scatterplot of PC I against PC II for a PCA on 10 counts (n = 34) including *Marcusenius kaninginii* (n = 31) and only *M. intermedius* (n = 3); *M. kaninginii*: ◆, holotype; □, other specimens; *M. intermedius*: ▲, holotype; △, other specimens.

(Snoeks, 2004; Vreven & Snoeks, 2009). In the latter case, the first principal component (PC I) can be considered as a size factor if the variable loadings are of the same size and sign (Humphries et al., l981; Bookstein et al., 1985). Therefore, the scores of the second (PC II) and third component (PC III) were used to explore the dataset. PCAs were performed using STATISTICA for Windows version 12. Two invariable meristics, i. e. the scales around the caudal peduncle and the pelvic-fin rays, and one meristic variable with incomplete data, i.e. the scales covered by the dark transversal bar (SDB), were excluded from the analyses. Univariate comparisons of the raw meristic data, and percentages (% HL, SL or CPL) were performed using the non-parametric Mann-Whitney U-tests with the Bonferroni correction for multiple comparisons (Rice, 1989; Vreven & Teugels, 2005). To prevent interference of allometric growth-effects, one very large specimen of *M. moorii* was excluded from the analysis on the measurements. The most diagnostic variables were identified by using the scatterplots to visualize the amount of overlap between species for the examined variables. All locality data have been translated in English.

The following abbreviations are used: CPL, caudal peduncle length; DRC, Democratic Republic of the Congo; HL, head length; LS: longueur standard; MWU-test, Mann-Whitney U-test; RMCA, Royal Museum for Central Africa, Tervuren, Belgium; SL, standard length. Abbreviations of museum collections follow Sabaj Perez (2014); as such the acronym MRAC is used for the collection numbers of the Royal Museum for Central Africa, Tervuren, Belgium.

Results

Analysis of the meristic characters. A PCA was carried out on 10 meristics (n = 57). The most important loadings on PC I are for the number of scale rows between the dorsal and anal fins, the number of dorsal and anal-fin rays, the number of scales between the dorsal fin and lateral line and the total vertebrae number. The highest loadings on PC II are for the number of scales between dorsal fin and lateral line, scale rows between the pelvic fin and lateral line and the number of teeth on the upper jaw (Table 1a).

A plot of the PCA scores of PC I against PC II shows two major groups with partial overlap in the centre (Fig. 1). The group situated mostly on the positive side of the PC I, contains the specimens of *M. kaninginii* and specimens of *M. intermedius*, while the other group, situated almost fully on the negative side of the PC I consists of *M. moorii* specimens.

Two additional PCAs have been carried out on the meristic characters, including *M. kaninginii* and each of the two other species separately. In the analysis with *M. intermedius*, the most important loadings on PC I are for the number of scales between the dorsal fin and lateral line, the number of pectoral-fin rays, the number of scale rows between the dorsal and anal fins and

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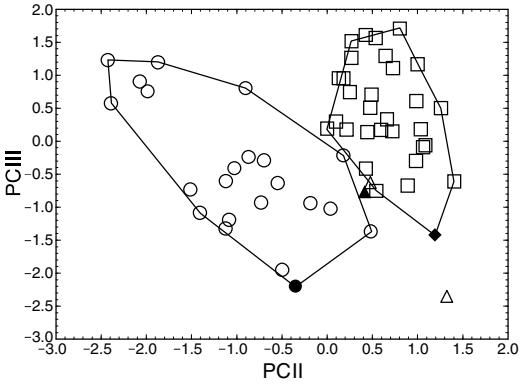


Fig. 3. Scatterplot of PCII against PCIII for a PCA on 27 log-transformed measurements for all examined specimens (n = 57); *Marcusenius kaninginii*: ◆, holotype; □, other specimens; *M. intermedius*: ▲, holotype; △, other specimens; *M. moorii*: ●, neotype; ○, other specimens.

those between the pelvic fin and the lateral line. The most important loadings on PCII are for the total vertebrae, the number of teeth in the lower jaw and dorsal-fin rays (Table 1b). Both species are clearly separated on PCII (Fig. 2) with the *M. intermedius* specimens situated on the positive side of PCII and all *M. kaninginii* specimens with lower values. In the second analysis, specimens of *M. kaninginii* were compared with *M. moorii* only. This resulted in a separation with partial overlap (results not shown), similar to the results of the first PCA.

MWU-tests, with sequential Bonferroni correction were performed on all meristics included in the PCA (Table 1c). Probably because of the low number of available specimens for *M. intermedius*, only one meristic, i. e. the total number of vertebrae, was found to be significantly different between *M. kaninginii* and *M. intermedius* ($p \leq 0.05$). Dorsal-fin ray and total vertebrae numbers were highly significantly different ($p \leq 0.001$) and anal-fin ray number was significantly different ($p \leq 0.05$) between *M. kaninginii* and *M. moorii*.

Comparison between *M. kaninginii* and *M. intermedius* revealed two important characters which allow a good diagnostic separation of both groups: the total vertebrae, 42–44 (median 43) vs. 40–41 (40), and the number of scales on lateral line, 43–47 (45) vs. 38–43 (41). Although three meristic variables are significantly different between specimens of *M. kaninginii* and *M. moorii*, there is still major or even complete overlap, preventing their use as good diagnostic meristic characters between both species.

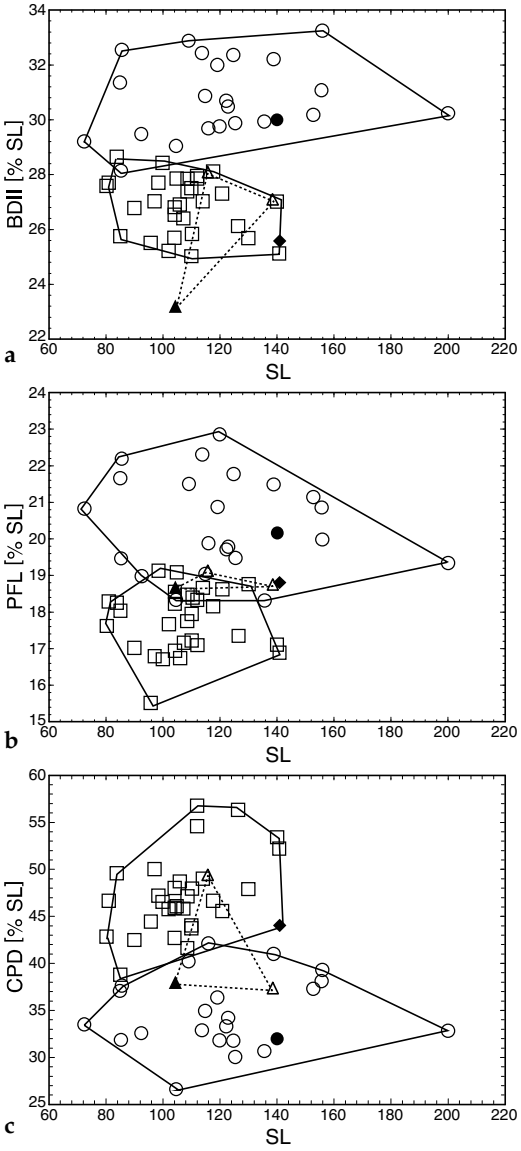


Fig. 4. Scatterplot of: **a,** body depth II, BDII (at anterior insertion of dorsal and anal fins in % SL) against SL (in mm); **b,** pectoral-fin length, PFL (in % SL) against SL (in mm); and **c,** caudal peduncle depth, CPD (in % caudal peduncle length against SL (in mm). *Marcusenius kaninginii*: ◆, holotype; □, other specimens; *M. intermedius*: ▲, holotype; △, other specimens; *M. moorii*: ●, neotype; ○, other specimens.

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Analyses of the measurements. A PCA was carried out on 27 log-transformed measurements (n = 57). The most important loadings on PC II are for the caudal peduncle depth, the middle caudal peduncle depth and the snout length. The most important loadings on PC III are for the distance between the nostril and eye, the caudal peduncle length and the eye diameter (Table 2a). In this analysis, the specimens of *M. kaninginii* are fully separated from the specimens of *M. moorii*, but slightly overlap with the *M. intermedius* specimens, which seem to occupy an intermediate position (Fig. 3).

No measurement was found to be significantly different between specimens of *M. kaninginii* and *M. intermedius* (Table 2b), while 12 of the 27 measurements were found to be significantly different ($p \leq 0.05$), nine of which highly significant ($p \leq 0.001$) between *M. kaninginii* and *M. moorii*. Even for these highly significant differences; a considerable overlap remained except for three, non allometric, diagnostic characters with only little overlap. The specimens of *M. kaninginii* have a shallower body at the anterior base of anal fin [25.0–28.6 (mean 26.8); vs. 28.1–33.5 (30.6) % SL in *M. moorii*] (Fig. 4a); a shorter pectoral-fin length [15.5–19.1 (17.7) vs. 17.7–22.8 (20.2) % SL)] (Fig. 4b); and a deeper caudal peduncle [39–57 (47) vs. 27–42 (34) % of its length] (Fig. 4c).

Marcusenius kaninginii, new species
(Fig. 5)

Holotype. MRAC B0-001-P-001, male, 141.0 mm SL; DRC: North-Kivu: Lowa River, Walikale, upstream waterfall and Lowa bridge in Walikale city, 221 km from Bukavu on Bukavu-Kisangani Road, 1°25'53" S 28°04'27" E; T. Kisekelwa, 15-20 February 2009.

Paratypes. MRAC B0-001-P-002–006, 5, 83.0–140.0 mm SL; same data as holotype.

Additional material. MRAC B0-025-P-0001–0013, 13, 80.3–110.0 mm SL; AMNH 264651 (ex MRAC B0-025-P-0023–0025), 3, 112.0–140.8 mm SL; BMNH 2016.1.12.1–3 (ex MRAC B0-025-P-0020–0022), 3, 110.0–117.6 mm SL; CUMV 98699 (ex MRAC B0-025-P-0017–0019), 3, 104.2–120.8 mm SL; ZSM 44645, 3 (ex MRAC B0-025-P-0014–0016), 3, 107.2–114.0 mm SL; same locality as holotype; March 2010.

Other material identified as *M. kaninginii*: MRAC B3-021-P-0033–0034, 2, 138.2–149.7 mm SL; DRC: North-Kivu: Itebero-Mpango, Luka River, Lowa basin, ± 1°40' 37" S 28°07'55" E; T. Kisekelwa & L. Dieudonné, 21 July 2013. – MRAC B3-021-P-0018, 1, 82.7 mm SL; MRAC B3-021-P-0019, 1, 136.0 mm SL; DRC: South-Kivu: Bunyakiri-Kambale, Bukavu-Hombo road, Shafu River, Lowa basin, 2°03'55" S 28°32'13" E; T. Kisekelwa, 18 July 2013. – MRAC B5-002-P-0001–0005, 5, 80.0–144.5 mm SL; DRC: North-Kivu: Isangi-KBNP, Elaimbi River,

Table 1. a, Principal component loadings for first three principal axes of a PCA performed on 10 counts for all three groups (n = 57): *Marcusenius kaninginii*, *M. intermedius* and *M. moorii*. Most important counts in bold. **b,** Idem for a PCA including *M. kaninginii* and *M. intermedius* only. **c,** Results of the MWU-tests comparing the 10 meristics included in the PCAs between the *M. kaninginii* (n = 31) and *M. intermedius* (n = 3) and between *M. kaninginii* and *M. moorii* (n = 23). Significance of differences based on sequential Bonferroni correction: NS, not significant; *, significant, $p \leq 0.05$; **, highly significant, $p \leq 0.001$.

	a. PC Loadings PCA I			**b.** PC Loadings PCA II			**c.** Results MWU-tests	
	PC I	PC II	PC III	PC I	PC II	PC III	*M. kaninginii* vs. *M. intermedius*	*M. kaninginii* vs. *M. moorii*
Scales on lateral line	−0.044	0.172	**−0.745**	−0.188	0.211	**0.658**	NS	NS
Dorsal-fin rays	**−0.452**	0.095	0.190	0.277	0.304	−0.125	NS	**
Anal-fin rays	**−0.382**	0.184	**−0.367**	0.295	0.163	**0.343**	NS	*
Pectoral-fin rays	0.232	0.262	−0.111	**−0.463**	0.137	−0.041	NS	NS
Scales between dorsal and anal fins	**−0.465**	−0.142	−0.142	**0.427**	0.173	0.285	NS	NS
Scales between dorsal fin and lateral line	**−0.428**	**−0.361**	−0.078	**0.498**	−0.121	0.014	NS	NS
Scales between pelvic fin and lateral line	0.110	**0.602**	−0.156	−0.328	−0.289	0.226	NS	NS
Teeth in upper jaw	0.275	**−0.473**	**−0.303**	0.076	−0.228	**0.391**	NS	NS
Teeth in lower jaw	0.112	−0.277	**−0.337**	−0.009	**−0.523**	**0.323**	NS	NS
Total vertebrae	0.308	−0.212	−0.074	−0.208	**0.603**	0.206	*	**

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Lowa basin, 1°49'30.8"S 28°11'06.6"E; T. Kisekelwa & M. Heri, 24 July 2014.

Diagnosis. *Marcusenius kaninginii* belongs to the group of *Marcusenius* species with eight large circumpeduncular scales. Within this group it can be distinguished from *M. dundoensis*, *M. fuscus* and *M. sanagaensis* by its lower number of lateral line scales (43–47 vs. 49–61). It differs from *M. ghesquierei* and *M. kutuensis* by having fewer dorsal-fin rays (18–23 vs. 27–34) and from *M. schilthuisiae* by fewer anal-fin rays (27–30 vs. 32–35). It is distinguished from *M. intermedius* by a higher number of lateral line scales (43–47 vs. 38–43) and higher total vertebrae number (42–44 vs. 40–41). Furthermore, it can be distinguished from *M. intermedius* by the terminal mouth position and the rounded shape of the snout (vs. mouth sub-terminal and the snout pointed). It is distinguished from *M. moorii* by the blackish longitudinal band along the lateral line; the shallower body at the anterior base of the anal-fin depth (25.0–28.4 % SL vs. 28.1–33.5; Fig. 4a), the shorter pectoral fin (15.5–19.1 % SL vs. 17.7–22.8; Fig. 4b); and the deeper caudal peduncle (39–57 % of its length vs. 27–42; Fig. 4c).

Description. Maximum size 141.0 mm SL. Counts and measurements are given in Table 3. Body elongate. Head profile straight and a little convex at head tip. Mouth terminal. Chin with a small sub-mental swelling. Upper jaw with 5 and lower jaw with 5–6 bicuspid teeth. One single row of teeth in both upper and lower jaws; in upper jaw,

Table 2. a, Principal component (PC) loadings for first three principal axes (PC) of a PCA performed on 27 measurements for all three groups (n = 57): *Marcusenius kaninginii*, *M. intermedius* and *M. moorii*. Most important measurements in bold. **b,** Results of MWU-tests comparing 27 measurements included in PCAs between *Marcusenius kaninginii* (n = 31) and *M. intermedius* (n = 3) and between the *M. kaninginii* and *M. moorii* (n = 22). Significance of differences based on sequential Bonferroni correction: NS, not significant; *, significant, $p \leq 0.05$; **, highly significant, $p \leq 0.001$.

	a. PC Loadings			**b.** MWU-tests	
	PC I	PC II	PC III	*M. kaninginii* vs. *M. intermedius*	*M. kaninginii* vs. *M. moorii*
Standard length	−0.194	0.004	−0.037	NS	NS
Head length	−0.183	0.009	0.003	NS	NS
Head depth	−0.206	−0.040	0.063	NS	NS
Head width	−0.196	0.013	−0.022	NS	NS
Snout length	−0.182	**0.322**	−0.091	NS	NS
Interorbital width	−0.171	0.090	−0.208	NS	NS
Eye diameter	−0.147	−0.228	**−0.325**	NS	NS
Postorbital length	−0.192	−0.019	−0.030	NS	NS
Distance between nostrils	−0.138	−0.126	0.105	NS	NS
Distance between nostril and eye	−0.203	−0.235	**−0.586**	NS	NS
Body depth I	−0.217	−0.052	0.225	NS	*
Body depth II	−0.218	−0.211	0.112	NS	**
Caudal peduncle depth	−0.168	**0.448**	0.072	NS	**
Middle caudal peduncle depth	−0.186	**0.430**	0.083	NS	**
Caudal peduncle length	−0.182	−0.257	**0.330**	NS	**
Predorsal distance	−0.187	0.136	−0.016	NS	**
Preanal distance	−0.190	0.125	−0.083	NS	**
Prepelvic distance	−0.182	0.126	−0.052	NS	*
Prepectoral distance	−0.176	0.074	−0.034	NS	NS
Dorsal-fin length	−0.208	**−0.286**	−0.064	NS	**
Dorsal-fin height	−0.196	−0.052	0.224	NS	*
Anal-fin length	−0.219	−0.177	−0.153	NS	NS
Anal-fin height	−0.205	−0.012	0.237	NS	NS
Pelvic-fin length	−0.206	−0.046	0.215	NS	**
Pectoral-fin length	−0.213	−0.213	0.241	NS	**
Distance between pelvic and anal fins	−0.205	0.150	−0.202	NS	NS
Distance between pectoral and anal fins	−0.200	0.153	−0.090	NS	NS

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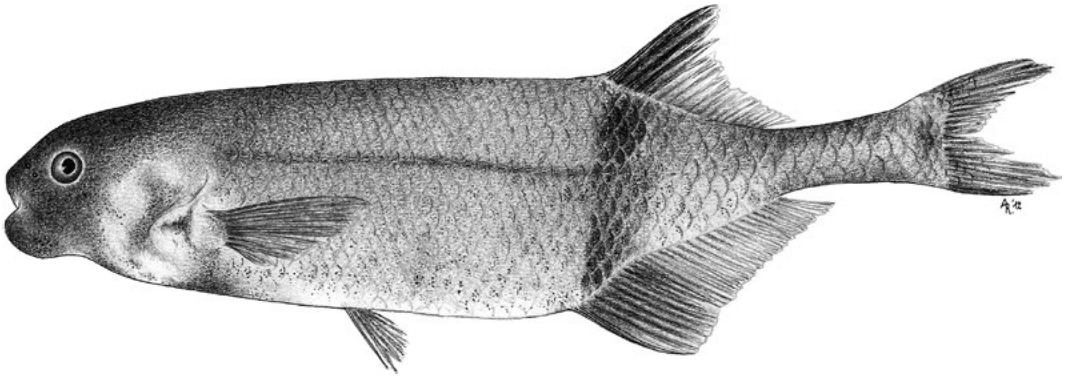


Fig. 5. *Marcusenius kaninginii*, MRAC B0-001-P-001, male, 141.0 mm SL; DRC: North-Kivu: Lowa River, Walikale. Drawing: Alain Reygel.

3 median teeth placed in a straight line, while 2 outer a little backwards; in lower jaw, teeth form a curved line. Eye small, situated in anterior half of head, somewhat nearer to dorsal margin of head. Upper and lower nostrils not in vertical plane, upper one more anteriorly than lower one.

Predorsal distance longer than preanal distance. Dorsal fin shorter than anal fin, originating slightly behind origin of anal fin; ratio dorsal-fin/anal-fin length 0.6–0.9 (mean 0.8). Body depth at middle of body equal to body depth at level of anal-fin insertion. Distance between pectoral and anal fins slightly shorter than pre-pelvic distance. Scales on the body just behind the head small, becoming larger more posteriorly.

Colour pattern. Live specimens dark-brown in overall colour. In addition, a transversal black bar between anterior parts of anal and dorsal fins covering 3–4 consecutive scales at level of lateral line, and extending on to anterior parts of dorsal and anal fins. Further, a black mid-longitudinal band from posterior edge of head near operculum to black transversal bar. Caudal peduncle dark. Often two additional vague black markings on fresh as well as on recently preserved specimens: a first one near anterior origin of lateral line; a second one towards posterior end of caudal peduncle. After preservation, dark background colour of body fading. As a result, black mid-longitudinal band standing out much more clearly.

Distribution and ecology. *Marcusenius kaninginii* is known from the middle Lowa basin and has been collected in the stretch downstream of the Wenga Falls and upstream of the Boboro Falls as well as in some tributaries draining this area (Fig. 6). All types and additional specimens have been collected upstream of the Lowa bridge. Other specimens have later been collected in two left bank affluents, Elaimbi River, an affluent of the Luka River, draining the north-eastern border of the Kahuzi-Biega National Park (KBNP) and in Shafu brook (Luhoho River tributary) near Bunyakiri.

Marcusenius kaninginii has mainly been collected in shallow water (±1–2 m), near the shores. It has been collected at stations with low water velocity, near roots of vegetation or dead wood and at places with foliage-shade. The following parameters were measured at the type locality on 30 December 2010: water temperature ±21.5 °C (at 10.00 a.m.); pH nearly neutral, 7.0; dissolved O_2

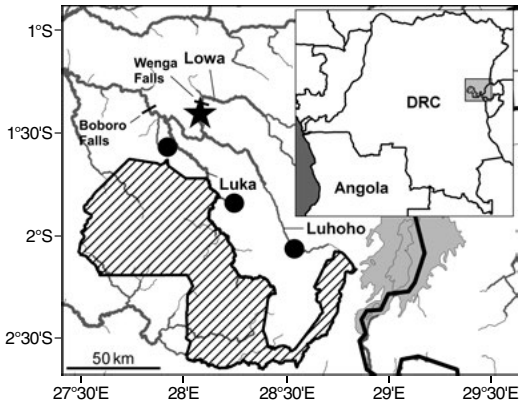


Fig. 6. Distribution of *Marcusenius kaninginii* within middle Lowa basin, DRC. ★, type locality; ●, other localities; striped area includes Kahuzi-Biega National Park.

Table 3. Measurements and counts for *M. kaninginii*, *M. intermedius* and *M. moorii*. H, holotype; N, neotype.

	M. kaninginii			*M. intermedius*			*M. moorii*		
	H	(n = 30)		H	(n = 2)		N	(n = 22)	
		mean ± SD	range		mean ± SD	range		means ± SD	range
Standard length (mm)	141		80.2–140.8	104		115–138	140		72–200
In percentage of head length									
Head depth	84	84.9 ± 4.7	77–93	70	81.4 ± 0.7	80–82	95	89 ± 6.0	78–102
Head width	50	49.8 ± 2.5	44–54	53	50.2 ± 1.0	49–51	53	49 ± 1.8	46–52
Snout length	28	29.7 ± 1.7	24.5–33	28	29.7 ± 1.6	28–31	28	26 ± 1.8	22–29
Interorbital width	36	39.4 ± 3.1	31–44	37	37.8 ± 3.4	34–41	40	36 ± 2.2	32–40
Eye diameter	14	16.6 ± 1.2	14–19	15	13.3 ± 2.4	10–15	17	17 ± 2.1	12–21
Postorbital length	57	57.5 ± 1.9	53–62	56	56.9 ± 1.3	55–58	56	58 ± 2.6	53–63
Distance between nostrils	6	6.8 ± 0.4	5–8	6	6.8 ± 0.3	6–7	6	7 ± 1.1	5–9
Distance between nostril and eye	6	8.2 ± 0.9	6–9	7	7.8 ± 0.5	7–8	6	8 ± 1.0	6–10
In percentage of standard length									
Head length	23.3	24.0 ± 0.8	22.0–26.2	23.4	23.3 ± 0.6	22.6–23.9	22.2	24.2 ± 1.1	22.1–26.5
Body depth I	23.0	23.6 ± 1.5	20.9–26.2	23.4	27.1 ± 0.9	26.2–28.1	29.8	25.3 ± 2.1	21.3–29.1
Body depth II	25.6	26.8 ± 1.0	25.0–28.6	23.2	27.5 ± 0.4	27.1–28.0	30.0	30.8 ± 1.3	28.1–33.2
Caudal peduncle length	15.8	15.1 ± 1.2	12.8–18.3	16.6	16.3 ± 0.8	15.5–17.1	19.1	17.2 ± 1.0	15.8–20.0
Predorsal distance	66.3	66.6 ± 1.4	63.7–69.7	68.3	68.2 ± 1.7	66.4–69.9	62.6	63.4 ± 2.0	59.3–66.5
Preanal distance	61.3	62.5 ± 1.3	59.5–65.3	61.5	61.7 ± 1.0	60.6–62.7	57.8	59.7 ± 2.0	54.1–63.2
Prepelvic distance	39.2	39.2 ± 1.0	37.1–41.0	39.6	40.2 ± 1.2	38.9–41.4	35.9	37.4 ± 1.6	33.2–40.7
Prepectoral distance	23.9	24.0 ± 0.8	22.2–25.4	23.2	24.0 ± 1.1	22.9–25.2	21.4	23.3 ± 1.1	20.6–25.9
Dorsal-fin length	17.2	18.7 ± 1.1	16.8–21.2	17.8	17.9 ± 1.4	16.5–19.3	20.8	20.7 ± 1.7	17.4–25.8
Dorsal-fin height	16.2	15.9 ± 0.9	14.0–17.6	15.8	17.0 ± 0.3	16.6–17.3	16.7	16.8 ± 1.0	14.6–18.2
Anal-fin length	25.9	24.6 ± 1.2	21.2–26.6	23.9	23.5 ± 0.6	22.9–24.2	24.2	26.5 ± 2.3	23.3–31.1
Anal-fin height	15.3	15.4 ± 0.9	13.6–17.3	15.6	16.0 ± 0.0	15.6–16.0	16.6	16.1 ± 1.0	14.0–18.3
Pelvic-fin length	9.8	9.4 ± 0.4	8.5–10.4	9.9	10.5 ± 0.2	10.2–10.7	9.9	10.2 ± 0.6	8.4–11.0
Pectoral-fin length	18.8	17.7 ± 0.8	15.5–19.1	18.7	18.9 ± 0.1	18.7–19.1	20.2	20.4 ± 1.2	18.3–22.8
Distance between pelvic and anal fins	23.1	24.1 ± 1.4	21.7–26.3	21.6	22.3 ± 0.5	21.8–22.8	23.1	22.9 ± 1.2	20.7–26.7
Distance between pectoral and anal fins	37.4	39.2 ± 1.4	36.5–43.0	37.2	38.3 ± 3.1	35.2–41.4	37.8	37.4 ± 2.1	32.7–41.3
In percentage of caudal peduncle length									
Caudal peduncle depth	44	48 ± 4	39–57	38	43 ± 9	37–49	32	35 ± 4	27–42
Middle caudal peduncle depth	42	44 ± 4	37–55	37	43 ± 9	37–49	31	34 ± 4	24–42
		median	range		median	range		median	range
Scales on lateral line	45	44	43–47	43	41	38–43	41	43	40–47
Dorsal-fin rays	22	21	18–23	21	22	21–22	23	25	22–29
Anal-fin rays	30	29	27–30	28	28	28	29	31	27–34
Pelvic-fin rays	6	6	6	6	6	6	6	6	6
Pectoral-fin rays	10	10	9–12	10	10	10	11	10	10–11
Scales around caudal peduncle	8	8	8	8	8	8	8	8	8
Scales between dorsal fin and anal fin	16	17	16–19	17	17	17	17	18	17–20
Scales between dorsal fin and lateral line	7	8	6–9	8	8	8	8	8	8–10
Scales between pelvic fin and lateral line	10	10	9–11	10	111	10–11	11	101	9–11
Teeth in upper jaw	5	5	4–6	5	5	5.0	5	4	1–6
Teeth in lower jaw	5	6	3–6	6	6	6	6	5	2–6
Total vertebrae	43	43	42–44	41	40 ± 0	40–41	41	42	40–43

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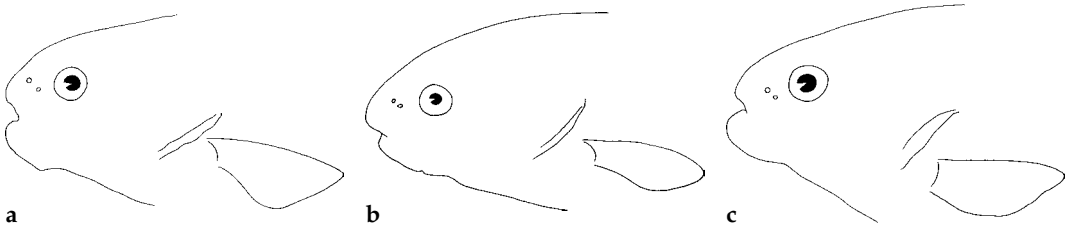


Fig. 7. Schematic illustration of head region of: **a,** *Marcusenius kaninginii*, MRAC B0-001-P-001-001, holotype, 141.0 mm SL; **b,** *M. intermedius*, MRAC 15164, holotype, 104.4 mm SL; **c,** *M. moorii*, MRAC 87685, neotype, 140.0 mm SL.

about 8.0 mg · l^{-1} (91 % of saturation); conductivity relatively low, 58.7 µS · cm^{-1}; total solid matter 173.2 mg · l^{-1}; a low concentration of nutrients: 0.134 mg · l^{-1} for phosphates, 0.007 mg · l^{-1} for nitrites and 0.068 mg · l^{-1} for ammonium, and a relatively high concentration of 1.354 mg · l^{-1} for silica.

Etymology. *Marcusenius kaninginii* is dedicated to Professor Boniface Kaningini Mwenyimali, Director of UERHA (Unité d'Enseignement et de Recherche en Hydrobiologie Appliquée) and Rector of ISP/Bukavu (DRC). He supported in various ways the sampling expeditions of TK in the Lowa system.

Local knowledge. *Marcusenius kaninginii* appears not to be abundant in the region and, as a result, is most of the time absent from the local markets. The fish is not well appreciated and most often only used as food for the fishers' families.

According to local fishermen, *M. kaninginii* is scarcely caught with hooks; therefore it is qualified as a crafty species. Hence, fykes baited with earthworm are used to catch this species. *Marcusenius kaninginii* is often caught syntopically with other Mormyrid species such as *Mormyrus caballus*, *Myomyrus macrops* and *Pollimyrus isidori osborni*.

Discussion

Within the group of *Marcusenius* species with eight large circumpeduncular scales, *M. kaninginii* is most similar to *M. intermedius* and *M. moorii*. The holotype of *M. intermedius* was collected at Kabambaie while the two additional specimens were collected at Koteli and at Komi, Lodja; all situated in the Kasaï basin. *Marcusenius moorii*, is more widespread. In addition to its occurrence in the Lower Guinea ichthyofaunal province, [from the Sanaga basin (Cameroon) in the north to the Kouilou basin (Republic of Congo) in the south], it is also known from the Congo ichthyofaunal province where its distribution is limited to the Lower and Middle Congo (Hopkins et al., 2007). Currently, *M. kaninginii* is only known from a small stretch of the Lowa basin. It is the first *Marcusenius* species with eight circumpeduncular scales to be reported from the Lualaba (Upper Congo) system, i. e. above the Wagenia Falls.

Within the Lowa basin, *M. kaninginii* is known from the main channel and its affluents in-between Wenga Falls and Boboro Falls. The upstream barrier, i. e. the Wenga Falls, are situated about ± 9 km upstream while the downstream one, i. e. the Boboro Falls, are situated about ± 30 km downstream of the type locality. These falls, probably form an effective barrier against upstream fish migration, and might well constitute the upper and lower edges of the natural distribution of *M. kaninginii* in the basin. Indeed some Mochokidae and Malapteruridae seem to be limited to the Lowa stretch downstream of the Boboro Falls and as such are absent from above the Boboro falls where *M. kaninginii* can be found (Kisekelwa et al., 2013).

The mouth of *M. kaninginii* is terminal with a rounded snout, a small sub-mental swelling, and a slightly convex upper head margin anteriorly, becoming rather straight more posteriorly (Fig. 7a). Instead, *M. intermedius* clearly has a sub-inferior mouth and a more pointed snout than *M. kaninginii*, and a more convex upper margin of the head for over its entirely head length (Fig. 7b). The mouth position and snout shape of *M. moorii* is very similar to that of *M. kaninginii* but with a more pronounced snout showing a more straight upper head margin (Fig. 7c).

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With regard to the colour pattern, the transverse black bar situated between the dorsal and anal fins is always present in *M. kaninginii* and covers 3–4 subsequent scales. This bar is only present in some *M. moorii* specimens where it covers about 3 subsequent scales. Boden et al. (1997) already reported this bar to be present in some *M. moorii* specimens and when present to cover 2 scales or more. In *M. intermedius*, likewise the transversal black bar is present on the holotype and one of the two additional specimens (MRAC 15164, 29260). In addition, *M. kaninginii* has a well-marked black longitudinal band on the lateral line scales between the posterior margin of the head and the transversal black bar. Although not reported by Pellegrin (1924), a longitudinal black band seems to be present on the holotype and one of the two additional specimens of *M. intermedius* (MRAC 15164, 29260). Observations on newly collected, live specimens are needed to further document the occurrence of both black markings in *M. intermedius*. In *M. moorii*, however, the latter band is clearly absent (see also Boden et al., 1997). Further, the general body profile of *M. kaninginii* is similar to that of *M. intermedius*, but it is quite different from that of *M. moorii*. The body depth of *M. kaninginii* reaches its maximum at level of pectoral-fin base and remains deep up to the dorsal- and anal-fin origins. The body depth of *M. moorii* instead reaches its maximum at the level of the dorsal- and anal-fin origins.

Some mormyrids exhibit sexual dimorphism formerly described as anal-fin indentation in males (Pezzanite & Moller, 1998). This is true also for the males of *M. kaninginii*, which present both the anal-fin ray extension and an indentation of the posterior ventral body wall (see Fig. 5).

Marcusenius kaninginii is found in the Lowa River basin near Walikale. The headwaters of the Lowa belong to the Albertine Highlands Ecoregion and extends downstream to the Upper Congo ecoregion, as defined by Thieme et al. (2005). The former ecoregion includes the Kahuzi-Biega National Park (KBNP), which partially includes the Lowa basin (Brown & Abell, 2005). Some of the *M. kaninginii* specimens have been collected in the Luka, a left bank tributary of the middle Lowa River and in its tributary, the Elaimbi River. The Luka forms the main river of the Lowa basin draining the north-eastern part of the KBNP (ICCN-PNKB, 2009). Brown & Abell (2005) already predicted that further exploration of the rivers in this ecoregion would reveal new fish species. The current description confirms their point of view. Mining exploitation, deforestation and poaching are the main threats that may impact the fish diversity in the Albertine highlands area (Brown & Abell, 2005). In addition, the KBNP, well known for the presence of the eastern lowland Gorilla currently still lacks a fish conservation protocol within its strategic management plan (ICCN-PNKB, 2009). It is hoped that the discovery of this new fish species, will generate new impetus for fish conservation in this ichthyologically under-studied area.

Comparative material. *Marcusenius intermedius*: MRAC 15164, holotype, 104.4 mm SL; DRC: Kabambaie, 5°45'S 20°49'E. – MRAC 23198, 1, 115.7 mm SL; DRC: Koteli, 2°51'N 24°34'E. – MRAC 29260, 1, 138.5 mm SL; DRC: Komi, Lodja, 3°30'S 23°23'E.

M. moorii: MRAC 87685, neotype, 140.0 mm SL; Gabon: Talagouga, Ogowe, 0°10'S 10°43'E. – MRAC 73-18-P-11, 1, 155.9 mm SL; Cameroun: Nyong, Ebogo River, 3°23'N 11°28'E. – MRAC 73-18-P-12, 1, 155.7 mm SL; Cameroon: Nyong, Ebogo River, 3°23'N 11°28'E. – MRAC 83-31-P-264–265, 2, 138.8–152.7 mm SL; DRC: Kisangani, Congo River, 0°30'N 25°12'E. – MRAC 189293, 1, 104.6 mm SL; DRC: Congo River, Water meadow langs, Lindi River, Kisangani, 0°33'N 25°05'E. – MRAC 137968, 1, 124.1 mm SL; DRC: Yangole, Lilanda River, 0°48'N 24°17'E. – MRAC 68689, 1, 92.0 mm SL; DRC: Yangambi, Boonde River, 0°47'N 24°28'E. – MRAC 77-25-P-34, 1, 59.2 mm SL; DRC: Lona River, 02°28'S 22°56'E. – MRAC 131495–131496, 1 of 2, 122.2 mm SL; DRC: Lomela territory, Yaekama, Biaka marsh, 0°47'N 24°18'E. – MRAC 21580, 1, 49.2 mm SL; DRC: Lomami, 02°23'S 23°34'E. – MRAC 19805, 1, 94.9 mm SL; DRC: Stanleyville, 0°30'N 25°12'E. – MRAC 73-23-P-862–864, 3, 101.9–107.7 mm SL; DRC: Boende, Tshuapa River, 0°14'S 20°50'E. – MRAC 98825–98838, 6 of 14, 113.7–200.0 mm SL; DRC: Boende, Tshuapa River, 0°14'S 20°50'E. – MRAC A1-088-P-0128–0129, 1, 115.9 mm SL; Gabon: Road Koulamoutou–Mbigou, Bouenguidi River, at Pangani, 1°12.94'S 12°26.74'E. – MRAC 98-029-P-4197–4234, 4 of 37, 72.4–109.0 mm SL; Gabon: Ivindo, mouth of the Nounat, 1°10'53"N 13°06'34"E. MRAC A0-048-P-4483–4491, 6 of 9, 85.3–122.9 mm SL; Gabon: Mbilaten, Ogowe River, mouth of Ananga River, ± 0°17'S 10°29'E.

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Notropis megalops (photograph by Kevin W. Conway)
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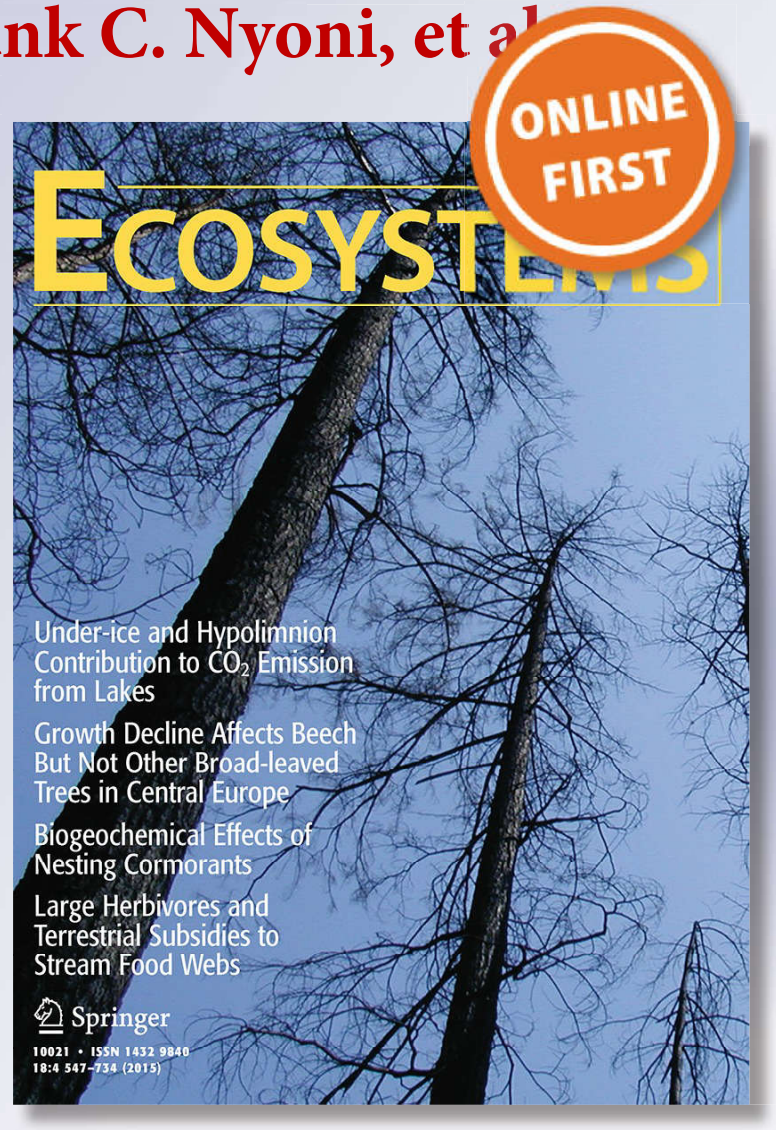
Landscape Control on the Spatial and Temporal Variability of Chromophoric Dissolved Organic Matter and Dissolved Organic Carbon in Large African Rivers

Thibault Lambert, François Darchambeau, Steven Bouillon, Bassirou Alhou, Jean-Daniel Mbega, Cristian R. Teodoru, Frank C. Nyoni, et al

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Landscape Control on the Spatial and Temporal Variability of Chromophoric Dissolved Organic Matter and Dissolved Organic Carbon in Large African Rivers

Thibault Lambert,[1]* François Darchambeau,[1] Steven Bouillon,[2] Bassirou Alhou,[3] Jean-Daniel Mbega,[4] Cristian R. Teodoru,[2] Frank C. Nyoni,[5] Philippe Massicotte,[6] and Alberto V. Borges[1]

[1]*Chemical Oceanography Unit, University of Liège, Liège, Belgium;* [2]*Department Earth and Environmental Sciences, K.U. Leuven, Leuven, Belgium;* [3]*University of Niamey, Niamey, Niger;* [4]*Institut de Recherches Agronomiques et Forestières du Gabon, Libreville, Gabon;* [5]*Integrated Water Resources Management Center, University of Zambia, Lusaka, Zambia;* [6]*Département des sciences de l'environnement, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, Quebec G9A 5H7, Canada*

Abstract

The characteristics of colored dissolved organic matter (CDOM) as well as the concentrations and stable isotope composition ($\delta^{13}C$) of dissolved organic carbon (DOC) were characterized in several large rivers of Africa including the Congo, Niger, Zambezi, and Ogooué basins. We compared the spatial and temporal patterns of dissolved organic matter (DOM) quantity and quality along with various environmental gradients, including hydrology, river size, catchment vegetation, and connectivity to land. The optical proxies used include the absorption coefficient at 350 nm, the specific ultra-violet absorbance, and the spectral slope ratio (S_R = 275–295-nm slope divided by 350–400-nm slope). Our results show that land cover plays a primary role in controlling both DOC concentration and optical properties of DOM in tropical freshwaters. A higher cover of dense forest in the catchment leads to a higher quantity of highly aromatic DOM in the river network, whereas an increasing savannah cover results in lower DOC concentrations and less absorptive DOM. In addition to land cover, the watershed morphology (expressed by the average slope) exerts a strong control on DOC and CDOM in tropical rivers. Our results also show that the percentage of C3 and C4 vegetation cover is not an accurate predictor for DOM and CDOM quality in rivers due to the importance of the spatial distribution of land cover within the drainage network. The comparison of our results with previously published CDOM data in temperate and high-latitude rivers highlights that DOM in tropical freshwaters is generally more aromatic, and shows a higher capacity for absorbing sunlight irradiance.

Key words: Carbon cycle; Colored dissolved organic matter; Tropical rivers; Land cover; Landscape; Carbon isotopes.

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*Corresponding author; e-mail: tlambert@ulg.ac.be

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Introduction

The role of inland waters in the global carbon (C) cycle is now widely recognized to go beyond the downstream transport of C, but includes production, mineralization, storage, and emission of C to the atmosphere (Cole and others 2007; Battin and others 2008; Ward and others 2013; Abril and others 2014; Fasching and others 2014). A significant amount of terrestrial dissolved organic matter (DOM) is degraded in freshwaters through photochemical and biological mineralization and returns to the atmosphere as CO_2 (Cole and others 2007). However, the mechanisms underlying the relationship between DOM and CO_2 concentrations reported in temperate and boreal ecosystems (Sobek and others 2005; Lapierre and del Giorgio 2012), and more recently in African tropical inland waters (Borges and others 2015) are still debated (Lapierre and others 2013; Fasching and others 2014; Borges and others 2015). To date, most studies focusing on sources, composition, and fate of DOM and its link with CO_2 emissions have been conducted in temperate and high-latitude rivers due to their susceptibility to global climate change and anthropogenic activities (Wilson and Xenopoulos 2009; Stedmon and others 2011). Tropical rivers exhibit the highest riverine dissolved organic carbon (DOC) flux to the oceans (Meybeck 1993) but DOM cycling at the terrestrial–aquatic interface in these systems is poorly constrained with the exception of the Amazon River (Mayorga and others 2005; Pérez and others 2011; Remington and others 2011; Ward and others 2013).

The characterization of the chromophoric or colored fraction of DOM (CDOM) has been proposed as a powerful approach to study the composition, sources, and reactivity of DOM in aquatic ecosystems for numerous reasons. First, as the chemical composition of DOM determines its optical properties (Stedmon and Markager 2005), optical measurements allow us to trace DOM sources and processing in the river network (Jaffé and others 2008). Secondly, the measurement of the optical properties of DOM via in situ continuous measurement technologies can be used to assess DOC content, quality, and transport in freshwaters at high spatial or temporal resolution (for example, Downing and others 2009; Prairie and others 2010). Thirdly, remote sensing of CDOM by optical sensors on satellites is a powerful tool to study the distribution of terrestrial DOM in inland waters (for example, Massicotte and others 2013), and its fate in river plumes of major world rivers (for example, Salisbury and others 2011). Fourthly, recent studies have shown that terrestrial inputs of CDOM are a relevant component of the C cycle in inland waters by demonstrating a causal link between CDOM and riverine fluxes of CO_2 through photodegradation and microbial degradation processes (Lapierre and others 2013; Fasching and others 2014). Finally, CDOM measurements are an ideal tool for the study of DOM through extensive sampling programs (Jaffé and others 2008). Such studies have recently been performed in temperate (Spencer and others 2012) and Arctic rivers (Stedmon and others 2011), but to date no equivalent effort has been made for tropical rivers.

Land cover, in particular the areal extent of wetlands at the catchment scale, has been identified as a strong predictor for DOM concentration and composition in rivers (Eckhardt and Moore 1990; Frost and others 2006), suggesting that the role of riverine processes in altering the terrestrial DOC signal at the annual scale was minimal except in river systems with long surface water residence times (Hanley and others 2013). However, such analyses are mostly limited to temperate and boreal catchments and very few studies have assessed the effect of land cover and other catchment features on DOM in tropical rivers. However, the situation is likely to be different in tropical basins, as (1) wetlands of tropical landscapes, comprising swamps, marshes and seasonally or permanently inundated floodplains, consist of forest and/or savannah vegetation (Mayaux and others 2004) and (2) tropical rivers have been shown to exhibit contrasting DOC concentrations and CDOM properties depending on if they flow on forest-dominated or savannah-dominated basins (for example, Bouillon and others 2014; Mann and others 2014). Moreover, watershed morphology also influences riverine DOM concentration and composition by affecting, in part, the preferential hydrological connectivity between the vegetation unit fringing the river channel and receiving waters (Bird and Pousai 1997; Marwick and others 2014) and the residence time of the hydrological flow path in DOC-rich upper soil layers (Rasmussen and others 1989). In addition, catchments with similar land cover but contrasting soil properties can exhibit small scale differences in both the concentration and the chemical composition of stream DOM (McClain and others 1997). Thus, the dominant land cover within a catchment may be inadequate to predict DOM content and quality in rivers and more detailed studies are warranted to assess how the landscape morphology in term of size, shape, and

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Table 1. Drainage Area, Discharge, Precipitation, and Dominant Soil Types of the Study Sites

Basin	Drainage area ($\times 10^6$ km^2)	Rank[1]	Discharge (km^3 yr^{-1})	Precipitation range (mm yr^{-1})	Dominants soil types[2]
Congo	3.7	1	1200	1400–2500	Ferralsols (50%), Arenosols (15%), Acrisols (10%), Gleysols (5%)
Niger	2.1	3	154	180–2100	Arenosols (25%), Leptosols (19%), Lixisols (13%), Plinthosols (14%)
Ogooué	0.2	14	150	1600–2200	Ferralsols (29%), Cambisols (29%), Plinthosols (14%), Arenosols (9%)
Zambezi	1.4	4	103	400–1500	Arenosols (26%), Leptosols (14%), Ferralsols (11%), Luvisols (11%)

[1]*Refers to the rank of the basin in terms of drainage area in the African continent.*
[2]*World Reference Base for Soil Resources (WRB) classification, from (Dewitte and others 2013).*

composition controls DOM concentration and composition in tropical ecosystems.

In this study, we aimed to assess how the various landscapes of major African basins affect DOC and CDOM patterns in rivers, and complements the need to explore the link between DOM and recent estimates of CO_2 emissions by African inland waters (Borges and others 2015). CDOM data were collected at biweekly frequency during a period between 1 and 2 years in several major tropical rivers of Africa, including the Congo, the Niger, the Zambezi as well as the Ogooué. In addition, our analysis integrates CDOM data obtained from several sampling campaigns aiming at describing spatial variability carried out during both wet and dry seasons in the Congo and the Zambezi. The dominant land cover of drainage basins ranged from typical rainforest (Ogooué River) to typical savannah (Niger River), with the Congo and Zambezi showing a mixture of both vegetation cover. Combined with data on the concentrations and the stable carbon isotope composition (δ^{13}C) of DOC, we used this large dataset to compare the spatial patterns of CDOM quality along a gradient of watershed morphology and vegetation cover. Finally, we compared this dataset with previously published CDOM data from temperate and Arctic rivers.

Material and Methods

Study Sites and Sampling Locations

The Congo, the Niger, the Zambezi and the Ogooué rivers are among the largest rivers of Africa, both in terms of drainage area and discharge (Table 1). The climatic conditions are highly variable between and within each basin: the Congo and the Ogooué drainage basins cross the Equator and have the highest precipitation level, whereas the Zambezi and the Niger basins, close to the Tropic of Capricorn and to the Tropic of Cancer, respectively, have a lower annual precipitation. Linked to precipitation patterns, the vegetation varies from typical tropical humid rainforest close to the Equator to grassland, woodland, shrubland, and desert area toward the Tropics (Figure 1). Consequently, the distribution of the different land cover classes is heterogeneous between large basins as well as within each of the drainage areas, especially for the Congo and the Zambezi Basins. One additional difference between the study sites arises from the soil types, being more heterogeneous in the Niger and in the Zambezi Basins compared to the Congo and the Ogooué Basins (Table 1; Supplementary Figure S1).

The Congo River was monitored at Kisangani (DR Congo, 0°31′N, 25°11′E). In the same area, water samples from the Tshopo River, a tributary draining a forest catchment, were also collected. Monitoring on the Niger River took place in Niamey (capital of Niger, 13°31′N, 2°06′E). The Zambezi River and its major tributary (the Kafue River) were monitored at Chirundu, about 100 km south of Lusaka (capital of Zambia, 15°25′S, 28°17′E). Both rivers were sampled upstream of their confluence and both rivers are influenced by the presence of dams further upstream (Teodoru and others 2015). The Ogooué River was monitored in Lambaréné (Gabon, 0°42′S, 10°14′E). Water samples were collected approximately biweekly for one to two years between 2011 and 2014 (Table 2), so that the sample collection encompassed the range of discharge conditions for each study site.

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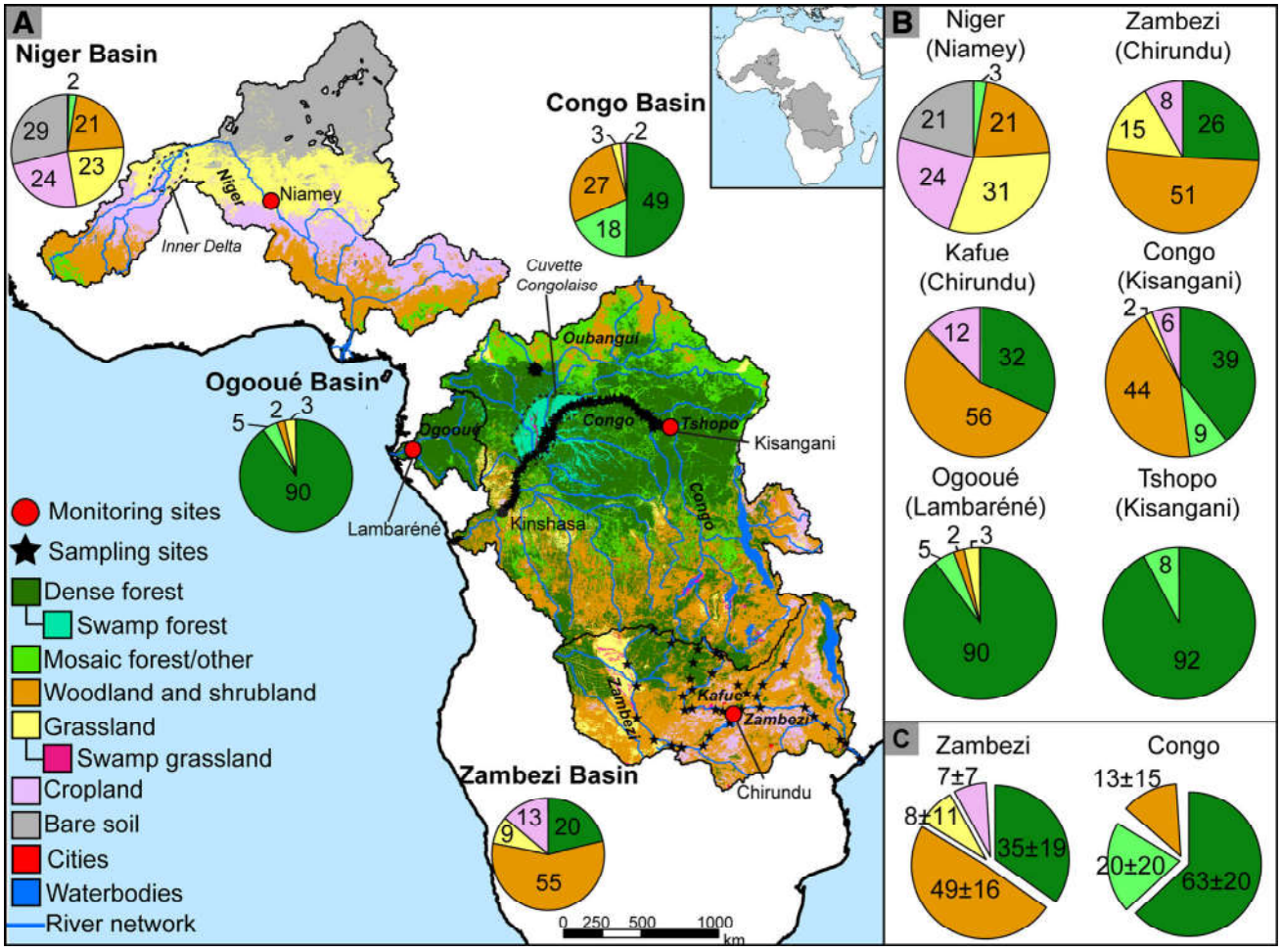


Figure 1. (**A**) Land cover map of study sites and sampling sites. *Red circles* indicate monitoring sites and *black stars* sites sampled during field campaigns. The pie charts show the fractional contribution (in %) of each land cover class for each drainage basin. (**B**) Fractional contribution (in %) of each land cover class in the catchment upstream of the sampling points for monitored rivers. (**C**) Mean and standard deviation of fractional contribution (in %) of each land cover class in the catchment upstream of the sampling points for samples collected during field campaigns. Land cover classes were defined from the Global Land Cover 2000 database (Mayaux and others 2004).

Additional CDOM data were obtained during field campaigns carried out during both dry and wet seasons along the Congo and the Zambezi rivers. A total of eight surveys were conducted in the Congo basin between 2010 and 2014 during which water samples were collected from the mainstem and from all tributaries encountered regardless of their size. Five of the campaigns were conducted from Kisangani to the confluence between the Congo and the Itimbiri tributary, located about 350 km downstream of Kisangani, during wet (November–December 2012, September 2013) and dry (May–June 2010, March 2014) periods (n = 103). Two extensive surveys were carried out during high waters (December 2013) and low waters (June 2014) along the 1700 km stretch from Kisangani to Kinshasa (n = 164). Two surveys (n = 16) were carried out in tributaries of the Oubangui catchment during dry (March 2011) and wet (November 2012) seasons mainly in the Lobaye province, Central African Republic (Bouillon and others 2014). The Zambezi River and its tributaries were sampled during wet (February–April 2012 and January–March 2013) and dry (October–November 2013) periods across the basin (n = 110) (Teodoru and others 2015). The dataset covers a wide range of geomorphological characteristics with respect to watershed morphology and land cover properties (Supplementary Figure S2).

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Table 2. Sampling Period, Morphological Attributes, and Hydrological Fluctuations for Rivers Monitored

Rivers (station)	Sampling period	*n*	Drainage area ($\times 10^3$ km^2)	Mean slope (°)	Mean elevation (m)	Q_{max}/Q^1_{min}
Niger (Niamey)	03/04/11–17/03/13	50	675	0.28	336	26.2
Zambezi (Chirundu)	19/02/12–22/11/13	20	685	0.51	1049	1.6[2]
Kafue (Chirundu)	20/02/12–24/11/13	20	157	0.49	1122	3.2[2]
Congo (Kisangani)	10/12/12–29/05/14	47	972	0.96	1062	3.0
Tshopo (Kisangani)	10/12/12–29/05/14	47	58	0.41	581	n.d.
Ogooué (Lambaréné)	19/04/12–18/04/14	55	214	0.50	446	3.9

[1]*Ratio between maximal and minimal discharge during the sampling period.*
[2]*Sampling stations are located downstream of dams that regulate river discharge.*

DOC and CDOM Sampling and Analyses

Sampling was mainly performed from dugout canoes, and in a few cases from a bridge or from the river shore. Approximately, 2 l of water were collected 0.25 m below the water surface, kept away from direct sunshine, and filtered and conditioned within 2 h of sampling. Filtrations were performed successively on pre-combusted GF/F glass fiber filters (0.7 µm porosity), then on 0.2 µm polyethersulfone syringe filters. Samples for the measurement of DOC concentration and $\delta^{13}C_{DOC}$ signatures were stored in 40 ml glass vials with polytetrafluoroethylene (PTFE) coated septa with 50 µl H_3PO_4 (85%). Samples for CDOM analyses were stored in 20 ml amber glass vials with PTFE-coated septa but without H_3PO_4 addition. DOC samples were stored at ambient temperature, while CDOM samples were stored at 4°C. Samples for Fe were stored in high-density polyethylene 20 ml vials with 50 µl HNO_3 (85%).

DOC and $\delta^{13}C_{DOC}$ were analyzed with an Aurora1030 TOC analyzer (OI Analytical) coupled to a Delta V Advantage IRMS. Typical reproducibility observed in duplicate samples was in most cases less than ±5% for DOC, and ±0.2‰ for $\delta^{13}C_{DOC}$. Quantification and calibration was performed with an aqueous solution of IAEA-C6 and in-house sucrose standards. Fe was measured by inductively coupled plasma spectrometry (Agilent 7700x ICP-MS).

Absorbance was recorded on a Perkin-Elmer UV/Vis 650S spectrophotometer using a 1 cm quartz cuvette. Absorbance spectra were measured between 190 and 900 nm at 1 nm increment and instrument noise was assessed measuring Milli-Q water as blank. After subtracting the blank spectrum, the correction for scattering and index of refraction was performed according to Johannessen and Miller (2001), by fitting the absorption spectra to the data over the 200–700 nm range according to the following equation:

$$A_\lambda = A_0 e^{-S(\lambda-\lambda_0)} + K, \quad (1)$$

where A_λ and A_0 are the absorbance measured at defined wavelength λ and at reference wavelength λ_0 = 375 nm, respectively, S the spectral slope (nm^{-1}) that describes the approximate exponential decline in absorption with increasing wavelength and K a background offset. The fit was not used for any purpose other than to provide an offset value K that was then subtracted from the whole spectrum.

The specific ultra-violet absorbance ($SUVA_{254}$) was calculated as the UV absorbance at λ = 254 nm (A_{254}) normalized to the corresponding DOC concentration and was used as an indicator of the aromaticity of DOC with high values (>4 l mgC^{-1} m^{-1}) indicating the presence of more complex aromatic moieties and low values (<3 l mgC^{-1} m^{-1}) indicative the presence of mainly hydrophobic microbial-dominated compounds (Weishaar and others 2003). The natural UV absorbance of Fe at λ = 254 nm was estimated based on measured Fe concentrations (according to Weishaar and others (2003), a concentration of 0.02 mg l^{-1} of Fe produces an additive absorbance of 0.002 cm^{-1}) and was then subtracted from the UV absorbance measured. The corrected value of A_{254} was then used to calculate $SUVA_{254}$. These corrections are critical to avoid significant overestimation of $SUVA_{254}$ values, especially at low DOC/Fe ratios (Supplementary Figure S3).

Napierian absorption coefficients were calculated according to

$$a_\lambda = 2.303 \times A_\lambda / L, \quad (2)$$

where a_λ is the absorption coefficient (m^{-1}) at wavelength λ, A_λ the absorbance corrected at wavelength λ and L the path length of the optical cell in meters (0.01 m). The Napierian absorption

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Table 3. DOC and CDOM Properties Measured at Monitored Rivers and During Campaigns

Basin	Monitoring/ campaigns	*n*	DOC (mg L^{-1})			$\delta^{13}C_{DOC}$ (‰)			a_{350} (m^{-1})			$SUVA_{254}$ (L mgC^{-1} m^{-1})			S_R		
			Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
Congo	Congo River	47	5.1 ± 1.2	2.7	8.1	−27.6 ± 1.1	−29.9	−25.0	20.5 ± 5.1	9.3	34.7	4.7 ± 0.4	3.5	5.5	0.77 ± 0.02	0.73	0.83
	Tshopo River	47	3.7 ± 0.8	2.5	5.8	−29.8 ± 0.5	−30.4	−28.3	14.9 ± 3.7	8.1	23.3	4.7 ± 0.4	3.9	5.6	0.75 ± 0.04	0.70	0.84
	Campaigns	272	10.8 ± 10.3	1.3	67.8	−29.0 ± 1.1	−30.7	−25.2	42.5 ± 41.1	3.6	249.4	4.7 ± 0.6	2.6	5.9	0.77 ± 0.04	0.61	0.93
Ogooué	Ogooué River	55	5.1 ± 1.9	1.6	9.5	−29.6 ± 0.3	−30.4	−28.9	21.3 ± 8.1	7.4	40.8	5.0 ± 0.5	3.3	5.7	0.74 ± 0.03	0.62	0.82
Zambezi	Zambezi River	15	2.5 ± 0.6	2.1	3.4	−23.1 ± 0.3	−23.4	−22.5	1.7 ± 0.4	1.0	2.5	1.8 ± 0.5	1.2	3.1	1.12 ± 0.18	0.97	1.53
	Kafue River	17	4.3 ± 1.0	3.2	6.5	−22.8 ± 0.7	−24.0	−21.1	6.6 ± 2.9	1.5	12.7	2.8 ± 0.5	1.3	3.4	1.00 ± 0.06	0.90	1.10
	Campaigns	110	3.1 ± 1.0	1.3	5.9	−22.5 ± 1.4	−28.1	−19.6	6.3 ± 3.6	1.2	16.6	3.1 ± 0.7	1.4	5.1	0.98 ± 0.17	0.69	1.47
Niger	Niger River	32	2.4 ± 0.5	1.7	3.4	−23.7 ± 0.9	−25.9	−21.7	4.5 ± 1.8	2.5	10.1	2.7 ± 0.8	1.3	4.2	1.07 ± 0.19	0.82	1.49

coefficient at 350 nm (a_{350}) was chosen to assess CDOM content in order to facilitate comparison with other studies (Stedmon and others 2011; Spencer and others 2012). Spectral slopes for the intervals 275–295 and 350–400 nm were determined from the linear regression of the log-transformed *a* spectra versus wavelength. The slope ratio S_R, calculated as the ratio of $S_{275–295}$ to $S_{350–400}$, is related to the molecular weight distribution of DOM with values less than 1 indicative of enrichment in high molecular weight compounds and high values above 1 indicative of an high degree of low molecular weight compounds (Helms and others 2008).

Landscape Analysis

For each sampling site, we calculated the mean slope, mean elevation, and total drainage area of the contributing watershed in the geographic information system (GIS) software ArcGIS, using the subwatershed polygons defined by the HYDRO1K global hydrologic data set (U.S.G.S. 2000). The fractional land cover type of the contributing watershed was determined from the Global Land Cover (GLC) 2000 database of Africa (Mayaux and others 2004). For our study, the 27 sub-classes defined by the GLC database were aggregated in seven first level classes based on vegetation structural categories: dense forest, mosaic forest/other, woodlands and shrublands, grasslands, agricultural lands, bare soils, and others (Supplementary Table 1). In this study, we used *savannah* as a generic term including grassland, wooded savannah, and shrubland vegetation biomes. The soil types for each study sites were derived from the soil map of Africa at the continent scale produced by Dewitte and others (2013).

Results

DOC concentrations and a_{350} varied over a wide range among the study sites, from 1.26 to 67.8 mg l^{-1} and from 1.05 to 264.0 m^{-1}, respectively (Table 3; Supplementary Table 2). For large rivers (that is, at monitoring sites), highest mean DOC concentrations (>5 mg l^{-1}) were measured in the Congo and the Ogooué and lowest (<2.5 mg l^{-1}) in the Zambezi and the Niger. The seasonal variability in DOC concentration was less pronounced in the Zambezi, the Kafue, and the Niger compared to the other rivers, and was not correlated with the hydrological fluctuations indicated by the ratio of the seasonal maximum and minimum of freshwater discharge (Q_{max}/Q_{min}).

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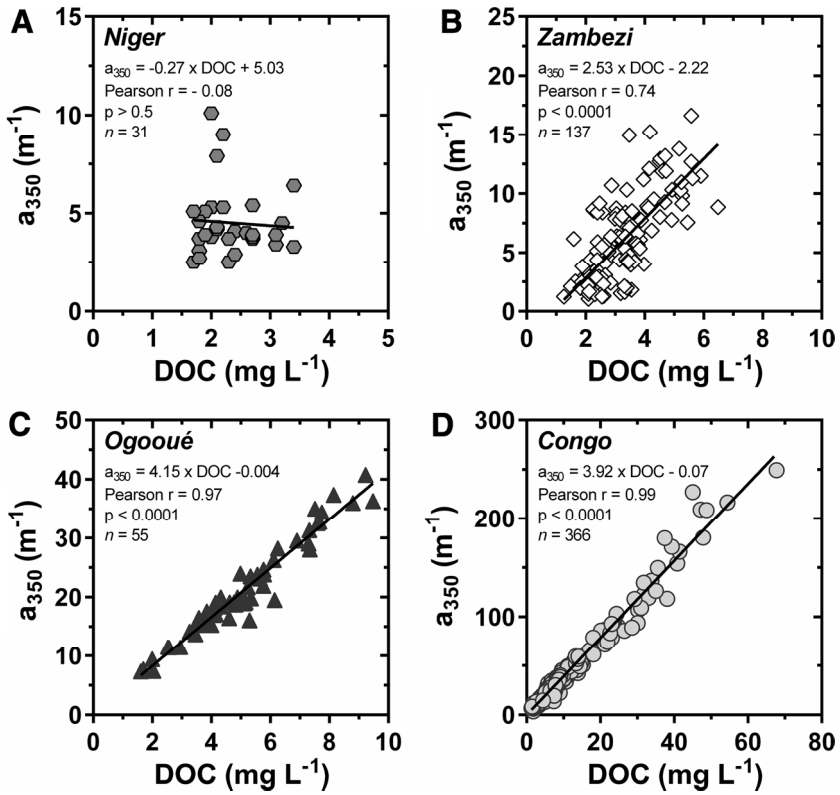


Figure 2. Relationships between a_{350} and DOC concentrations in (**A**) the Niger, (**B**) the Zambezi, (**C**) the Ogooué, and (**D**) the Congo basins.

Maximum DOC concentrations and highest variation were measured during campaigns carried out in the Congo basin, with a mean concentration of 10.8 ± 10.3 mg l^{-1} and values ranging from 1.3 to 67.8 mg l^{-1}. In contrast, the spatial variability in DOC concentrations was less pronounced in the Zambezi basin with a mean DOC concentration (3.1 ± 1.0 mg l^{-1}). In general, a_{350} values followed similar seasonal and spatial patterns across river basins as those of DOC. However, for an equivalent DOC concentration, a_{350} was about 50% lower in samples from the Zambezi and the Niger basin compared to those from the Congo and Ogooué. For example, while the Tshopo and Kafue rivers exhibited similar mean DOC concentrations (3.7 ± 0.8 and 4.3 ± 1.0 mg l^{-1}, respectively), a_{350} was much higher in the Tshopo River (14.9 ± 3.7 m^{-1}) than in the Kafue River (6.6 ± 2.9 m^{-1}). The correlations between a_{350} and DOC concentration were strong in the Congo and the Ogooué basins, weaker but still significant in the Zambezi basin, and not significant in the Niger basin (Figure 2). Overall, the slope of the relationship between a_{350} and DOC increased with increasing percentage of dense forest coverage (%DF), but this relationship levels off at %DF above 50% (Figure 3).

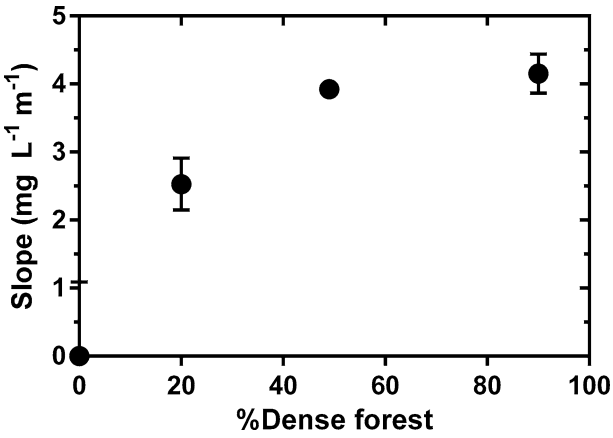


Figure 3. Correlation between the slope of the a_{350}/DOC relationship and the percentage of dense forest coverage at the basin scale. *Error bars* represent the 95% confidence intervals.

Based on DOC isotopic composition ($\delta^{13}C_{DOC}$) and optical properties of CDOM ($SUVA_{254}$ and S_R), the four studied basins can be grouped as follows: DOC-rich waters of the Congo and the Ogooué basins presented lower $\delta^{13}C_{DOC}$ signatures (averages from −29.8 ± 0.5 to −27.6 ± 1.1‰), higher aromaticity (mean $SUVA_{254} \geq 4.5$ l mgC^{-1} m^{-1}), and a higher contribution of high molecular weight

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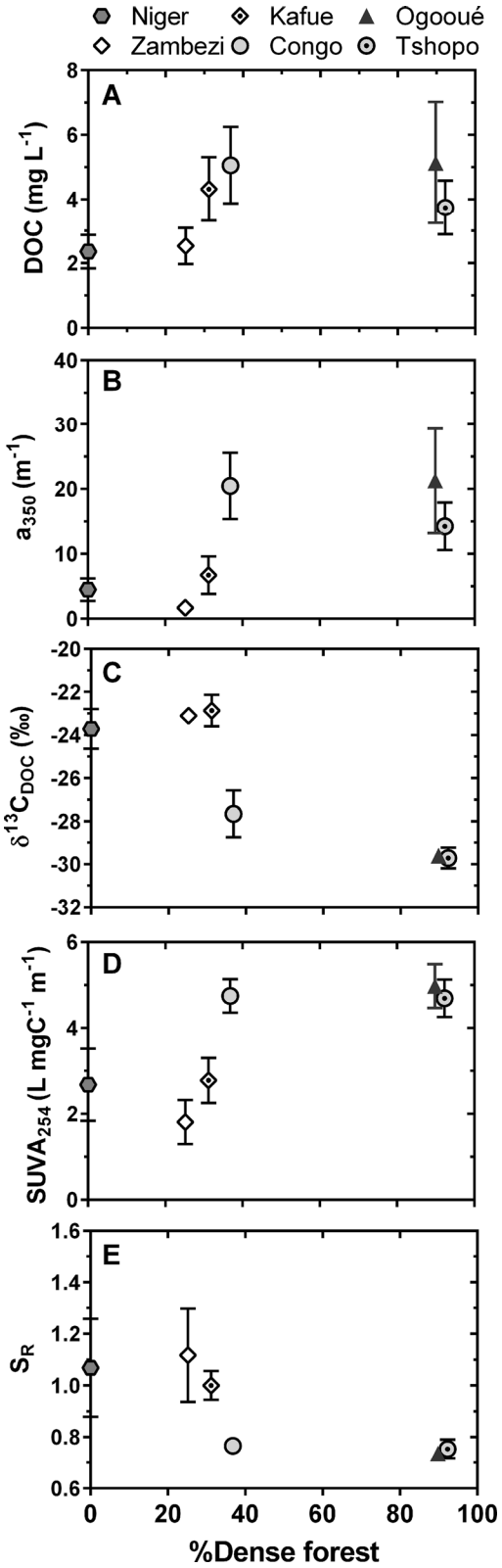


Figure 4. (**A**) DOC concentration, (**B**) a_{350}, (**C**) $\delta^{13}C_{DOC}$, (**D**) $SUVA_{254}$, and (**E**) S_R as a function of %DF at sampling points for large rivers.

compounds (mean $S_R < 0.8$), while DOC-poorer waters of the Zambezi and the Niger basins were characterized by higher $\delta^{13}C_{DOC}$ signatures (averages from -23.7 ± 0.9 to -22.5 ± 1.4‰), lower aromaticity (mean $SUVA_{254} < 3.5$ l mgC^{-1} m^{-1}), and a higher degree of low molecular weight compounds (mean S_R closed to 1). Seasonal variations in CDOM properties were more marked in the Zambezi and the Niger basins compared to the Congo and the Ogooué basins. No clear correlations were found between $\delta^{13}C_{DOC}$ and CDOM properties and changes in hydrological flow (Q_{max}/Q_{min}).

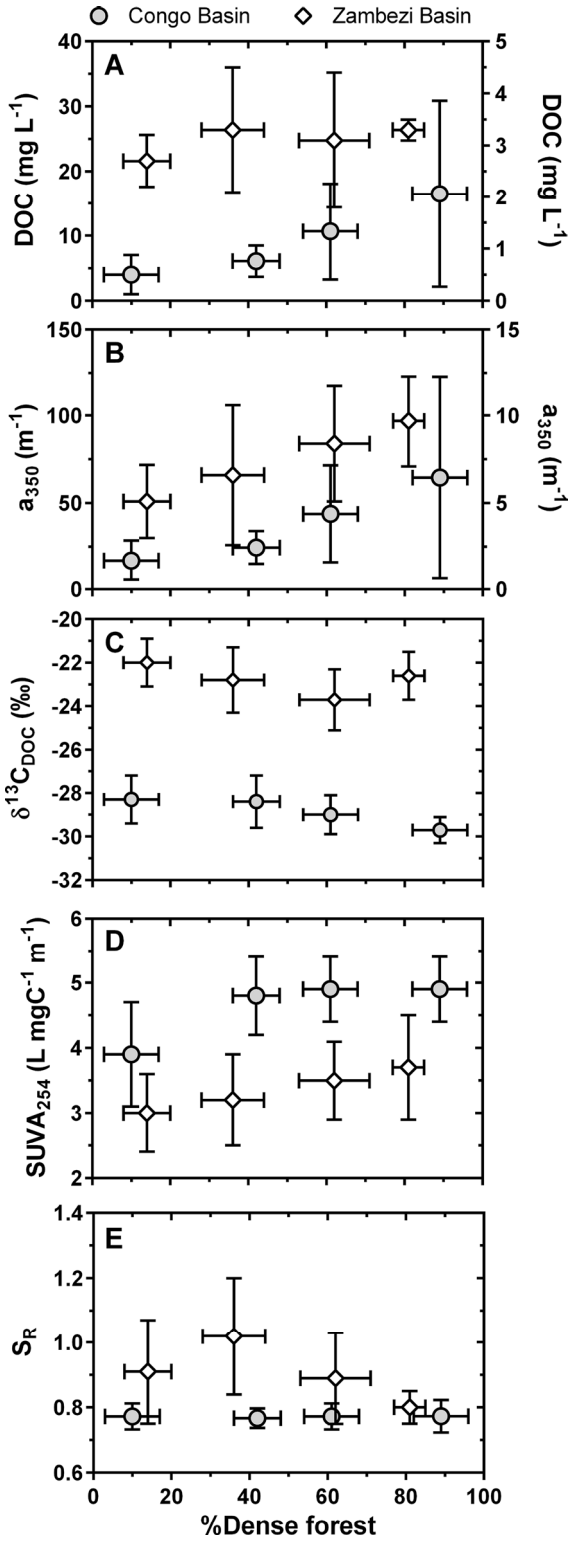
The %DF in the catchment was chosen to explore potential links between land cover and riverine DOM due to the greatest range of variation compared to the other land classes (Supplementary Figure S2). When considering large African rivers sampled, no clear relationships appear between DOC properties and %DF (Figure 4A, C), or between CDOM properties and %DF (Figure 4B, D, E). Significant differences in riverine DOM can be observed in rivers draining typical savannah- or forest-landscapes (for example, the Niger versus the Ogooué and the Tshopo). However, river basins having heterogeneous land cover (for example, the Zambezi, the Kafue, and the Congo) exhibited contrasting DOC and CDOM patterns, with values close to rivers draining typical savannah- or forest-catchment.

Significant correlations between %DF and DOC/CDOM properties were observed within the Congo and the Zambezi basins (Figure 5). DOC concentrations and a_{350} (Figure 5A, B) were positively correlated with %DF in the Congo basin ($r = 0.43$ and 0.41, respectively, $P < 0.0001$, $n = 211$), whereas this was only the case for a_{350} in the Zambezi basin ($r = 0.38$, $P < 0.005$, $n = 84$). Similarly, $\delta^{13}C_{DOC}$ signatures (Figure 5C) decreased with increasing %DF in the Congo basin ($r = -0.54$, $P < 0.0001$, $n = 206$) but not in the Zambezi basin ($r = -0.18$, $P > 0.05$, $n = 80$). Regarding DOM composition, $SUVA_{254}$ values were positively linked to %DF in both basins ($r = 0.27$, $P = 0.0001$, $n = 197$ for the Congo and Pearson' $r = 0.33$, $P = 0.0025$, $n = 83$ in the Zambezi), whereas no relationship was observed between S_R and %DF ($P > 0.05$ in both basins).

Discussion

Data from this study provide evidence that the largest African basins strongly differ in their DOC and CDOM properties. We argue that this wide

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variability results from several factors that interact in regulating DOC and CDOM in freshwaters, namely the land cover and the watershed morphology.

◂ **Figure 5.** (**A**) DOC concentration, (**B**) a_{350}, (**C**) $\delta^{13}C_{DOC}$, (**D**) $SUVA_{254}$, and (**E**) S_R as a function of the %DF at sampling point for tributaries and mainstem collected during campaigns. Data were bin-averaged for each 25% interval of dense forest cover and plotted against the mean %DF for these intervals. For (**A**) and (**B**), DOC concentrations and a_{350} are indicated on the *left axis* for the Congo basin and on the *right axis* for the Zambezi basin.

Effect of Land Cover on CDOM Released in the Fluvial Network

The effect of land cover can be assessed through the carbon isotopic composition of DOC by considering C_3 and C_4 vegetation as the dominant sources of DOM in these systems. In northern Australia, Bird and Pousai (1997) have shown that organic carbon in the surface soils of tropical forests (exclusively C_3) and tropical savannah (mixed C_3/C_4 woodlands and shrublands and exclusively C_4 grasslands) have contrasting isotopic signatures with values ranging from −29.4 to −27.2 ‰ and from of −26.6 to −14.4‰, respectively. Also, Kohn (2010) pointed to a decrease of the isotopic signature of C_3-plants with increasing mean annual precipitation, with values lower than −29‰ in environments receiving more than 2000 mm yr^{-1} of precipitation. The spatial distribution of $\delta^{13}C_{DOC}$ values in streams and rivers of the four studied basins appears to be in accordance with the spatial repartition of the dominant land cover. The high ratio between the concentration of particulate organic carbon (POC) and chlorophyll *a* (Chl *a*) measured in the Congo basin in this study (POC:Chl $a \sim 15000 \pm 45000$, $n = 223$, data not shown) also suggests that contribution to OM of in-stream phytoplankton production was negligible, as also reported in other African tropical rivers such as the Tana (Tamooh and others 2012). The low autochthonous contribution to the DOM pool in tropical rivers was already highlighted in basins of Madagascar (Marwick and others 2014) and in the Kafue Flats of the Zambezi basin (Zurbrügg and others 2013). Finally, even if phytoplankton DOC production has been shown in tropical lakes such as Lake Kivu, its rapid uptake by bacteria limits its accumulation and presence within the aquatic ecosystem (Morana and others 2014). For the above reasons, we assume that most the DOM is of allochthonous origin and that $\delta^{13}C_{DOC}$ values are representative of the relative inputs of DOM originating from C_3 and C_4 vegetation.

Clear distinctions emerge between C_3- and mixed C_3/C_4 dominated basins when DOC concentration and CDOM properties are plotted against

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$\delta^{13}C_{DOC}$ (Figure 6). To assess the effect on soil variability on these patterns, we compared DOM properties in streams and rivers draining (1) similar land cover but different soil types and (2) different land covers but similar soil type (Supplementary Figure S4). In accordance with previous studies (for example, McClain and others 1997), we found that the soil type impacts the concentration and the chemical composition of stream DOM (Supplementary Figure S4A). However, these differences are relatively small and are obscured by the variations in stream DOM content and composition imposed by vegetation (Supplementary Figure S4B), suggesting a large-scale control of land cover on riverine DOM concentration and composition in tropical freshwaters.

Catchments dominated by C_3-biomass export greater quantities of DOC than those where C_4 biomass is more important (Figure 6A). The higher riverine DOC concentrations in forest areas compared to savannah areas have already been reported from the catchment to the continental scale in Africa (for example, Laraque and others 2009) as well in the Amazon Basin (Seyler and others 2004), and results from higher carbon standing stocks in tropical forest were compared to savannah ecosystems (Bird and Pousai 1997). However, although the lower values of riverine DOC concentration are not significantly different between forest and savannah biomes, lowest CDOM content were found in rivers receiving DOM from mixed C_3/C_4 savannah vegetation (Figure 6B). This result shows that DOM released from savannah soils is less optically active (that is, less absorptive) compared to DOM released from forest soils. The less absorptive character of DOM in savannah-dominated basins is reflected in the lower slope of the relationship between DOC and a_{350} in the Zambezi Basin (2.53) relative to the Congo (3.92) and the Ogooué (4.15) basins and by the fact that the slope of the relationship between DOC and a_{350} increases with the %DF across basins (Figure 3).

The repartition of $SUVA_{254}$ and S_R values along the $\delta^{13}C_{DOC}$ gradient shows that C_3- and mixed C_3/C_4-dominated basins strongly differ in their stream DOM composition (Figure 6C, D). This is not surprising given that the specific absorption coefficient of DOM is related to its chemical composition (Yacobi and others 2003). $SUVA_{254}$ and S_R can be used as proxies to trace the sources of DOM as well as the processes that regulate its fate in freshwaters. In general, highest DOC concentrations and CDOM load found in streams and rivers during high flow periods are linked with greatest aromaticity (high $SUVA_{254}$) and average molecular weight (low S_R) compared to base flow periods. Inversely, lower $SUVA_{254}$ and higher S_R values in streams can be expected during base flow periods when water originates from deeper mineral horizons of the soil profile (for example, Striegl and others 2005). Both sorption and biotic processes occurring during the transport of DOM produced in surface layers into underlying soil layers may affect its chemical composition, leading to a decrease in its aromaticity

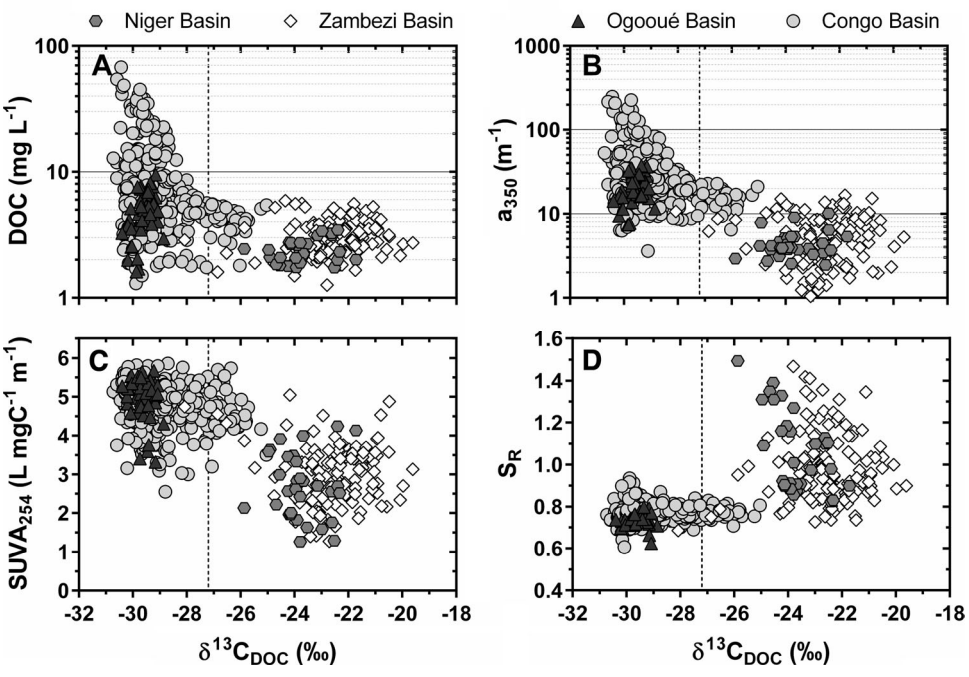


Figure 6. (**A**) DOC concentration, (**B**) a_{350}, (**C**) $SUVA_{254}$, and (**D**) S_R as a function of $\delta^{13}C_{DOC}$ values for all samples acquired in the Congo, the Ogooué, the Zambezi and the Niger basins. The *vertical dashed line* represents the upper $\delta^{13}C$ signature for organic carbon in the surface soils of exclusively C_3 tropical forests, according to Bird and Pousai (1997).

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and molecular weight (Striegl and others 2005; Lambert and others 2013). The seasonal changes in the properties of CDOM, reported in a large variety of rivers ranging in size and hydroclimatic zones, are attributed to the seasonal shift in dominant source from modern plant material in organic-rich soil horizons during the high flow to older, more degraded soil OM originating from lower soil horizons during low flow conditions (Striegl and others 2005; Neff and others 2006; Raymond and others 2007; Stedmon and others 2011; Bouillon and others 2014).

The high $SUVA_{254}$ values concomitant with low $\delta^{13}C_{DOC}$ (Figure 6) indicate that C_3 biomass releases DOM with a greater proportion of aromatic and higher molecular weight compounds compared to C_4 biomass. Moreover, the narrow range of variability of these optical proxies compared to previous studies (see references above) suggests that changes in DOM sources were minimal despite significant seasonal hydrological variability, and that inputs of less degraded plant material continuously fuelled stream DOC. Keeping in mind that the data presented in Figure 2 include samples that cover the seasonal variability, the strong correlations between DOC and a_{350} observed in the Congo and the Ogooué basins support this assumption. In contrast, the large range of variation in $SUVA_{254}$ and S_R values observed for $\delta^{13}C_{DOC}$ greater than $-26‰$ suggest more pronounced changes in DOM sources across the hydrological year in savannah-dominated basins. Thus, the larger range in both $SUVA_{254}$ and S_R we observed in C_3/C_4 environments are indicative of different DOM source pools mobilized along the hydrological cycle, according to the two-source model described above. The contribution of several sources differing in their optical properties to stream CDOM export in savannah-dominated basins is supported by the relatively weaker correlation between DOC and a_{350} observed in the Zambezi basin (Figure 2).

Our results highlight that the variability in the optical properties of DOM transported in tropical river systems can be directly related to the different types of land cover. Similar observations have recently been reported at the regional scale in the northwestern part of the Congo basin (Mann and other 2014), and likely reflect inherent differences in the molecular composition of DOM derived from C_3 or C_4 biomass (or, in more general terms, between woody vegetation and grassland vegetation). DOM in forest floors or superficial soil horizons is known to be substantially more humic and aromatic, and contained a greater proportion of large and complex molecules compared to other land cover types (Qualls and others 1991; Aitkenhead-Peterson and others 2003). In addition, several studies have reported differences in the lability between C_4-compounds derived from grassland vegetation and C_3-compounds derived from woody vegetation both in soils (Bird and Pousai 1997; Wynn and Bird 2007) and aquatic ecosystems (Quay and others 1992; Mayorga and others 2005). For example, C_4-grasses fuel disproportionately the production of dissolved CO_2 in streams and rivers of the Amazon Basin (Mayorga and others 2005), indicating that grasslands may be intrinsically more biodegradable due to an enrichment in labile compounds in soil organic matter build up under grasses (see also Quay and others 1992; Wynn and Bird 2007). Consequently, soil OM (SOM) derived from grassland can be more efficiently processed by soil microorganisms, thus enhancing the incorporation of microbial inputs in the SOM pools and ultimately in the DOM pool. As microbial inputs have a low aromaticity (for example, Lambert and others 2013), this DOM pool would lead to lowering $SUVA_{254}$ and higher S_R values. In line with this hypothesis, Jaffé and others (2012), who studied the molecular complexity of DOM from small streams across climatic regions by detailed molecular characterization methods, showed that DOM derived from C_4-grassland was enriched in microbial inputs compared to other environments.

Watershed Morphology Effects on CDOM Released in the Fluvial Network

The correlations between %DF as estimated from GIS analysis and DOC or CDOM properties were not obvious across basins (Figure 4) and within a given basin (Figure 5). Although the large heterogeneity in terms of land cover, size, and morphological attributes of the sampling sites may also complicate the relationships between %DF and DOC/CDOM, the lack of significant correlations likely reflects the influence of additional features of the landscape on the loading and fate of DOM in tropical rivers.

The first proposed explanation concerns the spatial repartition of the land cover within the drainage basin. Marwick and others (2014) recently highlighted that $\delta^{13}C_{DOC}$ in C_4-dominated basins was biased toward the isotopic fingerprints of the C_3-enriched riparian fringe due to preferential hydrological connectivity with stream channel (see also Bird and Pousai 1997). The same likely occurs in the Zambezi basin, where $\delta^{13}C_{DOC}$ values were not correlated with %DF (Figure 5C). Indeed, the Zambezi River crosses numerous and extensive

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floodplains characterized by substantial C_4 components that contribute to a great proportion of organic carbon export (for example, Zurbrügg and others 2013, Figure 1). By extension, we suggest that the difference in land cover between lowland and upland basins contribute to the discrepancies between GIS data and field measurements. For example, despite similar land cover partition at their sampling point (32 and 39% of %DF, respectively, Figure 1), the Kafue and the Congo rivers exhibited DOC and CDOM patterns matching those expected for rivers draining typical savannah- and forest-dominated catchments, respectively (Figure 4). However, while lowlands are covered by forest and the uplands by savannah in the Congo basin upstream of Kisangani, an opposite situation characterizes the Kafue catchment. Steeper slopes allow faster movement of water through the soils, which limits the residence time of hydrological flowpaths in the superficial layers of soils and thus reduces the amount of DOM reaching adjacent streams. Inversely, watersheds with low slopes have generally thicker organic horizons and longer residence times in soils, and are thus capable of supplying significant quantities of DOM (Rasmussen and others 1989). In line with this hypothesis, an altitude effect was observed in the Congo Basin for DOC and a_{350} with values decreasing with increasing the slope (Supplementary Figure S5). Our results highlight that the fractional distribution of C_3 and C_4 vegetation cover in tropical basins is not an accurate predictor for riverine DOM (and CDOM) quantity and quality. The spatial distribution of land cover types within the basin (along the riparian fringe and between lowlands and uplands) plays an integral role in controlling DOM in freshwaters, leading to discrepancies between GIS data and field measurements.

Additionally, photochemical and microbial degradation and the interaction between these two processes are known to strongly alter the optical properties of terrestrial DOM during its transport in freshwaters (for example, Spencer and others 2009; Fasching and others 2014). Measurements of pelagic respiration rates performed during field campaigns were 12.7 ± 5.8 and 14.5 ± 14.1 mmolC m^{-3} d^{-1} in the Congo and the Zambezi basins, respectively, which are close to the median value measured in African inland waters [~20 mmolC m^{-3} d^{-1}, Borges and others (2015)] and in the range of bacterial respiration rates reported in temperate and others tropical systems (Amado and others 2013). Microbial degradation of terrestrial DOM can efficiently consume aromatic compounds (for example, Ward and others 2013) and at the same time release new less aromatic compounds (Fasching and others 2014). Moreover, photochemical degradation has been shown to result in a preferential loss of CDOM compared to DOC due to the decay of photo-labile aromatic compounds (for example, Spencer and others 2009; Lapierre and del Giorgio 2014). This can lead to a quick alteration of DOM chemical composition and a significant shift in its optical properties at the first time of light exposure, as pointed by Spencer and others (2009) who irradiated DOM from the Congo River.

The water bodies that have extended water residence time, such as lakes, reservoirs or large floodplains, enhance the opportunities for these processes to decompose terrestrial DOC (Battin and others 2008). Thus, the low molecular weight and aromaticity of DOM in the Zambezi River at the outlet of the Kariba Reservoir (Figure 4) likely reflect photochemical and/or microbial degradation processes. Finally, increased solar radiation under open canopy conditions can also promote the loss of DOC exported downstream via photodegradation. For the Niger River at Niamey, the combination of (1) the slowdown of stream waters as the river enters the Inner Delta (Figure 1), (2) the lack of new inputs of terrestrial organic material by significant tributaries along the approximately 760 km stretch between the Inner Delta and Niamey, and (3) the total absence of shading along the same stretch may explain the lack of relationship between DOC and CDOM (Figure 2). Finally, the autochthonous production of uncolored DOM can impact the relationship between DOC and CDOM in lakes or estuarine systems (for example, Rochelle-Newall and others 2014). However, as discussed above, the autochthonous contribution to the DOM pool in our study sites is most probably low.

Tropical Rivers Versus Temperate and High-Latitude Rivers

To assess the variability of CDOM in streams and rivers along a climatic gradient, we compared our results with previous studies carried out in temperate (Spencer and others 2012), Arctic (Stedmon and others 2011; O'Donnell and others 2012; Spencer and others 2012), and other tropical systems (Yamashita and others 2010; Mann and others 2014) (data detailed in Supplementary Table 3). The comparison of the relationship between DOC and CDOM at the global scale highlights that while similar low slopes have been reported in all climatic biomes, maximum values tend to increase in the

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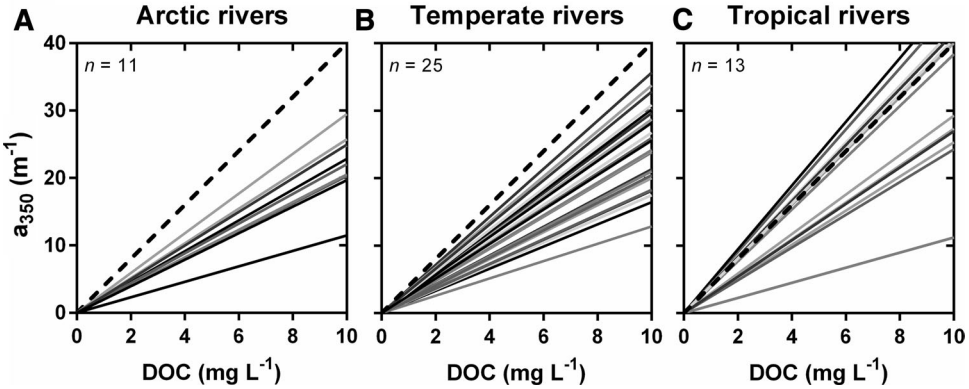


Figure 7. Relationships between CDOM (a_{350}) and DOC concentrations for published data in (**A**) Arctic rivers (Stedmon and others 2011; O'Donnell and others 2012; Spencer and others 2012), (**B**) temperate rivers (Spencer and others 2012), and (**C**) tropical rivers (Yamashita and others 2010; Mann and others 2014; this study). To simplify the comparison, the *y*-intercept was set to 0 and the relationship was plotted over the 0–10 mg L^{-1} [DOC] range. The *dashed line* represents the 4:1 slope. Data are detailed in Supplementary Table 3.

order Arctic < temperate < tropical rivers (Figure 7). The fact that the highest slope is found in tropical rivers can be related to the highly aromatic character of DOM in tropical ecosystems [Figure 8, see also Pérez and others (2011)], especially in tropical rainforest dominated basins (Table 3). The good correlation between the slope of the DOC/a_{350} relationship and $SUVA_{254}$ is not surprising, as $SUVA_{254}$ is by definition the specific absorption coefficient of DOM measured at 254 nm. In other words, higher $SUVA_{254}$ values are associated with a higher ability of DOM to absorb sunlight, supporting previous studies that show that aromatic molecules are key components of CDOM (for example, Spencer and others 2009).

The strong colored character of DOM can have strong potential implications for the reactivity of DOM and CO_2 emissions in tropical rainforest ecosystems. Indeed, recent studies have shown that fresh aromatic compounds can be extensively respired by microorganisms (Ward and others 2013; Fasching and others 2014; Lapierre and del Giorgio 2014) and that the photochemical susceptibility of CDOM is directly correlated with its initial aromatic nature (Lapierre and others 2013; Lapierre and del Giorgio 2014). For these reasons, recent studies have concluded that surface water CO_2 concentrations should increase as a function of CDOM (Lapierre and others 2013; Fasching and others 2014). However, a recent data compilation of CO_2 and respiration measurements in Sub-Saharan African inland waters, including the studied basin here, showed that less than 20% of CO_2 emissions were attributed to in-stream degradation of OM (Borges and others 2015). The fact that CO_2 river concentrations were correlated to above-ground terrestrial vegetation biomass, both within and across basins, lead these authors to conclude that the CO_2 oversaturation in rivers was to a large extent explained by lateral inputs of CO_2 from wetlands and groundwaters, and that an overall positive relationship between CO_2 and DOC concentrations is not necessarily causal. In absence of data on direct CO_2 production from photodegradation of CDOM, this contribution to CO_2 fluxes was not envisaged although it is unlikely that it could account for greater than 80% of CO_2 emissions that cannot be accounted by in-stream respiration. Indeed, in the Amazon black-waters (Rio Negro) photodegradation represents only a small part (<10%) of the total DOC mineralization (Amaral and others 2013), and explains less than 1% of its CO_2 emission (Remington and others 2011). Yet, we acknowledge that this pathway of CO_2 production needs to be further quantified in highly humic rivers such as the Congo.

It is possible that photodegradation of terrestrial CDOM in tropical rainforest ecosystems can be limited by shading of the dense canopy and light absorption by CDOM itself and that microbial degradation is limited by the low nutrient status (data not shown). This also could explain the strong relationship between DOC and CDOM in the Congo basin (Figure 2), where sampling encompassed both spatial and temporal dimensions. Degradation of terrestrially derived CDOM could, therefore, mainly be localized in areas of lowest canopy density with rivers or at sea in rivers

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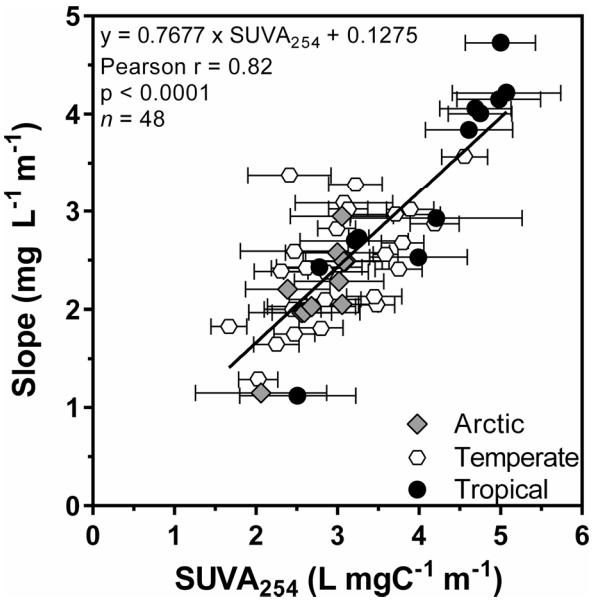


Figure 8. Correlation between the slope of the a_{350}/DOC relationship and mean $SUVA_{254}$ for stream waters at the global scale, including Arctic (Stedmon and others 2011; O'Donnell and others 2012; Spencer and others 2012), temperate (Spencer and others 2012), and tropical rivers (Yamashita and others 2010; Mann and others 2014; this study).

plumes. Further studies addressing biological and photochemical degradation rates at large scales are needed to better estimate the relative contribution of CO_2 emissions resulting from terrestrial inputs to those resulting from in-stream metabolism, especially in tropical rivers.

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Shift in the chemical composition of dissolved organic matter in the Congo River network

Thibault Lambert[1], **Steven Bouillon**[2], **François Darchambeau**[1], **Philippe Massicotte**[3], **and Alberto V. Borges**[1]

[1]University of Liège, Chemical Oceanography Unit, Liège, Belgium
[2]K.U. Leuven, Department of Earth and Environmental Sciences, Leuven, Belgium
[3]Aarhus University, Department of Bioscience, Århus, Denmark

Correspondence to: Thibault Lambert (tlambert@ulg.ac.be)

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Abstract. The processing of terrestrially derived dissolved organic matter (DOM) during downstream transport in fluvial networks is poorly understood. Here, we report a dataset of dissolved organic carbon (DOC) concentrations and DOM composition (stable carbon isotope ratios, absorption and fluorescence properties) acquired along a 1700 km transect in the middle reach of the Congo River basin. Samples were collected in the mainstem and its tributaries during high-water (HW) and falling-water (FW) periods. DOC concentrations and DOM composition along the mainstem were found to differ between the two periods because of a reduced lateral mixing between the central water masses of the Congo River and DOM-rich waters from tributaries and also likely because of a greater photodegradation during FW as water residence time (WRT) increased. Although the Cuvette Centrale wetland (one of the world's largest flooded forests) continuously releases highly aromatic DOM in streams and rivers of the Congo Basin, the downstream transport of DOM was found to result in an along-stream gradient from aromatic to aliphatic compounds. The characterization of DOM through parallel factor analysis (PARAFAC) suggests that this transition results from (1) the losses of aromatic compounds by photodegradation and (2) the production of aliphatic compounds by biological reworking of terrestrial DOM. Finally, this study highlights the critical importance of the river–floodplain connectivity in tropical rivers in controlling DOM biogeochemistry at a large spatial scale and suggests that the degree of DOM processing during downstream transport is a function of landscape characteristics and WRT.

1 Introduction

Dissolved organic matter (DOM) is composed of thousands of heterogeneous compounds that differ in origin and reactivity (Leenheer and Croué, 2003) and is a central component of the global carbon cycle (Battin et al., 2008). DOM in streams and rivers mainly originates from the terrestrial ecosystem, but can also be fueled by internal sources (Bertilsson and Jones, 2003; Battin et al., 2008). Recent experimental and field studies have demonstrated that sorption, photochemical and biodegradation processes continuously degrade and transform DOM throughout fluvial networks (Massicotte and Frenette, 2011; Ward et al., 2013; Cory et al., 2014; Fasching et al., 2014; Lapierre and del Giorgio, 2014; Lambert et al., 2016). Large surveys of boreal lakes have suggested that DOM was degraded along a gradient from aromatic to aliphatic compounds and that the chemical composition of DOM was the dominant control of overall DOM reactivity (Kothawala et al., 2014; Kellerman et al., 2015). Similarly, a large survey of temperate streams and rivers has reported a preferential loss of aromatic DOM and parallel gain in aliphatic DOM with increasing stream order, resulting in a diminution of the variability in dissolved organic carbon (DOC) concentration and DOM composition from small headwater streams to large rivers (Creed et al., 2015). However, the role of environmental factors (i.e., climatic variables, water chemistry, landscape properties) on DOM transformation in fluvial networks remains poorly constrained (Massicotte and Frenette, 2011; Marín-Spiotta et al., 2014).

The consideration of temporal dynamics in addition to the spatial dimension is poorly investigated, yet it represents a crucial step towards a better understanding of DOM transport and processing in fluvial networks. Temporal dynamics refer here to the changes of the hydrological state of catchments that occur between high-flow and low-flow periods and are susceptible to altering DOM dynamics for at least two reasons. First, the concentration, the composition and the reactivity of DOM in streams and rivers are largely determined by seasonal changes in water levels that control the hydrological connectivity between rivers and wetlands (Junk et al., 1989; Battin, 1998; Besemer et al., 2009; Osburn et al., 2009; Lambert et al., 2016). Secondly, the increase in water discharge during high-flow periods induces a decrease in water residence time (WRT) within catchments due to increasing water velocities. Beyond the role of external and intrinsic drivers on DOM degradation, WRT represents a major control that regulates the degree of DOM transformation in aquatic ecosystems (Cory et al., 2007; Battin et al., 2008; Weyhenmeyer et al., 2012). According to the recent pulse-shunt concept (Raymond et al., 2016) that builds off of the "active pipe" concept (Cole et al., 2007), the degree of DOM processing in fluvial networks should be reduced during high-flow periods as hydrological events favor the transport of DOM through the drainage network and therefore reduce the time in which dynamic processes can take place.

African tropical rivers have among the highest specific flux of DOC worldwide (Meybeck, 1993) and have an important role in the global carbon cycle (Borges et al., 2015a, b). Yet they remain largely underrepresented in large-scale studies on DOM processing. For example, only few studies have investigated DOM biogeochemistry in the Congo Basin (Spencer et al., 2010, 2012; Bouillon et al., 2012, 2014; Mann et al., 2014), despite the fact that the Congo River – the largest river in Africa and the second largest river worldwide after the Amazon in terms of drainage basin area and water discharge (Laraque et al., 2009) – is the second major exporter of terrestrial organic carbon to the oceans, 85–90 % being in the form of DOC (Coynel et al., 2005; Laraque et al., 2009). Downstream transport of DOM in the Congo River has not yet been investigated in the middle part of the Congo Basin where the second-largest tropical flooded forest wetland is located, the Congolese "Cuvette Centrale". Additionally, in the middle reach, the Congo River receives inputs from its major tributaries, such as the Kasai, Ruki and Oubangui (Fig. 1).

Emerging conceptual models that describe how inland waters transform DOM flowing down the fluvial network, such as the "chemostat" hypothesis (Creed et al., 2015) and the pulse-shunt concept (Raymond et al., 2016), need to be tested by empirical data acquired through extensive studies in tropical ecosystems. The Congo River and its tributaries were sampled along a 1700 km transect from the city of Kisangani to the city of Kinshasa during two contrasting hydrological periods (Figs. 1 and 2). DOM was characterized through its optical properties, its stable carbon isotope composition ($\delta^{13}C_{DOC}$) and its DOC concentration. Optical measurements (including absorption and fluorescence) have been underscored as an efficient tool for characterizing the source and the reactivity of specific fractions of the DOM pool at large spatial scales (Jaffé et al., 2008; Yamashita et al., 2010; Massicotte and Frenette, 2011; Cawley et al., 2012; Kothawala et al., 2014; Lambert et al., 2016), notably with the development of multicomponent deconvolution techniques such as the parallel factor analysis (PARAFAC) (Stedmon et al., 2003; Murphy et al., 2013). The aims of this study were (1) to characterize the longitudinal evolution of DOM in the Congo River in the middle reach of the basin, (2) to investigate the transformation of DOM during its downstream transport in the fluvial network and (3) to identify potential environmental drivers of DOM processing.

2 Materials and methods

2.1 Study area

The Congo is the largest river in Africa and the second largest river in the world after the Amazon in terms of drainage basin area ($\sim 3.7 \times 10^6$ km^2) and water discharge ($\sim 43\,000$ m^3 s^{-1}) (Laraque et al., 2009). The river originates in the southeastern part of the basin and is called the Lualaba until it crosses the city of Kisangani, after which it becomes known as the Congo. The Congo Basin straddles the Equator, with major tributaries located in both hemispheres (Fig. 1). Thus, the rainy season on the northern part is compensated for by the dry season on the southern part, and vice versa. Consequently, seasonal water height variations are largely attenuated (Runge, 2008), in stark contrast with the Amazon river, leading to marked differences in biogeochemistry (e.g., CH_4 dynamics; Borges et al., 2015b) and aquatic ecology (e.g., phytoplankton development; Descy et al., 2016) between these two rivers. The hydrological cycle of the Congo River at Kisangani and Kinshasa is similar, with a bimodal distribution: maximum water peaks occur in December and May and minimum flow in August and March (Fig. 2). The center of the basin is covered by evergreen forest (~ 50 % of the total area) and is surrounded by savannah on the northern and southern rims of the catchment. The Cuvette Centrale is located in the central part of the basin on both sides of the Equator and mainly consists of a vast permanently flooded forested area of 360×10^3 km^2 (Bwangoy et al., 2010). The core of the Cuvette Centrale corresponds to the net increase in the wetland fraction along the Congo River as the mainstem connects with large tributaries flowing through the flooded forest (Fig. 1 and Supplement Fig. S1). The most important tributaries of the Congo in terms of discharge are the Oubangui (4200 m^3 s^{-1}) and the Sangha (2220 m^3 s^{-1}) on the northern side, the Kasai (9000 m^3 s^{-1})

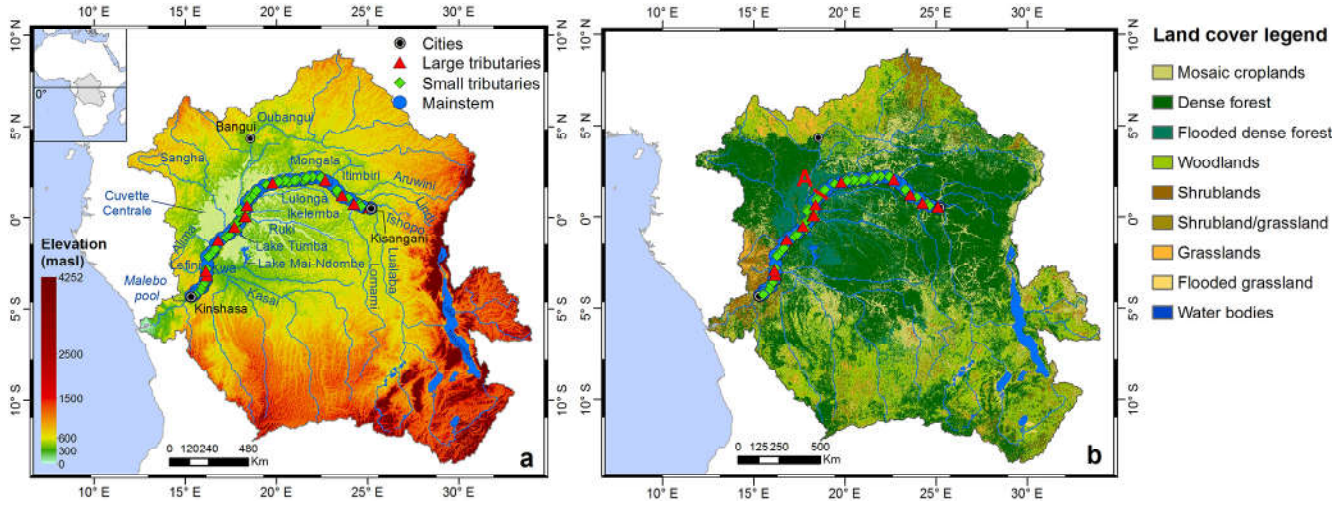


Figure 1. Maps of the Congo Basin. **(a)** The elevation (Lehner et al., 2008), the main hydrological network, the extent of the Cuvette Centrale (Lehner and Döll, 2004), and the distribution of sampling sites along the Kisangani–Kinshasa transect. **(b)** The dominant land cover (Bontemps et al., 2011). The red line "A" indicates the entrance of the Congo River within the core of the Cuvette Centrale (see text for details and Fig. S1).

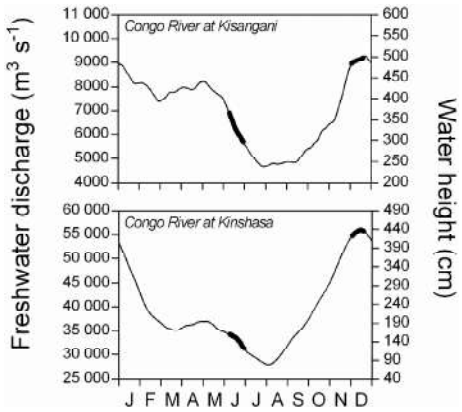


Figure 2. Average freshwater discharge of the Congo River, as well as corresponding water height at the gauging station at **(a)** Kisangani for the period 2013–2016 and **(b)** Kinshasa for the period 2003–2013. Timing of the two cruises is indicated by thicker lines.

on the southern side, and the Ruki (3950 $m^3 s^{-1}$) and the Lulonga (2040 $m^3 s^{-1}$) along the Equator (Bricquet, 1995; Coynel et al., 2005; Laraque et al., 2009).

2.2 Field data collection

Samples were collected during the yearly discharge maximum in December (3–19 December 2013; high waters (HW)) and during falling waters (FW) following the second discharge maximum occurring in March (10–30 June 2014) (Fig. 2). As the water level of the Congo River follows similar seasonal fluctuations at Kisangani and Kinshasa (Fig. 2), the timing of high and low waters was likely similar for the sites located along the mainstem. WRT between Kisangani and Kinshasa has been roughly estimated to increase by about ∼ 5 days during FW compared to HW (Descy et al., 2016) but is likely not uniform along the transect: because the main tributaries are all located downstream, the increase in WRT is probably more important upstream of the Oubangui. Water samples were collected in the Congo River itself as well as in small and large tributaries at the confluence (Table 1). Stations along the mainstem were located ∼ 50 km apart from Kisangani to Kinshasa. Major tributaries included the Tshopo, the Lindi, the Itimbiri, the Aruwimi, the Mongala, the Oubangui, the Sangha and the Lefini on the right bank of the Congo, and the Lomami, the Lulonga, the Ikelemba, the Ruki and the Kwa/Kasai on the left bank. The Lefini was sampled only during the first campaign (high waters).

Water sampling was performed from a 22 m boat on the mainstem and with a canoe in the tributaries. Approximately 2 L of water was collected 0.5 m below the surface, stored away from direct sunshine and filtered and conditioned typically within 15 min of sampling. Filtrations were performed successively on pre-combusted GF/F glass fiber filters (0.7 µm porosity), then on 0.2 µm polyethersulfone syringe filters. Samples for the measurement of DOC concentrations and $\delta^{13}C_{DOC}$ signatures were stored in 40 mL glass vials with polytetrafluoroethylene (PTFE)-coated septa with 50 µL of H_3PO_4 (85 %). Samples for colored DOM (CDOM) and fluorescent DOM (FDOM) analyses were stored in 20 mL amber glass vials with PTFE-coated septa but without H_3PO_4 addition. Samples for CDOM and FDOM were stored just after filtration at 4 °C in a refrigerator on the boat until transportation back to Belgium, where they were also stored at 4 °C until analysis within 1 week of arrival. Potential stor-

Table 1. Selected attributes (mean ± standard deviation, min–max) of sampling sites during the field campaigns: oxygen saturation level ($\%O_2$), pH, Secchi depth, vertical light attenuation coefficient (K_d), total suspended matter (TSM) and chlorophyll *a* (Chl *a*) concentrations (data from Descy et al., 2016).

Period	*n*	$\%O_2$ (%)	pH	Secchi (cm)	K_d (m^{-1})	TSM ($mg\,L^{-1}$)	Chl *a* ($\mu g\,L^{-1}$)
Maximum high waters							
Mainstem	35	60.3 ± 10.6 (48.4–89.2)	6.46 ± 0.22 (6.07–6.92)	54.6 ± 15.6 (25–80)	1.5 ± 0.4 (1.0–2.8)	29.4 ± 21.9 (14.0–99.8)	0.84 ± 0.42 (0.10–1.76)
Major tributaries	13	54.3 ± 33.3 (8.6–111.3)	5.67 ± 1.09 (3.91–6.87)	79.5 ± 60.7 (25–250)	1.5 ± 0.6 (0.4–2.5)	12.5 ± 13.3 (0.74–44.4)	0.54 ± 1.02 (0.01–3.57)
Minor tributaries	26	27.9 ± 30.2 (4.2–99.8)	5.33 ± 0.75 (3.91–6.17)	86.2 ± 29.7 (15–140)	1.5 ± 0.5 (0.9–2.8)	7.7 ± 13.4 (1.7–71.4)	0.35 ± 0.42 (0–1.85)
Falling waters after second peak water discharge							
Mainstem	34	84.8 ± 7.4 (54.2–93.4)	6.82 ± 0.32 (6.08–7.38)	46.8 ± 5.7 (35–62)	3.9 ± 0.6 (1.5–4.6)	31.9 ± 9.1 (4.0–45.4)	3.99 ± 1.54 (1.13–7.68)
Major tributaries	12	62.1 ± 31.2 (0.3–98.2)	5.77 ± 1.22 (3.63–7.05)	66.7 ± 23.4 (35–106)	3.3 ± 0.7 (2.4–5.1)	14.4 ± 12.5 (0.73–43.0)	1.65 ± 2.27 (0.02–6.39)
Minor tributaries	41	37.8 ± 35.6 (0.3–103.0)	4.56 ± 0.77 (3.6–6.1)	80.7 ± 42.2 (38–205)	3.3 ± 0.9 (1.5–5.2)	6.1 ± 6.5 (0.5–34.8)	0.55 ± 0.99 (0.01–5.12)

age and degradation effects were assessed by analyzing a series of contrasting samples after 3 months (Fig. S2). The differences in optical proxies (a_{350}, $SUVA_{254}$ and S_R) were less than 5 % ($n = 22$) and no significant changes were observed by comparing excitation–emission matrices (EEMs). Samples for major elements (including Fe) were stored in 20 mL scintillation vials and acidified with 50 µL of HNO_3 65 % prior to analysis.

Fe was measured by inductively coupled plasma spectrometry (Agilent 7700× ICP-MS). DOC and $\delta^{13}C_{DOC}$ were analyzed with an Aurora1030 total organic carbon analyzer (OI Analytical) coupled to a Delta V Advantage isotope ratio mass spectrometer. Typical precision observed in duplicate samples was in >95 % cases <±5 % for DOC and ±0.2 ‰ for $\delta^{13}C_{DOC}$. Quantification and calibration were performed with series of standards prepared in different concentrations, using both IAEA-C6 ($\delta^{13}C = -10.4$ ‰) and in-house sucrose standards ($\delta^{13}C = -26.9$ ‰). All data are reported in the δ notation relative to VPDB (Vienna Pee Dee Belemnite). Absorbance was recorded on a Perkin-Elmer UV–visible 650S spectrophotometer using a 1 cm quartz cuvette. Absorbance spectra were measured between 200 and 700 nm in 1 nm increments, and instrument noise was assessed measuring ultrapure (Type 1) Milli-Q (Millipore) water as a blank. After subtracting the blank spectrum, the correction for scattering and index of refraction was performed by fitting the absorbance spectra to the data over the 200–700 nm range according to the following equation:

$$A_\lambda = A_0 e^{-S(\lambda-\lambda_0)} + K, \quad (1)$$

where A_λ and A_0 are the absorbance measured at defined wavelength λ and at reference wavelength $\lambda_0 = 375$ nm, respectively, S is the spectral slope (nm^{-1}) that describes the approximate exponential decline in absorption with increasing wavelength, and K is a background offset. The fit was not used for any purpose other than to provide an offset value K that was then subtracted from the whole spectrum (Lambert et al., 2015). Fluorescence intensity was recorded on a Perkin-Elmer LS45 fluorescence spectrometer using a 1 cm quartz cuvette across excitation wavelengths of 220–450 nm (5 nm increments) and emission wavelengths of 230–600 nm (0.5 nm increments) in order to build EEMs. If necessary, samples were diluted until $A_{254} < 0.2\ m^{-1}$ to avoid problematic inner filter effects (Ohno, 2002). Before each measurement session (i.e., each day), a Milli-Q water sample was also measured and subtracted from EEMs.

Water temperature, $\%O_2$, and pH were measured in situ with portable field probes calibrated using standard protocols (YSI ProPlus probe). Pelagic respiration (R) was determined from the decrease in O_2 in 60 mL biological oxygen demand bottles over ~24 h incubation periods. The bottles were kept in the dark and close to in situ temperature in a cool box filled with in situ water. The O_2 decrease was determined from triplicate measurements at the start and the end of the incubation with an optical O_2 probe (YSI ProODO). The respiratory quotient (RQ), defined as the molar ratio of O_2 consumed to CO_2 produced by respiration, allows the conversion of respiration measurements from O_2 to C units. The RQ value is in theory equal to 1 for the oxidation of glucose but higher than 1 for more complex and reduced organic molecules, such as lipids and proteins (e.g., 1.3 in a temperate stream with a catchment dominated by pastures; Richardson et al., 2013), or lower than 1 for highly oxidized and oxygen-rich molecules (e.g., 0.8 in boreal lakes; Berggren et al., 2012).

Given the range of RQ values, we adopted a RQ value of 1.0. The vertical light attenuation coefficient, K_d (m^{-1}), was calculated from simultaneous measurements of surface irradiance with a Li-Cor LI-190 quantum sensor and underwater photosynthetically active radiation (PAR) measurements with a submersible Li-Cor LI-193SA spherical quantum sensor. K_d was derived from the slope of the semi-logarithmic regression between relative quantum irradiance and depth. Transparency of the water column was measured using a 20 cm diameter Secchi disk.

2.3 Characterization of DOM composition

The specific ultraviolet absorbance ($SUVA_{254}$) was calculated as the UV absorbance at $\lambda = 254$ nm (A_{254}) normalized to the corresponding DOC concentration (Weishaar et al., 2003). The natural UV absorbance of Fe at $\lambda = 254$ nm was estimated based on measured Fe concentrations and was then subtracted from the UV absorbance measured. The corrected value of A_{254} was then used to calculate $SUVA_{254}$. The $SUVA_{254}$ was used as an indicator of the aromaticity with higher values indicative of greater percent of aromaticity (Weishaar et al., 2003).

Napierian absorption coefficients were calculated according to

$$a_\lambda = 2.303 \times A_\lambda / L, \quad (2)$$

where a_λ is the absorption coefficient (m^{-1}) at wavelength λ, A_λ the absorbance corrected at wavelength λ and L the path length of the optical cell in meters (0.01 m). CDOM was reported as the absorption coefficient at 350 nm (a_{350}). Spectral slopes for the intervals 275–295 ($S_{275–295}$) nm and 350–400 nm ($S_{350–400}$) were determined from the linear regression of the log-transformed a spectra vs. wavelength. The slope ratio S_R was then calculated as the ratio of $S_{275–295}$ to $S_{350–400}$. S_R is inversely related to the molecular weight (MW) distribution of DOM (Helms et al., 2008). The fluorescence index (FI) was calculated as the ratio of the emission intensities at 470 and 520 nm at an excitation wavelength of 370 nm (McKnight et al., 2001). A higher FI value (e.g., 1.8) indicates an aquatic microbial DOM source, while a lower value (e.g., 1.2) indicates a terrestrial source. Intermediate values indicate a mixed DOM source.

2.4 PARAFAC modeling

EEM preprocessing (including removing first and second Raman scattering, standardization to Raman units, absorbance corrections and inner filter effects) was performed prior to the PARAFAC modeling. The scans were standardized to Raman units (normalized to the integral of the Raman signal between 390 and 410 nm in emission at a fixed excitation of 350 nm) with a Milli-Q water sample run the same day as the samples (Zepp et al., 2004). The PARAFAC model was built using MATLAB (MathWorks, Natick, MA, USA) and the drEEM toolbox version 1.0 (Murphy et al., 2013). Validation of the model was performed by split-half analysis and random initialization. The normalization step was applied to scale each EEM to its total signal, thus ensuring the model focused entirely on compositional rather than concentration gradients. Additional samples analyzed in the same manner and collected from the Kwa/Kasai River basin ($n = 104$), Lago Janauacá (a central Amazon floodplain lake, $n = 17$), the Niger River ($n = 19$) and the Okavango Delta ($n = 17$) were added to the dataset to increase the variability in DOM fluorescence signatures and help detect components that could have been present in insufficient quantity to be detected in our environment. The maximum fluorescence F_{Max} values of each component for a particular sample provided by the model were summed to calculate the total fluorescence signal F_{Tot} of the sample in Raman units. The relative abundance of any particular PARAFAC component X was then calculated as $\%C_X = F_{Max}(X)/F_{Tot}$.

The positions of maximum peaks established by the model were compared to the classical excitation–emission matrix nomenclature (Fellman et al., 2010; Coble et al., 2014) and with other reported PARAFAC models built in a large variety of freshwater ecosystems (Table 2). Additionally, each PARAFAC component was associated with a dominant molecular class based on recent studies aiming to correlate individual molecular formula with different PARAFAC components through Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS). Such studies have been carried out in Swedish lakes (Kellerman et al., 2015), boreal streams and rivers (Stubbins et al., 2014), and a subtropical wetland (Wagner et al., 2015). Although such a comparison has not been performed with our own samples, the relatively good consistency of associations between optical and molecular linkages observed in these contrasting environments suggests that PARAFAC components can track dominant DOM molecular composition similarly across different biomes in terms of DOM MW and enrichment in aliphatic or aromatic molecules (Wagner et al., 2015).

2.5 Landscape analysis

The total drainage area and the Strahler stream order (Strahler, 1957) were calculated at each station in the geographic information system (GIS) software ArcGis® (ESRI 2011, ArcGis Desktop 10.3.1), using the ArcHydro tools (v. 2.0) and the hydrological data and maps based on shuttle elevation derivatives at a 3 arcsec resolution (Lehner et al., 2008). The extents of wetland areas and flooded dense forest cover were extracted from the Global Lakes and Wetlands Database (Lehner and Döll, 2004) and from Global Land Cover 2009 database (Bontemps et al., 2011), respectively.

2.6 Statistical analysis

Mann–Whitney t tests were performed to investigate differences in DOM properties spatially (mainstem vs. tributaries) and temporally (HW vs. FW). A principal component analysis (PCA) was also performed to explore DOM evolution during its transport through the Congo fluvial network. The optical properties of DOM, including level of CDOM (a_{350}), bulk composition ($SUVA_{254}$, S_R, FI) and the relative abundance of PARAFAC components, were used as the variables. Given the different units of these variables, data were scaled to zero mean and unit variance as recommended by Borcard et al. (2011). The PCA was performed using the prcomp function in R software.

3 Results

3.1 DOC concentrations and DOM bulk composition

DOC concentrations in the mainstem were higher during HW (5.4–13.9 mg L^{-1}, average 8.2 ± 2.6 mg L^{-1}) compared to FW (4.2–9.8 mg L^{-1}, average 5.9 ± 1.8 mg L^{-1}) but showed similar longitudinal patterns during both hydrological periods (Fig. 3a, b): DOC increased slowly in the upper part of the transect (0.0012 mg DOC km^{-1} at HW and 0.0002 mg DOC km^{-1} at FW) and then faster as the Congo River evolved throughout the core of the Cuvette Centrale and mixed with the Kwa/Kasai River (0.0091 mg DOC km^{-1} at HW and 0.0085 mg DOC km^{-1} at FW) (Fig. 1). The breaking point in the DOC longitudinal increase in DOC concentrations was located around kilometer 700 during HW, and kilometer 500 during FW. DOC concentrations in tributaries were highly variable (from 1.8 to 67.8 mg L^{-1}) and were found to be correlated with the extent of flooded forest (Fig. 4), resulting in highest concentrations in tributaries draining the Cuvette Centrale and lowest concentrations in those draining savannah areas upstream of Kinshasa (Fig. 1). Tributaries located downstream of the Cuvette Centrale were also characterized by lowest DOC concentrations during FW compared to HW, while no clear pattern was observed for those located upstream.

$\delta^{13}C_{DOC}$ signatures in the mainstem were lower during HW (from −30.6 to −28.8 ‰, average -29.4 ± 0.3 ‰, $n = 35$) compared to FW (from −29.3 to −25.2 ‰, average -27.5 ± 0.9 ‰, $n = 34$). $\delta^{13}C_{DOC}$ during HW decreased about 0.7 ‰ from Kisangani to ca. kilometer 1200, remained stable until ca. kilometer 600 and then increased slightly towards Kinshasa. During FW, $\delta^{13}C_{DOC}$ decreased markedly about 3 ‰ between Kisangani and ca. kilometer 1600. Downstream, values were variable (-27.2 ± 0.6 ‰ between kilometers 600 and 1600, $n = 18$) and then showed ~ 1 ‰ drops at ca. kilometer 600 and ca. 200, coinciding with the confluence zones with the Oubangui and the Kwa/Kasai rivers, respectively. In tributaries, $\delta^{13}C_{DOC}$ values displayed

Table 2. Spectral properties (excitation and emission maxima (Ex_{max} / Em_{max})) of the six components identified using PARAFAC modeling, correspondence with peak classification, general assignment and comparison with previously identified components in different environments, dominant molecular association and possible source and reactivity. Dominant molecular association is based on FTICR-MS studies. Numbers in brackets refer to the second peak of maximal excitation.

Component	Ex_{max} (nm)	Em_{max} (nm)	Peak name[1]	General assignment[2]	Comparison with others environments						Dominant molecular association[4,6,10]	Potential sources and reactivity
					Arctic Rivers[3]	St. Lawrence River[4]	Boreal streams[5,6]	Boreal lakes[7,8]	Tropical wetland[9,10]	Zambezi Basin[11]	Tropical wetland[12]	
C1	<260 (375)	488	A+/C+	Terrestrial humic-like	C3	C3	C2	C3	C5	C2	C2	High aromaticity, high MW	T^{1-12}, P^{-5}
C2	305 (<260)	414	M	Microbial humic-like	–	C7	C5	C2	C4	C3	C3	Aliphatic, low MW	$M+^{8-10}$
C3	330 (<260)	444	A_C/C	Terrestrial humic-like	C1	C2	C3	C1	C6	C1	C1	High aromaticity, high MW	T^{1-12}, $P-^{5,9,10}$
C4	<260	444	A_C	Terrestrial humic-like	–	C1	–	C5	C2	C4	–	Aromatic, intermediate MW	T^{9-11}, $P+^{4}$, Pr^{10}
C5	350	424	C	Humic-like	–	–	C4	–	–	–	–	Low aromaticity, low MW	$P+^{5}$
C6	275	350	B/T	Tryptophan-like	C5	C4	C6	C6	C7	C5	C4	Aliphatic, low MW	$Au^{4,9,11}$, $M+^{3,7}$, $M-^{5}$

[1] Coble et al. (2014). [2] Fellman et al. (2010). [3] Walker et al. (2013). [4] Massicotte and Frenette (2011). [5] Lapierre and del Giorgio (2014). [6] Stubbins et al. (2014) (FTICR-MS). [7] Kothawala et al. (2014). [8] Kellerman et al. (2015) (FTICR-MS). [9] Yamashita et al. (2010). [10] Cawley et al. (2012). [11] Lambert et al. (2016). [12] Wagner et al. (2015) (FTICR-MS). Au: autochthonous production; T: terrestrial inputs; M+: microbial degradation; M−: biolabile; P+: photoproduct; Pr: photo-resistant; P−: photosensitive.

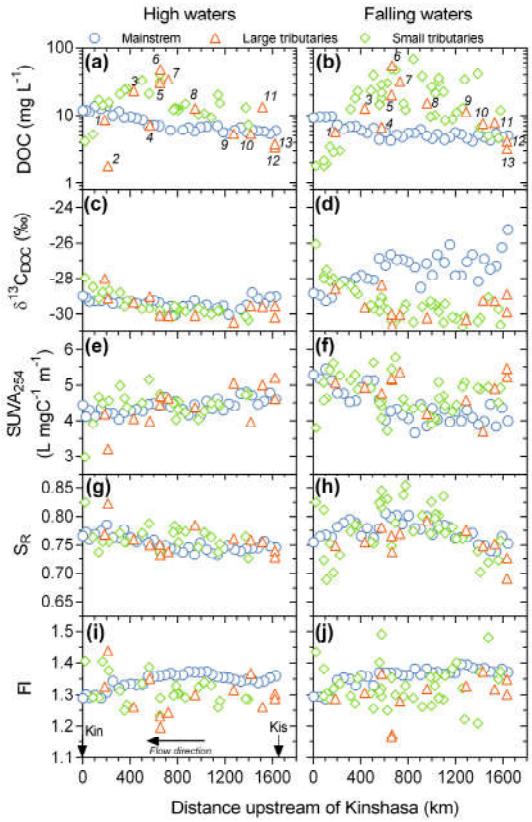


Figure 3. Longitudinal evolution of DOM properties in the mainstem, large and small tributaries along the Kisangani–Kinshasa transect during HW (left panels) and FW (right panels). From top to bottom, the panels represent DOC, $\delta^{13}C_{DOC}$, $SUVA_{254}$, S_R and FI. Numbers refer to large tributaries: (1) the Kwa/Kasai, (2) the Lefini, (3) the Sangha, (4) the Oubangui, (5) the Ruki, (6) the Ikelemba, (7) the Lulonga, (8) the Mongala, (9) the Itimbiri, (10) the Aruwimi, (11) the Lomami, (12) the Lindi and (13) the Tshopo.

a similar pattern during the two hydrological periods with low and relatively stable values (-29.7 ± 0.5 ‰, $n = 76$) in streams and rivers draining dense forest areas and higher signatures in those flowing savannah areas 0–400 km upstream of Kinshasa (-28.1 ± 0.8, $n = 14$). Stations of the mainstem located within or upstream of the Cuvette Centrale were characterized by higher $\delta^{13}C_{DOC}$ values than those measured in tributaries collected along the same transect, during both HW ($p < 0.004$) and FW ($p < 0.0001$). Downstream of the Cuvette Centrale, stations of the mainstem had lower $\delta^{13}C_{DOC}$ signatures than those measured in tributaries ($p < 0.0001$, all periods).

$SUVA_{254}$ and S_R during both periods varied mainly between 4.0 and 5.2 L mg C^{-1} m^{-1} and between 0.734 and 0.802 among all stations, respectively (10–90 % percentiles, $n = 160$), indicating that DOM in the Congo Basin was dominated by aromatic compounds of high MW during both periods (Fig. 3e–h). $SUVA_{254}$ generally decreased from Kisangani to Kinshasa in the mainstem during HW, with a slight increase between kilometers 500 and 800 upstream of Kinshasa, while S_R exhibited stable values from Kisangani and then started to increase towards Kinshasa at kilometer 700. Compared to HW, $SUVA_{254}$ during FW was relatively stable and lowest from Kisangani to kilometer 500 but higher between kilometers 0 and 500 as $SUVA_{254}$ increased markedly in this section ($p < 0.0001$). S_R exhibited a hump-shaped pattern during FW, with increasing values from Kisangani to kilometer 500 and decreasing value between kilometers 0 and 500. A slight decrease in $SUVA_{254}$ ($p = 0.0043$) associated with an increase in S_R ($p = 0.047$) was also observed between kilometers 200 and 400. Generally, $SUVA_{254}$ in tributaries were slightly higher at FW than at HW ($p = 0.035$), similar to the mainstem in HW but higher in FW ($p = 0.0113$). FI in the mainstem gradually decreased from Kisangani to Kinshasa during both hydrological periods, with higher values during FW than at HW ($p = 0.0006$), and were higher than in tributaries ($p < 0.0001$). No distinct seasonal variation was apparent in tributaries.

3.2 PARAFAC results

Six PARAFAC components were determined to adequately model our dataset (Table 2, Fig. S3). Components C1, C3, C4, and C5 are all classified as "humic-like" but differ in terms of sources, molecular association and reactivity (Table 2). C1 and C3 are commonly reported in freshwaters and are associated with a group of high MW and aromatic molecules of terrestrial origin (e.g., Wagner et al., 2015). Both are susceptible to photodegradation (Lapierre and del Giorgio, 2014). C4 is associated with terrigenous molecules of lower aromaticity and MW relative to C1 and C3 (Kellerman et al., 2015). C4 commonly originates from terrestrial inputs (Stedmon and Markager, 2005; Yamashita et al., 2010; Lambert et al., 2016) and has been shown to be resistant to photodegradation (Massicotte and Frenette, 2010; Ishii and Boyer, 2012). Among the humic-like compounds, C5 is associated with molecules characterized by lowest aromaticity and MW produced during the photodegradation of terrestrial DOM (Lapierre and del Giorgio, 2014; Stubbins et al., 2014). C2 and C6 are classified as microbial humic-like and tryptophan-like components, respectively (Fellman et al., 2010). In contrast to the other components, C2 and C6 are associated with low MW DOM fractions enriched in aliphatic molecules biologically produced within aquatic ecosystems (Kellerman et al., 2015; Wagner et al., 2015). Both C2 and C6 can be assigned to a fraction of DOM produced during the microbial degradation of terrestrial DOM within freshwaters (Stedmon et al., 2003; Walker et al., 2013), although autochthonous primary production represents another potential source for C6 (Yamashita et al., 2010).

The relative contribution of C1 and C3 showed similar patterns along the mainstem during both periods (Fig. 5). %C1 and %C3 during HW presented a slight downstream decrease

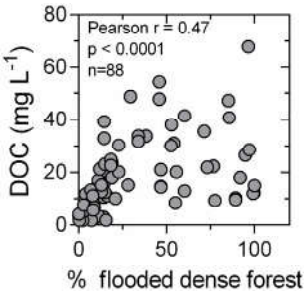


Figure 4. Relationships between DOC concentrations in tributaries and the extent of flooded dense forest.

followed by an increase with minimal contribution recorded around kilometer 1100. %C1 and %C3 were lowest during FW ($p = 0.017$ and $p < 0.0001$, respectively), with low variability upstream of kilometer 500 and highest contribution downstream. %C4 displayed a general increase along the transect at HW especially marked between Kisangani and ca. kilometer 1100 and between kilometers 600 and 150. During FW, the longitudinal evolution of %C4 showed an opposite pattern to those of %C1 and %C3, with a higher contribution than during HW ($p > 0.0001$). Overall, %C5 was higher during FW than during HW ($p < 0.0001$) and exhibited longitudinal patterns opposite to those of %C3 during both periods. %C2 was relatively stable along the mainstem during both periods, with higher contribution during FW compared to HW ($p = 0.0076$). C6 exhibited the lowest contribution to FDOM signal and %C6 tended to be lower during FW compared to HW. Longitudinal evolution of C6 was characterized by a strong drop along the mainstem, occurring around kilometer 800 at HW and kilometer 500 at FW.

Overall, tributaries were characterized by higher %C3 but lower %C2 and %C6 relative to the mainstem ($p < 0.0001$). %C4 and %C1 were higher ($p = 0.0007$) and lower ($p = 0.017$), respectively, in tributaries than in the mainstem during HW, and no difference was observed at FW. No difference was observed for %C5 between tributaries and the mainstem for both periods. The seasonal variability within tributaries was characterized by higher contribution of C4 ($p = 0.025$) and C5 ($p < 0.0001$) and lower contribution of C3 ($p = 0.0015$) and C6 ($p = 0.003$) during FW compared to HW.

3.3 PCA results

The first two principal components (PC) accounted for 57 % of the total variance (Fig. 6). The first PC (PC1) showed a transition from terrestrial aromatic DOM (%C3, $SUVA_{254}$, DOC, a_{350}, positive loadings) to aliphatic DOM (%C2, %C6, FI, negative loadings). Along the second PC (PC2), PARAFAC components are distributed with C1 and C3 as negative loadings and C4 and C5 as positive loadings. C1 and C3 have been identified as photosensitive (Yamashita et al., 2010; Lapierre and del Giorgio, 2014), while C4 and C5 are considered photo-resistant (Massicotte and Frenette, 2010; Ishii and Boyer, 2012) and produced by photodegradation (Lapierre and del Giorgio, 2014), respectively. Therefore, PC2 can be interpreted as being indicative of photodegradation (Cawley et al., 2012). The distribution of sampling stations for a given Strahler order was highly heterogeneous (Fig. 6a). However, a global pattern emerges along PC1 with stations collected in the mainstem showing mainly negative scores (Fig. 6b). Furthermore, stations of the mainstem collected during HW had negative scores along PC2 but positive scores during FW. Overall, stations collected during HW had mainly negative scores along PC2, while those sampled at FW showed large variability along PC2.

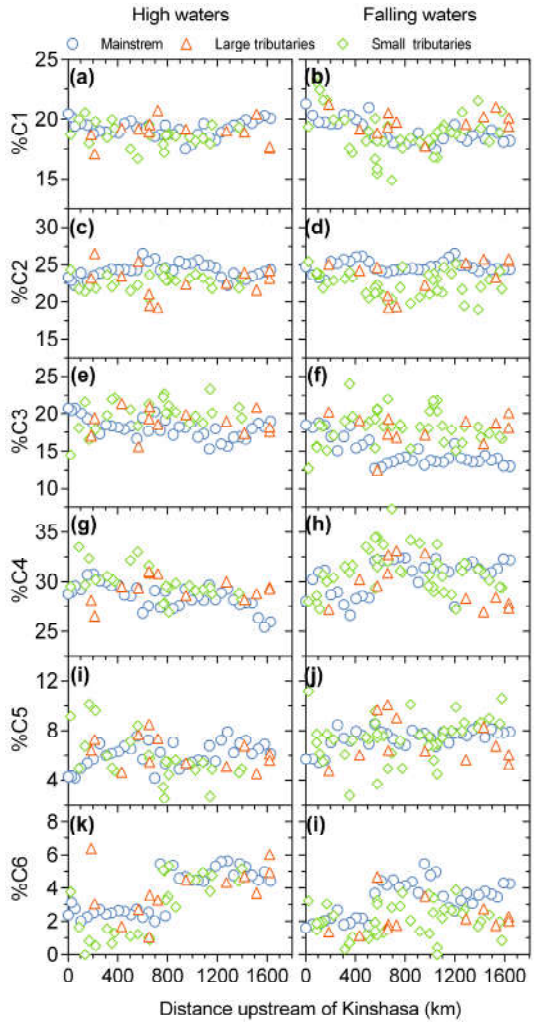


Figure 5. Longitudinal evolution of the relative contribution of PARAFAC component in the mainstem and large and small tributaries along the Kisangani–Kinshasa transect during HW (left panels) and FW (right panels).

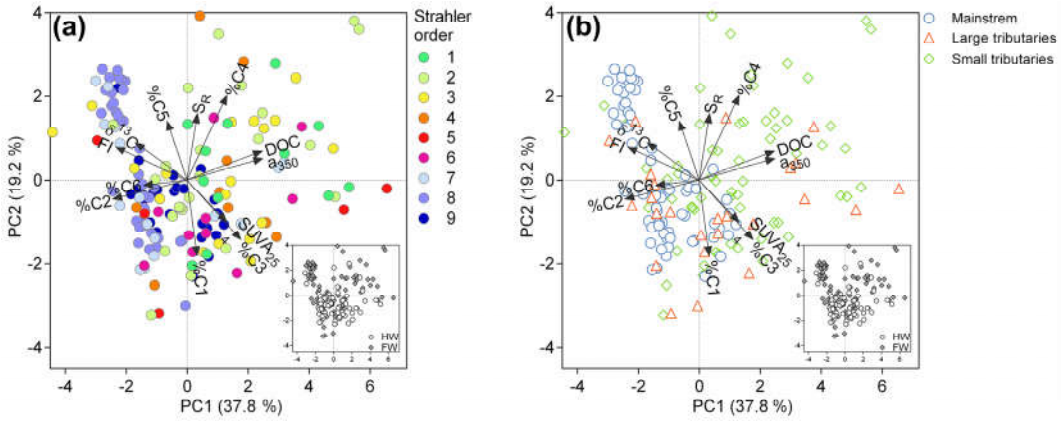


Figure 6. Graphical representation of PCA results, including a loadings plot for the input variables and a scores plot for stations based on **(a)** their Strahler stream order or **(b)** sampling location. PCA results based on the hydrological period is included in each plot.

4 Discussion

4.1 Longitudinal evolution of DOM

From Kisangani to Kinshasa the Congo River continually receives DOM inputs from tributaries flowing through the Cuvette Centrale (Fig. 4), resulting in a net longitudinal increase in DOC concentrations during both periods. However, the longitudinal evolution in DOM content and composition differed between the two campaigns due to the combination of several factors.

4.1.1 Seasonal changes in DOM sources mobilized in the upper basin

The large variation in $\delta^{13}C_{DOC}$ values in the mainstem at Kisangani between HW (−29.0 ‰) and FW (−25.2 ‰) can be related to a shift in the source of DOM mobilized in the upper part of the basin due to differences in water routing during the hydrograph. Thus, decreasing $\delta^{13}C_{DOC}$ signatures that occurred during high-flow periods have been attributed to the mobilization of fresh DOM from superficial soil horizons in a wide variety of catchments (Neff et al., 2006; Sanderman et al., 2009; Lambert et al., 2011; Bouillon et al., 2012). Higher $\delta^{13}C_{DOC}$ values during low-flow periods reflect the deepening of water flow paths and the subsequent mobilization of more degraded DOM from deeper soil horizons. This seasonal change in DOM composition at the start of the Kisangani–Kinshasa transect is further supported by 0ongoing high-frequency sampling carried out at Kisangani (own unpublished data).

4.1.2 Reduced lateral exchanges between the Congo River and the flooded forest during falling waters

The decreasing but higher $\delta^{13}C_{DOC}$ signatures in the mainstem compared to tributaries during FW indicate a reduced lateral mixing between the central water masses of the Congo River and DOM-rich waters from tributaries. Although we cannot rule out that lateral exchanges are likely driven by water level fluctuations in the Congo River, our data suggest that photodegradation of terrestrial DOM contributes to limit the downstream DOC enrichment during FW. Photodegradation is known to preferentially act on the colored, photosensitive molecules associated with high MW and aromaticity (Helms et al., 2008; Spencer et al., 2009; Lapierre and del Giorgio, 2014) and is expected to be highly efficient in aquatic ecosystems rich in light-absorbing CDOM such as the Congo River (Cawley et al., 2012; Cory et al., 2014). A stronger effect of DOM photodegradation in the mainstem during FW is supported by three lines of evidence in our data. First, C1 and C3 – both associated with highly aromatic molecules of high MW (Table 2) – were lower during FW compared to HW, and this decrease occurred along with a decrease in DOM aromaticity (lower $SUVA_{254}$) and increase in average MW (higher S_R) (Figs. 3 and 5). Second, the more drastic decrease in %C3 is consistent with the well-documented high photosensitivity of this component relative to other terrestrial humic-like components (Yamashita et al., 2010; Cawley et al., 2012; Lapierre and del Giorgio, 2014). Finally, the PCA clearly separates stations collected in the mainstem between HW (negative scores) and FW (positive scores) along PC2, which shows that photodegradation is an important driver of DOM composition in the Congo Basin.

A stronger degree of DOM photodegradation during FW implies a higher exposure of CDOM to solar irradiation, either spatially (i.e., in the water column) or temporally. The higher coefficient of light attenuation in the water column (K_d) and lower Secchi depths during FW (Table 1) indicate that the penetration of sunlight in the water column was reduced compared to HW. This was likely due to the greater total suspended matter (TSM) concentrations (Table 1) and phytoplanktonic development (Descy et al., 2016), although CDOM likely remained the main driver of light penetration. Thus, the degree of DOM photodegradation appears to be

mainly driven by changes in WRT that has been estimated to increase about ∼ 5 days in FW compared to HW (Descy et al., 2016). Although sunlight depth penetration was low and the variation in the WRT relatively small, rates of photodegradation can be elevated in CDOM-rich ecosystems (Cory et al., 2015), and this process can substantially alter terrestrial DOM over a short timescale (e.g., ∼ 12 h of sunlight alteration according to Cory et al., 2015). Further research is needed in order to quantify photodegradation rate and the relative importance of this process on C cycling in the Congo Basin, but this was beyond the scope of the present study.

4.1.3 Role of large tributaries and channel width in controlling the longitudinal evolution of DOM from Kisangani to Kinshasa

DOM enrichment was more pronounced within the core of the Cuvette Centrale (Fig. 2), which corresponds to the region where the major tributaries in terms of discharge (i.e., the Lulonga, the Ruki, the Sangha, the Oubangui and the Kwa/Kasai rivers) connect the mainstem. These tributaries are also very rich in terrestrial DOM from the large flooded forest (Coynel et al., 2005; Laraque et al., 2009) (Figs. 1 and S1). DOC concentrations increased faster immediately as the Congo enters this central part of the Cuvette Centrale during HW, reflecting the strong lateral mixing between water masses. However, the net rise in DOC concentrations during FW was found to occur first at ∼ 70 km downstream of the confluence zone with the Oubangui River, coinciding with a strong reduction of the channel width (Fig. S4). The ∼ 1 ‰ drop in $\delta^{13}C_{DOC}$ associated with changes in DOM composition (especially increase in $SUVA_{254}$ and %C3) at this station indicates that the reduction of the channel width favors the lateral mixing between the mainstem and waters from the Cuvette Centrale that travel along the river ridge lined by dense forest without being significantly impacted by photodegradation (Fig. S5). In fact, a "complete" lateral mixing with waters from the Cuvette Centrale occurs after the confluence zone with the Kwa/Kasai River (based on cross channel transects of conductivity and pH, not shown). In this part of the river, the high discharge of the Kwa/Kasai combined with a narrow mainstem channel devoid of sandbars and islands (Runge et al., 2008) forces lateral mixing. This is supported by the fact that $\delta^{13}C_{DOC}$ signatures of the Congo mainstem became typical of black waters only after connecting with the Kwa/Kasai during FW, and could also explain why the DOC increase is greater at this point, while DOC is typically higher in tributaries located more upstream (e.g., the Ruki River).

Large tributaries also controlled the general evolution of DOM composition from Kisangani to Kinshasa. DOM aromaticity ($SUVA_{254}$) decreased slightly along the transect during HW (from ∼ 4.6 to 4.2 mg C L^{-1} m^{-1} from Kisangani to Kinshasa), but increased significantly during FW (from ∼ 4.0 to 5.3 mg C L^{-1} m^{-1} from Kisangani to Kinshasa) due to an increase in DOM aromaticity in large tributaries flowing through or connected to the Cuvette Centrale.

4.2 DOM transformation during its downstream transport

Strahler stream order was used as an organizing concept for the individual characterization of stream reaches within the network (Strahler, 1957; Poole, 2010), and in order to investigate DOM composition across a gradient of streams and rivers. Although DOM in the Congo Basin is dominated by terrestrial inputs of aromatic DOM, the loadings plot along PC1 indicates a transition in the dominant DOM composition from aromatic (%C3, $SUVA_{254}$) to aliphatic (%C2, %C6, FI) compounds (Fig. 6). It is noteworthy that similar gradients have recently been reported in Swedish lakes (Kellerman et al., 2015) and US rivers networks (Creed et al., 2015), suggesting that the large-scale governing processes controlling DOM in freshwater are similar across biomes. However, the underlying mechanisms remain to be elucidated. Thus, the gain in aliphatic DOM has been attributed to the increasing influence of autochthonous sources (Creed et al., 2015) or to the degradation of terrestrial DOM (Kellerman et al., 2015), while external factors (i.e., not related to DOM composition) have been suggested to have little influence on this pattern (Kellerman et al., 2015). Our study supports the hypothesis that the degradation of terrestrial DOM is the main driver of DOM transformation in aquatic systems, but it also highlights the role of landscape morphology and environmental conditions in influencing the transition from an aromatic- to aliphatic-dominant composition.

4.2.1 Losses of aromatic DOM through photodegradation and biological activity as a producer of aliphatic DOM

The role of photodegradation in removing the aromatic fraction of DOM in the Congo Basin, strongly suggested by the PCA as well as by the changes in DOM composition along the mainstem between HW and FW, is also supported by patterns in C5. Indeed, %C5 was inversely related to %C3 (Fig. 7a), suggesting that C5 tracks molecules directly produced during the photodegradation of terrestrial aromatic molecules as previously demonstrated in boreal freshwaters (Lapierre and del Giorgio, 2014). In addition, %C5 was inversely related with measurements of pelagic community respiration (R) performed concurrently with DOM sampling (Borges et al., 2015a) and attributed to bacterial respiration since phytoplankton biomass is generally low (Descy et al., 2016) (Fig. 7b). Given that C5 is associated with molecules of low MW and aromaticity (Stubbins et al., 2014), this observation is consistent with experimental results showing that the aromatic and high MW fraction of terrestrial DOM can be photochemically converted into more labile substances

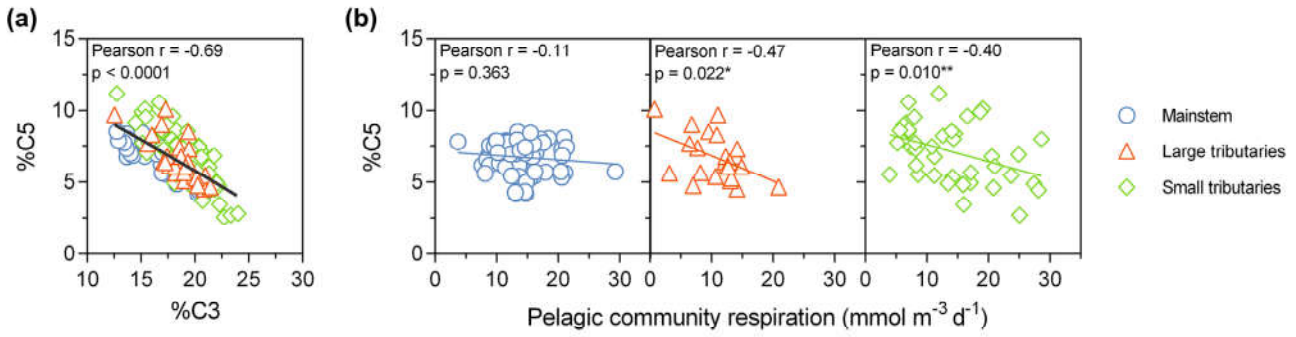


Figure 7. **(a)** Relationship between %C5 and %C3 and **(b)** relationships between %C5 and pelagic community respiration (R) in the Congo Basin.

of lower MW that in turn can support the aquatic bacterial metabolism (Bano et al., 1998; Tranvik and Bertilsson, 2001; Remington et al., 2011; Cory et al., 2014). The lack of correlation between %C5 and R in the mainstem likely indicates an additional source of labile DOM. The higher concentrations of chlorophyll a in the mainstem compared to tributaries (Table 1) suggest that this source could be phytoplanktonic exudates that have been shown to be very labile and rapidly assimilated by bacteria (Baines and Pace, 1991; Morana et al., 2014).

The dual role of microorganisms as consumers of terrigenous aromatic DOM and producers of novel compounds has recently been emphasized (Guillemette and del Giorgio, 2012; Ward et al., 2013; Fasching et al., 2014) and could explain the gain in aliphatic DOM in the Congo River. Components C2 and C6 – associated with aliphatic molecules (Table 2) – exhibited systematically higher contributions in the mainstem compared to tributaries, implying a permanent internal production. However, none of these components were correlated to chlorophyll a concentrations (data not shown), suggesting that the phytoplankton primary production in the Congo Basin was not controlling their distribution. On the other hand, several studies carried out in a wide variety of aquatic ecosystems have attributed the origin of components like C2 and C6 to the biological degradation of terrestrial DOM within the aquatic ecosystems (Stedmon et al., 2003; Yamashita et al., 2010; Walker et al., 2013; Fasching et al., 2014; Kellerman et al., 2015). Therefore, microbial reworking of terrestrial DOM during its transport could lead to the production of these components, an assumption supported by higher FI values in the mainstem inferring greater inputs of microbially derived DOM relative to tributaries (McKnight et al., 2001).

Our results contrast with previous investigations based on lignin biomarkers suggesting that DOM transformation in the Congo Basin is mainly driven by dynamic exchanges with the particulate organic carbon (POC) pool via sorption or leaching processes (Spencer et al., 2012; Mann et al., 2014). The $\delta^{13}C$ signatures of POC collected during our field campaigns spanned a much wider range but were poorly correlated with $\delta^{13}C_{DOC}$ ($R^2 = 0.20$, $p < 0.0001$; $n = 158$, data not shown), suggesting limited exchange between these pools. However, our results are in line with previous field studies showing that downstream changes in DOM composition can be largely controlled by photodegradation (Massicotte and Frenette, 2011; Cawley et al., 2012).

4.2.2 Role of external drivers on the transition from aromatic towards aliphatic

The distribution of data in the PCA (Fig. 6) shows that (1) DOM in the mainstem is enriched in the aliphatic fraction compared to its tributaries and (2) the distribution of tributaries for a given Strahler order is strongly heterogeneous. This highlights the combined effects of landscape morphology and water chemistry that can influence the downstream transformation of DOM independently of the size of the streams/rivers. Thus, land cover – here mostly forest and savannah – has a strong influence on the composition of DOM released in aquatic ecosystems (Mann et al., 2014; Lambert et al., 2015), and DOM photodegradation is likely more pronounced in catchments with large open areas as suggested by the lower %C3 in savannah-dominated catchments compared to forest-dominated catchments (Fig. S6). Also, a strong connectivity with terrestrial sources can maintain a greater aromatic character to DOM independently of the size of the rivers. This typically refers to the well-known role of wetland areas in delivering large quantities of aromatic DOM in inland waters (Battin et al., 1998; Hanley et al., 2013; Lambert et al., 2016) and thus obscuring variations in DOM occurring upstream. A multi-year monitoring illustrates, for example, that DOM in the Oubangui River at Bangui (i.e., upstream of the Cuvette Centrale, Fig. 1) experiences significant changes in composition between rising waters (June; DOM has few aromatic structures) and high waters (December; DOM is highly aromatic; Bouillon et al., 2014), while our data report highly aromatic DOM during both periods as the river becomes enriched in organic material from the flooded forest (Laraque et al., 2009). Finally, the positive correlations be-

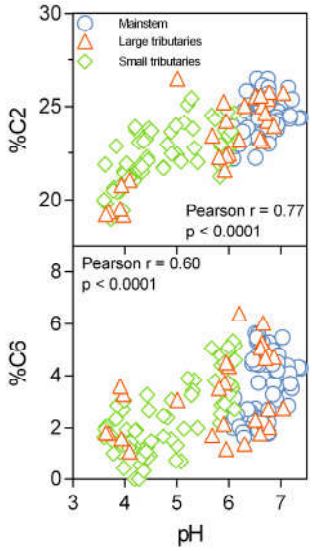


Figure 8. Relationship between the relative contribution of aliphatic components (C2 and C6) and pH of stream waters in the Congo Basin.

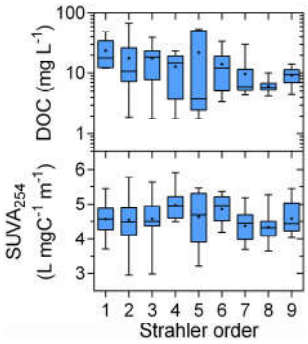


Figure 9. DOC concentrations and DOM aromaticity ($SUVA_{254}$) across a gradient of streams and rivers in the Congo Basin as a function of stream order. The box spans the interquartile range (25th–75th percentiles), whiskers correspond to min–max values, and the horizontal bar and cross correspond to the median and average, respectively.

tween %C2 and pH and between %C6 and pH (Fig. 8) advocate for a limited degree of bacterial degradation of DOM in very acidic environments as previously suggested by Borges et al. (2015a) based on the CO_2 and DOC relationship in the Congo Basin. Such streams and rivers typically correspond to the DOM-rich "black waters" originating from the Cuvette Centrale, with pH between 3.6 and 5.9 and an average of 4.4 (Fig. S7).

4.3 The chemostat hypothesis and the pulse-shunt concept

The chemostat hypothesis suggests a decrease in DOC concentrations and a convergence in DOM composition towards lower aromaticity with increasing stream order due to the increasing influence of in-stream processes that overwhelm terrestrial inputs from headwater catchments (Creed et al., 2015). The shift from a predominantly terrestrial influence to biogeochemical processing – assessed by the variation of $SUVA_{254}$ as a function of stream order – has been estimated to occur in third- or fourth-order streams in river networks across the United States (Creed et al., 2015). A net decrease in $SUVA_{254}$ associated with a decrease with DOC concentrations was only found to occur from sixth- to eighth-order streams in our study (Fig. 9), reflecting the influence of the Cuvette Centrale (i.e., strong connectivity with the flooded dense forest, acidic waters) on the DOM biogeochemistry in the Congo Basin. Although the seasonal variations of water level are modest in the Congo and that the Cuvette Centrale does not flush as floodplain lakes in the Amazon (e.g., Moreira-Turcq et al., 2003), the flooded forest is drained by several large rivers (Ruki, Sangha) that bring large amounts of water and DOM to the mainstem (the Ruki is the second tributary after the Kasai). This falls in line with the "flood pulse concept" of Junk et al. (1989) that highlights the critical importance of the river–floodplain connectivity in lowland tropical rivers (Battin, 1998; Zurbrügg et al., 2013; Lambert et al., 2016), while the chemostat hypothesis builds on the river continuum concept (Vannote et al., 1980) that is typically applicable to rivers at temperate latitudes and devoid of large wetlands. Patterns of other data acquired during the same cruises, such as CO_2 and CH_4, also showed the influence of inputs of these quantities from the Cuvette Centrale (Borges et al., 2015a). Also, an increase in DOM content and aromaticity was found to occur in ninth-order streams, reflecting the fact that DOM-rich waters from the Cuvette Centrale can travel along the ridge of the Congo River without completely mixing with the central water masses of the mainstem (Fig. S5).

Our study also supports the "pulse-shunt" conceptual model that states that the removal of terrestrial DOM in fluvial networks is a function of the hydrological regime of the basin (Raymond et al., 2016). It should be noted that the seasonal variation in water discharge is relatively low in the Congo Basin compared to other large rivers, yet apparently sufficient to significantly impact the degree of DOM photodegradation between FW and HW. The switch between active and passive pipes is likely to be more pronounced in large drainage basins in the Northern and Southern Hemisphere with more contrasting hydrological regimes, as recently shown in the adjacent Zambezi Basin (Lambert et al., 2016). Our results also suggest that the photodegradation pathway is more sensitive to changes in WRT compared to the biological pathway, but this hypothesis needs to be verified in other environments and by additional incubation experiments.

5 Data availability

The digital elevation model HydroSHEDS (Lehner et al., 2008) is available at http://hydrosheds.cr.usgs.gov/index.php. The Global Lakes and Wetlands database (Lehner and Döll, 2004) is available at http://www.worldwildlife.org/pages/global-lakes-and-wetlands-database. The Global Land Cover 2009 database is available at http://landcover.usgs.gov/landcoverdata.php. The dataset is available upon request from A. V. Borges.

The Supplement related to this article is available online at doi:10.5194/bg-13-5405-2016-supplement.

Author contributions. Alberto V. Borges, François Darchambeau, Steven Bouillon designed the study; Alberto V. Borges and François Darchambeau collected the field data; Steven Bouillon and Thibault Lambert performed sample analysis; Thibault Lambert carried out the geographical system information (GIS) analysis and performed the PARAFAC model with help of Philippe Massicotte; and Thibault Lambert analyzed the data and drafted the manuscript, which was revised and approved by all co-authors.

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A review of the Distichodus antonii assemblage (Characiformes: Distichodontidae) from the Congo basin

Tuur Moelants[ab], Victorine Mbadu Zebe[c], Jos Snoeks[ab] & Emmanuel Vreven[ab]

[a] Department of Vertebrates, Ichthyology, Royal Museum for Central Africa, Tervuren, Belgium

[b] Faculty of Science, Department of Biology, Laboratory of Biodiversity and Evolutionary Genomics, KU Leuven, Leuven, Belgium

[c] Département de Biologie, Laboratoire d'Hydrobiologie, Limnologie et Aquaculture, Université de Kinshasa, Kinshasa, Democratic Republic of the Congo

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A review of the *Distichodus antonii* assemblage (Characiformes: Distichodontidae) from the Congo basin

Tuur Moelants[a,b], Victorine Mbadu Zebe[c], Jos Snoeks[a,b] and Emmanuel Vreven[a,b]*

[a]*Department of Vertebrates, Ichthyology, Royal Museum for Central Africa, Tervuren, Belgium;* [b]*Faculty of Science, Department of Biology, Laboratory of Biodiversity and Evolutionary Genomics, KU Leuven, Leuven, Belgium;* [c]*Département de Biologie, Laboratoire d'Hydrobiologie, Limnologie et Aquaculture, Université de Kinshasa, Kinshasa, Democratic Republic of the Congo*

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The status of the *Distichodus antonii* assemblage, all large-sized, banded *Distichodus* species with a brownish-greenish colour pattern from the Congo basin, incorporating *Distichodus antonii*, *Distichodus atroventralis*, *Distichodus fasciolatus*, *Distichodus langi* and *Distichodus mossambicus*, has been revised. *Distichodus antonii*, *D. fasciolatus* and *D. langi* are found to be valid species, though the latter has long been considered a possible junior synonym of *D. antonii*. A detailed redescription for each of these three species is provided. Reports of *D. mossambicus* from the Congo basin are based on misidentifications; these specimens are assigned to the *D. atroventralis* complex, a seemingly polyspecific complex in need of further in-depth revision. An identification key to the large-sized *Distichodus* species from the Congo basin is provided.

Keywords: *Distichodus antonii*; *Distichodus atroventralis*; *Distichodus fasciolatus*; *Distichodus langi*; Congo basin

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Introduction

Distichodontidae is an endemic family of African freshwater fish that can be distinguished from other Characiformes by the unique combination of the presence of true ctenoid scales, i.e. with the ctenii formed by a series of independent ossifications along the margin of the scale, and the presence of at least some bicuspid teeth in each jaw (Boulenger 1909; Vari 2007). The family contains 15 genera (Vari 1979, 2007), among which the genus *Distichodus* Müller and Troschel, 1845. Species of this genus are characterized by a slightly mobile upper jaw, an edentulous maxilla not tightly applied to the posterior of the premaxilla (Vari 1979; 2007) and one or two series of small bicuspid teeth on both upper and lower jaw (Mamonekene and Vreven 2008), a mobile joint between the dentary and the angulo-articular, and a completely pored lateral line positioned along the midlateral surface of the body (Vari 1979; 2007).

Twenty-four valid species of *Distichodus* are recognized (Vari 2007; Froese and Pauly 2013), 14 of which are known from the Congo River basin. Boulenger (1909) divided *Distichodus* in two major groups based on their number of lateral line (LL) scales. One group consisted of species with a low LL scale count (37–57), including for the Congo basin, at that time: *Distichodus affinis*, *Distichodus altus*, *Distichodus noboli*

*Corresponding author. Email: emmanuel.vreven@africamuseum.be

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and *Distichodus notospilus*, and currently also *Distichodus decemmaculatus* and *Distichodus teugelsi*, and a second group with a higher LL scale count (60–110), including for the Congo basin, at that time: *Distichodus antonii*, *Distichodus atroventralis*, *Distichodus fasciolatus*, *Distichodus lusosso*, *Distichodus maculatus*, *Distichodus mossambicus* and *Distichodus sexfasciatus*, and currently also *Distichodus langi*. The first group corresponds to the smaller *Distichodus* species with a maximum TL ≤ c. 200 mm, while the second group includes the larger species – maximum TL ≥ c. 310 mm (Daget and Gosse 1984; own observations). Within the latter group, *D. maculatus* can easily be distinguished from the other species by its unique colour pattern, characterized by numerous large, dark spots all over the body. *Distichodus lusosso* can easily be distinguished by its distinctively prolonged snout and terminal mouth. *Distichodus sexfasciatus* can be separated from the other species by its strongly compressed snout that is deeper than long (versus feebly or not compressed) and an orange-reddish to red overall body colouration (only shared with *D. lusosso*). In addition it can be distinguished from all except *D. antonii* by its deep body (2¼ to 2½ versus > 2½ times the TL), and all but *D. lussoso* by its 10–14 teeth in the outer row of both the upper and the lower jaws (versus 15 or more) (Boulenger 1909; own observations). In contrast, identification of the five remaining nominal species within the large-size group from the Congo basin, i.e. *D. antonii*, *D. atroventralis*, *D. fasciolatus*, *D. langi* and *D. mossambicus*, remains far more problematic. These are all species with a light brownish to greenish colour pattern and a number of transverse bars; they are referred to here as the *D. antonii* assemblage. Indeed, in collections from the RMCA, misidentifications have been found for the first three species. In addition, the status of *D. langi* as a valid species has been questioned and synonymy with *D. antonii* has been suggested (Daget and Gosse 1984). This paper is a first contribution to the review of the status of these five nominal species.

Material and methods

A total of 126 specimens were studied using eight meristics and 28 measurements. The following counts were taken according to Zengeya et al. (2011): the number of scales on the lateral line, scales between dorsal fin and lateral line, scales between pelvic fin and lateral line, and total number of dorsal, anal, pectoral and pelvic fin rays. Following Vari and Ferraris (2004), the two posteriormost anal fin rays, when joined at their base, were counted as one ray only. In addition to these counts, the number of circumpeduncular scales was counted as the number of scales around the caudal peduncle just posterior to the end of the anal fin.

All measurements were taken according to the definitions by Teugels and Thys van den Audenaerde (1990), except for the 10 following measurements – head depth: vertical distance from the anteriormost point where the left and right branchiostegal membranes meet on the ventral side of the head towards the dorsal head margin; postorbital length: distance from the posterior bony edge of the orbit to the posteriormost point of the operculum; internasal distance: minimum distance in-between the nostrils; head width: minimum width of the head as measured on the level of the opercula; distance between the pectoral fin and the anus: distance from the dorsal articulation point of the pectoral fin base to the anterior edge of the anus; distance between the pelvic fin and the anus: distance from the most external articulation point of the pelvic fin base to the anterior edge of the anus; distance between the dorsal fin

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and the caudal peduncle: distance from the end of the dorsal fin base to the hypural joint of the caudal fin along the longitudinal line; pectoral fin base length: distance from the insertion point of the first pectoral fin ray to the insertion point of the last fin ray; pelvic fin base length: distance from the insertion point of the first pelvic fin ray to the insertion point of the last fin ray; distance between the distal tip of the pelvic fin and the anus: distance from the posterior tip of the longest pelvic fin ray when the fin is pressed against the abdomen, to the anterior edge of the anus. The number of vertical bars and qualitative features such as the mouth position, and body and fin colouration were also recorded.

Data were explored and analysed using principal components analysis (PCA) on the correlation matrix of the raw meristics and on the covariance matrix of the $\log(x + 1)$-transformed measurements. $\text{Log}(x + 1)$ transformation was necessary to be able to include values between zero and one. PCA is used here as a model and distribution-free technique for exploring multivariate data sets (Marcus 1990). Only specimens for which all data were available were included in the analyses. When analysing the $\log(x + 1)$ transformed measurements, the first principal component, which mainly accounts for size, is discarded allowing a size-free comparison of the specimens (Humphries et al. 1981; Bookstein et al. 1985). Dorsal and anal fin heights and the distance between the distal tip of the pelvic fin and the anus were not included in the PCA because of missing data for many of the examined specimens.

Non-parametric Mann–Whitney *U*-tests were used for univariate comparisons of the relative measurements (percentages) and raw meristics (Snoeks 2004; Vreven and Teugels 2005; Vreven and Snoeks 2009), with sequential Bonferroni corrections applied to correct for multiple comparisons (Rice 1989). Specimens of similar length classes (SL: p-value ≥ 0.5) were used to prevent interference of allometric growth effects. Data were analysed using STATISTICA 11.

Differential diagnoses for the species not belonging to the *D. antonii* assemblage are partly based on literature data. When part of the coordinates of a certain location is unknown, "??" has been used to replace the missing value(s).

Abbreviations used: CP, caudal peduncle; DRC, Democratic Republic of the Congo; HJ, hypural joint; HL, head length; LL, lateral line; PC, principal component; PCA, principal component analysis; SL, standard length; TL, total length.

Scientific institutions: AMNH, American Museum of Natural History, New York, USA; BMNH, Natural History Museum, London, UK; FUNDP, University of Namur, Namur, Belgium. MCZ, Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA; RMCA, Royal Museum for Central Africa, Tervuren, Belgium; RBINS, Royal Belgian Institute of Natural Sciences, Brussels, Belgium. Following common standards in ichthyology, the acronym MRAC was used for the collections of the Royal Museum for Central Africa.

Results

Analyses of the meristic data

A first PCA on eight meristics was conducted on all Congo species of the *D. antonii* assemblage ($n = 115$). Scale counts mainly determined PCI with highest loadings for the number of scales between the LL and the dorsal fin, the number of circumpeduncular scales and the number of LL scales in front of the HJ (Table 1). Conversely,

Table 1. Factor coordinates of variables on the first three axes of a principal components analysis on eight meristics for all specimens examined.

Meristics	PCI	PCII	PCIII
LL scales in front of HJ	**−0.8127**	−0.2322	0.0297
Scales LL – dorsal fin	**−0.9097**	0.0178	−0.1141
Scales LL – pelvic fin	−0.5930	0.3042	**−0.5402**
Circumpeduncular scales	**−0.8175**	0.1130	0.3028
Total dorsal fin rays	−0.5855	0.0446	**0.6620**
Total anal fin rays	−0.4071	**0.5905**	−0.1480
Total pectoral fin rays	−0.3849	**−0.3926**	**−0.4345**
Total pelvic fin rays	−0.2520	**−0.8554**	−0.0308

Most important coordinates in bold.

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highest loadings on PCII were found for the total number of pelvic, anal and pectoral fin rays.

The scatterplot of PCI versus PCII shows four groups with minor overlap (Figure 1). A first group, well separated from the others and containing only two specimens, is situated on the negative side of PCI and PCII and corresponds to *D. langi*. A second group is situated fully on the positive part of PCI and corresponds to *D. antonii*, including three misidentified syntypes of *D. fasciolatus*. A third group, *D. fasciolatus*, is found on the negative part of PCI, and almost completely on the positive side of PCII. It includes one specimen previously identified as *D. langi*. A fourth group is found on the negative side of PCII and with positive and negative values on PCI. This group contains the examined syntype and additional specimens of *D. atroventralis*, and all Congo specimens that were misidentified as *D. mossambicus*. This group will be referred to as the *D. atroventralis* complex as preliminary results suggest this group to be polyspecific.

Pairwise tests with sequential Bonferroni correction were performed between *D. antonii*, the *D. atroventralis* complex and *D. fasciolatus* (Table 2). *Distichodus langi* was not included in the Mann–Whitney *U*-tests due to the lack of specimens ($n = 2$). Between *D. antonii* and the *D. atroventralis* complex, four out of eight meristics were significantly different, three of which were highly significant (p-value < 0.001 after Bonferroni correction); between *D. antonii* and *D. fasciolatus*, six were found to be highly significantly different, and between the *D. atroventralis* complex and *D. fasciolatus*, five.

Analyses of the measurements

A second PCA on 25 log-transformed measurements was conducted on the *D. antonii* assemblage ($n = 119$). Highest loadings on PCII were found for the CP length, internasal distance and distance between the dorsal and adipose fins. Highest loadings on PCIII were found for the CP length, internasal distance and head depth (Table 3).

The resulting scatterplot of PCII versus PCIII shows three somewhat overlapping groups (Figure 2). A first group is completely situated on the negative side of PCII. This group corresponds to *D. antonii* and includes again the three misidentified syntypes of *D. fasciolatus*. A second group is found almost completely on the positive side of PCII, and corresponds to *D. fasciolatus*. It overlaps to some extent with the third group (*D. atroventralis* complex), situated mainly on the positive part of PCII and PCIII. One

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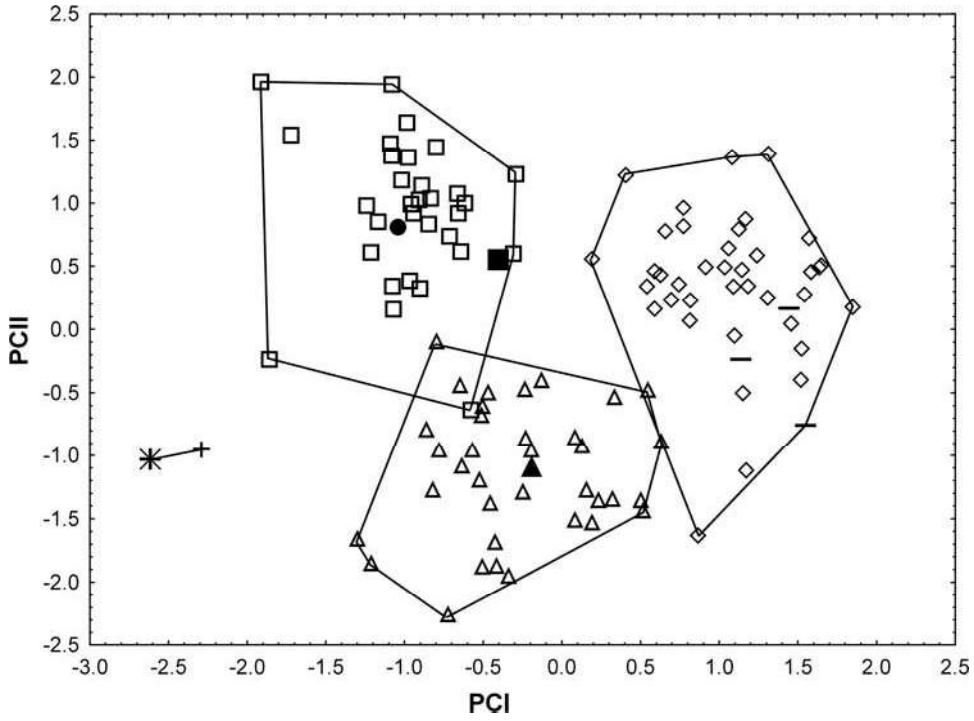


Figure 1. Scatterplot of principal component (PC) I against PCII for a principal components analysis carried out on eight meristics.
Note: *Distichodus antonii*: (♦) holotype, (◊) other specimens and (–) misidentified syntypes of *Distichodus fasciolatus*; *Distichodus atroventralis* complex: (▲) syntype *D. atroventralis* and (Δ) other specimens; *D. fasciolatus*: (■) lectotype, (□) other specimens and (●) misidentified *Distichodus langi* specimen; *D. langi*: (✳) holotype and (+) additional specimen.

specimen of *D. langi* is well separated from all other taxa on the positive side of both PCII and PCIII, but the type falls within the *D. atroventralis* complex.

Pairwise Mann–Whitney *U*-tests with sequential Bonferroni correction were performed between *D. antonii*, the *D. atroventralis* complex and *D. fasciolatus* (Table 2). Again, *D. langi* was not included because of the lack of specimens. A high number of highly significantly different ($p < 0.001$ after Bonferroni correction) variables were found between *D. antonii* and both the *D. atroventralis* complex and *D. fasciolatus*, i.e. respectively 10 and 14 out of 28 variables. In contrast, between the *D. atroventralis* complex and *D. fasciolatus*, only three out of 28 variables were significantly different, two of which highly significantly.

Taxonomic account

A redescription of *D. antonii*, *D. fasciolatus* and *D. langi* is given below. Diagnoses are based on our own counts, measurements and observations, complemented with literature data. An identification key to all large-sized *Distichodus* taxa from the Congo basin has been added below.

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Table 2. Results of Mann–Whitney *U*-tests on eight meristics and 28 measurements as percentages.

Morphometrics	*antonii* × *atrov*		*antonii* × *fascio*		*atrov* × *fascio*	
	Significance	*n*	Significance	*n*	Significance	*n*
Standard length (mm)	NS	50 × 29	NS	48 × 33	NS	26 × 33
As % of head length						
Head depth	NS	50 × 29	NS	48 × 33	NS	26 × 33
Snout length	NS	50 × 29	NS	48 × 33	NS	26 × 33
Eye diameter	NS	50 × 29	NS	48 × 33	NS	26 × 33
Postorbital length	NS	50 × 29	*	48 × 33	NS	26 × 33
Head width	NS	50 × 29	*	48 × 33	NS	26 × 33
Interorbital distance	NS	50 × 29	**	48 × 33	NS	26 × 33
Internasal distance	**	50 × 29	**	48 × 33	NS	26 × 33
As % of standard length						
Head length	NS	50 × 29	**	48 × 33	NS	26 × 33
Dorsal–pelvic fin distance	**	50 × 29	**	48 × 33	NS	26 × 33
Predorsal distance	NS	50 × 29	**	48 × 33	NS	26 × 33
Prepectoral distance	NS	50 × 29	**	48 × 33	NS	26 × 33
Prepelvic distance	NS	50 × 29	NS	48 × 33	NS	26 × 33
Preanal distance	**	50 × 29	**	48 × 33	NS	26 × 33
Pectoral–anus distance	**	50 × 29	**	48 × 33	NS	26 × 33
Pelvic–anus distance	**	50 × 29	**	48 × 33	NS	26 × 33
Caudal peduncle length	**	50 × 29	*	48 × 33	**	26 × 33
Caudal peduncle height	**	50 × 29	**	48 × 33	NS	26 × 33
Dorsal–adipose distance	**	50 × 29	**	48 × 33	*	26 × 33
Dorsal–CP distance	**	50 × 29	**	48 × 33	NS	26 × 33
Dorsal fin base length	*	50 × 29	NS	48 × 33	NS	26 × 33
Dorsal fin height	NS	34 × 28	NS	32 × 23	NS	25 × 23
Anal fin base length	NS	50 × 29	**	48 × 33	**	26 × 33

(*Continued*)

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Table 2. (Continued).

Anal fin height	NS	27 × 19	*	25 × 30	NS	17 × 30
Pectoral fin base length	NS	50 × 29	*	48 × 33	NS	26 × 33
Pectoral fin length	NS	50 × 29	NS	48 × 32	NS	26 × 32
Pelvic fin base length	NS	50 × 29	NS	48 × 33	NS	26 × 33
Pelvic fin length	NS	49 × 26	*	47 × 33	NS	24 × 33
End pelvic fin–anus distance	**	48 × 26	**	46 × 33	NS	24 × 33
Meristics	Significance	n	Significance	n	Significance	n
LL scales in front of HJ	**	50 × 40	**	50 × 34	NS	40 × 34
Scales LL–dorsal fin	**	50 × 39	**	50 × 34	**	39 × 34
Scales LL–pelvic fin	NS	50 × 38	**	50 × 33	NS	38 × 33
Circumpeduncular scales	**	49 × 39	**	49 × 34	**	39 × 34
Total dorsal fin rays	NS	44 × 40	**	44 × 34	**	40 × 34
Total anal fin rays	NS	50 × 40	**	50 × 34	**	40 × 34
Total pectoral fin rays	NS	50 × 40	NS	50 × 34	NS	40 × 34
Total pelvic fin rays	**	50 × 40	NS	50 × 34	**	40 × 34

Note: antonii: *Distichodus antonii*; atrov: *Distichodus atroventralis* complex; fascio: *Distichodus fasciolatus*. (NS) not significant, (*) significant values ($p < 0.05$ after Bonferroni correction), (**) and highly significant values ($p < 0.001$ after Bonferroni correction). SL has been tested for significance at $\alpha = 0.5$. Range of specimens: antonii × atrov: $24.8 \leq SL \leq 416.0$ mm; antonii × fascio: $43.7 \leq SL \leq 416.0$ mm; atrov × fascio: $46.5 \leq SL \leq 357.0$ mm.

Table 3. Factor coordinates of variables on the first three axes of a principal components analysis on 25 morphometrics for all specimens examined.

Log-measurements	PCI	PCII	PCIII
Standard length	−0.2905	0.0095	−0.0104
Head length	−0.2412	−0.0111	−0.0009
Head depth	−0.2670	−0.0125	**0.0322**
Snout length	−0.2787	−0.0152	0.0087
Eye diameter	−0.1628	−0.0142	−0.0171
Postorbital length	−0.2342	0.0024	0.0149
Head width	−0.2809	−0.0238	0.0092
Interorbital distance	−0.2897	−0.0390	0.0174
Internasal distance	−0.2604	**−0.0691**	**0.0417**
Dorsal–pelvic fin distance	−0.3151	−0.0053	−0.0197
Predorsal distance	−0.2882	0.0013	−0.0079
Prepectoral distance	−0.2451	−0.0077	−0.0029
Prepelvic distance	−0.2775	0.0033	−0.0081
Preanal distance	−0.2935	−0.0035	−0.0127
Pectoral–anus distance	−0.3136	−0.0064	−0.0154
Pelvic–anus distance	−0.3137	−0.0335	−0.0125
Caudal peduncle length	−0.2547	**0.0877**	**0.0619**
Caudal peduncle height	−0.3024	−0.0205	0.0018
Dorsal–adipose distance	−0.2896	**0.0506**	−0.0189
Dorsal–CP distance	−0.3014	0.0416	−0.0031
Dorsal fin base length	−0.3038	0.0017	−0.0210
Anal fin base length	−0.2703	0.0165	−0.0268
Pectoral fin base length	−0.2416	−0.0001	0.0167
Pectoral fin length	−0.2673	0.0107	−0.0017
Pelvic fin base length	−0.2687	0.0139	0.0101
Pelvic fin length	−0.2706	0.0229	−0.0208

Most important coordinates in bold.

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Identification key to large-sized (max. size ≥ c. 310 mm TL) Distichodus *species from the Congo basin*

1. Mouth terminal 2
   Mouth inferior 3
2. Snout distinctively prolonged; six to eight vertical dark bars on the flanks; *in vivo* colour orange to reddish ..... *D. lusosso*
   Snout not prolonged; 9–14 dark vertical bars on the flanks, starting to fade in specimens from c. 200 mm SL and almost completely disappearing at about 250 mm SL; *in vivo* olive-greenish body colouration ..... *D. antonii*
3. Large, dark spots all over the body; 16–21 dorsal fin rays ..... *D. maculatus*
   No large, dark spots; 6–9 or 13–20 dark vertical bars on the flanks, starting to fade in specimens from c. 150 mm SL and almost completely disappearing at about 300 mm SL; 22–29 dorsal fin rays 4

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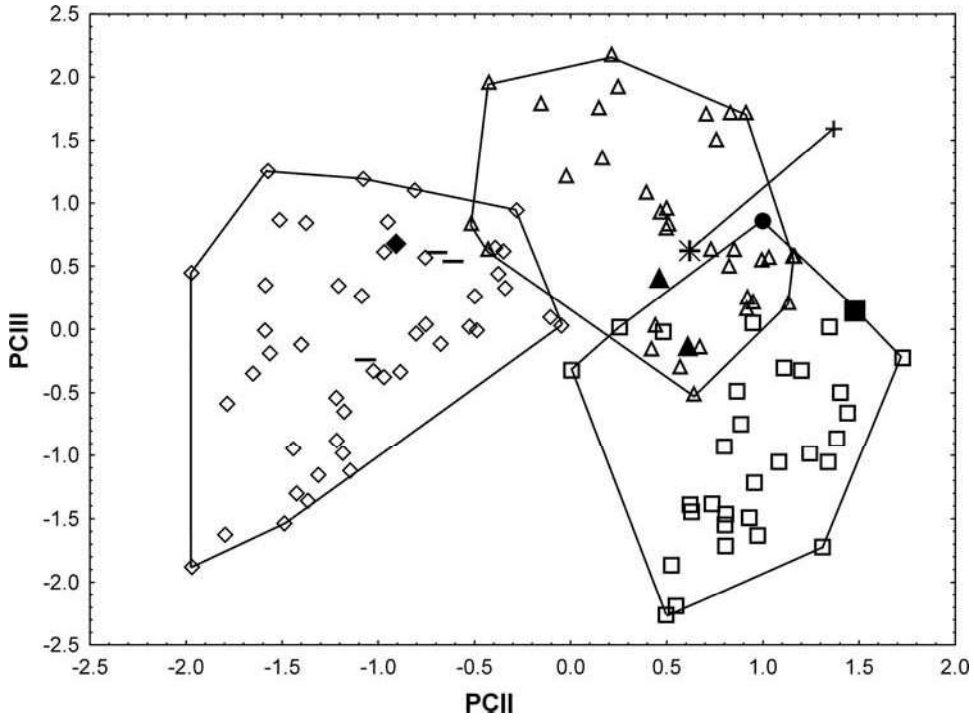


Figure 2. Scatterplot of principal component (PC) II against PCIII for a principal components analysis carried out on 25 log-transformed measurements.
Note: *Distichodus antonii*: (♦) holotype, (◊) other specimens and (–) misidentified syntypes of *Distichodus fasciolatus*; *Distichodus atroventralis* complex: (▲) syntypes of *D. atroventralis* and (Δ) additional specimens; *D. fasciolatus*: (■) lectotype, (□) other specimens and (●) misidentified *Distichodus langi* specimen; *D. langi*: (✳) holotype and (+) additional specimen.

4. Twelve to fourteen teeth in the outer row of the upper and lower jaw; body depth 2¼ to 2½ times in TL; snout very short and obtuse; in life, reddish body colouration ..... *D. sexfasciatus*
   At least 15 teeth in the outer row of the upper and lower jaw; body depth > 2½ times in the TL; snout longer, feebly or not compressed; in life, brown-yellowish body colouration 5

5. Six to nine dark vertical bars on the flanks, fading in specimens from c. 200 mm SL and almost completely disappearing at about 300 mm SL; pelvic fins greyish to black ..... *D. atroventralis* complex
   From 13 to 20 dark vertical bars on the flanks, fading in specimens from c. 200 mm SL and almost completely disappearing at about 300 mm SL: pelvic fins generally pale to yellow-orange, sometimes with slightly darker distal parts 6

6. From 24 to 26 dorsal fin rays; 17–20 pectoral fin rays; 10, rarely 11 pelvic fin rays ..... *D. fasciolatus*
   From 26 to 28 dorsal fin rays; 21 pectoral fin rays; 11 pelvic fin rays .... *D. langi*

Redescriptions of D. antonii, D. fasciolatus *and* D. langi

Distichodus antonii Schilthuis 1891
(Tables 4 and 5; Figures 3A and 4)

Distichodus antonii Schilthuis, 1891: 89.
Distichodus fasciolatus Boulenger, 1898 (*partim*): 27, pl. XIV.
Distichodus langi Nichols and Griscom, 1917 (*partim*): 687–688.

Type material
Holotype. BMNH 1899.9.6.10, Kinshasa, Stanley Pool (DRC) (± 4°15′02″ S, 15°25′ 00″ E), coll. Greshoff, 1899 (103.3 mm SL).

Note on the holotype. *Distichodus antonii* was described by Schilthuis in 1891 based on a single specimen from "Bayari Sea", collected by Greshoff (Schilthuis 1891). From the introduction of Schilthuis's article, it is clear that "Bayari Sea" is either situated near Kinshasa, or near Boma [Lower Congo (DRC)]. Curiously, two specimens are labelled as type in the BMNH: a "holotype" BMNH 1898.11.17.10 from "cataracts of Manyanga" with a size of 513.0 mm SL and a "type" BMNH 1899.9.6.10 from "Kinshasa, Stanley Pool" with a size of 103.3 mm SL (125.5 mm TL). The latter specimen and not the former clearly has to be considered as the holotype as it corresponds to the size (12.8 cm TL) given in the original description by Schilthuis (1891). We were unable to trace back the exact location of "Bayari Sea" and to the best of our knowledge this locality has nowhere else been used in the ichthyological literature of the Congo basin. Possibly, the widening of the Congo River channel at Pool Malebo (Stanley Pool) has been called a "Sea" by the collector.

Table 4. Diagnostic characteristics of the species of the *Distichodus antonii* assemblage, with associated ranges, means (continuous variables) and medians (categorical variables).

Characteristic	*D. antonii*	*D. fasciolatus*	*D. langi*	*D. atroventralis* complex
Mouth position	terminal	inferior	inferior	inferior
Internasal distance (%HL)	10.1–26.5 (16.5)	8.1–21.0 (11.0)	14.4 & 15.9	7.6–21.3 (11.5)
Head depth (%HL)	35.2–67.2 (46.0)	35.9–62.9 (45.1)	67.4 & 69.4	35.1–55.7 (46.5)
LL scales in front of HJ	52–64 (58)	61–70 (65)	68 & 70	61–76 (65–66)
Scales LL–dorsal fin	10–12 (11)	13–16 (14)	15 & 16	12–15 (13)
Scales LL–pelvic fin	10–11 (10)	10–12 (11)	12	9–11 (11)
Circumpeduncular scales	20–23 (22)	23–26 (24)	24 & 25	20–24 (22)
Total dorsal fin rays	21–25 (23)	24–26 (25)	26 & 28	22–29 (23)
Total pectoral fin rays	16–20 (18)	17–20 (18)	21	17–20 (18)
Total pelvic fin rays	10–11 (10)	10–11 (10)	11	10–11 (11)
Number of vertical bars	9–14 (11)	13–20 (16)	13	6–-9 (7)

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Table 5. Morphometric and meristic data for *Distichodus antonii*, *Distichodus fasciolatus* and *Distichodus langi*.

Morphometrics	*Distichodus antonii*				*Distichodus fasciolatus*				*Distichodus langi*	
	Holotype	All specimens			Lectotype	All specimens			Holotype	MRAC 2878
		min–max	*n*	mean		min–max	*n*	mean		
Standard length (mm)	103.3	24.8–416.0	50	120.2	278.2	55.7–445.0	34	135.6	364.0	300.0
Head length	27.2	8.4–98.6	50	30.7	57.2	14.9–106.6	34	31.1	75.6	65.8
As % of head length										
Head depth	48.9	35.2–67.2	50	46.0	52.8	35.9–62.9	34	45.1	67.4	69.4
Snout length	33.1	22.6–41.9	50	32.2	36.2	24.9–40.4	34	31.5	42.6	41.5
Eye diameter	36.5	25.1–50.4	50	37.8	31.2	24.4–44.5	34	36.4	23.7	22.0
Postorbital length	32.9	26.3–39.2	50	31.7	34.3	29.5–42.9	34	34.8	38.2	35.0
Head width	46.4	36.1–63.5	50	47.5	50.6	34.6–58.5	34	44.2	52.5	55.6
Interorbital distance	42.3	18.1–52.4	50	38.0	39.0	23.6–42.8	34	32.4	44.7	46.1
Internasal distance	18.3	10.1–26.5	50	16.5	13.4	8.1–21.0	34	11.0	15.9	14.4
As % of standard length										
Head length	26.3	22.5–34.0	50	26.7	20.6	20.3–26.7	34	23.7	20.8	21.9
Dorsal–pelvic fin distance	37.9	31.6–46.4	50	39.8	34.5	33.2–43.7	34	37.4	48.3	42.5
Predorsal distance	55.2	49.6–59.5	50	54.8	52.2	48.5–57.6	34	52.5	57.9	55.5
Prepectoral distance	27.2	23.1–33.3	50	27.2	21.8	21.8–28.4	34	24.6	20.4	22.0
Prepelvic distance	53.4	50.4–59.9	50	54.6	52.6	48.8–57.2	34	53.2	53.2	53.2
Preanal distance	82.0	77.3–91.3	50	83.9	79.2	73.0–90.5	34	79.8	77.8	80.3
Pectoral–anus distance	52.2	45.1–64.0	50	53.8	52.9	45.1–57.9	34	50.7	58.0	54.9
Pelvic–anus distance	25.5	21.0–33.6	50	26.8	21.1	18.6–25.8	34	21.7	25.9	23.4
Caudal peduncle length	8.6	3.7–10.3	50	7.2	10.9	5.9–11.9	34	9.0	9.9	13.8
Caudal peduncle height	13.7	11.9–19.0	50	13.9	11.0	10.5–15.8	34	11.9	17.4	15.9
Dorsal–adipose distance	11.1	8.3–13.1	50	10.9	14.4	12.6–17.1	34	14.5	15.0	14.9
Dorsal fin–CP distance	25.3	21.2–29.2	50	25.3	31.8	24.9–33.7	34	29.0	28.1	34.5

(*Continued*)

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Table 5. (Continued).

Morphometrics	*Distichodus antonii*				*Distichodus fasciolatus*				*Distichodus langi*	
	Holotype	All specimens			Lectotype	All specimens			Holotype	MRAC 2878
		min–max	*n*	mean		min–max	*n*	mean		
Dorsal fin base length	26.5	22.9–29.7	50	26.3	23.6	22.5–30.4	34	26.0	29.8	30.9
Dorsal fin height	–	14.6–23.1	34	19.2	19.9	14.1–26.5	34	21.1	17.1	16.4
Anal fin base length	10.8	8.8–12.1	50	10.7	11.8	10.1–14.4	34	12.1	10.7	10.3
Anal fin height	–	8.5–15.4	27	13.5	–	7.4–14.7	31	11.7	13.9	9.6
Pectoral fin base length	4.7	4.0–5.4	50	4.8	4.3	3.7–6.1	34	4.5	5.1	5.7
Pectoral fin length	15.8	12.3–20.4	50	18.2	16.2	13.7–20.5	33	17.7	19.5	19.7
Pelvic fin base length	4.0	2.8–6.0	50	4.3	4.6	3.2–5.6	4.5		5.3	5.3
Pelvic fin length	17.0	14.2–19.7	49	17.6	17.3	15.7–22.0	33	19.2	17.1	19.9
End pelvic fin–anus distance	6.4	3.9–18.0	48	8.7	2.9	-2.1–5.7	33	2.0	7.8	1. 6
Meristics		min–max	*n*	mean		min–max	*n*	mean		
LL scales in front of HJ	54	52–64	50	58	67	61–70	34	65	70	68
Scales LL–dorsal fin	10	10–12	50	11	13	13–16	34	14	16	15
Scales LL–pelvic fin	10	10–11	50	10	10	10–12	33	11	12	12
Circumpeduncular scales	–	20–23	49	22	24	23–26	34	24	24	25
Total dorsal fin rays	24	21–25	44	23	25	24–26	34	25	28	26
Total anal fin rays	13	12–14	50	13	14	12–16	34	14	14	14
Total pectoral fin rays	18	16–20	50	18	18	17–20	34	34	21	21
Total pelvic fin rays	10	10–11	50	10	10	10–11	34	10	11	11
Number of vertical bars	12	9–14	40	11	–	13–20	23	16	-	13

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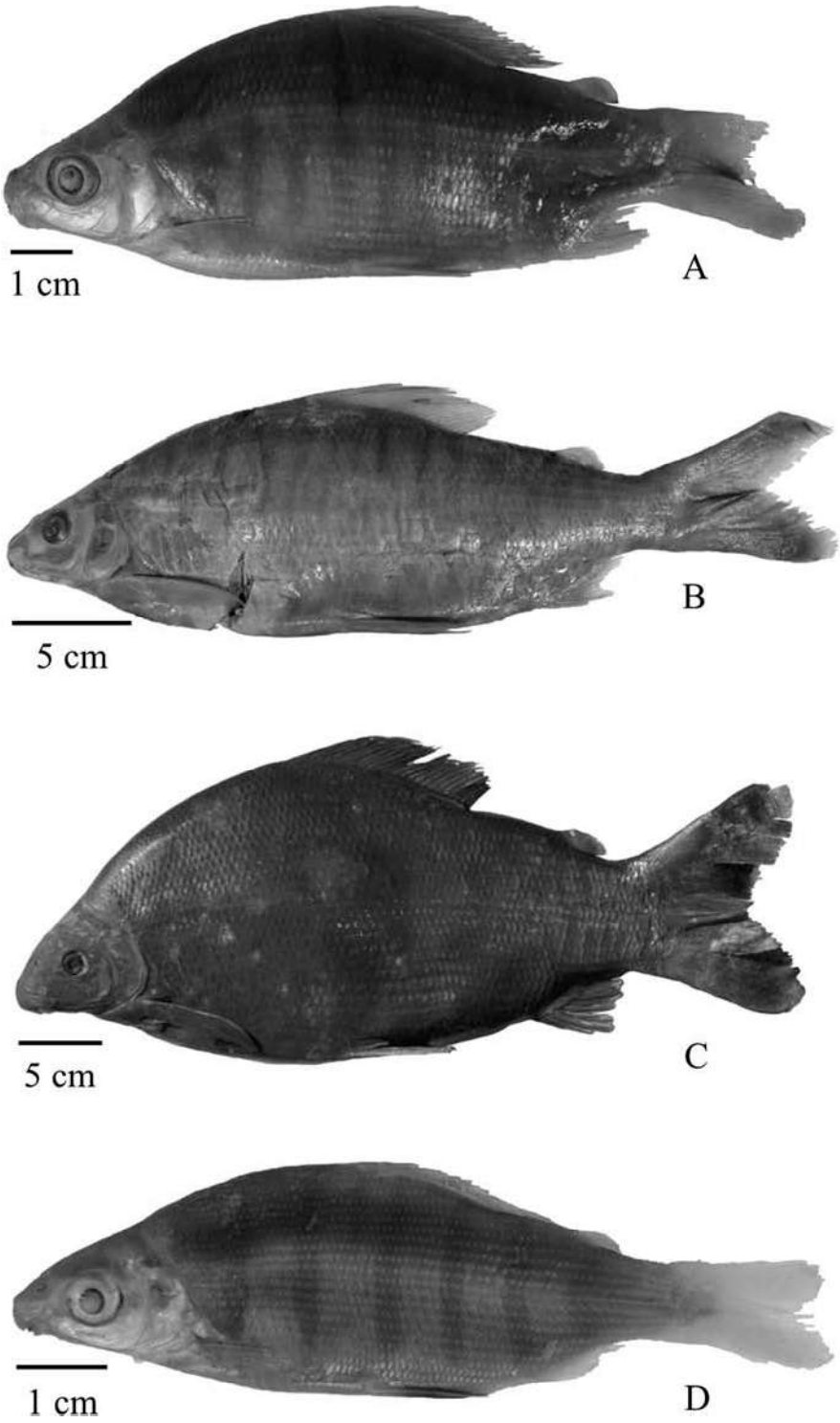


Figure 3. Illustrations of type specimens of the *Distichodus antonii* assemblage. (A) Holotype of *D. antonii* (BMNH 1899.9.6.10), 103.3 mm SL; (B) Lectotype of *Distichodus fasciolatus* (MRAC 23), 278.2 mm SL; (C) Holotype of *Distichodus langi* (AMNH 5915), 364 mm SL. Picture taken by M.L.J. Stiassny (AMNH); (D) Syntype of *Distichodus atroventralis* (BMNH 1891.12.29.13), 63.5 mm SL.

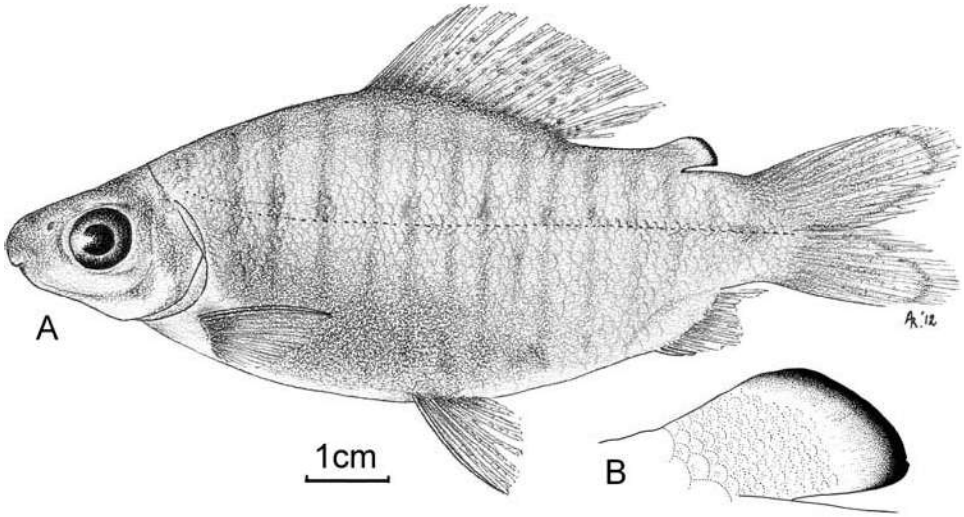


Figure 4. *Distichodus antonii* (B0-021-P-0873-0874) from the Loboya River, affluent of the Maiko River, Upper Congo (DRC), 89.4 mm SL. (A) Lateral view; (B) Detail of the colour pattern on the adipose fin.

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Other material examined (all lengths are SL)

Distichodus antonii Schilthuis, 1891. **Democratic Republic of the Congo (DRC).** MRAC 88 Manyanga (± 4°54′ S, 14°23′E), coll. Wilverth 1896 (279.9 mm). MRAC 96, Léopoldville (± 4°18′ S, 15°18′ E), coll. Wilverth 1896 (106.4 mm). MRAC 2148, Léopoldville (± 4°18′ S, 15°18′ E), coll. Christy 30/06/1912 (363.0 mm). MRAC 2263, Léopoldville (± 4°18′ S, 15°18′ E), coll. Christy 7/05/1912 (416.0 mm). MRAC 2649, Stanley Pool (± 4°18′ S, 15°18′ E), coll. Dubois 1912 (148.7 mm). MRAC 2650. Léopoldville (± 4°18′ S, 15°18′ E), coll. Dubois 1912 (362.0 mm). MRAC 40938–40941, Léopoldville (± 4°18′ S, 15°18′ E), coll. Tinant 1/01/1934–24/04/1934 (59.5–73.8 mm). MRAC 67448–449, Léopoldville (± 4°18′ S, 15°18′ E), coll. van Moorsel 07/1944 (98.8–102.2 mm). MRAC 48072, Kalamu river, near Boma (± 5°49′ S, 13°03′ E), coll. Dartevelle 01/01/937–4/08/1937 (181.2 mm). MRAC 96207, Near Léopoldville (± 4°18′ S, 15°18′ E), coll. Van de Weyer 1954 (58.4 mm). MRAC 117317, Stanley Pool, Nduka indigenous dam (± 4°20′ S, 15°24′ E), coll. Mission Brien-Poll-Bouillon, 23/09/1957 (211.3 mm). MRAC 177320, Stanley Pool, meadows near Kingabwa (± 4°19′ S, 15°21′ E), coll. Mission Brien-Poll-Bouillon, 25/9/1957 (103.8 mm). MRAC 177381, Stanley Pool (± 4°20′ S, 15°23′ E), coll. Van Orshoven 21/05/1964 (96.3 mm). MRAC 177604–607, Stanley Pool (± 4°18′ S, 15°18′ E), coll. Brichard 1967 (46.8–85.9 mm). MRAC 73-22-P-1333, Stanley Pool (± 4°06′ S, 15°15′ E and 4°20′ S, 15°23′ E), coll. Mandeville 22/09/1954 (24.8 mm). MRAC 73-22-P-1335, Stanley Pool (DRC) (± 4°06′ S, 15°15′ E and 4°20′ S, 15°23′ E), coll. Mandeville 24/11/1954 (27.0 mm). MRAC 73-22-P-1336-337, Stanley Pool (± 4°06′ S, 15°15′ E and 4°20′ S, 15°23′ E), coll. Mandeville 10/01/1955 (47.2–55.8 mm). MRAC 73-22-P-1352, Stanley Pool (± 4°06′ S, 15°15′ E and 4°20′ S, 15°23′ E), coll. Mandeville 1/02/1958 (250.3 mm). MRAC 86-21-P-30, Inga (± 5°39′ S, 13°39′ E), coll. Mutambwe Shango 1967 (43.7 mm). MRAC A7-014-P-

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0002-0008, Pool Malebo at Kinkole, Molondo Island (± 4°16′47.8″ S, 15°27′59.7″ E), coll. Mbadu Zebe 29/12/2004 (85.4–139.7 mm). MRAC B0-021-P-0873-0874, Loboya River, right bank, Bambondji II, site 115 (± 0°11′33.1″ N, 25°31′50.1″ E), coll. Danadu and Moelants 31/08/2010 (85.5–89.4 mm). MRAC B0-021-P-0875, Lobaye River, right bank, Djabir, Site 6B (± 0°29′28.1″ N, 24°10′42.8″ E), coll. Danadu and Moelants 9/08/2010 (153.2 mm). MRAC B0-021-P-0876-0877, Lomami River, left bank, Djabir surroundings, site Lom2G2 (± 0°30′40.7″ N, 24°10′30.9″ E), coll. Danadu and Moelants 13/08/2010 (99.6–119.9 mm). MRAC B0-021-P-0878, Congo River, right bank, village Batikamondji I, site 101 (± 0°08′36.1″ N, 25°31′42.0″ E), coll. Danadu and Moelants 30/08/2010 (134.7 mm). MRAC B0-021-P-0879-0882, Loboya River, right bank, site 119B (± 0°11′27.0″ N, 25°31′39.7″ E), coll. Danadu and Moelants 31/08/2010 (115.3–130.3 mm). Paralectotypes *D. fasciolatus* Boulenger, 1898: MRAC 28, Boma (DRC) (± 5°50′ S, 13°03′ E), coll. Wilverth 1896 (86.3 mm). MRAC 63, Matadi (DRC) (± 5°49′ S, 13°27′ E), coll. Wilverth 1896 (62.1 mm). MRAC 102, Léopoldville (DRC) (± 4°18′ S, 15°18′ E), coll. Wilverth 1896 (76.7 mm).

Republic of the Congo (Brazzaville). MRAC A4-046-P-1399-1407 (1/8), Kintelé, Congo River, (± 4°9.12′ S, 15°21.44′ E), coll. Mamonekene, Mady-Goma, Opoya *et al.* 19/6/2003, (95.7 mm). MRAC A4-046-P-1438, Djoué River, tributary of Congo River, downstream from hydroelectric dam (± 4°19′ S, 15°14′ E), coll. Mamonekene, Bakabana, Ibala Zamba 24/7/2003 (180.5 mm).

Differential diagnosis

Within the Congo basin, *D. antonii* can be distinguished from *D. affinis*, *D. altus*, *D. decemmaculatus*, *D. noboli*, *D. notospilus* and *D. teugelsi* by its higher total number of LL scales, i.e. 52–64 (versus < 46 in the six other species) and from *D. maculatus* by the absence of large, dark spots all over the body (versus 9–14 vertical dark bars instead) and a higher number of dorsal fin rays, i.e. 21–25 (versus 19–21). *Distichodus antonii* can be separated from all remaining Congo species by its terminal (versus inferior) mouth, with the exception of *D. lusosso* (which has a distinctive elongate snout and only six to eight vertical, dark bars) and by its low number of scales between the LL and the dorsal fin, i.e. 10–12 (versus 13–17) (Table 4).

Description

Morphometric and meristic data are given in Table 5. Body relatively deep (within *D. antonii* assemblage). Dorsal head profile straight, dorsal body outline concave from posterior to head to end of dorsal fin, straight from end of dorsal to adipose fin, and convex from adipose to caudal fin. Ventral head profile straight, concave from posterior to head to end of anal fin, and convex from end of anal to caudal fin. Head broad with nasal openings widely set apart (10.1–26.5 %HL), and head relatively shallow (35.2–67.2 %HL), although these characteristics are positively allometric. Mouth terminal. Posterior edge of maxillary not passing nostrils. Two rows of bicuspid teeth in each jaw. Origin of dorsal fin slightly in front of pelvic fin origin along vertical axis. Distal margin dorsal fin straight or slightly concave, distal margin of anal fin straight to slightly convex. Base and distal end of pelvic fin relatively far from vent; these distances are positive allometric, i.e. 21.0–33.6 %SL and 3.9–18.0 % SL, respectively. Pectoral and pelvic fin rays decreasing in length from outer to inner fin margin. Adipose fin situated approximately half-way between dorsal and caudal

fins. Caudal peduncle deeper than long. Caudal fin forked with two rounded lobes covered with numerous small scales except for translucent distal area.

Maximum reported total length 550 mm (Boulenger 1909). Duren (1943) stated it can reach a size of more than 800 mm.

Colouration

Body *in vivo* dark brown-olive, belly whitish. Dorsal fin scattered with numerous small, dark spots. Caudal fin dark brown or blackish. Adipose fin with a well-marked black distal margin (Figures 3A and 4). Pectoral fins uniform pale. Pelvic fins uniform pale or with slightly darker fin ray borders. Between 9 and 14 more or less distinct vertical dark bars on each flank that disappear with increasing size, starting to fade at c. 200 mm SL, disappearing at about 250 mm SL. Preserved specimens brownish with a lighter belly. The black margin of the adipose fin and vertical bars can be absent because of poor and/or long-term conservation.

Ecology

According to Gosse (1963), the fry and juveniles of *D. antonii* live in aquatic prairies and in plant fringes. Adults feed in *Echinochloa* patches and live at the edge of streams along islands and even on the bottom in the middle of the stream. The species is a phytophagous species; stomachs almost always contain plant fragments, with easily recognizable twigs and leaves of *Echinochloa*, which comprises the largest part of their diet. The same author stated that this species has two annual periods of reproduction, and that like *Citharinus* and all other *Distichodus* species, spawning and egg deposition have to take place at the border of the stream, just before the flood. Recent results from stomach content analyses in Pool Malebo confirmed the all year round herbivorous diet of *D. antonii*, mainly feeding on leaves, complemented with some detritus (Mbadu Zebe et al. 2010a). Matthes (1964) reported the species as occurring in lakes and large rivers. Mbadu Zebe et al. (2010b) reported that low water levels during the dry season in Pool Malebo leads to the formation of scale annuli due to the scarcity of macrophytes (its main food source).

Distribution

This species is a widespread Congo basin endemic (Figure 5). Poll (1976) reported the species to be widespread in the Congo basin, including the Lualaba River, but absent from Luapula-Mweru. However, we found one specimen (MRAC 121238) from "Kasenga, Luapula River", identified as *D. antonii* by Poll in 1959. We checked the identification and found it to be correct. As this specimen was originally registered as originating from "Kasenga, Lualaba", a labelling error probably occurred. Therefore, we agree with Poll (1976) that *D. antonii* is absent from the Luapula system. *Distichodus antonii* seems to be sympatric with the *D. atroventralis* complex, *D. fasciolatus* and *D. langi*. In recent collections (2010–2011) from the region of Kisangani, the first three taxa were even found syntopically in the same gillnet in all three possible combinations of two species.

Etymology

Distichodus antonii was most probably named by Schilthuis (1891) in memory of Anton Greshoff 1855–1905 (Nieuw Letterkundig Magazijn 2010) who collected the holotype.

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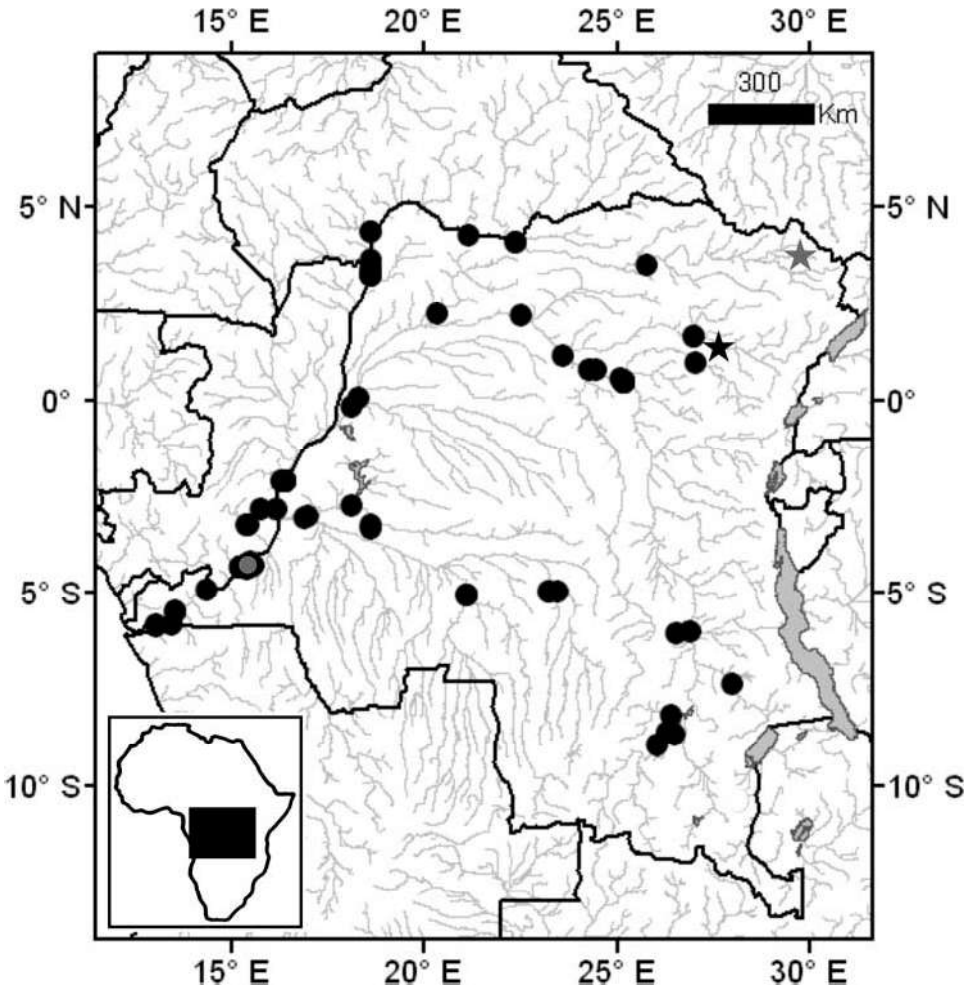


Figure 5. Distribution map of *Distichodus antonii* and *Distichodus langi* based on the specimens identified at the AMNH and MRAC.
Note: *Distichodus antonii*: (◦) holotype and (•) other specimens; *D. langi*: (⋆) lectotype and (⋆) specimen.

Distichodus fasciolatus Boulenger, 1898
(Tables 4 and 5; Figures 3B and 6)

Distichodus fasciolatus Boulenger, 1898: 27, pl. XIV.
Distichodus langi Nichols and Griscom, 1917 (*partim*): 687–688.

Type material (all lengths are SL)
Lectotype. MRAC 23, Boma (DRC) (± 5°50′ S, 13°03′ E), coll. Wilverth 1896 (278.2 mm SL)

Paralectotypes. MRAC 98, 99, Léopoldville (DRC) (± 4°18′ S, 15°18′ E), coll. Wilverth 1896 (113.9 mm; 111.0 mm). MRAC 150, Upoto (DRC) (± 2°09′ N, 21°30′

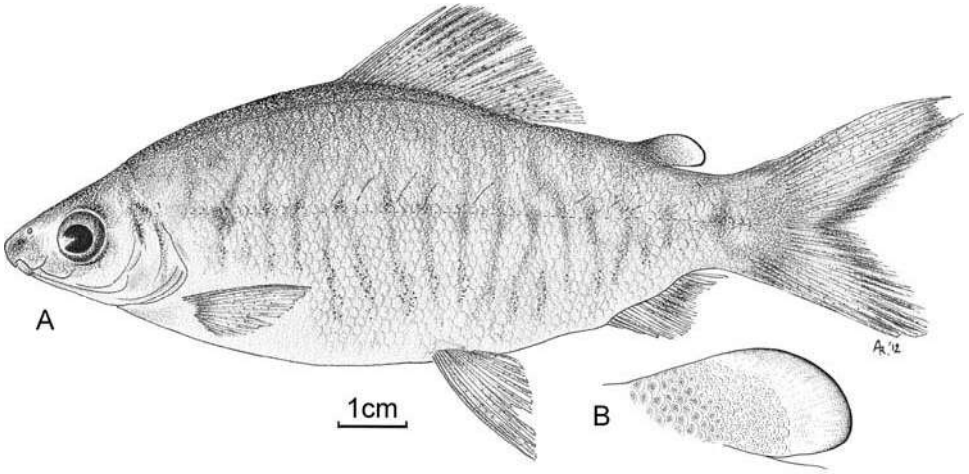


Figure 6. *Distichodus fasciolatus* (A7-014-P-0009-0018) from Pool Malebo (DRC), 100.9 mm SL. (A) Lateral view. (B) Detail of the colour pattern on the adipose fin.

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E), coll. Wilverth and Wagenaar 1896 (88.0 mm). MRAC 737, Léopoldville (DRC) (± 4° 18′ S, 15°18′ E), coll. Delhez 1899 (86.3 mm). BMNH 1891.12.29.22–23, Congo River (DRC), coll. received from Utrecht university museum 1891 (55.7 mm; 61.1 mm). BMNH 1898.7.9.19, Congo River, Monsémbé (DRC) (± 1°15′ S, 18°38′ E), coll. Weeks 1898 (114.4 mm). BMNH 1898.11.17.13, Boma, Lower Congo (DRC), coll. received from the Secretary of State Congo Free State 1898 (211.8 mm). BMNH 1898.11.17.14, Cataracts of Manyanga (DRC) (4°54′ S, 14°23′ E), coll. received from the Secretary of State Congo Free State 1898 (170.3 mm).

Note on the type specimens. The 13 syntypes of *D. fasciolatus* housed at RMCA and the BMNH originate from the Lower Congo (Matadi, Boma and Manyanga cataracts), and the Central Congo [Kinshasa (formerly Léopolville), Monsembé and Upoto]. Three syntypes (MRAC 28, 63 and 102) were found to be *D. antonii* and are therefore listed with *D. antonii*.

Because of the polyspecificity of the type series, the syntype MRAC 23, used in the original description of Boulenger (1898) to illustrate *D. fasciolatus*, is here designated as the lectotype. As such, all other syntypes of *D. fasciolatus* become paralectotypes.

Other material examined (all lengths are SL)

Distichodus fasciolatus Boulenger, 1898. **Democratic Republic of the Congo (DRC).** MRAC 744/A, Kutu (± 2°44′ S, 18°08′ E), coll. Dehlez 1899 (445.0 mm) – MRAC 2439, Léopoldville (± 4°18′ S, 15°18′ E), coll. Christy 1913 (77.7 mm) – MRAC 99075, Stanley Pool (± 4°06′ S, 15°15′ E and ± 4°20′ S, 15°23′ E), coll. Mandeville 10/7/1955 (116.2 mm). MRAC 117371, Stanley Pool, channel in front of Maluku (± 4° 04′ S, 5°33′ E), coll. Mission Brien-Poll-Bouillon 4/10/1957, Poll M., 1958 (66.5 mm). MRAC 91-013-P-0574-0575, Nsele River (Benzale) a tributary of Congo River near Kinshasa (± 4°08′ S, 15°40′ E), coll. Tshibwabwa Sinaseli 1985 (93.8 mm; 101.4 mm). MRAC A7-14-P-0009-0018, Pool Malebo at Kinkole, Japon island (± 4°18′26.9″ S, 15°30′33.7″ E), Mbadu Zebe 22/2/2007 (63.3–139.2 mm). MRAC A7-14-P-0019-0021. Pool Malebo at Kinsuka, Mimosa island (± 4°20′ S, 15°13′ E), coll. Mbadu Zebe 15/

8/2006 (208.1–252.8 mm). MRAC A7-14-P-0022-0024, Pool Malebo at Kinkole, Japon island (± 4°22′17.7″ S, 15°30′46.0″ E), Mbadu Zebe 25/2/2007 (98.4–119.6 mm). *Distichodus langi* Nichols and Griscom, 1917. AMNH 7010, Faradje (± 3°44′ N, 29°43′ E), coll. Lang-Chapin 1909–1915 (250.7 mm).

Republic of Angola. MRAC 158807, Dundo, in the rapids of the Luachimo River (± 7°21′ S, 20°50′ E), coll. Indigenous collect 20/10/1955 (316.0 mm).

Differential diagnosis

Within the Congo basin, *D. fasciolatus* can be distinguished from *D. affinis*, *D. altus*, *D. decemmaculatus*, *D. noboli*, *D. notospilus* and *D. teugelsi* by its higher total number of LL scales, i.e. 61–70 (versus < 46 in the six other species) and from *D. maculatus* by the absence of large, dark spots all over the body (13–20 vertical dark bars instead) and a higher number of dorsal fin rays, i.e. 24–26 (versus 19–21). It can be distinguished from *D.antonii* by its inferior mouth (versus terminal) and its higher number of scales between the LL and the dorsal fin, i.e. 13–16 (versus 10–12); from *D. lusosso* by its inferior mouth (versus terminal), its feebly compressed snout (versus distinctively prolonged), and its higher number of dark vertical bars along the body, at least in specimens ≤ 150 mm SL, i.e. 13–20 (versus 6–8); from *D. sexfasciatus* by its higher number of teeth on the outer row on both jaws, i.e. 20–30 (versus 12–18); its higher number of dark vertical bars on the flanks, at least in specimens ≤ 150 mm SL, i.e. 13–20 (versus 6–8) and its brownish-yellowish colouration (versus orange-reddish to red). Moreover, *D. fasciolatus* can be differentiated from both the *D. atroventralis* complex and *D. langi* by its lower number of pelvic fin rays, i.e. 10, exceptionally 11 (versus 11, exceptionally 10 for the *D. atroventralis* complex and always 11 for *D. langi*); from the *D. atroventralis* complex by its higher number of dark vertical bars, at least in specimens ≤ 150 mm SL, i.e. 13–20 (versus 6–9) and presence of pale pelvic fins, at least in specimens of ≤ 200 mm SL (versus blackish) and from *D. langi* by a combination of characteristics: 24–26 total dorsal fin rays (versus 26–28) and a lower head depth, i.e. 35.9–62.9% HL for specimens of comparable size (versus 67.4–69.4 % HL) (Table 4).

Description

Morphometric and meristic data are given in Table 5. Body relatively shallow (within *D. antonii* assemblage). Dorsal head profile straight, dorsal body outline concave from posterior to head to end of dorsal fin, straight from end of dorsal to adipose fin, and convex from adipose to caudal fin. Ventral head profile straight, concave from posterior to head to end of anal fin, and convex from end of anal to caudal fin. Head compressed with nasal openings relatively closely set (8.1–22.0 %HL), and head relatively shallow (35.9–62.9 %HL), although these characteristics are positively allometric. Mouth inferior. Posterior edge of maxillary not passing nostrils. Two rows of bicuspid teeth in each jaw. Origin of dorsal fin well in front of pelvic fin origin along vertical axis. Distal margin of dorsal fin straight or slightly concave, distal margin of anal fin straight to slightly convex. Base and distal end of pelvic fin relatively close to vent, although these distances are positive allometric, i.e. 18.6–25.8 %SL and – 2.1–5.7 %SL, respectively. Pectoral and pelvic fin rays decreasing in length from outer to inner fin margin. Adipose fin approximately half-way between dorsal and caudal fins. Caudal peduncle deeper then long or as deep as long. Caudal

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fin forked with superior lobe slightly pointed and inferior lobe rounded, covered with numerous small scales except for the translucent distal area.

Maximum recorded length: 600 mm TL (Daget and Gosse 1984).

Colouration

Small-sized specimens (c. 122–150 mm SL) *in vivo* are brown-yellowish on the flanks with copper-coloured reflections and with 13–20 dark vertical bars on the flanks. Dorsal fin scattered with many small, dark spots. Caudal fin with a discrete black outer edge. Adipose fin with no or only a very thin greyish distal margin (Figures 3B and 6). Pectoral fins pale. Pelvic fins generally pale, sometimes with slightly darker distal margins. Dark spot above the pectoral fin and at the base of the caudal fin. Larger specimens (c. 300 mm SL) are brownish, the transverse bars on the flanks as well as the dark spots above the pectoral fin and the root of the caudal fin can become vague but the marks on the dorsal fin clearly remain. Small preserved specimens are brownish with a silvery whitish belly; larger specimens (c. 450 mm SL) are uniformly brownish.

Ecology

According to Gosse (1963), *D. fasciolatus* is a bottom-dweller in streams. The fry are born near the river banks just before the April or December floods and feed on zooplankton and insect larvae in the flooded zones; adults mainly feed on insect larvae, aquatic plants, leaves and seeds. Fry were observed on the border of the stream just before the flood, spending the first few months in the flooded forest, before returning to the *Echinochloa* fields. Matthes (1964) considered this species as mainly herbivorous; the specimens he examined had sand, filamentous algae and plant debris in their stomachs, with some nematodes and insect debris (Ephemeroptera larvae, Trichoptera and small Dytiscidae). He also stated that *D.fasciolatus* is found near the muddy bottom in lakes, large rivers and their dead-ending branches. Recent stomach content analyses on specimens from the Lomami and Maiko rivers (Kisangani region) confirmed this mainly herbivorous diet, though insects were also found in the stomachs (Vanstallen, pers. obs.).

Distribution

This species is a widespread Congo basin endemic (Figure 7). One specimen (RBINS no. 9067) from a market in Albertville (Kalemie), Lake Tanganyika has been identified as *D. fasciolatus* by Poll (1953) and its identification confirmed by us. Poll (1953) stated that the species' discovery at Albertville is probably caused by a recent and rare migration through the Lukuga River. Another plausible explanation, however, might be that since the specimen was bought at a local market, it has not been caught in Lake Tanganyika itself, but in adjacent rivers from the Congo basin, such as the Lukuga River.

Etymology

The species name "*fasciolatus*" is derived from the Latin word "*fascia*", meaning "band, bandage, girdle, zone, strip, stripe" with "*fasciola*" as a diminutive (Brown 1956) and most probably refers to the well-marked transverse bars on the flanks.

Distichodus langi Nichols and Griscom, 1917
(Tables 4 and 5; Figures 3C and 8)

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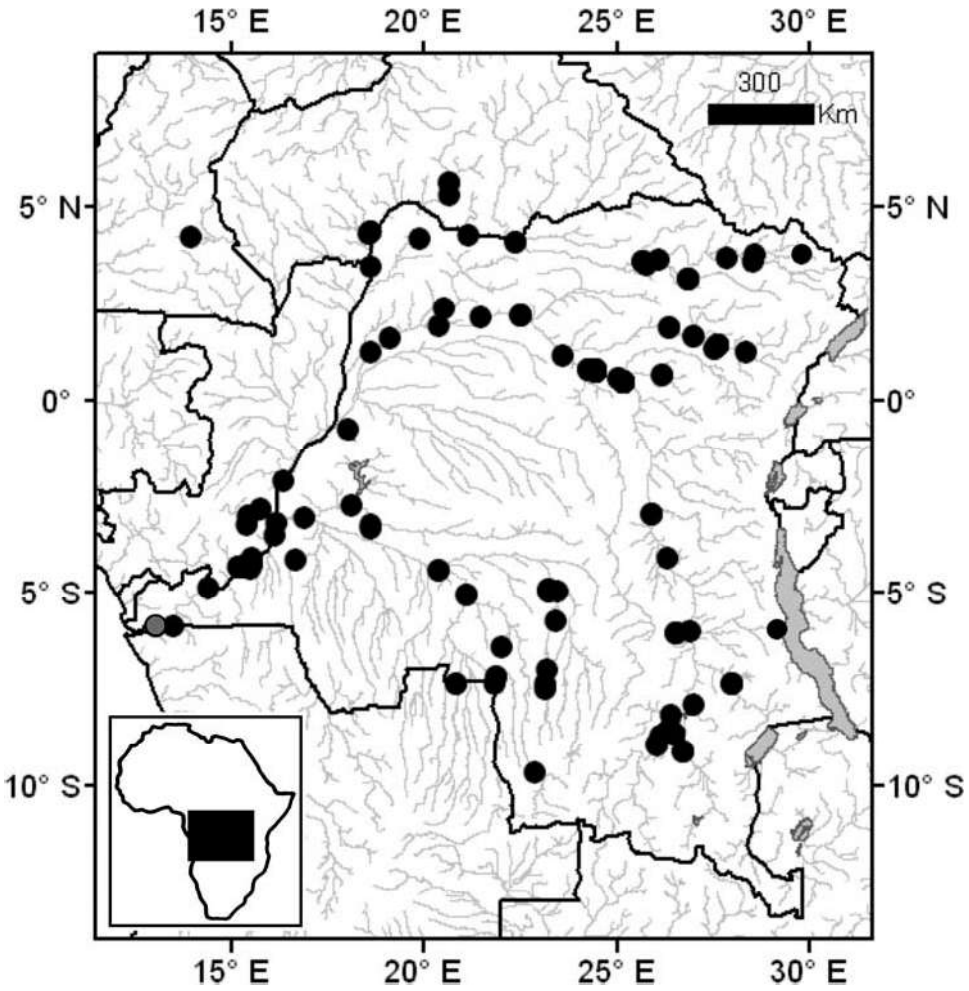


Figure 7. Distribution map of *Distichodus fasciolatus* based on the specimens identified at the AMNH and MRAC. (◉) lectotype and (●) other specimens.

Type material

Holotype. AMNH 5915, Faradje, Dungu region (DRC) (± 3°44′ N, 29°43′ E) (364 mm SL).

Note on the original description. Distichodus langi has been described by Nichols and Griscom in 1917 based on one type from Faradje and one other specimen with the same data. The current holotype has a size of 364 mm SL (472 mm TL), which does not correspond to the 510 mm SL mentioned by Nichols and Griscom (1917). This latter SL should be considered a lapsus because after verification of the Lang and Chapin collection, no *D. langi* specimen of this size has been found. The other specimen mentioned in the original description is reported to measure 325 mm SL, but this also does not correspond to the size of the other specimen (AMNH 7010), which measures 250.7 mm SL, 299.0 mm TL. This specimen has been wrongfully assigned paratype

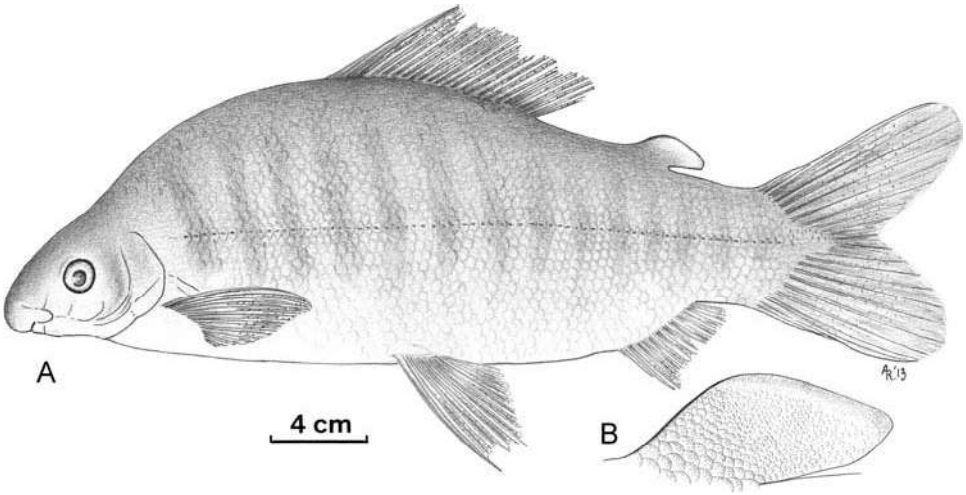


Figure 8. *Distichodus langi* (MRAC 2878) from Avakubi (DRC), 300.0 mm SL. (A) Lateral view; (B) Detail of the colour pattern on the adipose fin.

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status (Eschmeyer 2013) (see ICZN 1999: article 72.4.6). It has been re-identified as *D. fasciolatus* in this review (see above). In the original description, the size of the illustrated specimen (Nichols and Griscom 1917: plate LXIX) is mentioned as 560 mm TL, which corresponds to a recalculated standard length of 466 mm SL. This does not correspond to either of the two specimens mentioned by Nichols and Griscom (1917). In addition, the drawing does not resemble either of the two specimens. Based on the following counts and observations made on the illustration: 22 circumpeduncular scales; 13 scales between the LL and the dorsal fin, 24 dorsal fin rays and clearly black ventral fins, we assume this illustration represents a specimen from the *D. atroventralis* complex.

Other material examined
MRAC 2878, Avakubi (DRC) (1°19′ N, 27°33′ E) (300 mm SL).

Differential diagnosis
Within the Congo basin, *D. langi* can be distinguished from *D. affinis*, *D. altus*, *D. decemmaculatus*, *D. noboli*, *D. notospilus* and *D. teugelsi* by its higher total number of LL scales, i.e. 68–70 (versus < 46); from *D.maculatus* by the absence of large, dark spots all over the body and a higher number of dorsal fin rays, i.e. 26–28 (versus 19–21); from *D. antonii* by its inferior (versus terminal) mouth, its higher number of scales between the LL and the dorsal fin, i.e. 15–16 (versus 10–12) and its higher number of LL scales in front of HJ, i.e. 68–70 (versus 52–64); from *D. fasciolatus* by its higher number of dorsal fin rays, i.e. 26–28 (versus 24–26); its higher number of pelvic fin rays, i.e. 11 (versus generally 10) and its deeper head, 67.4–69.4 %HL (versus 35.9–62.9 %HL); from *D.lusosso* by its inferior (versus terminal) mouth and feebly compressed snout (versus distinctive prolonged); from *D. sexfasciatus* by its feebly compressed snout (versus strongly compressed), its higher number of dark vertical bars along the body, i.e. 13 (versus 6–7) and its higher number of teeth on the outer row on both jaws (> 20 versus 12–14) and from the *D. atroventralis* complex

by a higher number of scales between the LL and pelvic fin, i.e. 12 (versus 9–11), its higher number of pectoral fin rays, i.e. 21 (versus 17–20); its higher number of circumpeduncular scales, i.e. 24–25 (versus 20–24) and its deeper head, 67.4–69.4 % HL (versus 35.1–55.7 %HL) (Table 4).

Description

Morphometric and meristic data are given in Table 5. Body relatively deep (within *D. antonii* assemblage). Dorsal head profile and dorsal body outline concave to end of dorsal fin, straight from end of dorsal to adipose fin, and convex from adipose to caudal fin. Ventral head profile straight, concave from posterior to head to end of anal fin, and convex from end of anal to caudal fin. Head compressed with nasal openings relatively closely set (14.4 %HL and 15.9 %HL), but deep (67.4 %HL and 69.4 %HL). Mouth inferior. Posterior edge of maxillary not passing the nostrils. Two rows of bicuspid teeth in each jaw. Origin of dorsal fin well in front of pelvic fin origin along vertical axis. Distal margin dorsal fin straight or slightly concave, distal margin of anal fin straight to slightly convex. Base and distal end of pelvic fin relatively close to vent, i.e. 23.4 %SL and 25.9 %SL, and 1.6 %SL and 7.8 %SL, respectively. Pectoral and pelvic fin rays decreasing in length from outer to inner fin margin. Adipose fin little further removed from dorsal fin than from caudal fin. Caudal peduncle deeper then long. Caudal fin forked with two rounded lobes covered with numerous small scales except for translucent area distally. As only two specimens are currently known from *D. langi*, allometry could not be assessed.

Maximum recorded length: 364 mm SL, 472 mm TL.

Colouration

Lang provided information on the presumable life colour pattern based on five specimens (Nichols and Griscom 1917). It is however unsure whether these specimens are conspecific (see above), and therefore this information is not presented here.

The body of the two preserved specimens is uniformly brown-yellowish, with a slightly lighter belly. Dorsal fin scattered with many small, dark spots. Caudal fin brownish, with slightly darker median rays. Adipose fin with translucent distal margin (Figures 3C and 8). Pectoral fins pale to yellow-orange. Pelvic fins generally pale, while in the additional specimen the distal edges are darker coloured. Thirteen vague dark vertical bars have been observed on the flanks of the additional specimen, whereas the holotype has none.

Ecology

Not much is known about the ecology of *D. langi*. In the original description of Nichols and Griscom (1917), Lang is cited mentioning the following: "Evidently swimming in schools, as five of them have been killed by one explosion". However, since this original description is based upon three different species (see above), the relevance of this information is difficult to assess.

Distribution

This species is only known from two specimens from two different locations, Faradje in the Dungu region, Uele River basin (DRC) and Avakubi, Aruwimi River basin (DRC) (Figure 5).

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Etymology

The species name "*langi*" refers to the collector Herbert Lang (1879–1957), who collected fish in the Congo basin from 1909 to 1915 for the AMNH, in collaboration with James Chapin (Slack 2003).

List of specimens examined of the D. atroventralis *complex (all lengths are SL)* (Figure 3D).

Democratic Republic of the Congo (DRC): Syntypes: MRAC 30. Boma (DRC) (± 5°50′ S, 13°03′ E), coll. Wilverth 1896 (70.9 mm) – BMNH 1891.12.29.13. Congo River, Lower Congo (DRC), received from Utrecht University Museum 1891 (63.5 mm); Specimens: AMNH I-243645. Lulua River, Katende 2, Dipumu (± 6° 00′27.0″ S, 22°23′43″ E), coll. Mbimbi 01/12/2007 (64.2 mm; 90.2 mm) – AMNH I-243646. Lulua River, Nsanga Nyembo (± 5°56′53.41″ S, 22°20′29.40″ E), coll. Mbimbi 03/12/2007 (76.7 mm; 123.3 mm) – AMNH I-247823. Lulua, Bunkonde (6°14′49.2″ S, 22°28′48.0″ E), coll. Mbimbi 30/06/2008 (34.4 mm) – AMNH I-247825. Lulua, Katende 1, Nkombua (6°00′16.2″ S, 22°23′25.8″ E), coll. Mbimbi 21/05/2008 (37.0 mm) – MRAC 158797. Dundo, affl. Luachimo (± 7°21′ S, 20°50′ E), coll. De Barros Machado 02/06/1949 (225.5 mm) – MCZ 50439. Zaire River near Inga, a few km upstream and on the opposite side of river from Inga hydroelectric dam (5°27′30″ S, 13°36′??″ E), coll. Roberts and Stewart 1973 (53.2 mm; 62.8 mm) – MRAC 738. Banana (6°00′ S, 12°24′ E), coll. Delhez 1899 (53.5 mm) – MRAC 739. Matadi (5°49′ S, 13°27′ E), coll. Delhez, 1899 (246.6 mm) – MRAC 740. Matadi (5°49′ S, 13°27′ E), coll. Delhez 1899 (66.0 mm) – MRAC 741. Dolo (4°19′ S, 15°19′ E), coll. Delhez 1899 (357.0 mm) – MRAC 742. Kutu, (± 2°44′ S, 18°08′ E), coll. Delhez 1899 (158.3 mm) – MRAC 67444. Léopoldville (± 4°18′ S, 15°18′ E), coll. Henri van Moorsel 7/1944 (127.7 mm) – MRAC 117366. Stanley Pool, Yankau channel, N'Duka indigenous dam (± 4°20′ S, 15°24′ E), coll. Mission Brien-Poll-Bouillon 23/9/1957 (199.5 mm) – MRAC 73-22-P-1195-1197. Stanley Pool, Nsele river (± 4°15′ S, 15°33′ E), coll. Mandeville 29/10/1957 (23.8–41.2 mm) – MRAC 73-22-P-1201-1202. Stanley Pool, Ndjili river (± 4°20′ S, 15°22′ E), coll. Mandeville 30/10/1957 (67.8 mm; 76.8 mm) – MRAC 73-22-P-1223. Stanley Pool, Mbamu island (± 4°14′ S, 15°22′ E to ± 4°18′ S, 15°30′ E), coll. Mandeville J., 28/10/1957 (33.1 mm) – MRAC A7-014-P-0025. Pool Malebo at Kinkole, Mipongi island (± 4°16′26.9″ S, 15°30′29.6″ E), coll. Mbadu Zebe 26/1/2006 (128.0 mm) – MRAC A7-033-P-267. Congo River close to the Kinsanga channel, Inga (± 5°39′ S, 13°39′ E), coll. Hanssens 2007 (142.6 mm) – MRAC B0-017-P-0020. Lomami River, affluent Congo River, village Lieki, coll. Congo 2010 2/06/2010 (343.0 mm) – MRAC B0-017-P-0021-0022. Isangi, rive gauche fleuve Congo, coll. Congo 2010 5/06/2010 (261.9 mm SL; 325.0 mm SL) – MRAC B0-017-P-0093. Lomami River, left bank, just downstream of village Lieki (± 0°40′55″ N, 24°11′08″ E), coll Exp. Congo 2010 27/05/2010 (31.5 mm) – MRAC B0-017-P-0094. Little marsh on Lomami River, left bank, upstream of village Yandjambi (± 0°41′57.44″ N, 24°13′36.0114″ E), coll Exp. Congo 2010 28/05/2010 (31.4 mm) – MRAC B0-017-P-0095. Lomami River, left bank, upstreamof village Lieki (rive gauche riv. Lomami, en amont du village Lieki (± 0°40′55″ N, 24°11′08″ E), coll Exp. Congo 2010 01/06/2010 (61.6 mm) – MRAC B0-017-0096-0097. Lomami River, right bank, downstream of village Lieki (± 0°40′ 55″ N, 24°11′08″ E), coll. Exp. Congo 2010 30/05/2010 (21.7 mm; 25.9 mm) – MRAC B0-017-P-0098. Lomami River, downstream of village Lieki (± 0°40′55″ N, 24°11′

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08″ E), coll Exp. Congo 2010 27/05/2010 (32.0 mm) – MRAC B0-017-0099-0100. Lomami River, downstream of village Lieki (± 0°40′55″ N, 24°11′08″ E), coll. Exp. Congo 2010 03/06/2010 (27.6 mm SL; 36.2 mm) – MRAC B0-017-P-0101. Lomami River at confluence with Koli River, right bank, downstream of village Lieki (± 0°40′ 55″ N, 24°11′08″ E), coll. Exp. Congo 2010 01/06/2010 (25.6 mm) – MRAC B1-009-P-0022. Lobaye River, left bank, Site 2 (± 0°28′28.8″ N, 24°11′57.9″ E), village Bambondji II, coll. Danadu and Moelants 08/05/2011 (93.0 mm).

Republic of the Congo (Brazzaville): MRAC A8-020-P-0386-0390 (2/4). Léfini River, "Massala ma soso" rapids, right bank (2°55.93′ S, 16°07.42′ E), coll. Vreven and Ibala Zamba 23/8/2008 (46.5 mm; 68.5 mm).

Discussion

On the status of Distichodus mossambicus *in the Congo basin*

All *D. mossambicus* from the Congo basin were found to belong to the *D. atroventralis* complex, highlighting the existing confusion in the collections and literature on the presumed presence of *D. mossambicus* in the Congo basin.

Distichodus mossambicus Peters, 1852 was originally described from Tette (±16° 10′ S, 33°36′ E, Mozambique) along the Lower Zambezi River and is currently known from the Middle and Lower Zambezi, Pungwe and Buzi systems (Skelton 2001). Specimens from the Lower Congo (see Roberts and Stewart 1976) and the Kasai system in the Central Congo basin, (see Poll 1967 and the AMNH collection) have also been identified as *D. mossambicus*. Lowenstein et al. (2011) referred to the Lower Congo specimens in their analyses as *D.* cf. *mossambicus*, probably referring to the problematic status of this species in the Congo basin. Indeed, a comparison of *D. mossambicus* specimens from the Central Zambezi basin [Mlolo River (Zimbabwe)] with specimens identified as *D. mossambicus* from the Lower (Inga) and Central (Kasai) Congo basin clearly showed that they are not conspecific. Zambezi specimens have a terminal (versus inferior) mouth (see illustration by Poll 1967: 106, fig. 42), a clearly protruding snout (versus an obtuse, short snout), and a lower head depth (Figure 9). Hence, *D. mossambicus* has to be omitted from the list of the Congo basin species.

On the Distichodus antonii *assemblage from the Congo basin*

The material used for the description of *D. langi* was revealed to be polyspecific. This included the so-called paratype (see above) re-identified as *D. fasciolatus* and the specimen illustrated in the original description, which belongs to the *D. atroventralis* complex. Consequently, *D. langi* is currently only known from two specimens, i.e. the holotype and one additional specimen, which can be well distinguished from all other *Distichodus* species from the Congo basin. A third specimen, labelled as *D. langi* (AMNH 12379) from Kananga (formerly Luluabourg), is in bad condition and was not included in our analyses. It has been re-identified as part of the *D.atroventralis* complex.

Also the type series of *D. fasciolatus* revealed to be polyspecific with three syntypes belonging to *D. antonii*. These misidentifications have added to the confusion concerning the distinctive characters between *D.antonii* and *D. fasciolatus*. In

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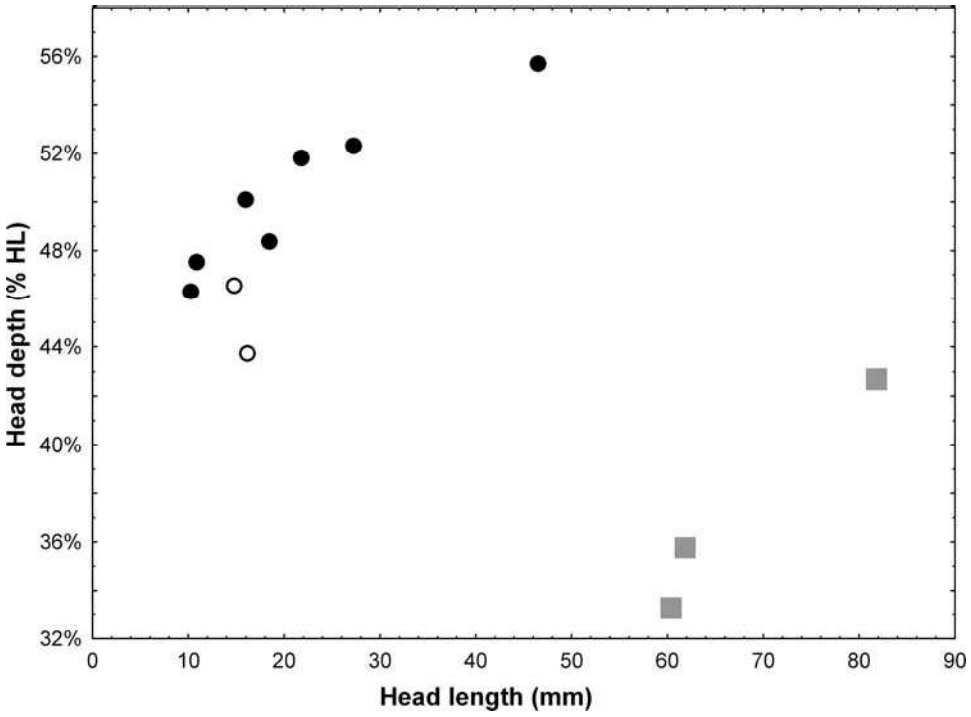


Figure 9. Scatterplots of HL (mm) against head depth (% HL) for specimens identified as *Distichodus mossambicus* from: (○) Lower Congo; (●) Kasai and (■) Zambezi.

addition, a specimen identified by Boulenger in 1896 as *D. antonii* had to be re-identified as *D. fasciolatus*, illustrating long-time misidentifications in both directions. Further verification of the *Distichodus* collections in the RMCA revealed numerous misidentifications among *D. antonii*, *D. fasciolatus* and the *D. atroventralis* complex, and this in all directions. According to Poll (1939), in all *D. fasciolatus* specimens from Kinshasa (Leopoldville) he examined, including the original syntypes of *D. fasciolatus* and in some from the Lower Congo, the distance between the dorsal and adipose fin fits two times or more in the dorsal fin base length. He further stated that in other specimens from some localities in the Lower Congo, this distance is regularly longer and does not fit twice into the dorsal fin length. Based on these observations, Poll (1939) suggested the possible presence of two subspecies in *D. fasciolatus*, but added that at several other localities such as Kunungu, Stanley falls (now Wagenia falls) in Stanleyville (now Kisangani), Kiambi and Nyonga both forms were found together. Because of their sympatry, these forms cannot be regarded as subspecies (Mayr and Ashlock 1991). The confusion between *D. antonii* and *D. fasciolatus* is clearly the result of misidentifications and the subspecies scenario that is based on a mixture of both species. Indeed, even the specimen from Léopoldville (Central Congo) illustrated by Poll (1939) as *D. fasciolatus* is clearly a *D. antonii* specimen.

Moreover, *D. antonii* and *D. fasciolatus* also occur sympatrically with the *D. atroventralis* complex. During recent sampling by one of us (TM 2010–2011),

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D. antonii, *D. fasciolatus* and specimens from the *D. atroventralis* complex were even found syntopically in the Congo River near its mouth with the Lomami and Maiko Rivers and in the Lomami River itself. *Distichodus fasciolatus* was found syntopically with *D. antonii* in the Loboya River, an affluent of the Maiko River.

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Diploneis fenestrata sp. nov. (Bacillariophyta), a new aerophilic diatom species from Zambia, Africa

JONATHAN C. TAYLOR[1, 2, 3], BALASUBRAMANIAN KARTHICK[1, 4*], CHRISTINE COCQUYT[3], PAULINE LANG[5, 6]

[1]*Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa*
[2]*South African Institute for Aquatic Biodiversity, Grahamstown, South Africa*
[3]*National Botanic Garden of Belgium, Meise, Belgium.*
[4]*Division of Plant Sciences, Agharkar Research Institute, GG Agarkar Road, Pune - 411004, India*
[5]*Ecology Assessment Unit, Scottish Environment Protection Agency, North Lanarkshire, Scotland, UK*
[6]*Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, Scotland, UK*
**Corresponding author: E-mail: karthickbala@aripune.org*

Abstract

Diploneis fenestrata, a new aerophilic diatom is described from Lumangwe falls, Zambia. This novel species occurs as an epiphyte on the roots and stems of trees located within the spray zone of the falls. Its morphology was examined with both light and scanning electron microscopy. *Diploneis fenestrata* is distinguished from other taxa in the genus *Diploneis* by its prominent square openings ranging in number from 4-14 occurring within the area of the longitudinal canal only at the outer cell wall. The occurrence of these characteristic square openings found in the genus *Diploneis* is further discussed.

Key words: Diatoms, *Diploneis fenestrata,* cell wall structure, Lumangwe falls

Introduction

African benthic diatoms from lotic ecosystems and especially central and southern African diatoms have received relatively little scientific attention given the scale of these territories. Recently, however, samples were collected from two of the largest and most under-studied catchment basins in Africa: the Zambezi and the Congo basins. The Zambezi catchment basin has received very little attention in terms of the study of rivers and streams, with only three studies in the last 60 years (Cholnoky 1954, Cholnoky 1970, Compère & Delmotte 1988, the latter authors also highlighted the scarcity of diatom records from Zambia). A countrywide survey of the diatoms of Zambia was recently undertaken and included both the Zambezi and Congo River drainage basins. The main focus of the EU ACP funded Southern Africa River Assessment Scheme (SAFRASS) project (www.safrass.com/), together with the BELSPO funded Congo Zambezi Diatom Monitoring (COZADIMO) project, is sampling rivers and streams with the aim of developing a diatom-based monitoring protocol for the inference of water quality (Cocquyt *et al*. 2014). However, samples were also collected from other moist or periodically wet habitats such as waterfalls, their associated spray zones and stream edges. It was in one such sample, collected from the spray zone of a waterfall located in northern Zambia, that an unidentified species of *Diploneis* (Ehrenberg) Cleve (1894: 76; basionym: *Pinnularia didyma* Ehrenberg 1845: 144) was discovered, its chief distinguishing character was the square openings in the outer cell wall in the longitudinal canal adjacent to the raphe. Only a few taxa from *Diploneis* have been recorded from the (sub-) tropics and, furthermore, most have probably been identified using concepts based on European taxa such as *D. elliptica* (Kützing) Cleve (1894: 92; basionym: *Navicula elliptica* Kützing 1844: 98), *D. oblongella* (Nägeli ex Kützing) Cleve-Euler in Cleve-Euler & Osvald 1922: 57; basionym: *Navicula oblongella* Nägeli ex Kützing 1849: 890), *D. ovalis* (Hilse) Cleve (1891: 44; basionym: *Pinnularia ovalis* Hilse 1860: 82) (Gasse 1986, Cocquyt 1998; for a recent example of misidentified species of *Diploneis* from Lake Ohrid see Jovanovska *et al*. 2013).

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Diploneis is a large genus occupying marine, brackish and freshwater habitats across both hemispheres; comprising more than 500 described taxa (Guiry & Guiry 2014) the majority of species are marine. Apart from aquatic habitats, some species of *Diploneis* (e.g. *D. minuta* Petersen, 1928: 381) are also known from aerophilic environments such as mosses, colonizing wet rock in the spray zone of water falls (Idei & Kobayashi 1986).

Diploneis is characterized by having very complex areolae. On each side of the raphe there is a continuous longitudinal canal within the valve structure whose inner wall projects into the cell. The canal is not apparently open to the cell interior but is open to the exterior (Round *et al.* 1990). This canal is usually visible in LM and is one of the key distinguishing features of *Diploneis*. It is the structure of longitudinal canal that the characteristic regular square openings in the outer cell wall occur. The nature of these openings is discussed and illustrated below.

Material and Methods

Material used in this study was collected on 31st May 2012 from heavily shaded roots and stems of trees growing in the spray zone of Lumangwe Falls on the Kalungwishi River located in Northern Province, Zambia (9.5422° S, 29.3874° E) at an elevation of 1128 m above sea level. The sample material (12-388) was preserved in the field with ethanol (20% v.v.). pH was measured using a Hanna HI 98129 portable meter.

The sample was digested using potassium permanganate ($KMnO_4$) with Hydrochloric acid and centrifuged, then rinsed with distilled water several times to remove the acid (Taylor *et al.* 2005). The cleaned material was used for preparation of permanent slides, with the specimens mounted in Pleurax (Von Stosch 1974).

Light microscope (LM) studies were carried out using a Nikon Eclipse 80i microscope equipped with differential interference contrast optics (DIC) and a 1.45 N.A. 100x oil immersion objective. Photographs were taken with Nikon DS-Fi1 digital camera. For scanning electron microscope (SEM) studies, aliquots of the cleaned material were air dried on a fragment (5mm x 5mm) of vinyl phonograph record (MacGillivary & Ehrman 2011). The vinyl fragment was fixed to aluminium stubs using double sided carbon tape, sputter-coated with gold palladium and specimens observed using a FEI Quanta 2000 F-SEM (10 kV, WD 10 mm) at the Laboratory for Electron Microscopy, North-West University, Potchefstroom, South Africa.

All raw and cleaned samples, permanent slides and SEM stubs (with the exception of the holotype specimen) are housed at South African National Diatom Collection at the North-West University, Potchefstroom, South Africa.

Ross *et al.* (1979) and Idei & Kobayasi (1986, 1988, 1989) is followed for valve terminology.

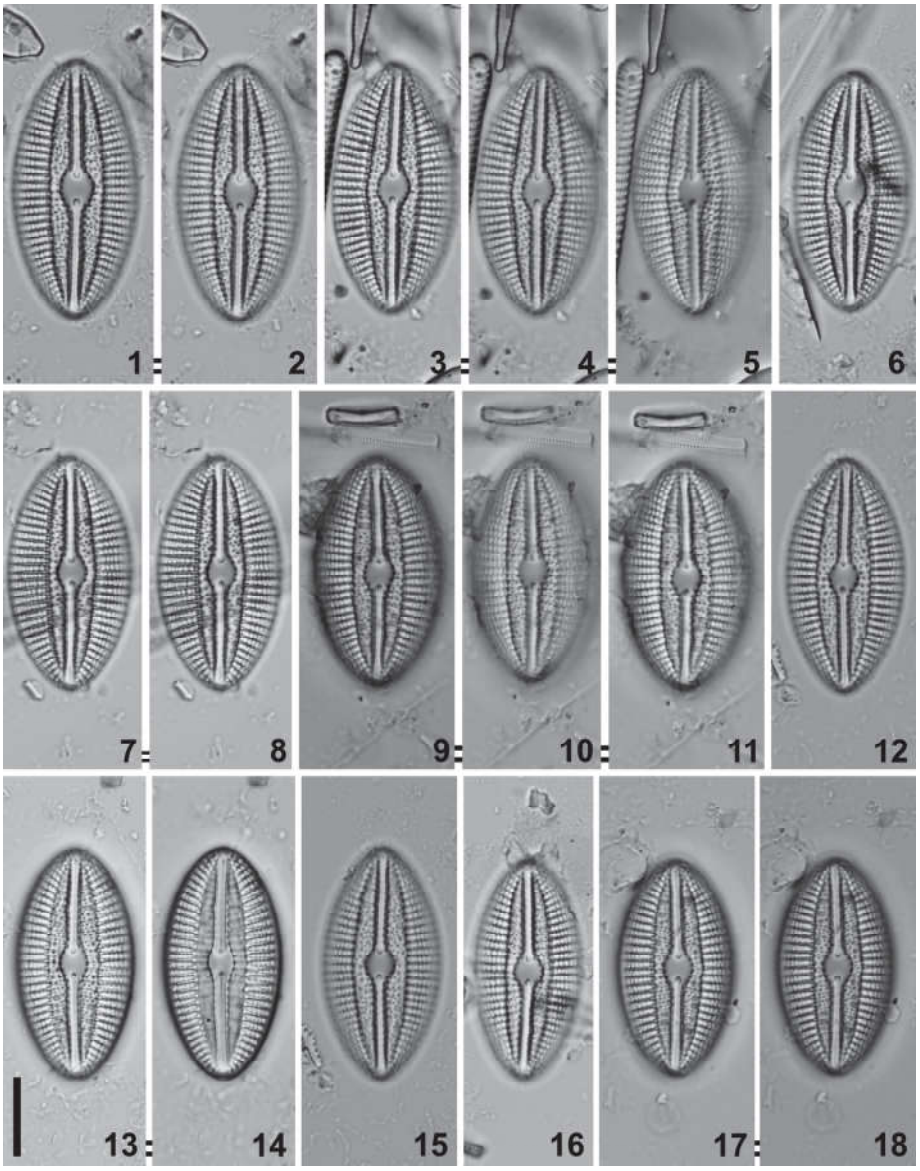
Results

Division Bacillariophyta
Class Bacillariophyceae Haeckel (1878) emend. D.G. Mann in Round *et al.* (1990)
Order Naviculales Bessey (1907)
Family Diploneidaceae D.G. Mann in Round *et al.* (1990)
Genus *Diploneis* Ehrenberg *ex* Cleve (1894)

Diploneis fenestrata J.C. Taylor & B. Karthick *sp. nov.* (Figs 1–48)

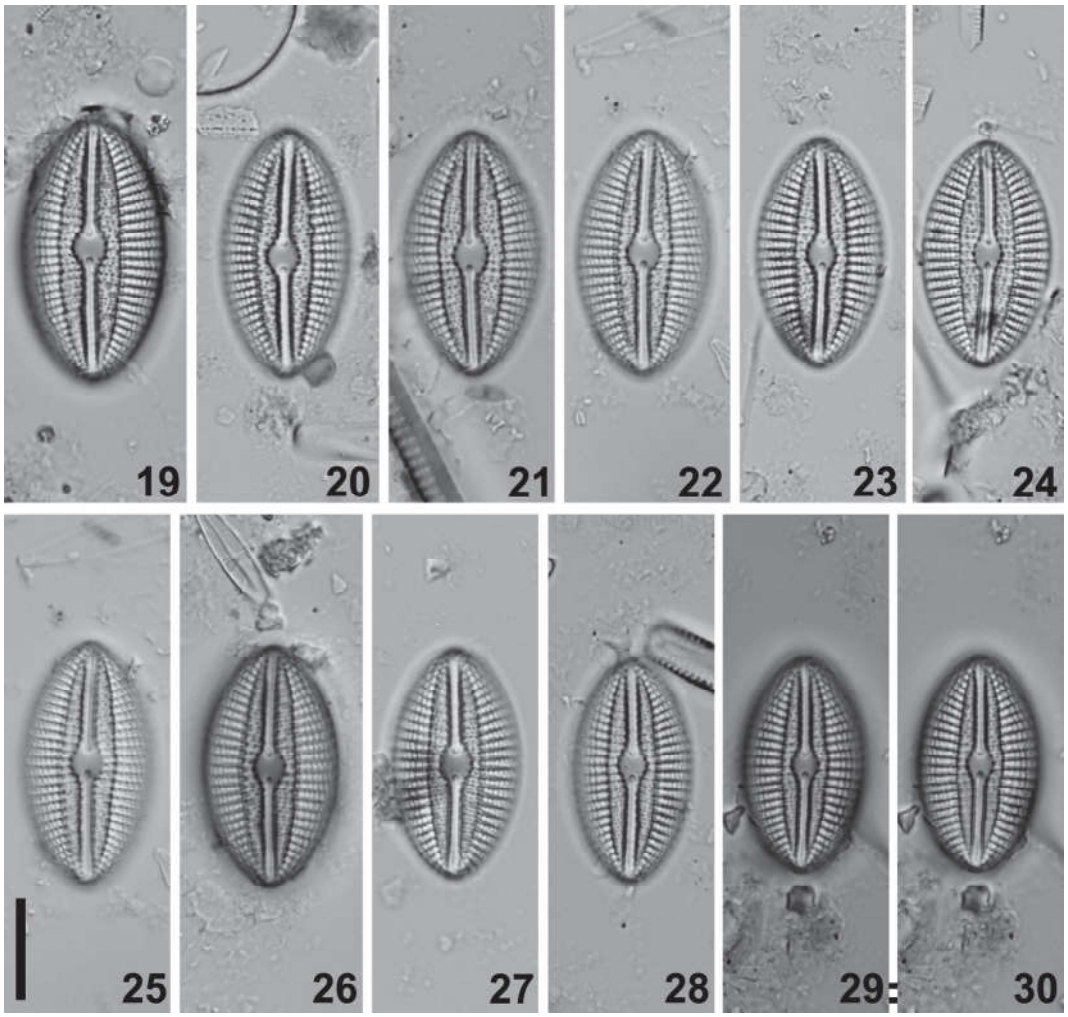
Valves broad, elliptical, with obtusely rounded ends, 21.0–39.0 µm long, 12.0–18.0 µm wide. Axial area narrow. Central area distinct and round to oval in shape. Longitudinal canals broad, lanceolate in flat median portion, occupying half of the width of each side of the valve (5.5–8.0 µm). Longitudinal canal ornamented with irregularly spaced areolae. Square to rectangular shaped openings present in between regularly spaced areolae in the longitudinal canal area numbering from 4 to 14. Biseriate striae, 9–12 in 10 µm nearly parallel in center becoming gradually radiate toward the valve apices. Areolae distinct near the valve margin.

Type:—ZAMBIA. Northern Province, Lumangwe Falls on the Kalungwishi River, 9.5422° S, 29.3874° E, epiphytic samples collected by Jonathan Taylor (collector number 12-388) by scraping roots and stems growing in spray zone of the falls (BR 4331, sample number D-NWU 12-388, holotype! = Fig. 13), **holotype designated here**; PUC 12-388, isotype!, sample number PUC 12-388, **isotype designated here.**



FIGURES 1–18. *Diploneis fenestrata:* LM of valves from specimen on holotype (BR 4331; Fig. 13 = holotype specimen) (Lumangwe Falls on the Kalungwishi River in Zambia); specimens at different focal depths, showing size range in valve view. Scale bar = 10 μm.

Externally, the valve surface is almost flat and the mantle is deep (Figs 31–34). The striae are composed of regularly arranged areolae (Fig. 32). The boundary between the longitudinal canal and the rest of the valve can be recognized by a thin hyaline band and by the shape, size and arrangement of the areolae (Figs 32, 33). Axial area is very narrow. The central area is distinct and round to oval in shape. Areolae above the longitudinal canal are large, densely arranged near the poles (35–40 /10 μm) and sparsely arranged near the central area (26–30 /10 μm) (Fig. 32). These areolae are cribrate with 2–15 openings per areola (Fig. 39). Above the alveoli the areolae are small and regularly arranged without clear boundaries between the alveolate striae (Fig. 40). Nearly round-shaped openings occur in between granulate structures (Fig. 36). The outer edge of the valve margin is finely serrated (Fig. 36). Silica depositions between the alveolate striae are evident with numerous granulate structures (Fig. 40). The characteristic feature of this species is the square openings (ranging from 4-14 per valve) that are present only above the longitudinal canal. The periphery of the opening is demarcated by regularly spaced small openings identical to those composing the areolae (Figs 41– 43). The external raphe fissure is slightly widening towards the central raphe endings and lies in a shallow round depression (Figs 31, 32). The central raphe endings are straight to slightly bent (Fig. 38). The terminal raphe fissures are bent over the valve mantle (Figs 32, 34–35, 37). Both distal raphe endings turn in the same direction (Figs 32, 34). The cingulum is composed of two open bands: one valvocopula and a pleura with a ligula (Fig. 35).



FIGURES 19–30. *Diploneis fenestrata*: LM of valves from holotype (BR 4331) (Lumangwe Falls on the Kalungwishi River in Zambia); specimens at different focal depths, showing size range in valve view. Scale bar = 10 µm.

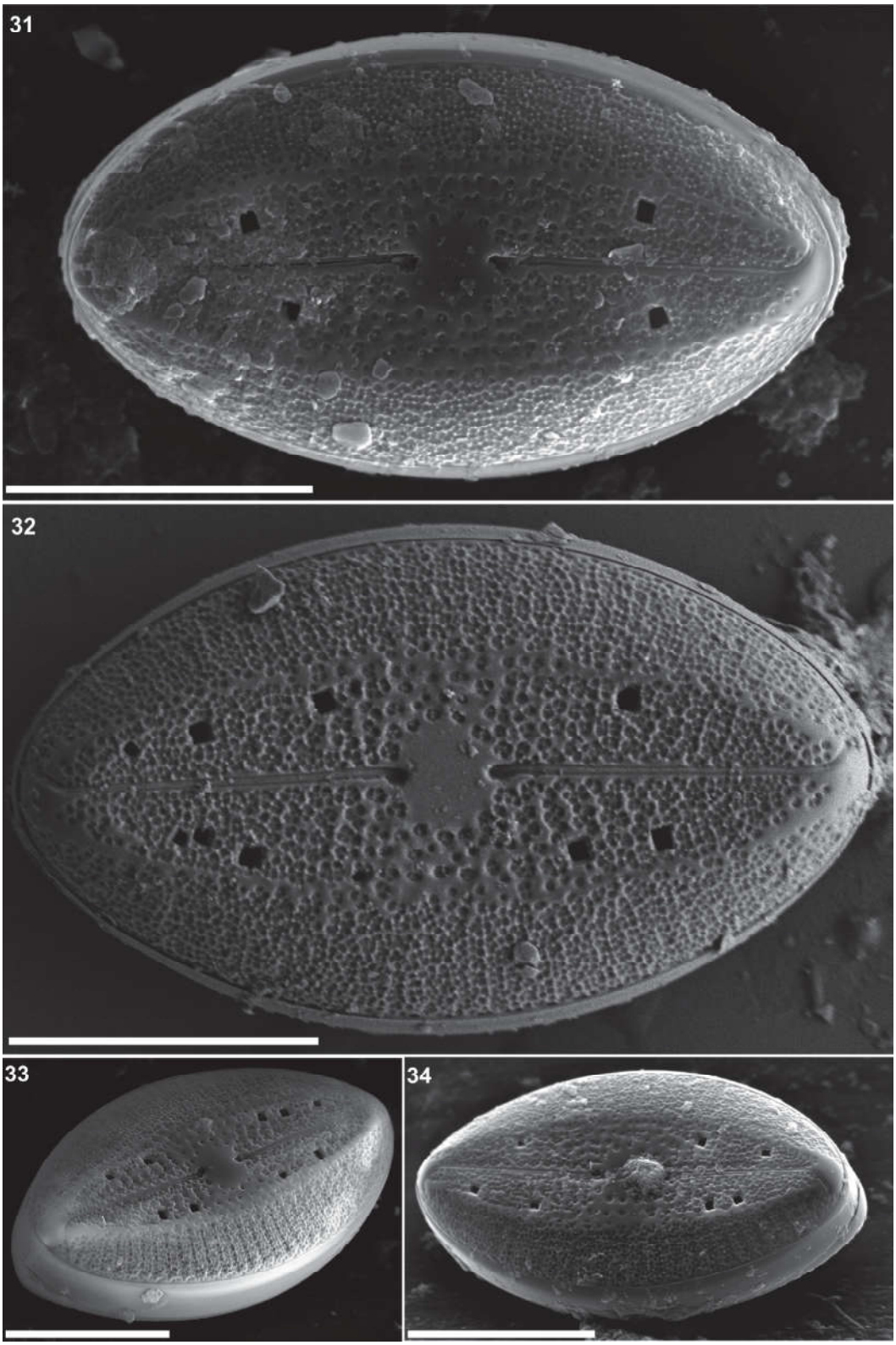
Internally, the longitudinal canals occupy the same width as externally (Fig. 44). Transapical costae on both sides on the longitudinal canal ends are smooth, and becoming narrower towards the poles (Figs 44–48). The central area is characterized by elevated silica thickenings (Fig. 46). Striae are comprised of two rows of areolae (Fig. 47). The raphe is straight, placed in a 'trench' below the axial plate with slightly bent and elevated proximal ends (Figs 45, 46). The valvocopula is an open band with a finely serrated valve mantle (Fig. 47). Distally the raphe ends in a bend on an elevated structure (Fig. 48). Distal and proximal ends bend in the same direction (Figs 44, 46).

Etymology:—The specific epithet is derived from the Latin word fenestrate, meaning 'window' and refers to the characteristic window-like "square openings" observed in this taxon.

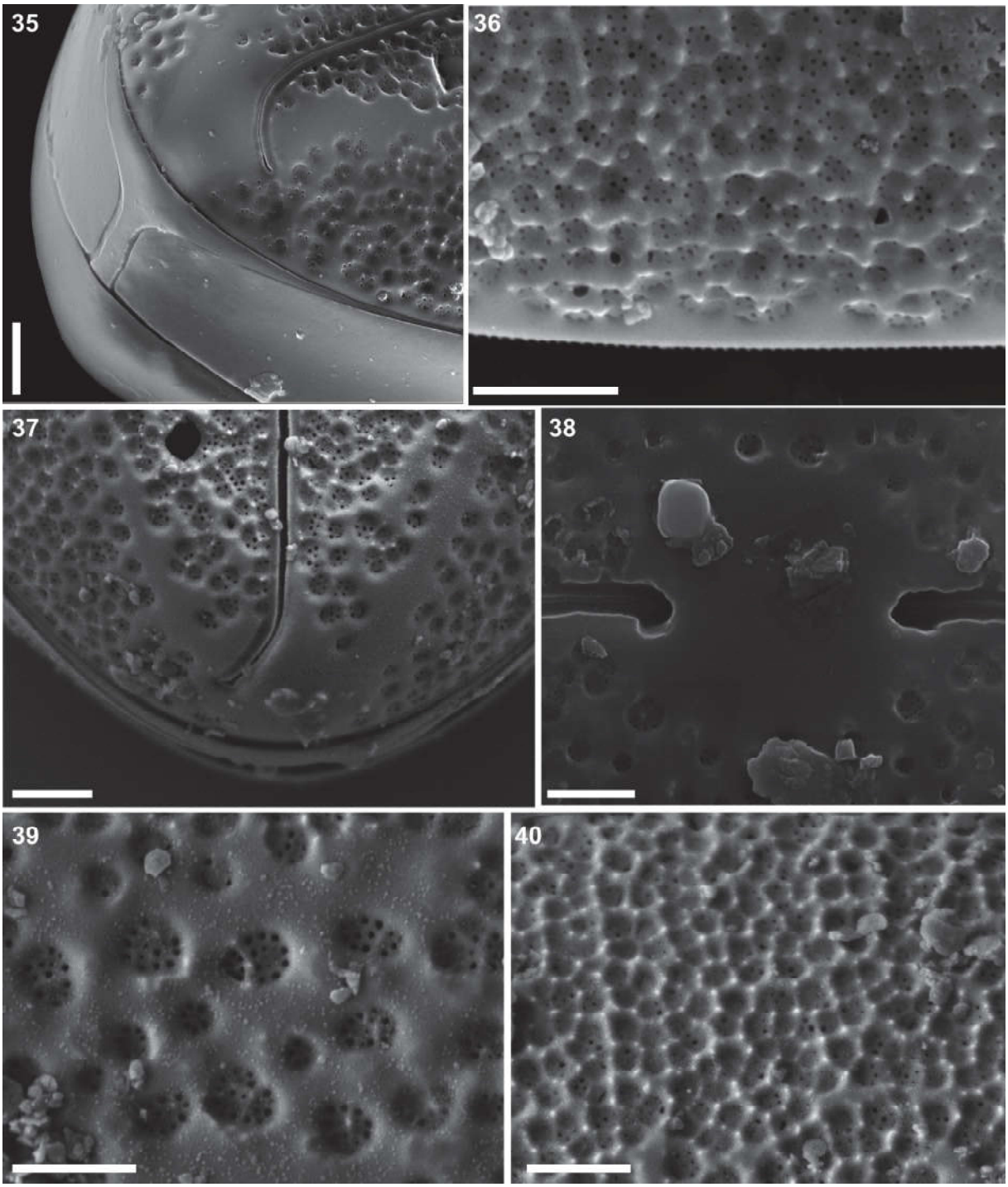
Ecology:—*Diploneis fenestrata* occurs sporadically in the type material. Samples were collected from very shallow trickling water over the roots and stems of trees. The area is periodically wetted depending on the volume of water in the river and the direction of the wind. The sample area was constantly in deep shadow for most of the day. Water quality parameters in the adjacent river were: pH 6.5, electrical conductivity 16 $\mu s.cm^{-1}$, PO_4-P 0.005 $mg.l^{-1}$ and NO_3-N 0.009 $mg.l^{-1}$.

Observations:—*Diploneis fenestrata* can be differentiated from some of the commonly occurring members of the genus such as *D. ovalis* and *D. subovalis* Cleve (1894: 96) by the valve outline and the shape, central area and size of the longitudinal canal (Lange-Bertalot & Reichardt 2000). Furthermore, the characteristic square openings on the longitudinal canal area of *D. fenestrata* can easily be distinguished under light microscope when samples are mounted in a high refractive index media. Similarly, *D. finnica* (Ehrenberg) Cleve (1891: 43), a species reported from Central Africa (Cocquyt 1998), also possess square opening on the outer valve face (Idei & Kobayasi 1989, figs 2, 5, 8, 20, 27). It is possible, however, to discriminate between *D. fenestrata* and *D. finnica* as the square

openings of the latter are present in the alveolate region and not in longitudinal canal region (Table 1). Additionally, the number of square openings in *D. finnica* is more restricted and ranges from 0–5 whereas the Zambian species has 4–14 square openings per valve; no cells were found to be without these characteristic openings. In case of *D. finnica*, members of the same population have valves with opening as well as without openings; this has been documented in populations from both Europe and Japan (Idei & Kobayasi 1989).

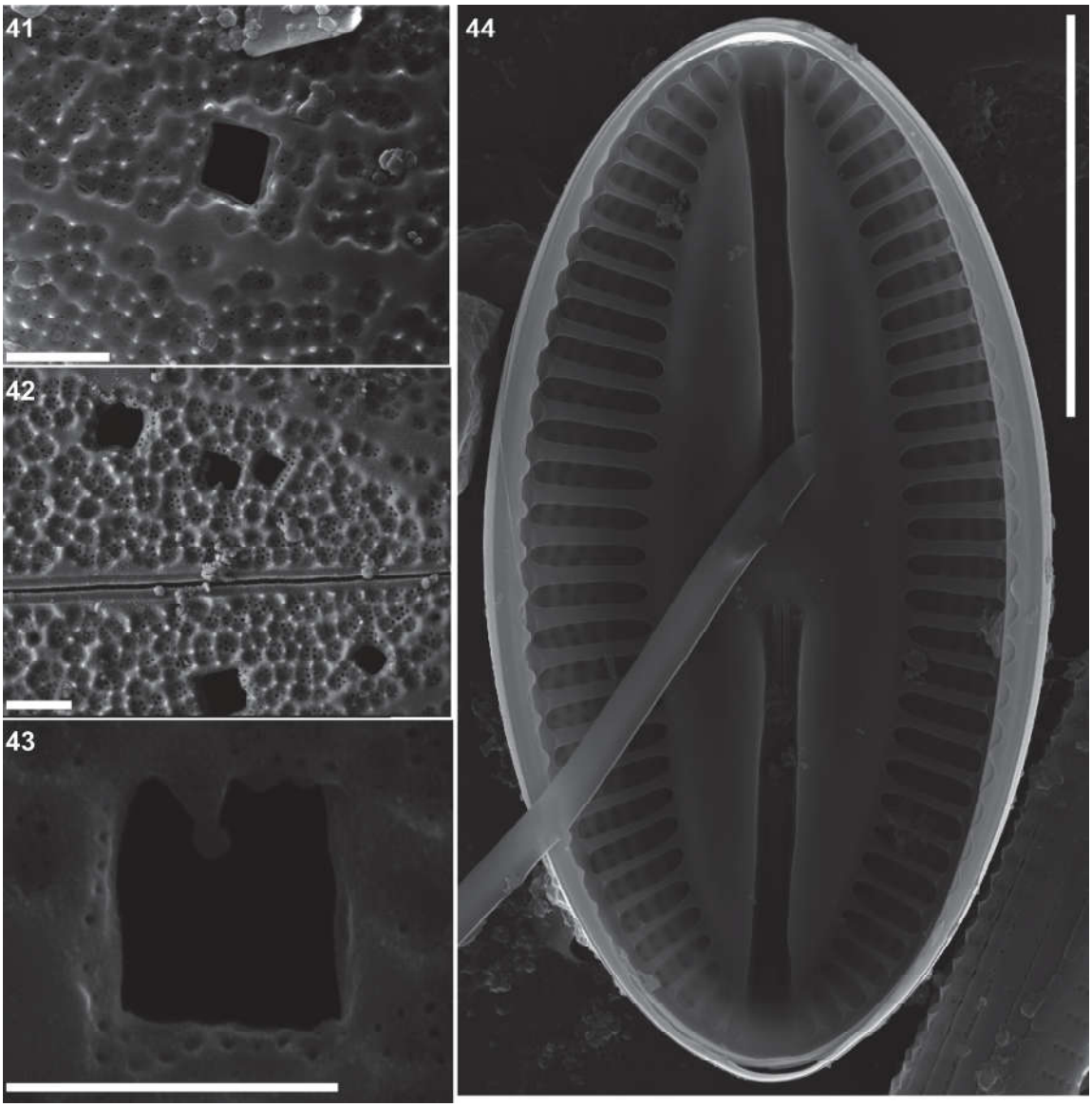


FIGURES 31–34. *Diploneis fenestrata* SEM: type material (sample D-NWU 12-388). External view of whole valves, showing flat valve surface and square opening on valve surface. Scale bar = 10 µm.



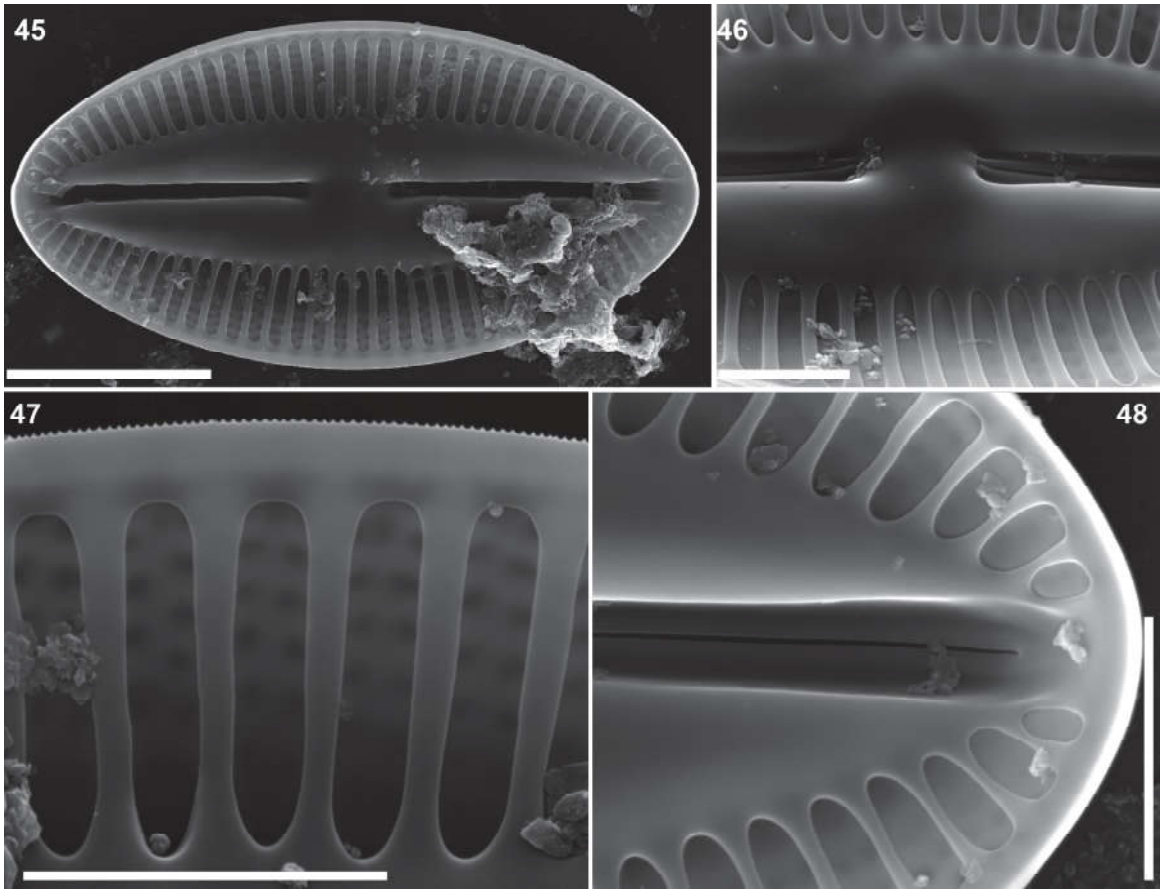
FIGURES 35–40, *Diploneis fenestrata* SEM: type material (sample D-NWU 12-388), external valve view. Fig. 35. Cingulum and valvocopula. Fig. 36. Illustrating small round shaped openings occurring between papillae-like structures in areolae. Fig. 37. Illustrating curved distal raphe ends. Fig. 38. Illustrating straight central raphe ends sunk in a round depression. Fig. 39. Illustrating cribrate areolae above longitudinal canal. Fig. 40. Illustrating silica depositions in between alveolate striae. Scale bar = 1µm.

Diploneis fenestrata can be distinguished from *D. marginestriata* Hustedt (1922: 236) by valve shape and longitudinal canal outline. In LM *D. fenestrata* can be easily distinguished from *D. marginestriata* and *D. finnica* by the valve shape as well as striae density (9–12 in 10 µm versus 16–18 in 10 µm respectively). Further the openings of *D. marginestriata* are either rectangular or triangular in shape and occur only near poles and central area (Idei & Kobayasi 1988, figs 3, 8, 10, 11), where as the opening in *D.* fenestrata occurs only above the longitudinal canal.



FIGURES 41–44. *Diploneis fenestrata* SEM: type material (sample D-NWU 12-388). Figs 41–43. External valve view showing square openings present only above longitudinal canal and periphery of opening demarcated by areolae-like structures. Fig. 44. Internal view of whole valve. Scale bars = 1 μm (Figs 41–43), 10 μm (Fig. 44).

Idei & Kobayasi (1989) mentioned that the square openings cannot be considered as dissolved valve structures or artifacts from, for example, parasitism, as the boundaries of the openings are clearly demarcated with small areolae like structures. Gordon & Brodland (1990) proposed that these square holes might be due to an accumulation of cubic crystals during silica deposition with the subsequent dissolution of crystals leading to the formation of the opening. However, their hypothesis was based on the fact that the square openings of *D. finnica* were found near the valve mantle, and, in their opinion, this was evidence that as the valve was deposited out from the axial region extraneous substances formed crystals near the margin towards the end of valve morphogenesis. In *D. fenestrata*, as well as in *D. marginestriata*, the openings are found adjacent to the axial area (the central area for *D. marginestriata*), and thus are formed early on during valve morphogenesis, which may possibly contradict the hypothesis of Gordon & Brodland (1990), although this cannot be confirmed, as we have not observed valve morphogenesis. Observations under laboratory conditions will help understand the origin and function of these characteristic square openings.



FIGURES 45–48. *Diploneis fenestrata* SEM: type material (sample D-NWU 12-388). Fig. 45.View of whole valve. Fig. 46. View of central area with proximal raphe ends. Fig. 47. Illustration of transapical costae and serrated advalvar edge. Fig. 48. Illustrating distal raphe ends. Scale bars = 10 μm (Fig. 45), 2 μm (Figs 46–48).

TABLE 1. Comparison of characters for selected members of *Diploneis* related to *D. fenestrata* sp. nov.

	D. fenestrata sp. nov.	*D. ovalis*	*D. subovalis*	*D. finnica*	*D. marginestriata*
Valve length (μm)	21.0–39.0	20.0–100.0	10.0–50.0	16.0–34.0	17.0–48.0
Valve width (μm)	12.0–18.0	10.0–35.0	9.0–20.0	7.0–8.0	6.5–11.0*
Valve shape	Broad, rhombic–elliptical	Elliptic	Elliptical to linear-elliptical	Broadly elliptical	Broad oval
Striae/10 μm	9–12	10–19	10–12	9–12	16 –22
Longitudinal canal	Broad, lanceolate (half the breadth of the valve)	Narrow, becoming broader in middle	Narrow	Lanceolate (one-third the breadth of the valve)	Broad
Axial area	Narrow	Narrow and occupied completely by raphe sternum	Narrow at the ends, becoming broader towards the center	Narrow and occupied completely by raphe sternum	Narrow
Central area	Distinct, round to oval	Large, orbicular	Round	Small and elliptical	Short striae at the central portion of longitudinal canal
Number of openings	4–14	None	None	0–5	1–2
Shape of openings	Square	-	-	Square	Rectangular or triangular
Location of opening	Only above the longitudinal canal.	-	-	Close to valve margin in the median and polar regions	Only above the longitudinal canal and mostly near central area or poles
Reference	Present Study	(Lange-Bertalot & Reichardt 2000). Hustedt (1930)	Krammer & Lange-Bertalot (1986)	Idei & Kobayasi (1989)	Idei & Kobayasi (1988)

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Actinellopsis murphyi gen. et spec. nov.: A new small celled freshwater diatom (Bacillariophyta, Eunotiales) from Zambia

JONATHAN C. TAYLOR[1,2,3*], BALASUBRAMANIAN KARTHICK[1,4], J. PATRICK KOCIOLEK[5], CARLOS E. WETZEL[6] & CHRISTINE COCQUYT[2]

[1]*North-West University, Private Bag X 6001, Potchefstroom 2520, South Africa*
[2] *Botanic Garden Meise, Nieuwelaan 38, 1860 Meise, Belgium*
[3]*South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown, South Africa*
[4]*Division of Plant Sciences, Agharkar Research Institute, Pune 411004 India*
[5]*University of Colorado Museum of Natural History and Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado, USA*
[6]*Public Research Centre – Gabriel Lippmann, Department of Environment and Agro-biotechnologies (EVA), 41 rue du Brill, 4422 Belvaux, Grand-duchy of Luxembourg*
**Corresponding author: E-mail: Jonathan.Taylor@nwu.ac.za*

Abstract

In this paper we describe *Actinellopsis murphyi gen. et spec. nov.* a small heteropolar and dorsiventral diatom from a seep habitat in Zambia. This novel taxon has the chief distinguishing characteristic, along with its cell symmetry, of having the raphe positioned wholly in the valve face and not extending onto the mantle. It is further placed within the Peroniaceae as it possesses a straight raphe on the valve face and rimoportulae, and the frustules are heteropolar with regard to the length of the raphe. We also transfer the fossil diatom *Actinella giraffensis* to *Actinellopsis* as the morphological characteristics of this taxon are consistent with those of the generitype.

Key words: Zambia, Africa, new genus, *Actinella, Peronia*

Introduction

Given the size and diversity of climatic and geomorphological features, the diatom flora of (sub)tropical Africa has been poorly investigated. This holds true in particular for Zambia with only one detailed study of a section of its diatom flora (Cholnoky 1970). This country has a relatively high rainfall for the region and there are a multitude of aquatic resources and habitats including lakes, swamps (wetlands), streams and rivers, often having waterfalls, which in turn are responsible for the creation of a variety of moist sub-aerial habitats. All of these water bodies provide a varied and diverse habitat for diatoms. From 2010 to 2012 a project was undertaken to survey and document various aquatic organisms from rivers and streams around Zambia. The SAFRASS project (www.safrass.com) has as its main aim the development of capacity building tools for river quality monitoring in Zambia. Diatoms were one of the water quality indicator groups chosen for this project and as such were sampled from more than 150 localities around the country forming the most complete survey of the diatom flora of Zambia to date. Although rivers were the primary target habitat for sample collection, periodically wetted and sub-aerial habitats were also sampled and it is in this type of habitat in which an interesting member of the Eunotiales was found.

The order Eunotiales P.C. Silva (1962: 835) is a well-defined lineage of raphe bearing diatoms, the only known group to possess both a raphe system along with one or more rimportulae (Round *et al.* 1990). The order has two families, the Eunotiaceae Kützing (1844: 32), which includes the vast majority of the described diversity of the order (Round *et al.* 1990), and the Peroniaceae (G. Karsten) Topachevs'kyj & Oksiyuk (1960: 301; basionym: Peronioideae G. Karsten 1928: 203,268), a small group into which a single genus, *Peronia* Brébisson & Arnott ex Kitton (1868:16), is placed. *Peronia*, like members of the Eunotiaceae, has a raphe system and rimoportulae, but unlike the members of that family, *Peronia* has a raphe system completely on the valve face (as opposed to having a short portion of the raphe on the valve face, and the majority of the raphe on the valve mantle) and the valves are dissimilar, with one having a longer raphe system than the other (Hustedt 1930, Patrick & Reimer 1966).

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The Eunotiales has been thought to be a primitive lineage (Hustedt 1952, Kociolek 2000), representing a "link" between the raphe-less pennate diatoms that have rimoportulae and the rest of the raphid diatoms that lack such rimoportulae. This early lineage placement amongst the raphid diatoms has been substantiated with molecular data (Sims *et al.* 2006). Some authors (Hustedt 1952, Kociolek 2000) have argued that *Peronia*, with its raphe placed near the median portion of the valve (similar to the naviculoid and other major groups of raphid diatoms), may be similar to the early raphe-bearing taxa. Character evolution from the *Peronia* type to that seen in the Eunotiaceae, would require the transference of the raphe system from the face to the mantle, and only a few candidates for what that might look like have been identified (see Burliga *et al.* 2013). Diversity of genera within Eunotiales is best represented in the southern hemisphere, where many genera, such as *Perinotia* Metzeltin & Lange-Bertalot (2007: 108) and *Bicudoa* C.E. Wetzel, Lange-Bertalot & Ector (Wetzel *et al.* 2012: 5), and many species have been described (e.g. Hustedt 1965, Metzeltin & Lange-Bertalot 1998, 2007).

In this paper we present light and scanning electron microscope observations of a unique member of the Zambian diatom flora, and describe the specimens as a genus new to science with the name *Actinellopsis murphyi gen. et spec. nov.* We discuss its systematic position and compare its morphology with a similar, fossil species described from the Eocene of the Canadian Arctic, which is also transferred to this new genus.

Material and Methods

The sample containing specimens of *Actinellopsis murphyi* gen. et sp. nov. was obtained from the surface of rocks covered with detrital material in a seep supplied with water from the spay zone next to the main cataract of Ntumbachushi Falls (09.853736°S, 28.944683°E), Luapula Province, Zambia by J.C. Taylor. The sample was fixed in a 20 % v.v. final concentration ethanol solution. The material was treated using a modified version of the hot potassium permanganate and hydrochloric acid method (Taylor *et al.* 2005). pH was measured using a Hanna HI 98129 portable meter. Temperature, electrical conductivity and oxygen concentration were measured using a YSI 556 MPS multiparameter meter.

Light microscope (LM) images were obtained using an Olympus BX51 microscope equipped with DIC optics and a 100x 1.4 N.A. oil immersion objective and an Olympus ColorView III digital camera. The stria density and valve size were determined based on measurements of 18 valves. For scanning electron microscopy (SEM), parts of the oxidized material was filtered and rinsed with additional deionized water through a 3-µm Isopore™ polycarbonate membrane filter (Merck Millipore). Filters were mounted on aluminum stubs and coated with platinum using a BAL–TEC MED 020 (BAL–TEC AG, Balzers, Liechtenstein) for 30 s at 100 mA. An ultra-high-resolution analytical field emission (FE) scanning electron microscope (SEM) Hitachi SU–70 (Hitachi High-Technologies Corporation, Tokyo, Japan) operated at 5 kV and 10 mm distance was used for the analysis. SEM images were taken using the high (SE-H) detector signal. Supplementary SEM studies were carried out using a FEI QUANTA FSEM at the Laboratory for Electron microscopy North-West University, South Africa.

The terminology used is that outlined in Anonymous (1975) and Ross *et al.* (1979).

Results

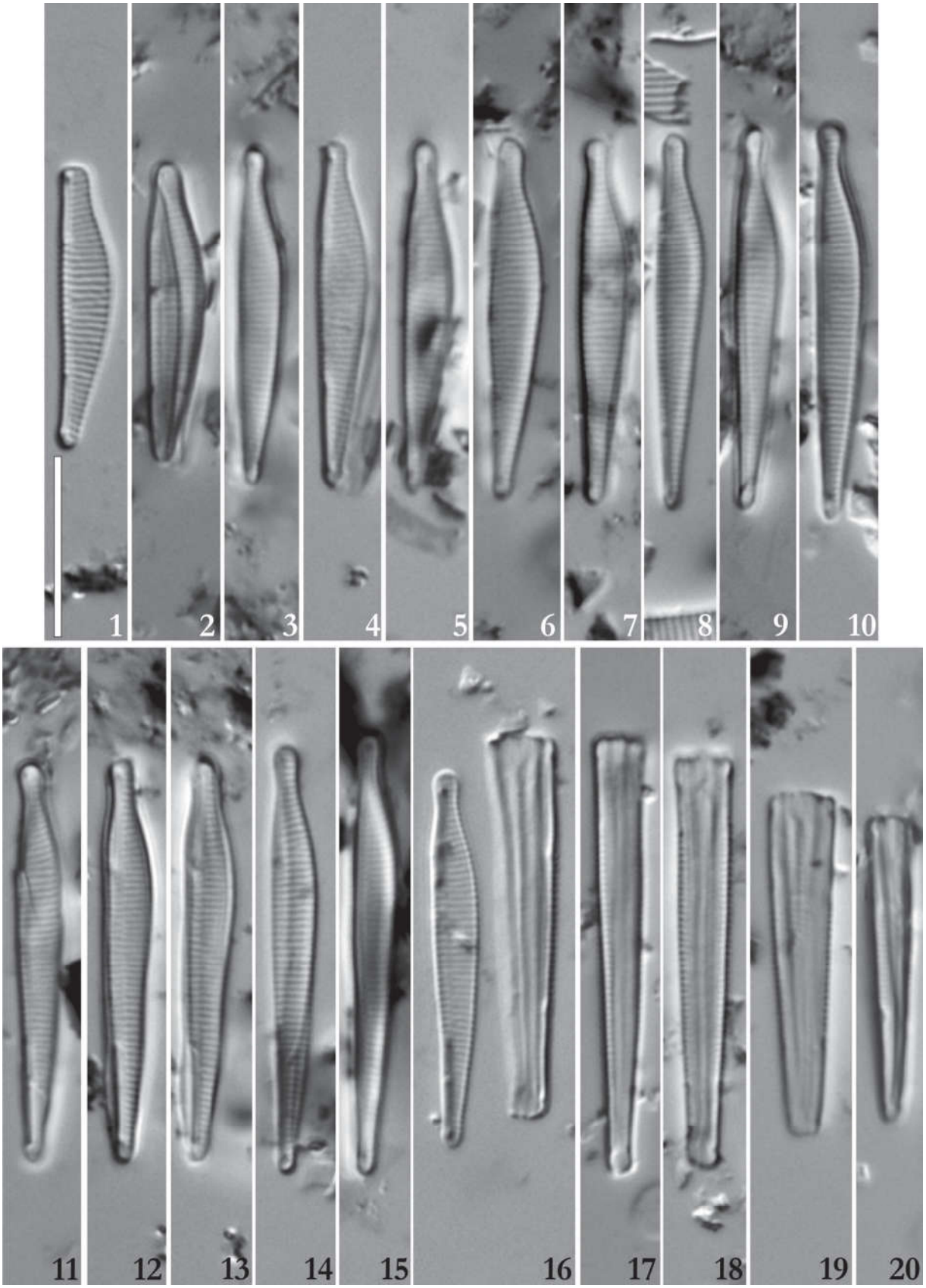
Division Bacillariophyta
Subdivision Bacillariophytina Medlin & Kaczmarska 2004
Class Bacillariophyceae Haeckel emend. Medlin & Kaczmarska 2004
Subclass Eunotiophycidae D.G. Mann in Round *et al.* 1990
Order Eunotiales P.C. Silva 1962
Family Peroniaceae (G. Karsten) Topachevs'kyj & Oksiyuk 1960

Genus *Actinellopsis* J.C. Taylor, B. Karthick & Kociolek *gen. nov*. (Figs 1–45)

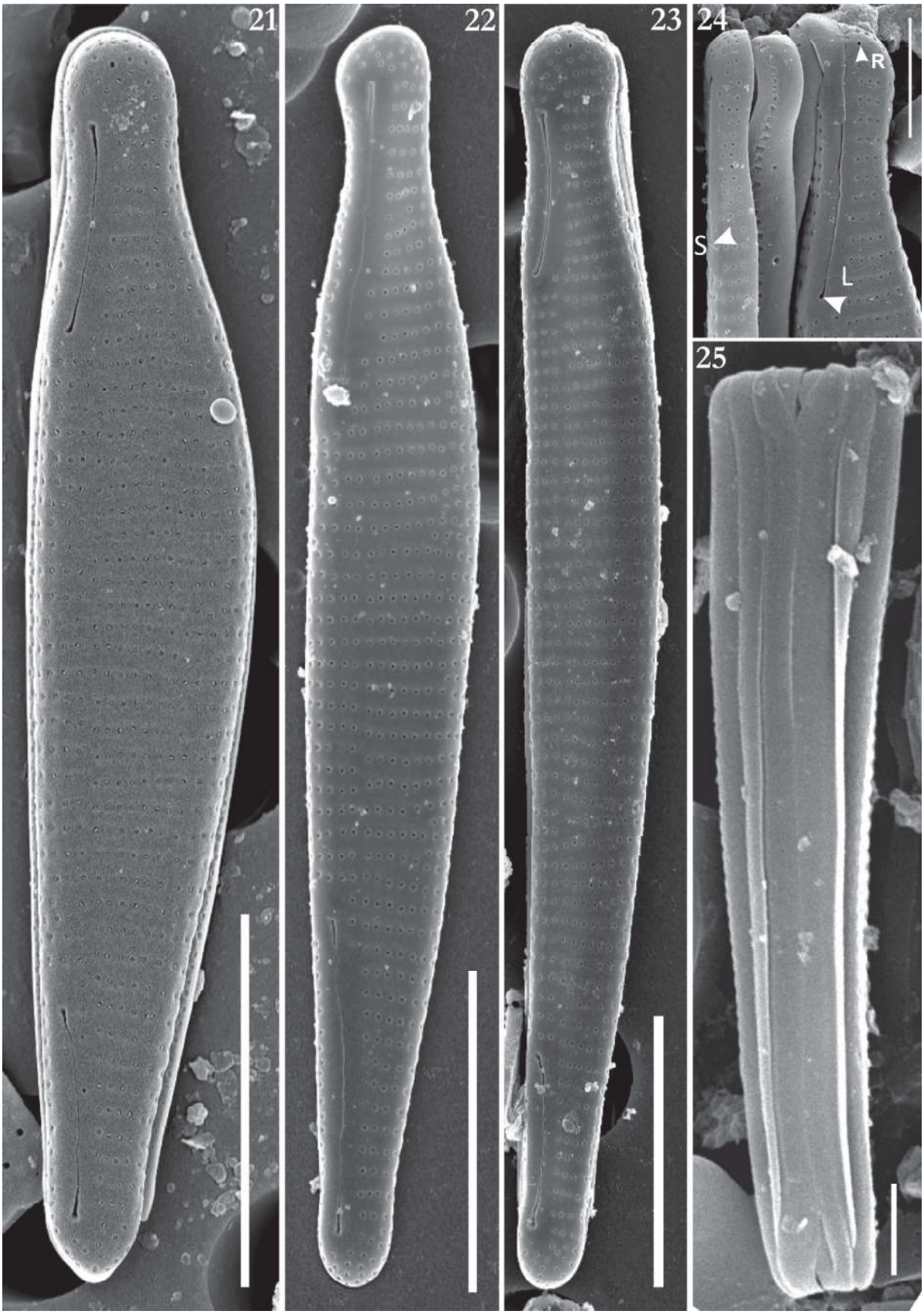
Cells clavate, small, asymmetrical to the apical axis, Valves weakly clavate and dorsiventral with ventral margin straight to slightly convex and dorsal margin convex, raphe positioned on the valve face towards one margin, short and curved in one valve and long and almost straight on another valve, single rimoportula per valve oriented parallel to apical axis. Spines wanting.

Generitype:—*Actinellopsis murphyi* J.C. Taylor, B. Karthick & Kociolek

Etymology:—The generic epithet *Actinellopsis* refers to this taxon's close morphological resemblance to *Actinella* F.W. Lewis (1864: 343).



FIGURES 1–20. LM images of *Actinellopsis murphyi gen. et. spec. nov.*, valves from holotype D-NWU 12-349, Ntumbachushi Falls on Ngona River, in Zambia, showing the size range in valve view. Figs 1–16. Valve view. Figs 17–20. Girdle view. Scale bar = 10 μm.

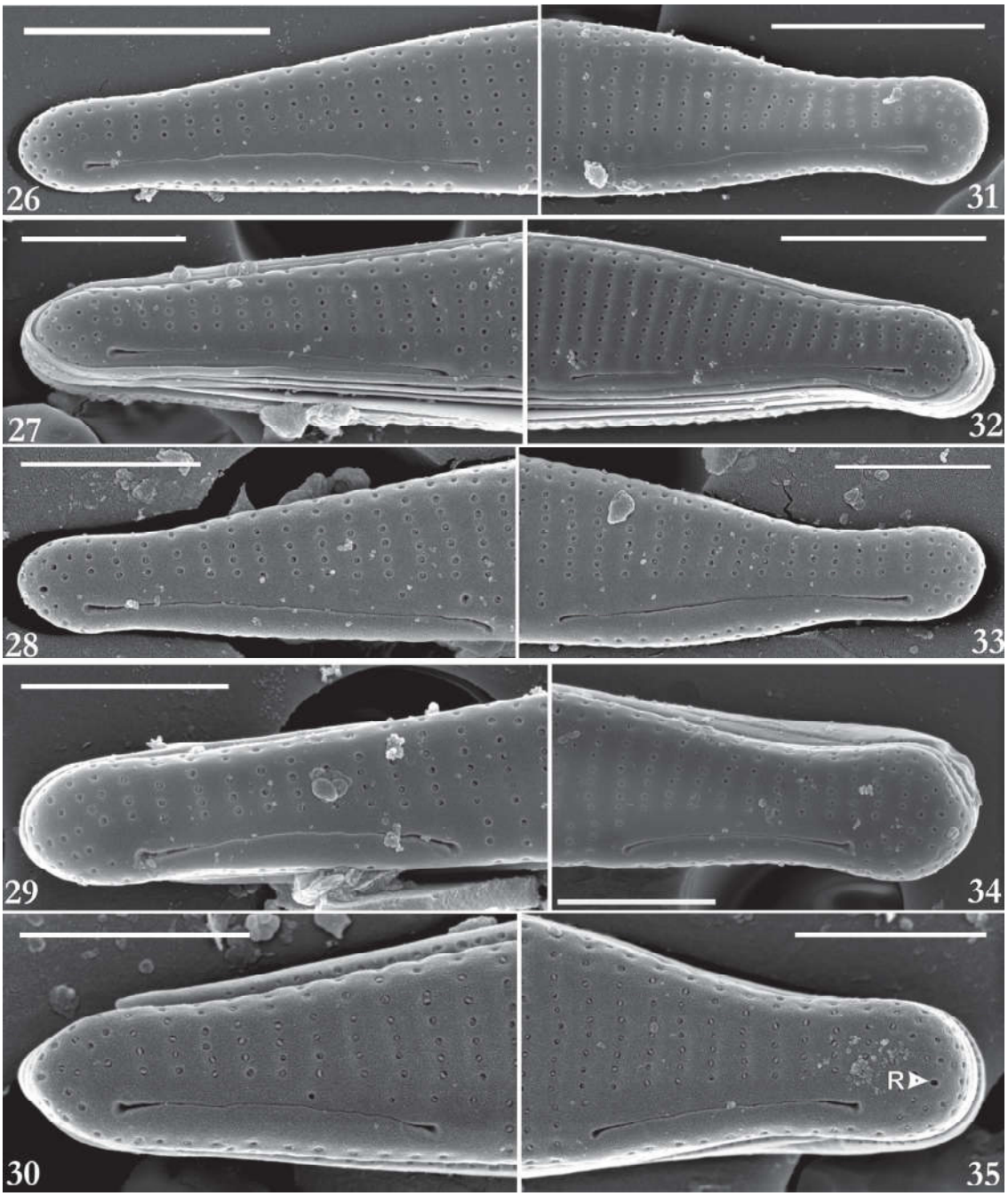


FIGURES 21–25. SEM images of valve exterior of *Actinellopsis murphyi gen. et spec. nov.* Figs 21–23. SEM images showing the external valve view. Fig. 24. SEM image of external girdle view showing epi and hypovalves with longer (L) and shorter (S) raphe slits External rimoportulae (R) opening indicated. Fig. 25. SEM image of the external girdle view of dorsal margin. Scale bar = 5 μm (21, 22, 23), 2 μm (24, 25).

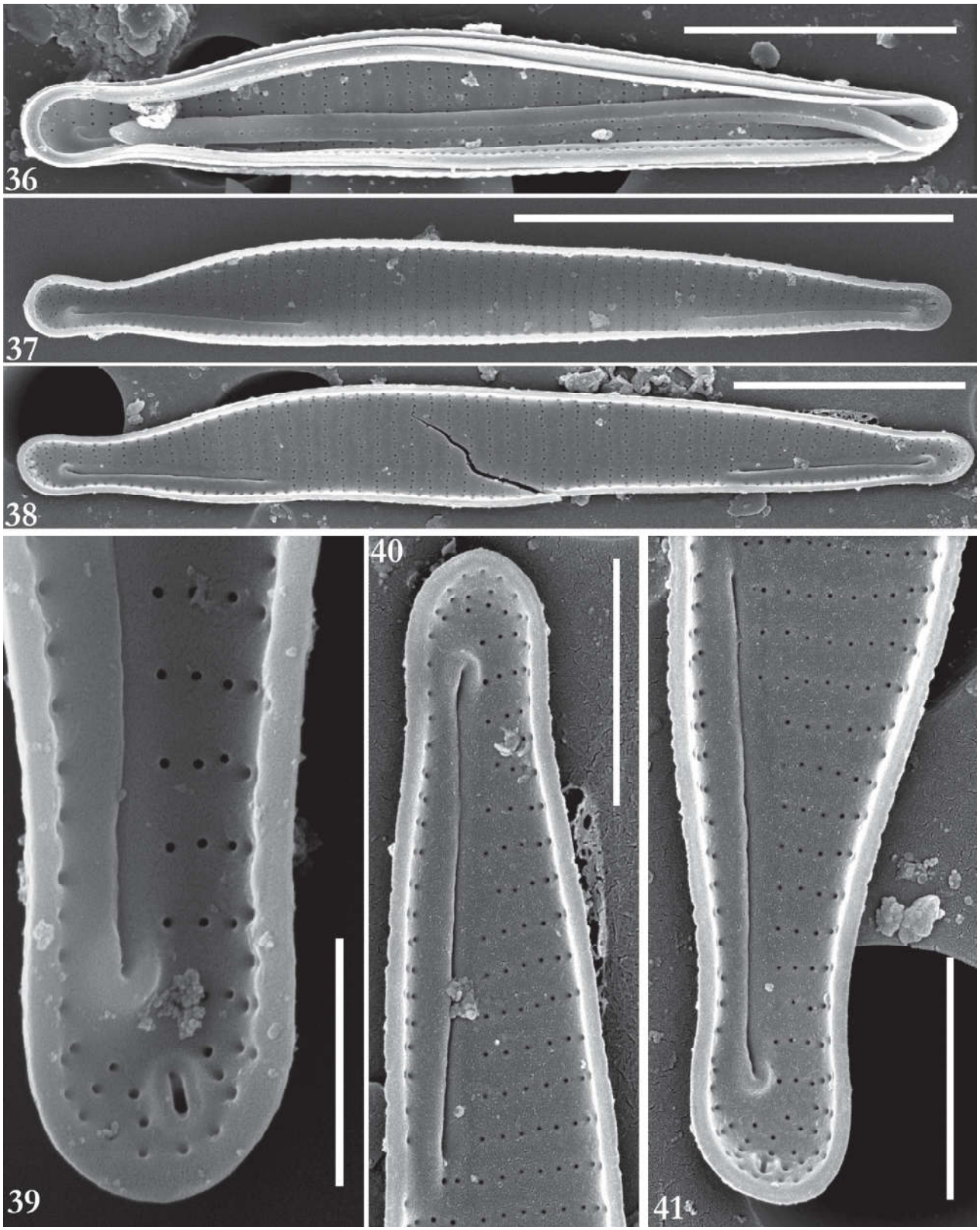
Actinellopsis murphyi J.C. Taylor, B. Karthick & Kociolek *spec. nov*. (Figs 1–45)

Type:—ZAMBIA, Luapula Province, Ntumbachushi Falls on Ngona River near the town of Kawambwa, (09.853736°S, 28.944683°E). Sample collected by J.C. Taylor from detrital material on rocks wetted by seeping water from spray zone of main falls. Slide BR 4375, Botanic Garden Meise, **holotype designated here** (the valve representing the holotype is here illustrated as Figure 12) ; D-NWU 12-349, South African National Diatom Collection, **isotype designated here**.

Cells clavate in girdle view 15.5–23.0 μm long. Plastid structure unknown. Valves weakly clavate and dorsiventral with ventral margin straight to slightly convex and dorsal margin convex, 2.2–3.1 μm wide at widest point of valve. Head pole subcapitate, foot pole not tumescent. Striae rather difficult to resolve in LM, 28–30 in 10 μm, parallel in the centre of the valve becoming radiate towards the apices. Areolae round, occluded by vela. No marginal spines. One valve possessing a short and arched raphe, the other valve possessing a longer, straight raphe. Distal and proximal ends slightly rounded but otherwise unmodified and situated at the junction of valve face and mantle. Helictoglossae indistinct. Rimoportula on headpole and footpole, orientated parallel to apical axis. Cingulum composed of 4 open, porous copulae.



FIGURES 26–35. SEM images of external head and foot poles of *Actinellopsis murphyi gen. et. spec. nov.* Figs 26–30. SEM images of external view of head pole. Figs 31–35. SEM images of external view of foot pole. Rimoportula opening (R). Scale bar = 3 μm (26, 27), 2 μm (28, 29, 30, 31, 32, 33, 34, 35).



FIGURES 36–41. SEM images of valve interior of *Actinellopsis murphyi gen. et. spec. nov.* Figs 36–38. SEM images showing the internal valve view. Figs 39–41 SEM images of internal pole showing rimoportulae, helictoglossa and internal areolar openings. Scale bar = 10 μm (37), 5 μm (36, 38), 2 μm (40, 41), 1 μm (39).

Etymology:—The specific epithet refers to the project co-coordinator of the SAFRASS project, Dr. Kevin Murphy of the University of Glasgow and is named after him to honour his contributions to the extension of the knowledge of the Zambian fauna and flora.

Distribution and ecology:—*Actinellopsis murphyi* has been found only from the type locality. It is found rarely in a sample that was collected from detrital material in seeping water flowing over the stones comprising the steps of a foot path next to the main cataract of Ntumbachushi Falls. The path was heavily shaded and covered in leaf litter. The seep water had its origin in the spray from the main falls, the permanency of this seep is uncertain. The main stream of the falls had the following characteristics pH 6.56, 18.13°C, 4 μS cm^{-1} electrical conductivity and oxygen concentration of 9.70 mg l^{-1}.

Observations:—*Actinellopsis murphyi* is a small taxon distinguished from other similar species by its distinct valve shape and size, the shape of the head pole and the striae density. It is comparable to the fossil species *Actinella giraffensis* Siver, A.P. Wolfe & Edlund (Siver *et al.* 2010: 343), being similar in valve symmetry (dorsiventral and heteropolar with an almost straight ventral valve margin). It also has epi and hypovalves with longer and shorter raphe slits. It appears that the epivalve of *Actinellopsis murphyi* bears the longer raphe (Fig. 24) and the hypovalve the shorter more strongly curved raphe (a characteristic somewhat similar to *Peronia*). *Actinellopsis murphyi* differs from *Actinella giraffensis* in regard to striae density and the shape of the headpole. These two species are very similar in girdle view and the positioning of the raphe slits at the valve margin is almost identical. It is also comparable to *Actinella indistincta* Vyverman & E.A. Bergey (in Sabbe *et al.* 2001: 328), the striae density is similar but the outline of the cell (arcuate) and structure of the apices is very different. In addition the raphe is almost entirely situated in the mantle and the cells are isovalvar.

Actinella disjuncta Metzeltin & Lange-Bertalot (2007: 28) is another species with a similar valve outline was described from a fossil samples of Brazilian Amazon (Lago Calado) and from a cataract in Sarawako Bako (Borneo, Malaysia). *Actinella disjuncta,* however, shows conspicuous spines, a raphe canal located on the valve mantle and coarser interstriae with the areolae placed deeply in the valve face (see Metzeltin & Lange-Bertalot 2007, pl. 41: figs 1–24 and pl. 42 figs 1–7).

Discussion

Actinellopsis is considered part of the Eunotiales, by virtue of its possession of both a raphe system and rimoportulae (Round *et al.* 1990). It has a suite of features that distinguish it from others in the Eunotiales. For example, *Actinellopsis* has a nearly straight raphe positioned entirely on the valve face—no part of the raphe is on the mantle. The arrangement of its raphe precludes assigning it to the Eunotiaceae (Round *et al.* 1990). The only genus within this family that has a majority of its raphe on the valve face is the recently described *Eunotioforma* Kociolek & Burliga (in Burliga *et al.* 2013: 49), which has a highly curved raphe system positioned close to the margin of the valve. Possession of a straight raphe on the valve face along with the presence of rimportulae suggests *Actinellopsis* belongs in the Peroniaceae, only the second genus assigned to this family (Round *et al.* 1990). While it shares symmetry features with *Peronia*, it differs by having the raphe positioned close to one margin, not near the median line (Round *et al.* 1990). Thus, we conclude that at this time *Actinellopsis* is best placed in the Peroniacae. It may reflect a transition from the raphe near the median portion of the valve to the raphe on the mantle. We can diagnose this family as those raphid diatoms with rimoportulae with a raphe in a sternum on the valve face. *Actinellopsis* might represent a condition where the raphe has begun to migrate from the valve face (as seen in *Peronia)* to the mantle, as seen in most members of the Eunotiaceae (Hustedt 1952, Kociolek 2000).

Actinella giraffensis, mentioned above, described from fossil material in the Canadian Arctic (Siver *et al.* 2010), has symmetry features and a rimoportula which provide evidence for its placement in *Actinella.* Based on the suite of features presented by Siver *et al.* (2010) we propose the transfer of their new species to *Actinellopsis*.

Actinellopsis giraffensis (Siver, A.P. Wolfe & Edlund) J.C. Taylor, B. Karthick & Kociolek, *comb. nov.*

Basionym: *Actinella giraffensis* Siver *et al.* (2010: 343, figs 1A–I, figs 2A–F, figs 3A–F).

Actinellopsis superficially resembles *Actinella*, which is also cuneate and asymmetrical about the transapical axis. The two differ in the type of raphe system and that *Actinella* is also asymmetrical about the apical and transapical axes. The superficial similarities in symmetry are due to independent acquisition of asymmetry about both the transapical and apical axes. Valve symmetry and other frustule organization (amphoroid organization) appear to have evolved independently many times (e.g. Cox 1979, Medlin 1991, Medlin & Round 1986, for *Eunophora* Vyverman, Sabbe &

D.G. Mann in Vyverman *et al.* 1998: 96; Stepanek & Kociolek 2014). Table 1 compares features of *Actinellopsis* with *Eunotia*, *Peronia* and *Actinella*.

TABLE 1. Comparison of characters for selected members of *Eunotiales* related to *Actinellopsis gen. nov*. (summarized from Round *et al.* 1990).

	Eunotia	***Peronia***	***Actinella***	***Actinellopsis gen. nov.***
Valve shape	Lunate	Cuneate in valve and girdle view	Elongate, lunate, cuneate in girdle view	Valve view - linear ventral margin, curved dorsal margin, cuneate in girdle view
Valve symmetry	Dorsiventral	Heteropolar	Heteropolar and dorsiventral, swollen at one end	Heteropolar and dorsiventral, swollen at one end
Spines	Rare	Junction of valve face and valve mantle	Junction of valve face and valve mantle or absent	Absent
Striae	Uniseriate, small round poroids	Uniseriate, small round poroids	Uniseriate, small round poroids	Uniseriate, small round poroids
Raphe length	Short	Short and well developed on R valve, diminutive often absent at head pole on P valve	Short	One valve bears a longer raphe and the hypovalve the other a shorter more strongly curved raphe
Raphe shape	Strongly curved	Straight	Strongly curved	Very slightly curved (long) to more strongly curved (short)
Raphe origin	Valve face ending close to edge of valve mantle	Slightly excentric on valve face	Valve face ending close to edge of valve mantle	Continuous along junction of valve face and mantle
Raphe endings	Raphe continuous past helictoglossa externally as short terminal fissure	Central raphe ending expanded curved to one side	Raphe continuous past helictoglossa externally as short terminal fissure	Both endings very slightly expanded curved towards ventral side
Rimoportulae	1 per valve near apex, RP of epivalve and hypovalve at opposite ends of cell	Valve with well developed raphe with rimoportula at each end, valve with diminutive raphe slits with only one at base pole	Present, one at each pole	Present, one at each pole, oriented towards apical axis
Sterna	Near edge of valve face	Between raphe slits	Poles with narrow sterna	Absent?
Cingulum	6–10 similar porous open bands	5 porous open bands	Open at apical end, 2 or more rows of poroids	Open at apices, perforated by 2 rows of poroids

Actinellopsis murphyi differs from *A. giraffensis* in the outline of the valve, having a more protracted 'headpole' as seen in valve view.

The disjunct distribution between *Actinellopsis murphyi* from Zambia and the 50 million year old fossil *Actinellopsis giraffensis* from the Canadian Arctic is puzzling. The Zambian locality is subtropical and an acid habitat (pH 6.5). The Canadian Arctic of the Eocene was quite different from today, with many subtropical to tropical species known from fossil localities (Eberle & Greenwood 2012). Freshwater fossil deposits of Eocene age are relatively rare (e.g. Benson *et al.* 2012), thus the temporal and spatial extent of *Actinellopsis* is not well understood. It is possible that the genus was much more widely distributed, and the species from Zambia represents a relict. Alternatively, an African-South American connection may have existed (as has been documented for many taxa, see Renner 2004 for a compilation of plant species), followed by a north-south connection in the new world between South and North America.

This genus appears to be an example of a Lazarus taxon, where a taxon occurs in the fossil record, there is a gap in its occurrence, and it "reappears" again (Jablonski 1986). Such taxa are known in plants (e.g. Chaney 1948), invertebrates (Fraiser & Bottjer 2005; Skelton & Gilli 2012) and vertebrates (Friedman & Coates 2006; Dawson *et al.* 2006). They are the result of observational gaps, due to sampling or loss via the fossilization process.

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REGULAR PAPER

New and interesting *Eunotia* (Bacillariophya) taxa from the Democratic Republic of the Congo, tropical central Africa

Jonathan C. Taylor[1, 2, 3,*], Christine Cocquyt[1] & Shigeki Mayama[4]

[1]Botanic Garden Meise, 38 Nieuwelaan, Meise 1860, Belgium
[2]School of Biological Sciences, North-West University, P Bag X6001, Potchefstroom 2520, South Africa
[3]South African Institute for Aquatic Biodiversity (SAIAB), P Bag 1015, Grahamstown 6140, South Africa
[4]Department of Biology, Tokyo Gakugei University, Koganei, Tokyo 184-8501, Japan
*Author for correspondence: Jonathan.Taylor@nwu.ac.za

Background and aims – Large-celled *Eunotia* species from tropical central Africa are documented in order to define morphological variation within species and to describe new or poorly documented taxa.
Methods – Samples were collected from the Congo River between Kisangani and Bumba in the Democratic Republic of the Congo. These samples were prepared for diatom analysis and examined according to standard methods and documented using both light and scanning electron microscopy.
Key results – A single population of *Eunotia zygodon* is used to demonstrate the high degree of change in gross cell morphology during its life cycle, changes previously observed in cultures of this genus. Cells of this taxon from each extremity of the reproductive cycle could be, and probably often were, placed in discrete taxa during sample analysis, especially as this taxon occurs rather infrequently. We also describe *Eunotia leonardii* as new to science. It has an undulate valve margin and is closely related to *Eunotia serra* and *Eunotia georgii* but differs in respect to overall cell size and the structure of the terminal raphe endings. The bi-undulate *Eunotia papilio* var. *africana* is raised to the rank of species and named *Eunotia fuseyi* as it is clearly a discrete taxon from *E. papilio* s. str.

Key words – Diatoms, D.R. Congo, *Eunotia fuseyi*, *Eunotia leonardii*, *Eunotia zygodon*, new species, taxonomy, tropical Africa.

INTRODUCTION

Reports on benthic diatoms from the Democratic Republic of the Congo (D.R. Congo) have been rather limited in the past with the notable exception of Zanon (1938), Kufferath (1948, 1956a, 1956b), Hustedt (1949), Cholnoky (1970), Compère et al. (1989), Compère (1995) and Golama (1996) and with several recent papers as discussed in Cocquyt & Taylor (2015). Recently, intensive studies, including diatom collection, were initiated in the Congo Basin downstream Kisangani, thanks to the Boyekoli Ebale Congo 2010 expedition, organised by the Congo 2010 Consortium composed of the University of Kisangani, the Royal Museum for Central Africa, the Royal Belgian Institute of Natural Sciences and the National Botanic Garden of Belgium – now Botanic Garden Meise (e.g. Cocquyt et al. 2013, Cocquyt & Taylor 2015, Karthick et al. 2016, Taylor et al. 2016). After this initial large scale effort, several smaller field expeditions, during which diatom samples were also collected, were undertaken. The material used for the present studied was sampled during such a campaign in the frame of the COBAFISH project (Congo Basin: From carbon to fishes) financed by the Belgian Science Policy.

Members of the genus *Eunotia* Ehrenb. form a large percentage of the diatom populations from tropical African waters of lower pH as observed during our investigations. *Eunotia* taxa have been historically subject to 'force-fitting' in order to ascribe extant names to taxa encountered in the tropics. Often, much confusion arises with taxa that are either rare or that have seemingly obvious characteristics such as multiple dorsal undulations (e.g., *Eunotia serra* Ehrenb.). The genus *Eunotia* is also problematic as its valves may vary quite widely in structure over the cell cycle with the gametangial mother cell and the first vegetative cell having very little similarity in terms of valve outline (e.g. *E. tropica* Hust., Mayama 1995). Although this phenomenon has been demonstrated on a number of occasions in cells grown in culture, the comparative rarity of large-celled *Eunotia* in environmental samples has made this difficult to demonstrate. In this paper we describe and discuss three relatively large-celled *Eunotia*

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species from tropical (moderately to very) acidic waters of rivers of D.R. Congo.

MATERIAL AND METHODS

Several samples collected in tributaries of the Congo River, downstream of Kisangani (D.R. Congo), between 2010 and 2014 were studied. Although the newly described *Eunotia* taxa are observed in many of the samples, the present paper especially deals with one sample in particular, CCA 2066 obtained on 24 Nov. 2012 from the submerged parts of *Nymphaea lotus* L. in the Lobaye River (0.48970°S 24.17728°E), a tributary of the Lomami River, Tshopo Province (formerly part of the Oriental Province), D.R. Congo by François Darchambeau and Ernest Tambwe. The Lomami River is a major tributary of the Congo River discharging in it downstream of Kisangani. The Lobaye as well as the Lomami River are large rivers, the latter a major tributary of the Congo River. Their considerable catchment area contains many villages and mining areas. As wastewater flows directly into the rivers, some impact and nutrient enrichment of the river water is to be expected. The studied sample CCA 2066 contains *Eunotia zygodon*, *Eunotia leonardii* and *Eunotia fuseyi*. The sample was fixed in a 20% v.v. final concentration ethanol solution. Subsamples for LM and SEM observations were prepared by oxidizing the material with H_2O_2 (37%) and heating at 100°C for 1 to 2 hours. Following digestion the material was rinsed several times with distilled water. A subsample from the organic–free material was mounted in Naphrax® (refractive index 1.71) for diatom community studies.

Physical parameters (temperature, conductivity and pH), collected concurrently with the diatom sample from the Lobaye River, were measured in situ with an YSI Pro-Plus multiprobe field meter equipped with a pH sensor 1001 ProSeries. Samples for nutrient analyses (NH_4, NO_3, NO_2 and SRP) were filtered and acidified in situ, and kept frozen until spectrophotometric analysis could be carried out in the laboratory of the Unité d'Océanographie Chimique, Département d'Astrophysique, Géophysique et Océanographie, University of Liège, Belgium. NO_3^- and NH_4^+ concentrations were estimated by spectrophotometry, using the dichloroisocyanurate-salicylate-nitroprussiate colorimetric method for NH_4^+ (Westwood 1981), the sulfanilamide colorimetric after vanadium reduction method for NO_3^- (APHA 1998). SRP was determined by spectrophotometry using the ammonium molybdate-potassium antimonyl tartrate method (APHA 1998). The detection limits were 0.30, 0.15, 0.03 and 8.0 µmol L^{-1} for NO_3^-, NH_4^+ and SRP respectively. Light microscope (LM) images were collected using an Olympus BX51 microscope equipped with Differential Interference Contrast optics and a 100x 1.4 N.A. oil immersion objective. An Olympus UC30 digital camera was used for photomicrographic imaging. For scanning electron microscopy (SEM), oxidized materials were rinsed with additional deionized water and isolated with micropipette on 6 mm × 6 mm cover slip. Cover slips were mounted on aluminium stubs and coated with osmium by an OPC 40A osmium plasma coater (Filgen, Nagoya, Japan). A field emission scanning electron microscope S-8020 (Hitachi, Tokyo, Japan) operated at 5 kV and 8 or 12 mm working distance was used for the analysis. The terminology used is that outlined in Anonymous (1975) and Ross et al. (1979). Cells were traced for illustration by selecting a number of cells over the size range illustrated and then tracing the exact outline of each cell, during this process the cells photographs were all maintained at a magnification of 1500 x in order to allow accurate comparison, aspect ratio of the original drawings was also maintained at 1:1 during the tracing process.

RESULTS

Eunotia zygodon Ehrenb.

Figs 1–5

Synonyms – *Eunotia tropica* Hust. sensu Hustedt (1927: 159, pl. 5, fig. 1) **synon. nov.**; *Eunotia monodon* var. *tropica* (Hust.) Hust. sensu Hustedt (in Schmidt 1933: pl. 371), **synon. nov**. – Type: Cayenne, French Guyana, sample of Ehrenberg's material nr 1108; marked valve on slide Nr R-7, Ehrenberg Collection in Museum für Naturkunde, Berlin, Germany (lecto-: STU).

Morphologic characteristics – Type, valve 53.3 µm long, 10.0 µm broad, 10–11 striae in 10 µm, marked lectotype (Reichardt 1995: 18, plate 1, fig. 16). Congo River specimens, 37.0–153.3 µm long, 11.0–14.3 µm broad, 11–15(–21) striae in 10 µm.

Taxonomic remarks – *Eunotia zygodon*, originally described from the island of Cayenne by Ehrenberg (1843), occurs rather commonly in tropical African regions (Foged 1966, Carter & Denny 1982, Cholnoky 1970). The original illustration (Ehrenberg 1843) was of a cell with two dorsal undulations, a concept that has remained rather stable. Jahn in Reichardt (1995) examined the type material of *E. zygodon* and illustrated a single cell conforming well to the original diagnosis by Ehrenberg. Metzeltin & Lange-Bertalot (1998) illustrated *E. zygodon* from tropical South America and commented that it conforms well to the type. Later, Ferrari et al. (2007) reported this taxon from Brazil, illustrating it by ten figures and providing a discussion (in Portuguese). Thus the established concept of this species seems to be rather stable, it is comparatively large, has two pronounced dorsal undulations and is therefore rather simple to identify. When we examined a sample from tropical Africa we found cells of a diatom conforming well to the description of *E. zygodon* as well as to the LM and SEM illustrations provided by Metzeltin & Lange-Bertalot (1998), but we also noted several cells of a *Eunoti*a taxon larger (longer) than *E. zygodon*, and with many more dorsal undulations. The angle of the raphe as it passed from the mantle onto the valve face was also somewhat different. In addition, the number of areolae occurring between the raphe ending and the dorsal margin of the valve face were consistently different. The shape of the apices was also different being more bluntly rounded than the established concept of *E. zygodon*. Thus we initially considered these two seemingly separate morphological entities as two separate species i.e. *E. zygodon* sensu stricto and a taxon previously identified by workers on African material (e.g. Gasse 1986, Zanon 1938) as *E. monodon* var. *tropica*. The latter taxon was a synonym of the earlier described *E. tropica* (Hustedt 1927) collected from Lake Aokiko in Japan. We considered that this morphological form deserved

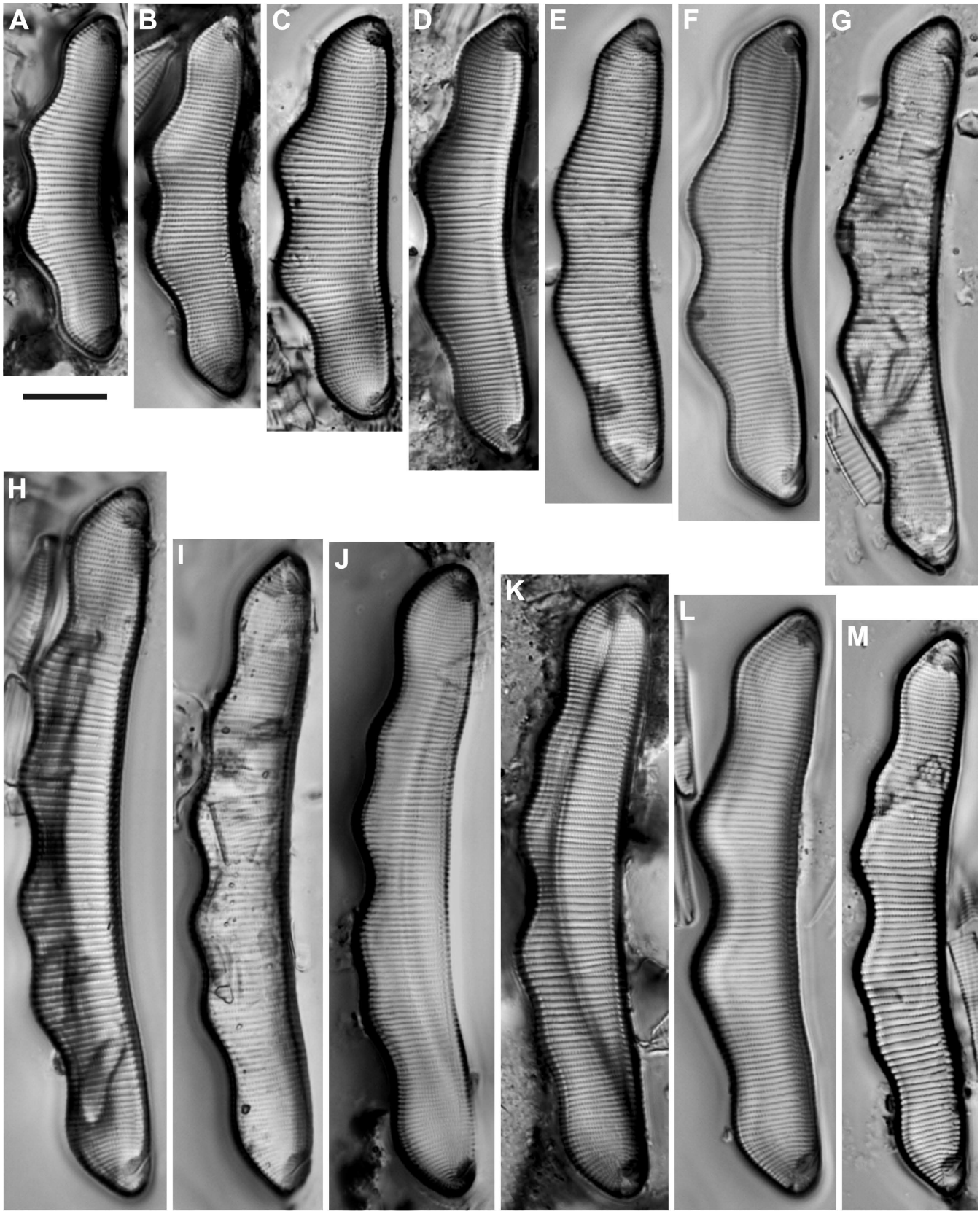


Figure 1 – *Eunotia zygodon*, LM. Size diminution series from a single population. Note especially G and M which are transitional forms between the bi-undulate and multi-undulate cells. Scale bar = 10 µm.

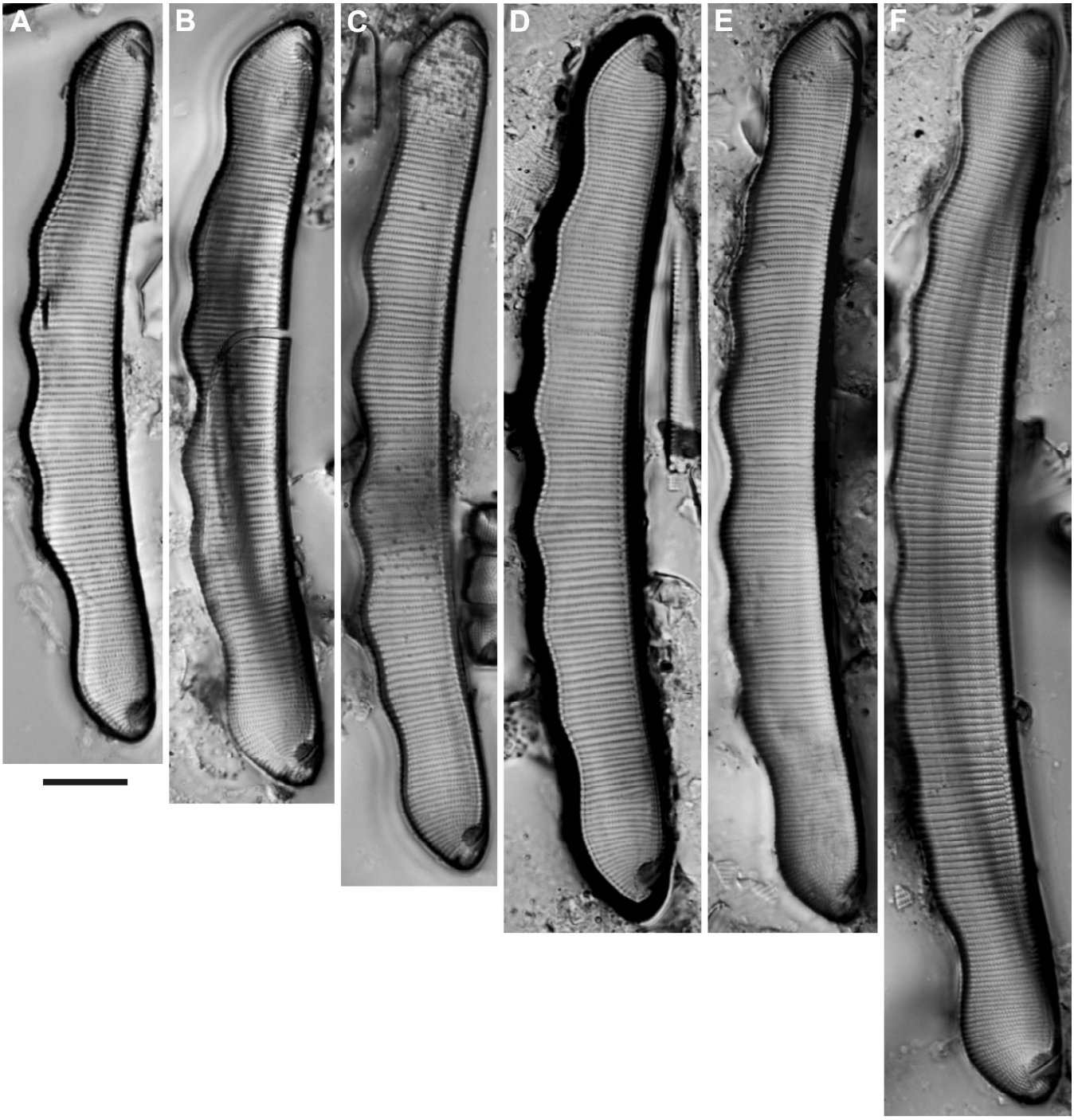


Figure 2 – *Eunotia zygodon*, LM. Size diminution series from a single population continued from Figure 1. Multi-undulate cells. Scale bar = 10 µm.

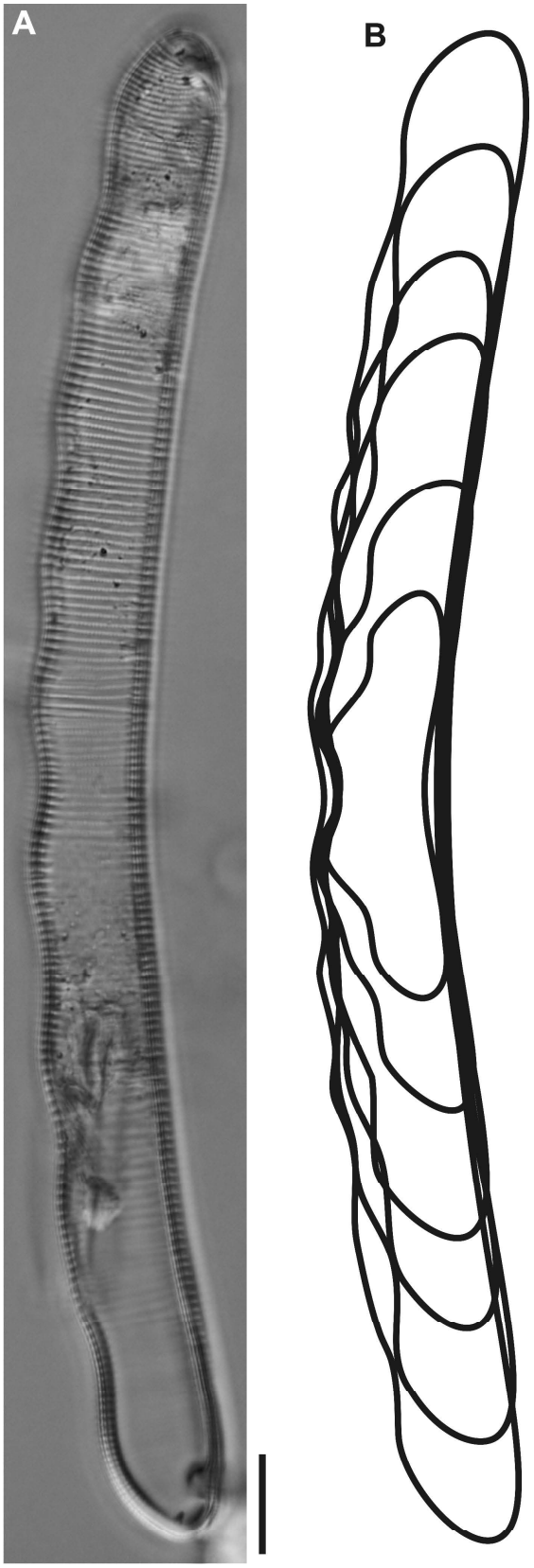


Figure 3 – *Eunotia zygodon*, LM: A, multi-undulate auxospore initial cell; B, traced outlines of selected cells from figures 1–3 showing geometric size reduction and reduction in number of dorsal undulations over the cell cycle. Scale bar = 10 µm.

the rank of species as it differed from *E. tropica,* as illustrated by Mayama (1995), and thus began an intensive investigation of the sample in question to determine the size range and other key characteristics. The more valves we examined though it became clear that we in fact were observing a single taxon rather than two discrete entities; we found a very clear diminution series showing the transition between the typical *E. zygodon* like cells to the *E. monodon* var. *tropica* like cells (figs 1–3).

What also helped to elucidate this problem was the digital tracing of the valve outline, with the inclusion of the raphe. By drawing a large cell and proportionally decreasing the size including shape of the valve apices and the angle of the raphe, the cell changed to conform to that of the smaller cells. No other digital manipulation than the proportional shrinking of the superimposed outline of the cell was used, which may be taken to replicate, to some extent at least, the geometrical progression in size reduction during asexual reproduction. We also then traced a number of cells of different sizes (fig. 3); after they were superimposed on each other that the cell width at the centre remains more or less the same and the way in which the undulations of the dorsal margin decrease in number over the cell cycle is also illustrated. The cells illustrated here (figs 1–3) therefore all belong to the same taxon i.e. *E. zygodon*. Manguin (1949) also illustrated *E. zygodon* cells with a broad ranging morphology from Madagascar, including a rather large cell that he identified as *E. zygodon* var. *elongata* Hust. In a later study from Madagascar Metzeltin & Lange-Bertalot (2002) lumped these different length cells with differing numbers of dorsal undulations under the epithet *E. tropica*. We also examined characteristics other than the valve length and outline under SEM. In cells of approximately 40, 80 and 130 µm in length, we determined the number of areolae between the valve margin and the mantle margin at the centre of the raphe. Mayama (2001) demonstrated that the characteristics of this region remain stable during the cell cycle. We consistently found 3–5 areolae above the raphe and 6–8 areolae below the raphe even though the smallest and largest cells measured differed in length by almost 90 µm.

Other characteristics were examined under the SEM and found to be consistent over the cells; these included the shape of the raphe and axial area on the valve mantle, the structure of the raphe and the raphe ending on the valve face, the position of the apical spine (fig. 5D) and the position of the rimoportula (fig. 5F). We also noted small foramina lips, after corrosion of the occluding membranes (cf. *E. pseudopectinalis* Hust.) (fig. 5C).

Eunotia leonardii J.C.Taylor & Cocquyt, **sp. nov.**

Figs 6–10

Type: D.R. Congo, Lobaye River, sample CCA 2066 (holo-: slide BR 4400, BR; the valve representing the type is illustrated here in fig. 6N; iso-: slide PUC–13-567, PUC; iso-: slide Zu 10/63 Friedrich Hustedt Diatom Collection, BRM). – Type locality: D.R. Congo, Tshopo Province (part of the formerly Oriental Province), Lobaye River, 0.48970°S, 24.17728°E, tributary of the Lomami River which is a major tributary of the Congo River.

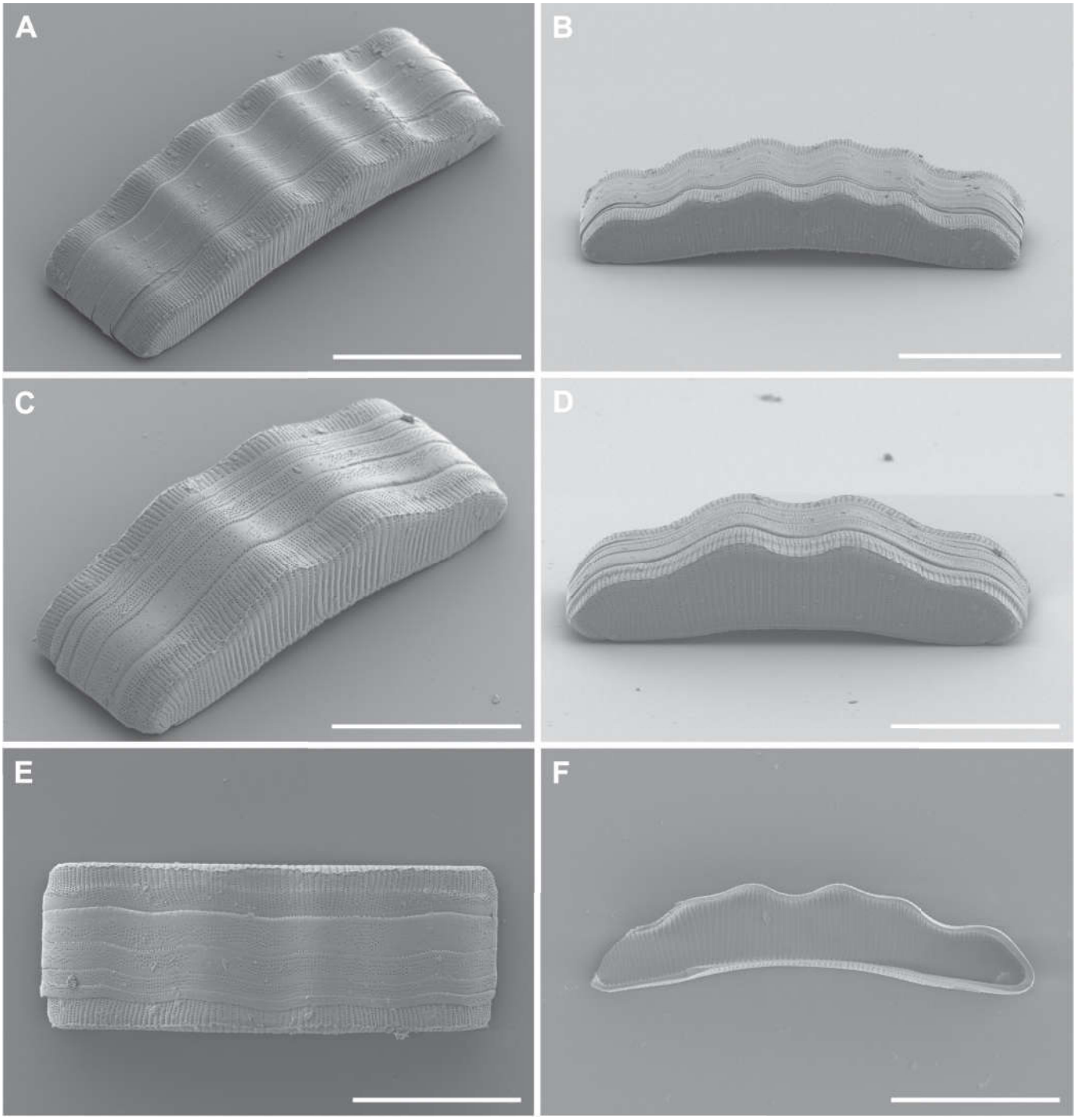


Figure 4 – *Eunotia zygodon*, SEM: A & B, external view of multi-undulate valve; C & D, external view of bi-undulate vale of *E. zygodon*; E, external view of the dorsal copulae of *E. zygodon*; F, internal view of valve. Scale bars: A & B = 30 µm; C–F = 20 µm.

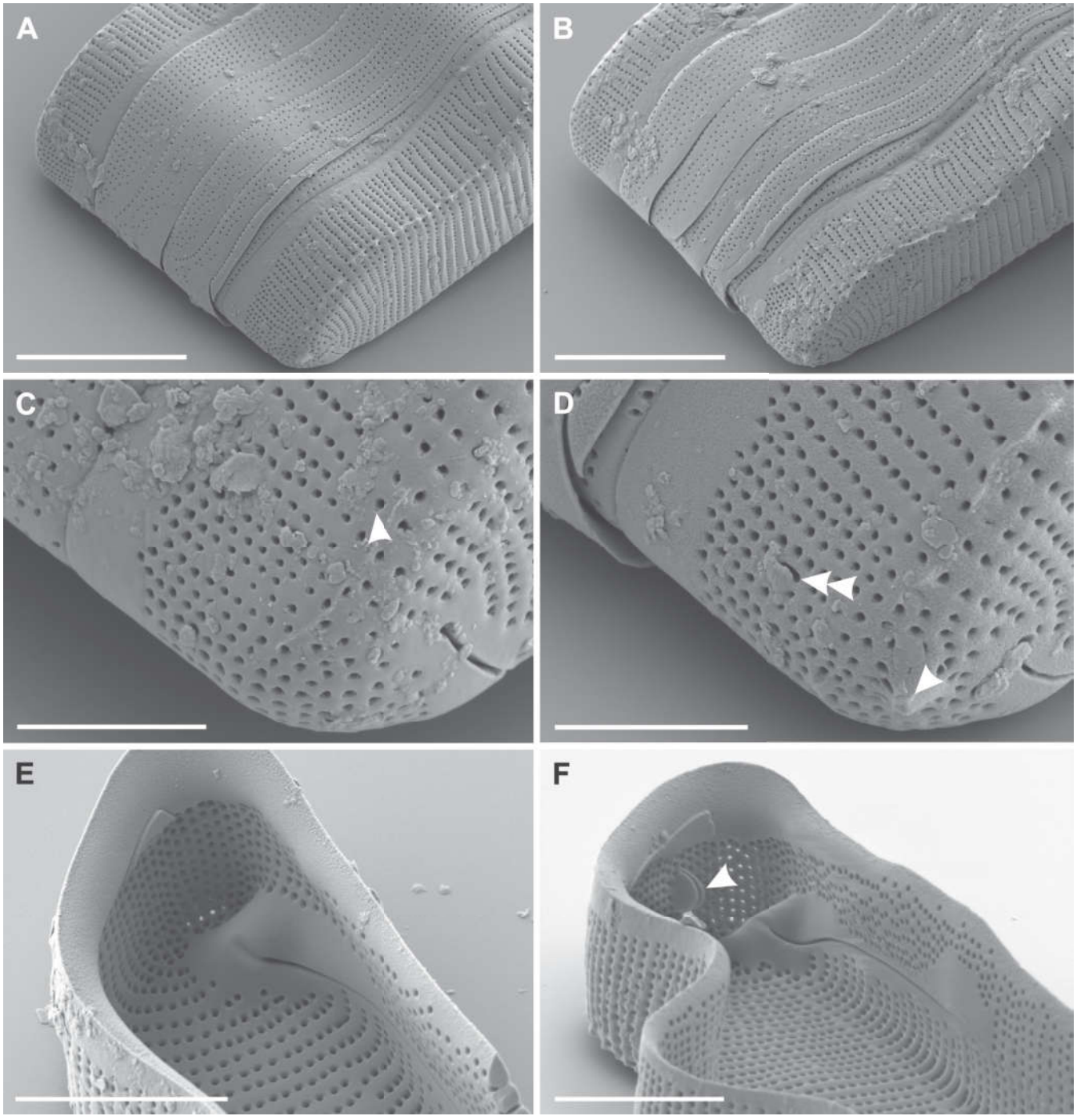


Figure 5 – *Eunotia zygodon*, SEM: A & B, external view of valve apices; C & D, detailed view of cell apices, note foramina lips (arrow C) apical spine (arrow D) and external opening of the rimoportula (double arrow D); E & F, internal view of the valve apices. Note the thickened terminal nodule and the position of the internal opening of the rimoportula (arrow F). Scale bars: A & B = 10 µm; C & D = 3 µm; E & F = 5 µm.

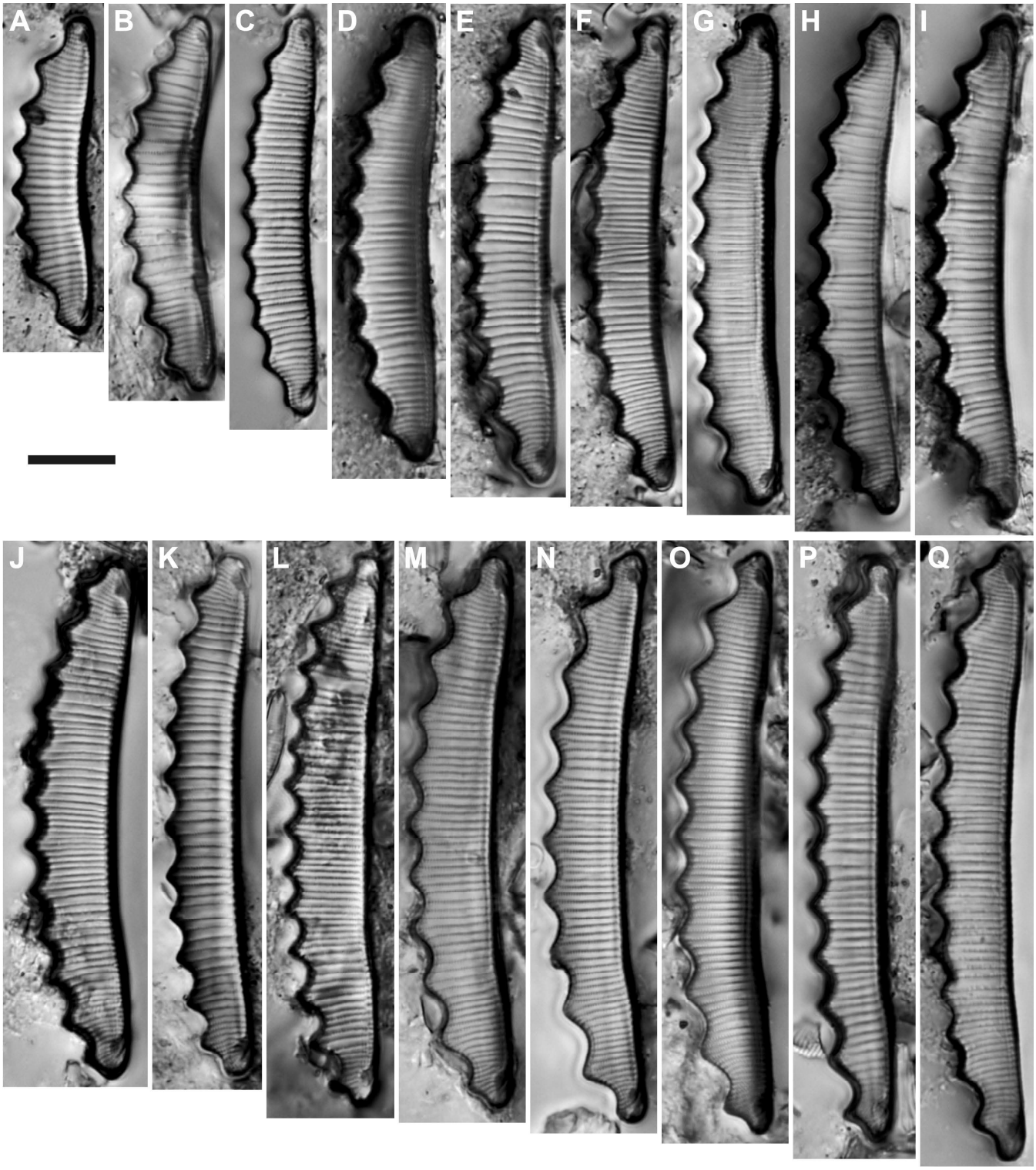


Figure 6 – *Eunotia leonardii* sp. nov., LM from sample CCA 2066, slide BR 4400. Size diminution series. Scale bar = 10 µm.

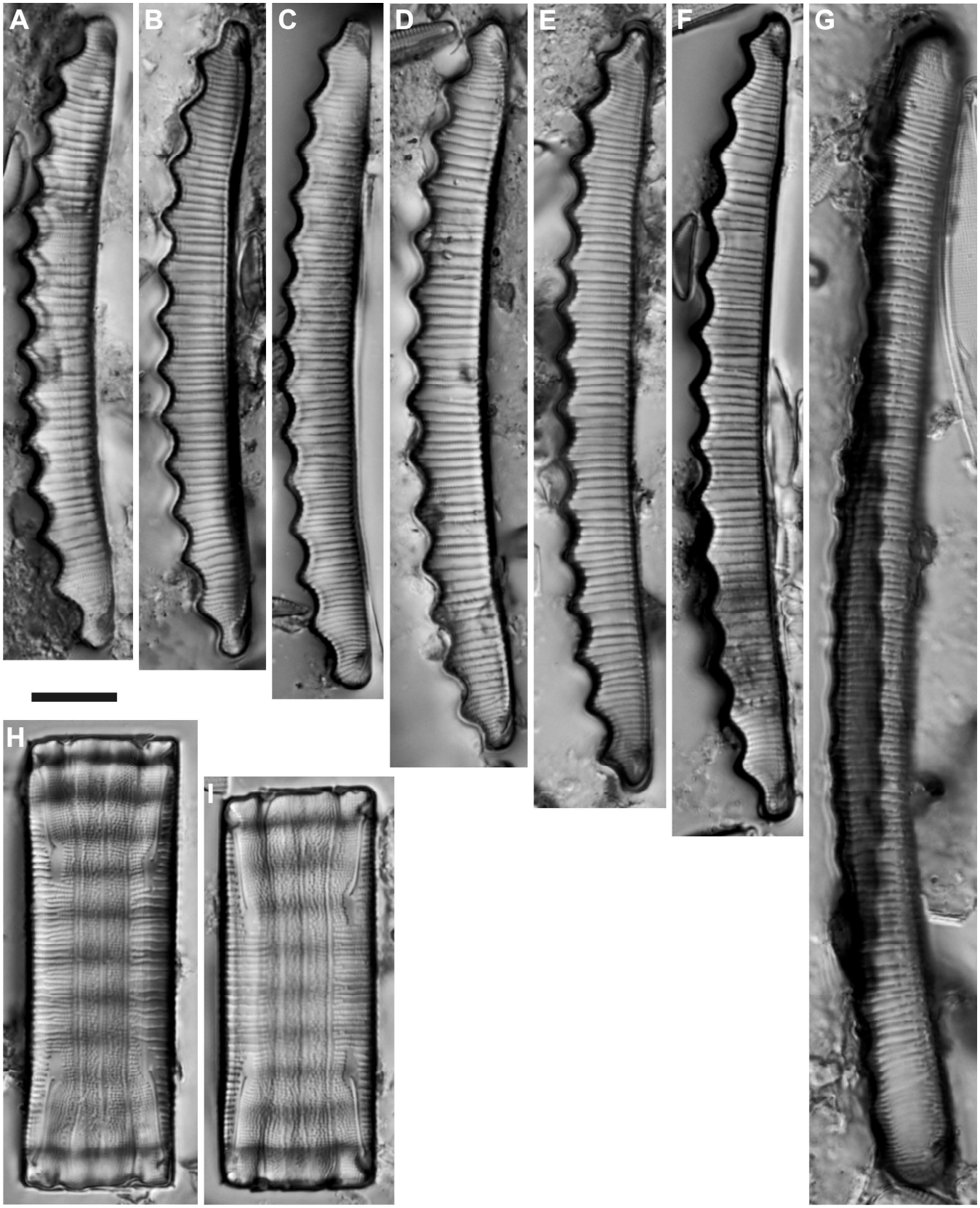


Figure 7 – *Eunotia leonardii* sp. nov., LM from sample CCA 2066, slide BR 4400. Size diminution series continued from figure 6. G, post auxospore initial cell; H–I, girdle view of ventral side of cell. Scale bar = 10 µm.

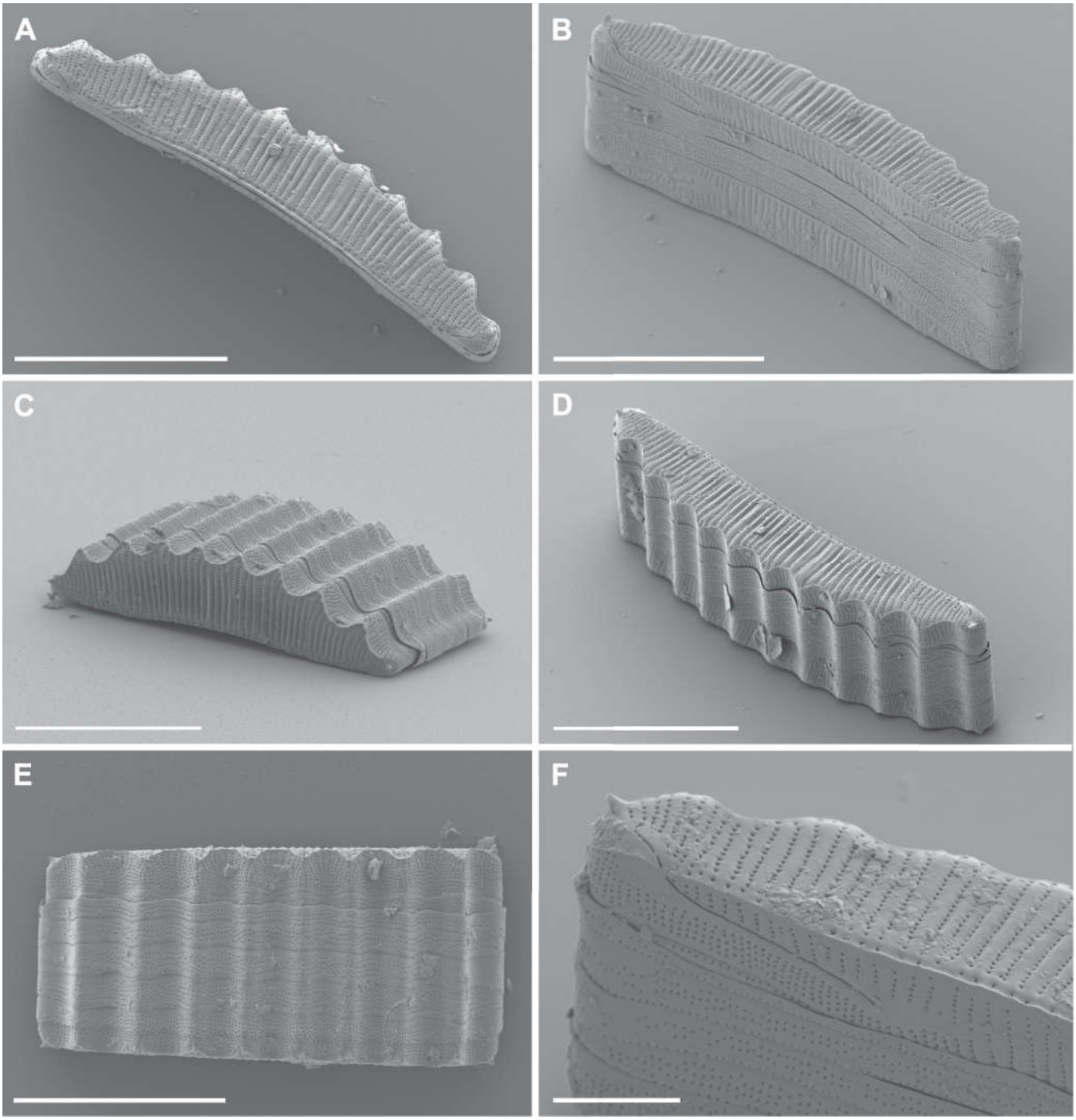


Figure 8 – *Eunotia leonardii* sp. nov., SEM from sample CCA 2066: A–E, external view of complete frustules; F, external detail of apex, note structure of raphe and the apical spine. Scale bars: A–E = 20 µm; F = 5 µm.

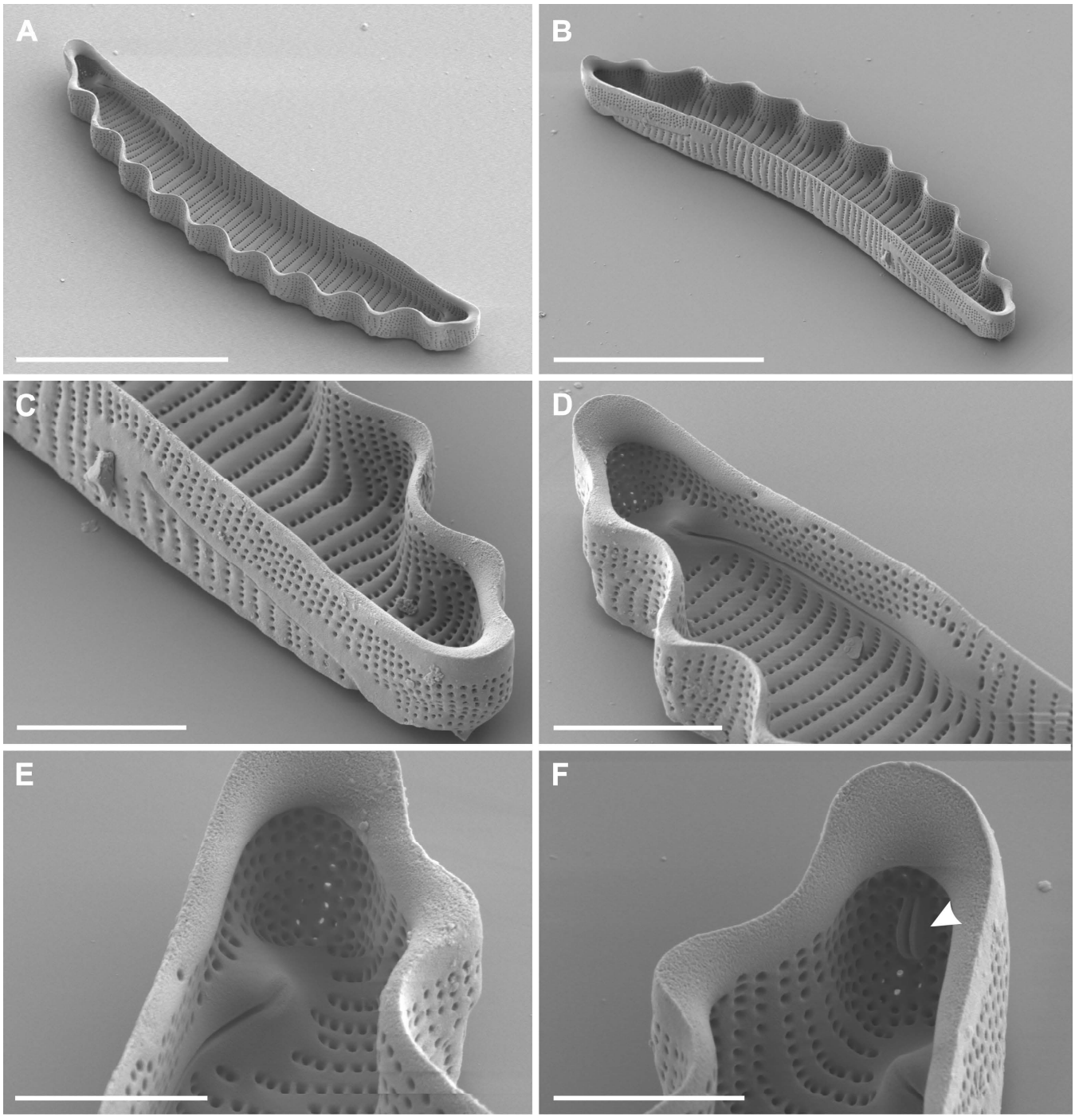


Figure 9 – *Eunotia leonardii* sp. nov., SEM from sample CCA 2066: A & B, internal oblique views of valve; C & D, internal view of detail of apices; E, internal view of the helictoglossa; F, internal view of the rimoportula (arrow). Scale bars: A & B = 20 µm; C & D = 5 µm; E & F = 3 µm.

LM: description – Valves with weakly arched dorsal margin, with many undulations (16–24 in 100 µm), parallel to slightly convex ventral margin. Ends often heteropolar, bluntly to acutely rounded. Length 36.0–137.0 µm, breadth 7.5–11.0 µm. Terminal raphe ends extend far onto the valve face and are more or less straight. Striae 9–16 in 10 µm. Puncta of transapical striae indistinct. Chain-forming frustules.

SEM: description – Striae evenly or unevenly spaced 9–16 in 10 µm becoming more densely spaced near the ends in all specimens, 14–20 in 10 µm. Few or no shortened striae intercalated at the dorsal margin. Striae composed of rounded areolae, 40–44 in 10 µm. Areolae occluded externally by hymenes anchored at 3 points giving the areolae a slightly triangular appearance (fig. 10D). Terminal nodules distinctly distant from the ends lying at the junction between valve mantle and valve face (fig. 9E). Terminal raphe fissures curved and extending with an angle of mostly c. 90–110° onto the valve face, more or less halfway towards the dorsal margin (fig. 8F). Internally the raphe terminates apically in a small helictoglossa with the cell wall rather thickened at this point (fig. 9E). A single rimoportula is found at one of the apices (fig. 9F) and a single terminal spine is found at both apices (fig. 8A). Girdle composed of multiple copulae, with a maximun of five observed (fig. 8E).

Etymology – The epithet *leonardii* is in honour of Jean Léonard, a Belgian botanist who worked at the Institut National pour l'Etude Agronomique du Congo (INEAC) and who collected algae material in the vicinity of Kisangani which is deposited at the herbarium of the National Botanic Garden of Belgium (BR) housed at the Botanic Garden Meise, Belgium.

Ecology – Temperature of 24.2°C, pH of 5.01, conductivity of 17.4 µS.cm^{-1}, 0.054 mg.l^{-1} NH_4^+, 0.0009 mg.l^{-1} NO_2^-, 0.16 mg.l^{-1} NO_3^-, and 0.038 mg.l^{-1} SRP.

Distribution – Lobaye and Lomami River basins in the Democratic Republic of the Congo, tropical Africa.

Taxonomic remarks – The chief differences between *Eunotia leonardii* and *E. georgii* Metzeltin & Lange-Bert. lie in the shape of the apices (bluntly to acutely rounded as opposed to acutely), the curvature of the ventral margin, which is never strongly concave in *E. leonardii*, the shape and angle of the raphe ending on the valve face, and the smaller range of striae in *E. georgii* (12–14 in 10 µm as opposed to 9–14 in 10 µm for *E. leonardii*). The largest difference lies perhaps in

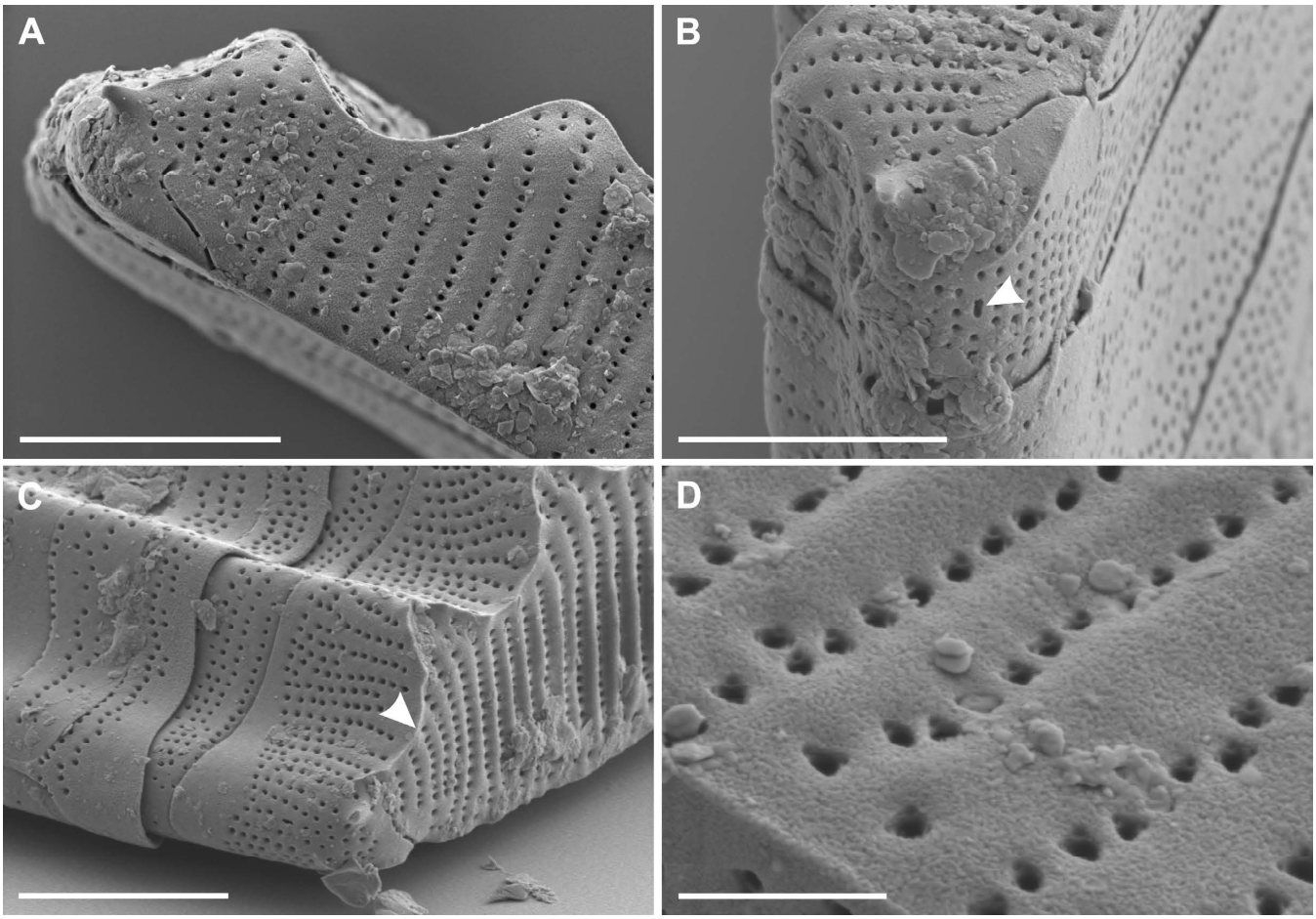


Figure 10 – *Eunotia leonardii* sp. nov., SEM from sample CCA 2066: A, external view of apex showing structure of the terminal raphe ending; B, external view of apex, note position of rimoportula (arrow); C, external view of apex and copulae, note marginal ridge of silica (arrow); D, external view of areolae, occlusion has been corroded away during cell treatment. Scale bars: A, C & D = 5 µm; B = 3 µm.

the number of areolae: *E. georgii* has 27–30 in 10 µm while *E. leonardii* has 40–44 in 10 µm.

Eunotia leonardii differs from *E. serra* in the shape of the valves, which have a weakly arched dorsal margin with many undulations in *E. leonardii* opposed to the rather strongly arcuate valves with more or less concave ventral margins and strongly convex dorsal margins with even undulations in *E. serra*. In *E. serra* the valve ends are rounded like the wave crest or obliquely truncated in the case where it fuses with a distal undulation. The number of undulations varies in *E. serra* from 6 to 18; the valves are 40–150 µm long and 12–16 µm broad. Striae and areolae of *Eunotia leonardii* are distinctly more densely spaced than in *E. diadema* Ehrenb. and *E. tetraodon* Ehrenb., areolae 24–27 in 10 µm.

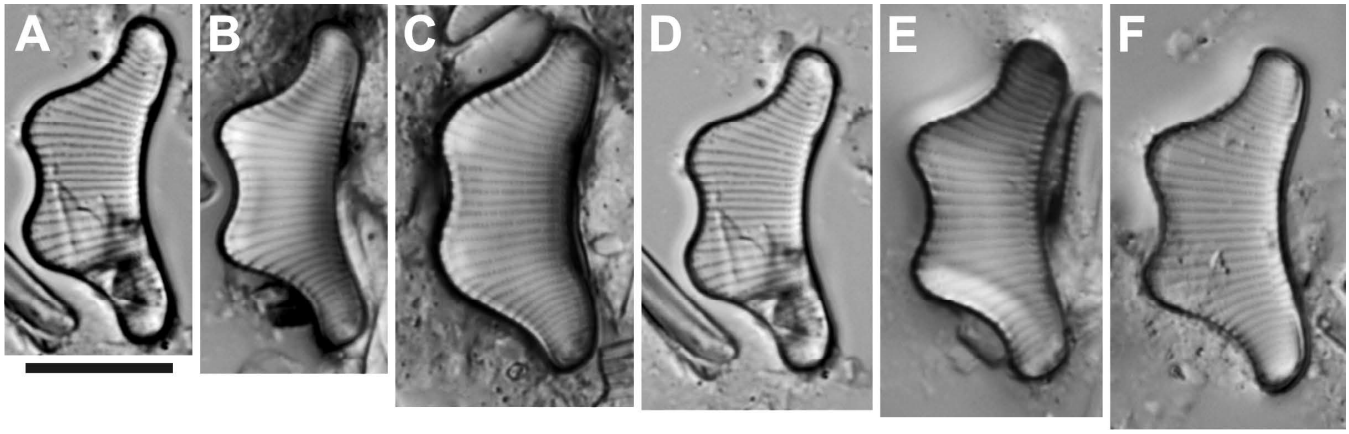


Figure 11 – *Eunotia fuseyi* sp. nov., LM from sample CCA 2066, slide BR 4401. Size diminution series. Scale bar = 10 µm.

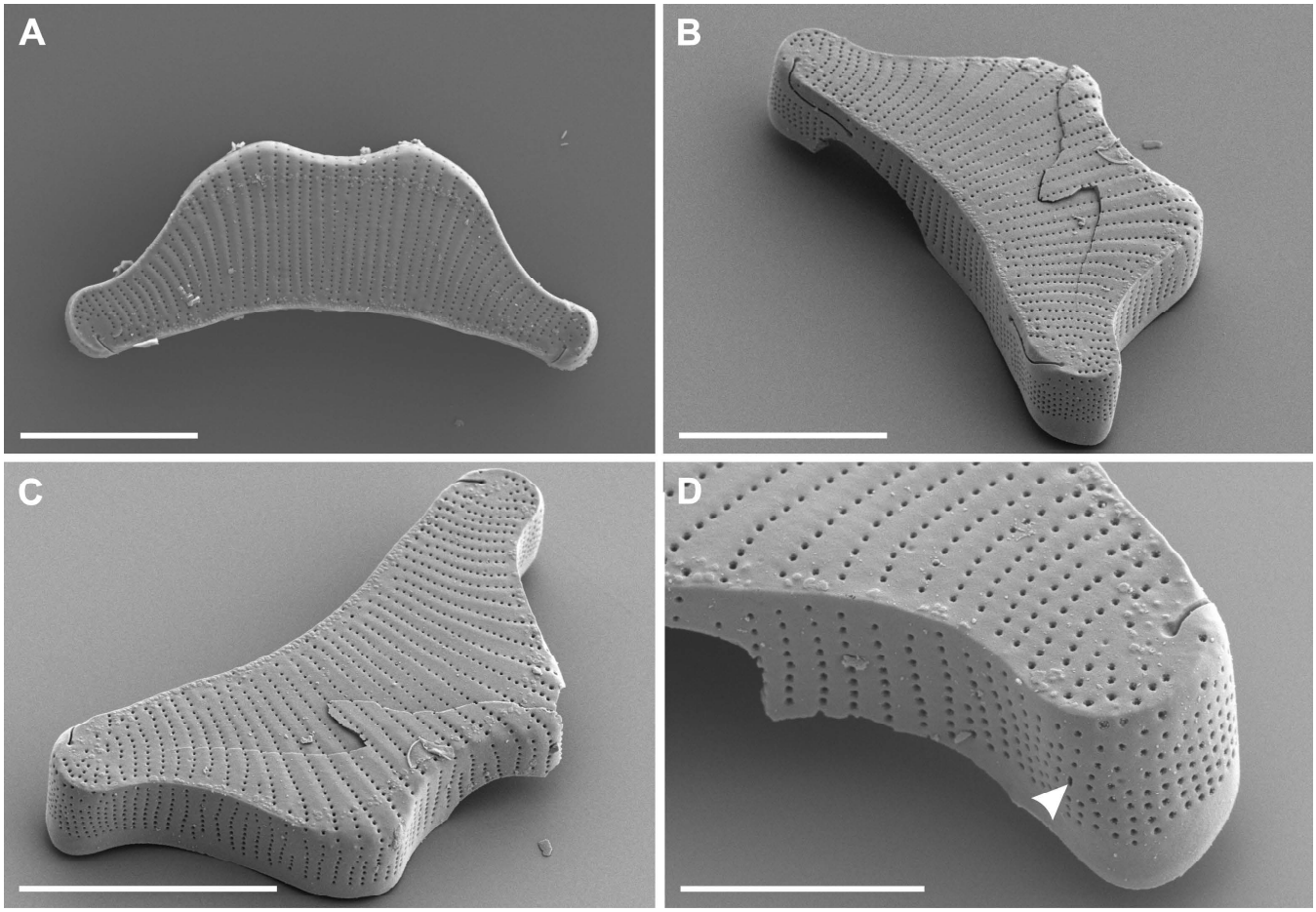


Figure 12 – *Eunotia fuseyi* sp. nov., SEM from sample CCA 2066: A–C, external view of complete valves; D, external detail of apex, note external opening of rimoportula (arrow). Scale bars: A–C = 10 µm; D = 4 µm.

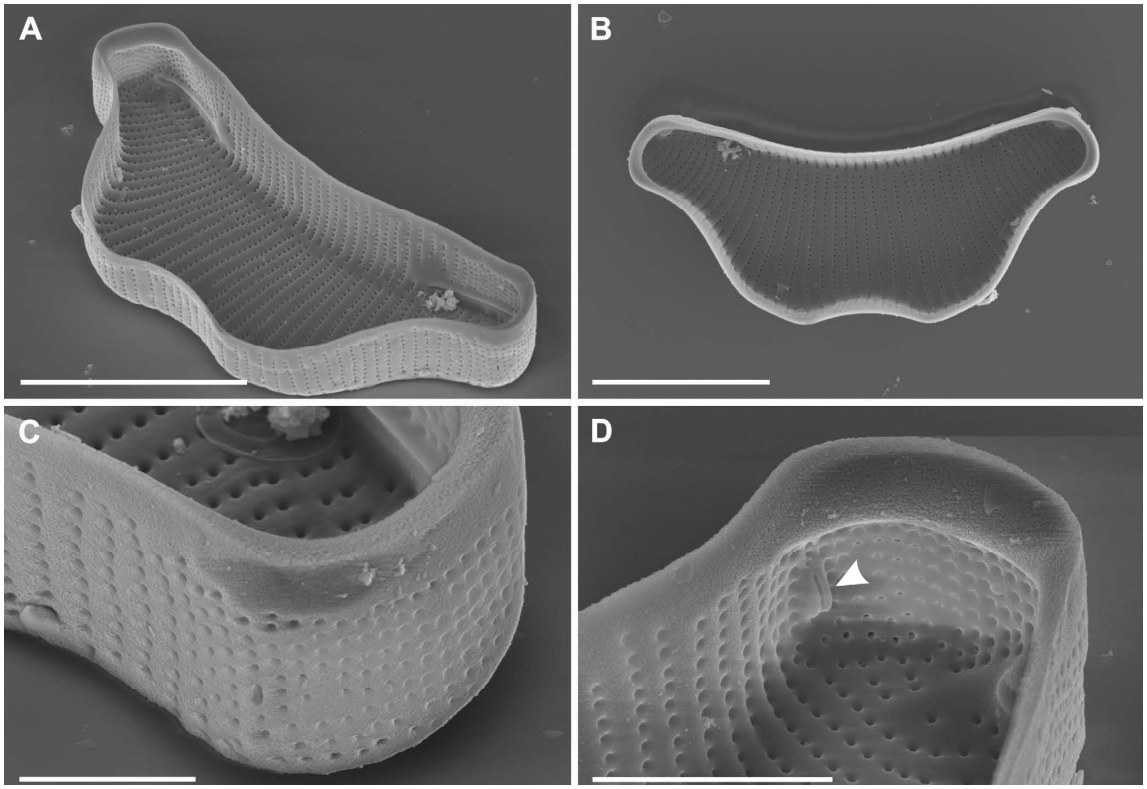


Figure 13 – *Eunotia fuseyi* sp. nov., SEM from holotype sample CCA 2066: A & B, internal view of complete valve; C, external view of apex showing position of external opening of rimoportula; D, internal view of the apex showing the rimoportula (arrow). Scale bars: A–B = 10 µm; C = 2 µm; D = 3 µm.

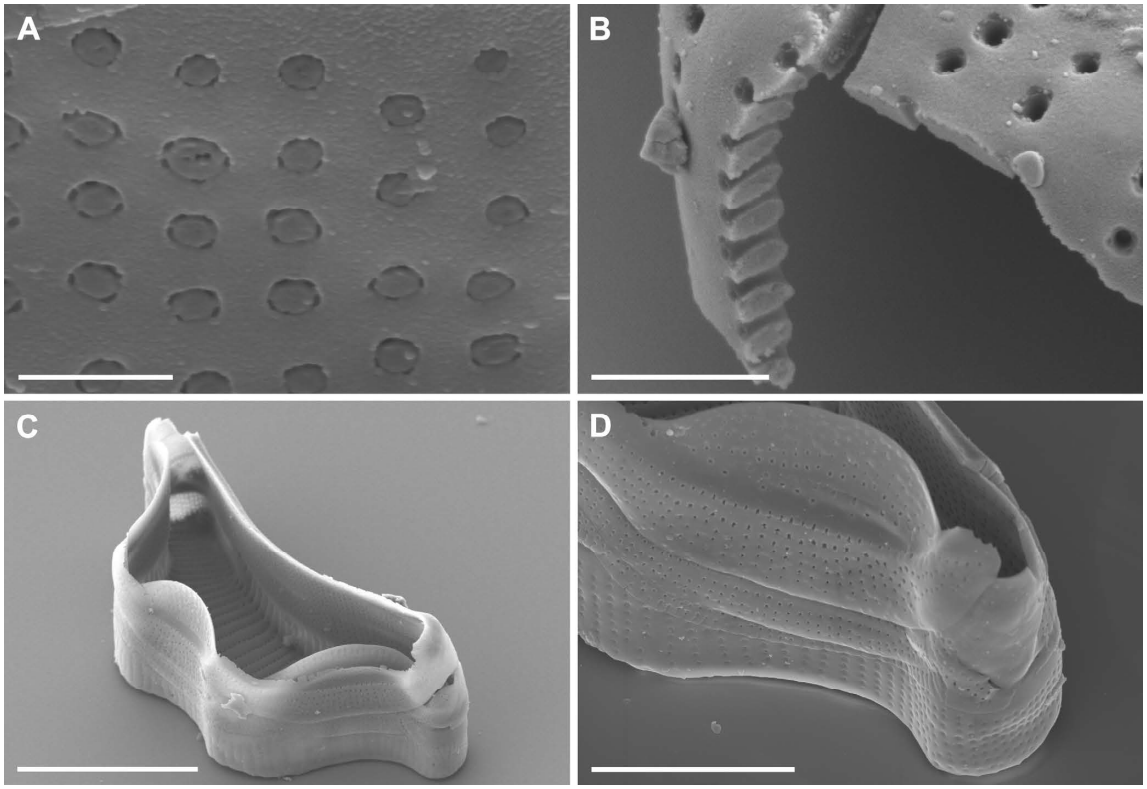


Figure 14 – *Eunotia fuseyi* sp. nov., SEM from sample CCA 2066: A, external view of areolae, note external occlusions; B, broken cell showing structure of areolae; C, valve with copulae; D, detail of copulae. Scale bars: A = 0.5 µm; B = 1 µm; C = 10 µm; D = 5 µm.

Mayama & Kobayasi (1990) examined materials from Degernä diatomite, the material from which *E. serra* was originally described. They found specimens with the following characteristics: valves 96–128 µm long and 13–16 µm broad with about 11 striae in 10 µm.

Eunotia fuseyi J.C.Taylor & Cocquyt, **sp. nov.**

Figs 11–14

Synonym – *Eunotia papilio* [as *papillo*] var. *africana* (Fusey) Fusey, **nom. inval.** in Fusey (1966: 61, pl. 1, figs 1– 4). – Type: D.R. Congo, Lobaye River, sample CCA 2066 (holo-: slide BR 4401, BR; the valve representing the type is illustrated here in fig. 11F; iso-: slide NWU–13-567, PUC; iso-: slide Zu 10/64 Friedrich Hustedt Diatom Collection, BRM). – Type locality: Democratic Republic of the Congo (DRC), Tshopo Province (part of the formerly Oriental Province), Lobaye River, 0.48970°S, 24.17728°E, tributary of the Lomami River which is a major tributary of the Congo River.

LM: description – Valves bigibbous. Ventral margin more or less concave. Dorsal margins slightly to strongly convex with respect to the two humps, separated by a depression of variable depth. Ends abruptly to strongly protracted, wide to narrow. Sometimes obliquely capitate and reflexed dorsally, never broadly rounded. Length 15.0–40.0 µm, breadth at widest parts 6–10 (12) µm. Terminal nodules offset from the poles. Terminal raphe fissures rather short on valve face, more or less 1/3 of the way towards the dorsal margin. Proximal raphe endings straight, running parallel and just below the junction of the valve face and mantle. Striae variable, 14–20 in 10 µm becoming only slightly more densely spaced near the ends. Areolae about 32 in 10 µm.

SEM: description – Striae evenly spaced, 14–20 in 10 µm becoming only slightly more densely spaced near the apices in all specimens. Very few (1–2) or no shortened striae intercalated at the dorsal margin. Striae composed of rounded areolae, 32 in 10 µm. Areolae occluded externally by hymenes anchored at 4 to 6 points and thus appearing more or less round (fig. 14A). Terminal nodules relatively close to the ends and not distinct (fig. 13A & B). Terminal raphe fissures short, straight and extending with an angle of mostly c. 110° onto the valve face, extending more or less ¼ to ⅓ of the distance towards the dorsal margin (fig. 12C & D). Internally the raphe terminates apically in a very small helictoglossa with the cell wall only slightly thickened at this point (fig. 13A & B). A single rimoportula is found at one of the apices located towards the dorsal side (fig. 13D). Terminal spines are absent. Girdle composed of at least two or more copulae (fig. 14C & D).

Etymology – The epithet *fuseyi* is in honour of Pierre Fusey, "chef de Travaux au Laboratoire de Cryptogamie du Muséum national d'Histoire naturelle" at Paris, France in the 1960s, and who studied algal samples from the Central African Republic. He described about 35, unfortunately invalid, new diatom taxa.

Ecology – Temperature of 24.2°C, pH of 5.01, conductivity of 17.4 µS.cm^{-1}, 0.054 mg.l^{-1} NH_4^+, 0.0009 mg.l^{-1} NO_2^-, 0.16 mg.l^{-1} NO_3^-, and 0.038 mg.l^{-1} SRP.

Distribution – Lobaye and Lomami River basins in the Democratic Republic of the Congo, tropical Africa, also more widely in tropical Africa including the Republic of the Congo.

Taxonomic remarks – Fusey described *E. papilio* var. *africana* as a variety from the Central African Republic, first in 1964 with a diagnosis in French and again in 1966, this time with a Latin diagnosis. However, under article 9.1 of the ICBN (McNeill et al. 2012) this name is invalid as two or more collections are mentioned and neither a type slide is designated nor a type is indicated in either of the descriptions. Reference is made to a number of photographs without date and locality information within the caption. We are however sure that this species is conspecific with the newly described *E. fuseyi* as it matches the measurements given by him as well as his illustrations (Fusey 1964, 1966). We believe that this variety deserves the rank of species as it is very different from the concept of *E. papilio* (Ehrenb.) Grunow sensu stricto. *Eunotia papilio* was originally described as *Himantidium papilio* Ehrenb. in 1843 and later-transferred to *Eunotia* by Grunow (1868). In his examination of the type material Reichardt (1995) recorded the following characteristics of *E. papilio* from the lectotype: 22–31 µm long, broadest region 11–16 µm, 8–12 in the narrowest part; 6 striae per 10 µm near the dorsal side to 12 near the ventral side, raphe short and curves onto the mantle; 18–26 puncta in 10 µm. *Eunotia fuseyi* has a higher number of areolae, a higher number of striae, the ventral margin is generally less convex, cells are often less constricted in the centre of the dorsal margin and the raphe runs along the junction of the valve face and valve mantle and is almost straight before curving onto the valve face.

DISCUSSION

Initially, during the examination of the *Eunotia zygodon* cells, we were convinced that there were two or even more distinct taxa present in our samples. We examined these different valves repeatedly grouping them according to length or breadth or number of undulations on the dorsal margin. We also examined the raphe endings on the valve face and could find no clear differences in these features. The more cells we photographed and examined the less clear the apparent differences became. This led us to trace the cells (fig. 3B) and then to superimpose the traced images over one another. By doing this it became obvious that we had a rather complete representation of the cell line and associated cell size reduction. Many *Eunotia* species have been initially described with reference to the undulations on the dorsal valve and this is evidenced by the names chosen for the species (e.g. *Eunotia triodon* Ehrenb. *E. diodon* Ehrenb. etc.), thus intrinsically linking the number of dorsal undulations to the species concept. This has always been the case for *Eunotia zygodon*, represented in literature as having two dorsal undulations; this is also concurrent with its initial description and illustration. As discussed in the introduction it may be possible that this species is rarely found in samples and when it is found there are only a few cells present. Also as earlier discussed this species forms long chains with the cells of more or less the same size in terms of cell length so that if only a

single chain or fragment of chain is sampled it will give the impression that cell size (length) and number of dorsal undulations are rather stable characters, however these are stable within the chain of cells but other chains may have a different valve morphology and it is only when numerous chains are sampled that this becomes obvious. This has been very well documented in cultures of the closely related (in terms of morphology) *Eunotia tropica* by Mayama (1995) but as far as we are aware has not been documented in naturally occurring *E. zygodon* populations. We do not propose that dorsal valve margin undulations will always be variable in number across all taxa of *Eunotia*, but it seems that several taxa have a stable number of undulations, e.g. *E. papilio* and *E. fuseyi* described herein. Caution is necessary when using this as a key character for identification (see discussion of *E. pectinalis* in Lange-Bertalot et al. 2011). In the case of *E. zygodon* we concur with Mayama (2001) that more stable regions of the valve face be chosen for confirmation of species identification.

The chain-forming *Eunotia leonardii* has multiple valve undulations and occurs *en-masse* in tropical African waters. At first view it shares some similarity with *Eunotia serra* but the similarities are very superficial. *Eunotia leonardii* has 8 to 18 valve undulations, compared to 6–18 undulations of *E. serra*, but the chief distinguishing character is the shape of the apices and curvature of the ventral margin.

Eunotia fuseyi was originally invalidly described as *E. papilio* var. *africana* as discussed above. As with many other taxa given the rank of variety the ascribed variety has little relation to the nominate variety, in this case there is a similarity to *E. papilio* var. *papilio*: both taxa have two dorsal undulations. However, other key characters are distinctly different, e.g., cell shape, striae density and areolae density, and this coupled with the original invalid description has led us to describe this entity as a distinct taxon requiring the rank of species. Ascribing taxa the rank of variety is problematic in modern diatom systematics as this rank is deemed unnatural. This is a problem worldwide and now many taxa, formerly at the rank of variety, are given the rank of species. Many taxa from Africa were ascribed the rank of variety, sometimes forma, in particular the rank '*africana*'. This was a convenient systematic tool for those working on African diatoms. If the taxon in question bore a superficial resemblance to one previously described from Europe or elsewhere, then the most convenient solution was to create a variety or forma. No blame is ascribed for this as species concepts changed over time and past studies were carried out without the benefit of scanning electron microscopy and other tools allowing for the detailed examination of diatom frustules. However, those interested in the study of the African diatom flora will have to systematically raise to the rank of species many of the taxa bearing epithets such as 'var. *africana*' or 'f. *africana*', but always after a thorough investigation of the concerned taxa.

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Navicula nielsfogedii J.C. Taylor & Cocquyt sp. nov., a new diatom (Bacillariophyta) from tropical and sub–tropical Africa

Jonathan C. Taylor[1,2, 3*], Christine Cocquyt[1] & Shigeki Mayama[4]

[1]*Botanic Garden Meise, 38 Nieuwelaan, Meise 1860, Belgium*
[2]*School of Biological Sciences, North–West University, P Bag X6001, Potchefstroom 2520, South Africa; *Corresponding author e–mail: Jonathan.Taylor@nwu.ac.za*
[3]*South African Institute for Aquatic Biodiversity (SAIAB), P Bag 1015, Grahamstown 6140, South Africa*
[4] *Department of Biology, Tokyo Gakugei University, Koganei, Tokyo 184–8501, Japan*

Abstract: In this paper we describe *Navicula nielsfogedii* sp. nov., a commonly occurring diatom through central and southern Africa and distributed from the sub–tropical zones of South Africa to tropical central Africa. We have examined the material of the most closely related species described from Africa, *Navicula feuerbornii* f. *africana* Foged and suggest that this species may be conspecific with *Navicula heimansioides* Lange–Bertalot and to have no link to the taxon *N. feuerbornii* Hustedt sensu Simonsen. With reference to *Navicula feuerbornii* f. *africana* we also discuss the changes over time in diatom species concepts, what we have dubbed 'species concept drift', and the consequences this holds for accurately identifying diatom taxa. As the newly described species is widely distributed and common in some samples from tropical and subtropical Africa we conclude that it may be a useful additional indicator organism for water quality monitoring in the region.

Key words: Congo River, diatoms, DR Congo, *Navicula nielsfogedii*, new species, taxonomy, tropical Africa

Introduction

Navicula Bory sensu lato was considered for the majority of the 20th century as a large genus into which many biraphid diatoms with bi–lateral symmetry around the transapical axis could be placed. In the last two decades an increasing number of taxa have been split from *Navicula* and placed in new genera (e.g., *Geissleria* Lange–Bertalot et Metzeltin), or re–ascribed to previously described genera (e.g., *Placoneis* Mereschowsky, *Sellaphora* Mereschowsky) and the concept of *Navicula* as a genus has been restricted to *Navicula* sect. *lineolatae* (Round et al. 1990). Even with this restricted delimitation *Navicula sensu stricto* remains a source of taxonomic and nomenclatural confusion (e.g., the various names and combinations of species such as *Navicula symmetrica* R. M. Patrick and *N. schroeteri* F. Meister). As with all diatom species, the naviculoid diatoms undergo what may be termed as 'species concept drift'. By this we mean that the original concept of the taxon established by the describing author becomes misinterpreted and changes over time, possibly due to the difficulty of interpreting line drawings. Often the species concept may also not have been made entirely clear by the author, for example *N. feuerbornii* Hustedt (see discussion). For this reason many workers in the field of diatom studies are now referring directly to type materials in order to reduce taxonomic and nomenclatural confusion. In and of itself taxonomic and nomenclatural accuracy is important, however, when the diatoms are being used for water quality monitoring studies it is important that the names be applied to taxa in a consistent manner, this is especially true for taxa which dominate diatom assemblages.

Recent work in the field of biomonitoring and water quality studies in tropical and sub–tropical Africa have brought to light many such instances which have proved a major hindrance in the successful application of biomonitoring techniques, based on diatoms, in Africa. In this paper we describe and discuss the distribution of *Navicula nielsfogedii* sp. nov. This is a rather commonly occurring taxon across sub–tropical and tropical Africa and has been found to dominate diatom assemblages in the Democratic Republic of the Congo (DRC) and thus accurate delimitation of this taxon is necessary.

MATERIALS AND METHODS

The sample (CCA 2071) containing *Navicula nielsfogedii* spec. nov. was obtained from wood substratum in the Lomami River at 0.49339° N and 24.19960° E, Tshopo Province (part of the formerly Oriental Province), Democratic Republic of the Congo by François Darchambeau and Ernest Tambwe on 24 November 2012. The sample was fixed in situ in a 20% v.v. final concentration ethanol solution. Diatom samples for LM and SEM observations were prepared by oxidizing the material using 37% H_2O_2 and heated to 100 °C for 1 to 2 hours. Following digestion the material was rinsed with distilled water. A subsample from the organic–free material was mounted in Naphrax® to obtain permanent diatom slides. Some physical parameters (temperature, conductivity and pH) were measured in situ with an YSI ProPlus multiprobe field meter equipped with a pH sensor 1001 ProSeries. Samples from nutrient analyses (NH_4, NO_3, NO_2 and SRP) were filtered and acidified in situ, and kept frozen until spectrophotometric analysis in the laboratory of the Unité d'Océanographie Chimique, Département d'Astrophysique, Géophysique et Océanographie, University of Liège, Belgium.

The type slide of *Navicula feuerbornii* f. *africana* FOGED (FOGED 1966, Ghana No. 119/1961) was obtained from the Museum Botanicum Hauniense, Copenhagen, Denmark.

Light microscope (LM) images were collected using a Olympus BX 51 microscope and a Nikon 80i microscope equipped with differential interference contrast optics and 100× 1.4 N.A. oil immersion objective. LM micrographs were taken with a UC30 digital camera at the Botanic Garden Meise, and a Nikon DS Fi1 at the North–West University. The striae density and valve size were determined based on measurements of 70 valves.

For scanning electron microscopy (SEM), parts of the oxidized material was rinsed with additional deionized water and isolated with micropipette on 6×6 mm cover slip. Cover slips were mounted on aluminium stubs and coated with osmium by OPC 40A osmium plasma coater (Filgen, Nagoya, Japan). A field emission scanning electron microscope S–8020 (Hitachi, Tokyo, Japan) operated at 5 kV and 8 or 12 mm working distance was used for the analysis. The terminology used is that outlined in ANONYMOUS (1975) and ROSS et al. (1979).

RESULTS

Navicula nielsfogedii J.C. Taylor et Cocquyt sp. nov. (Figs 1–22, 34–51)

Description

Light Microscopy (Figs 1–22): Valves linear–elliptic to linear lanceolate with cuneate, more or less obtusely rounded, slightly protracted ends, length 22–43 μm, width 5.3–6.7 μm. Raphe filiform, central raphe endings close, both deflected to the primary side, to which the asymmetrically enlarged central nodule is also directed. Axial area very narrow; central area varying from rounded to elliptic, more or less lanceolate expanded on the secondary side and thus distinctly asymmetric. Striae radiate in the central area, becoming parallel round the Voigt discordance and finally convergent towards the apices, 16–18 in 10 μm. Areolae comparatively dense, not possible to resolve in LM.

Scanning Electron Microscopy (Figs 34–51): Externally the raphe distinctly curved and central raphe endings deflected slightly to the primary side. A small structure, described as a silicate tongue in other *Navicula* taxa (Lange–Bertalot 2001) is present and appears to split the slightly expanded drop shaped central pores (Fig. 35–37) (cf. *Navicula tripunctata* (O. F. Müller) Bory, *N. jakovljevicii* Hustedt). However when this structure is examined more closely (Fig. 37) it becomes clear that it is a thin twisted bar of silica that extends down through the width of the cell wall and links the two sides of the central pore. Thus the structure cannot be accurately termed a silica tongue as it is linked at two locations. Axial area externally not thickened into a sternum. Voigt discordance clearly visible on the secondary side (Figs 34, 40). The stria closest to the apices consists of only a single areola from which a diagonal transapical slit extends giving the stria an inverted V–shape (Figs 38–39). A number of suture–like structures, similar to cranial sutures, (Figs 46–48) run from the raphe to the junction of the valve face and valve mantle on the external surface over the virgae (Figs 34–35), but not continuing on the valve mantle. These suture–like structures are dispersed from the central area up to the apices and are present in all of the geographically separate populations examined (DRC, Zambia and South Africa). Such structures were not reported in other diatoms and their value as taxonomic characteristics is unknown. Externally and internally the transapical striae are clearly broader than the virgae. Perforate hymenes occlude the areolae on the inside of the valve (Fig. 45), 40 areolae in 10 μm. The raphe sternum and additional rib are comparatively narrow (Figs 40–42). The raphe turns to a lateral position just behind the central nodule and up to the helictoglossae (Figs 43–44) (cf. *N. capitatoradiata* H. Germain and *N. arkona* Lange–Bertalot et Witkowski). Copulae are open without any perforation or ornamentation (Figs 49–51). Valvocopulae consist of a longer band and complementary short band restricted to the pole.

Holotype (designated here): slide BR 4397 from sample CCA 2071, Botanic Garden Meise, Belgium (BR). The valve representing the holotype is here illustrated in Figure 4.

Isotypes (designated here): slide PUC–13–572 from sample CCA 2071, South African Diatom Collection housed at North–West University, Potchefstroom, South Africa; slide ZU 10/29 from sample CCA 2071, Diatom Collection, Alfred–Wegener–Institue für Polar– und Meeresforschung, Bremerhaven, Germany (BRM).

Type locality: Lomami River, a major tributary of

the Congo River downstream Kisangani, Tshopo Province (part of the formerly Oriental Province), DRC. 0.49339° N and 24.19960° E

Etymology: The specific epithet honours the diatomist Niels Foged for his contribution to the knowledge of the diatom flora of Africa.

Ecology and Distribution: This taxon occurs commonly throughout the tropics and sub–tropics of southern and central Africa in freshwater rivers and streams. In general it occurs in slightly acidic to circumneutral, oligo– to mesotrophic waters with moderate conductivity. The type locality had the following characteristics at the time of collection: temperature of 25.7 °C, pH of 6.06, conductivity of 12.2 µS.cm^{-1}, 0.06 mg.l^{-1} NH_4, 0.009 mg.l^{-1} NO_2, 0.45 mg.l^{-1} NO_3, and 0.09 mg.l^{-1} SRP.

Discussion

Taxonomic comments

Navicula nielsfogedii is at the first sight close to or similar to a number of previously described taxa. *Navicula leptostriata* E. G. Jørgensen (Jørgensen 1948) overlaps *N. nielsfogedii* in terms of length (25–35 µm long), but is narrower (4.5–5.5 µm broad as opposed to 5.3–6.7 µm). The main difference lies in the central pores of *N. leptostriata* which are very closely placed and distinct. *Navicula notha* J. H. Wallace (Wallace 1960) also overlaps with *N. nielsfogedii* in terms of length (19–32 µm) but is generally narrower (breadth 4–5.5 µm). *N. notha* may be also slightly coarser in striation and areolation (15–17 in 10 µm and c. 38 in 10 µm respectively). In addition, the smaller central area as well as the shape of the valve separates it from *N. nielsfogedii*. *Navicula heimansioides* Lange–Bertalot (Lange–Bertalot 1993) differs by having less striae (14–16 in 10 µm), a lower number of areolae (32–35 in 10 µm) and different shaped valve endings. *N. heimansioides* was identified in Sierra Leone by Carter (1982) as *N. feuerbornii*. *Navicula feuerbornii* sensu Simonsen (1987) has abruptly rounded ends, is 33–46 µm long and 6–7 µm broad. The striae in the centre are slightly radial (never bent as in *N. nielsfogedii*) to weakly convergent near the apices, 10 in 10 µm in the centre to 14 in 10 µm towards the ends. Areolae are clearly visible, and it inhabits alkaline waters. None of the above taxa possess a silica structure (‘appears superficially as a tongue’) in the central pores as does *Navicula nielsfogedii*.

Taxonomic remarks on *Navicula feuerbornii* f. *africana*

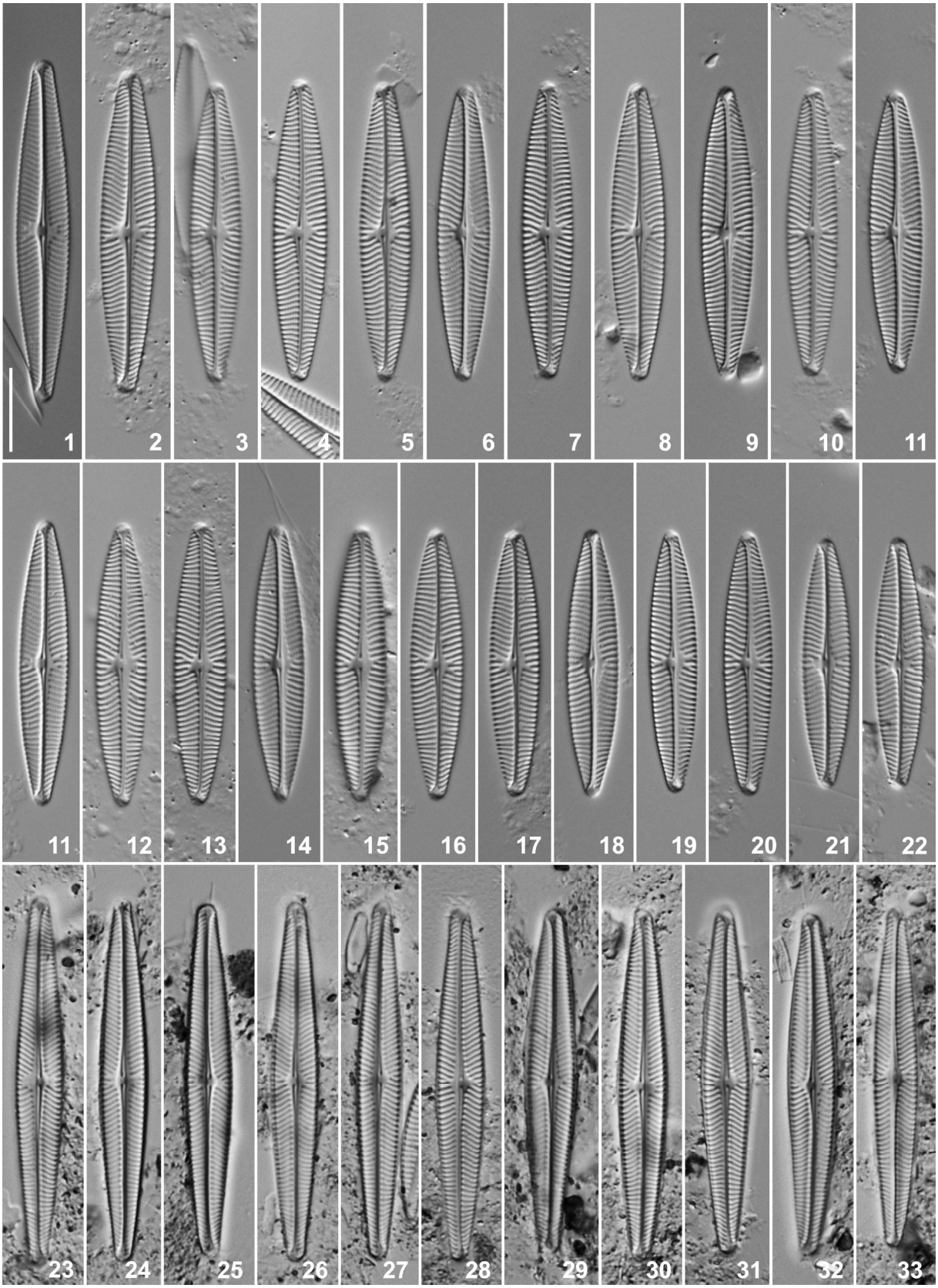
Based on studies of the type material of *Navicula feuerbornii* f. *africana* (Figs 23–33, taken from the type slide Ghana No 119/1961) we would suggest that this species may be conspecific with *Navicula heimansioides*. The name *Navicula feuerbornii* f. *africana* indicates a link to *N. feuerbornii*. As discussed below this form has little relation to the species *N. fuerbornii* sensu Hustedt. We postulate that *Navicula feuerbornii* f. *africana* and *Navicula heimansioides* may be conspecific based on our examination of the type material of *N. feuerbornii* f. *africana*. We measured 27 cells on the type slide of *N. feuerbornii* f. *africana* from Ghana and found that the populations of *N. heimansioides* discussed by Lange–Bertalot (2002) overlap in respect to length, breadth and number of striae (see Table 1) as well as in the shape of the valve apex. The cells we measured on the type slide differed in one respect from Foged’s initial description; he stated that the cells had 15 striae in 10 µm but his drawing shows a valve with 17 striae in 10 µm. Our measurements of cells from the type population also show an average of 17 striae in 10 µm.

Species concept drift

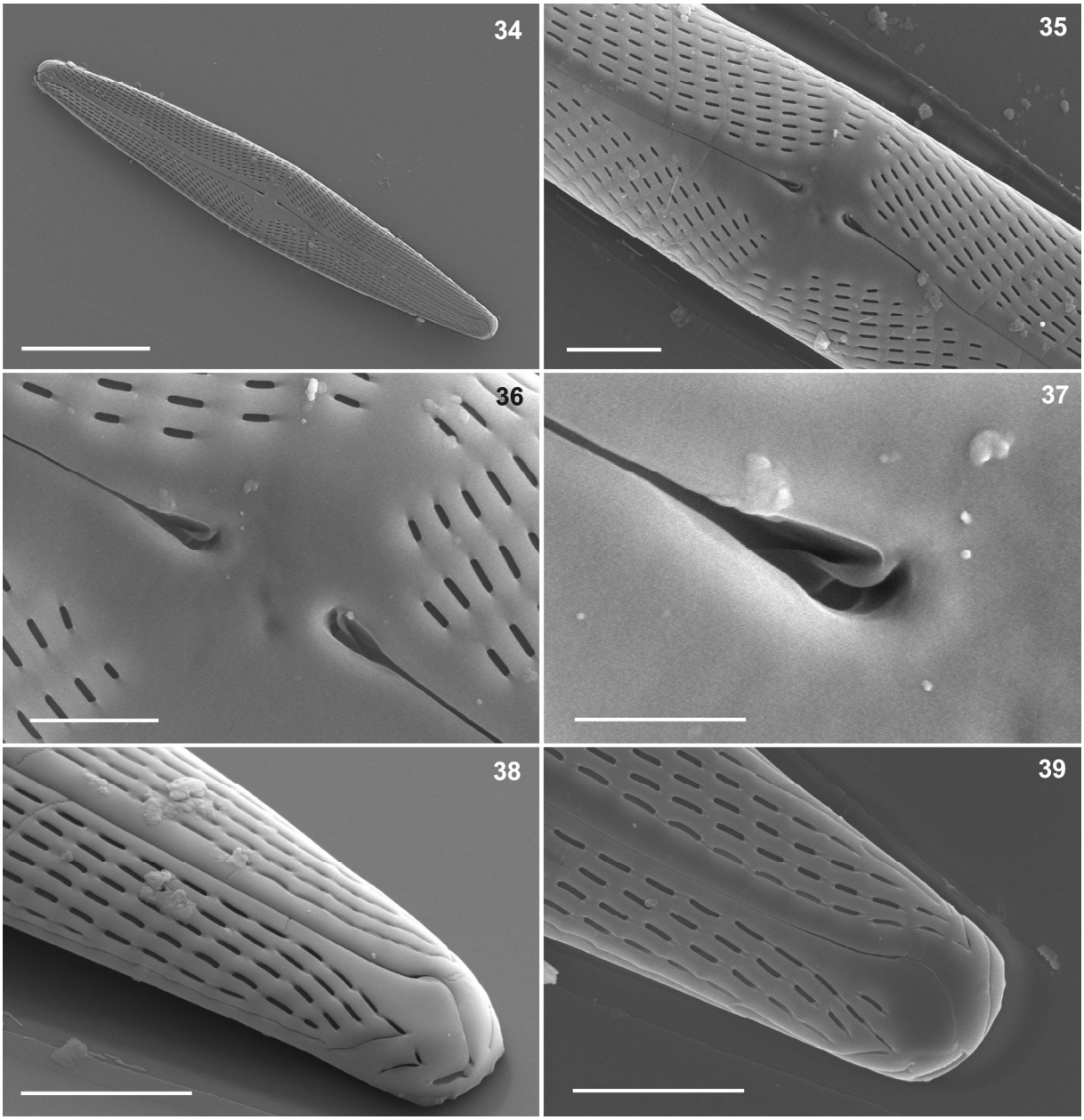
When diatoms were first systematically studied and documented, a brief description was accompanied by an often minute drawing (e.g. Kützing 1844). These drawings were also made from freshly collected material or material dried to thin sheets of mica. Surprisingly, despite this some species concepts remain remarkably stable (see discussion in Taylor & Coc-quyt 2015). However, other concepts can prove to be far less stable, especially when the original author of

Table 1. Morphometric data on the studied population from Lange-Bertalot (2002), Ghana (119/1961) and DR Congo (CCA 2071).

Taxon	Number of cells measured	Valve length (L) (µm)	Valve breadth (B) (µm)	L:B ratio	Striae in10µm
Navicula heimansioides (Lange-Bertalot 2002)	unknown	30–50	5–6	6.9	14–16
Navicula feuerbornii f. *africana* (Foged type Ghana No. 119/1961)	27	40–50	5.3–6.3	7.4	16–17
Navicula nielsfogedii sp. nov. (DRC population CCA 2071)	70	23–43	5.5–6.7	5.4	17–18

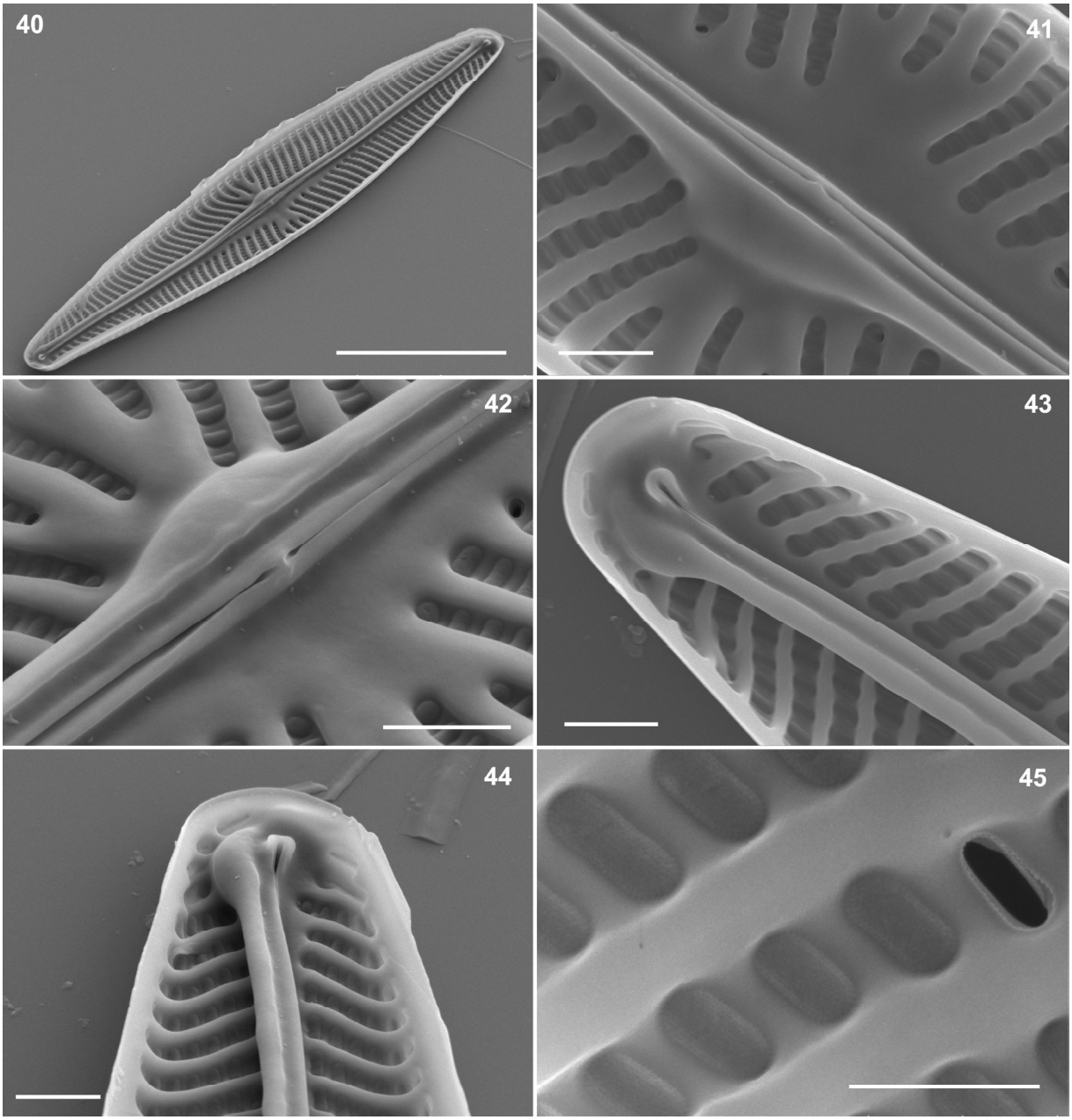


Figs 1–22. LM of type material of *Navicula nielsfogedii* from sample CCA2071.
Figs 23–33. LM of type material of *Navicula feuerbornii* f. *africana* from sample Ghana 119/1961. Scale bar 10 µm.



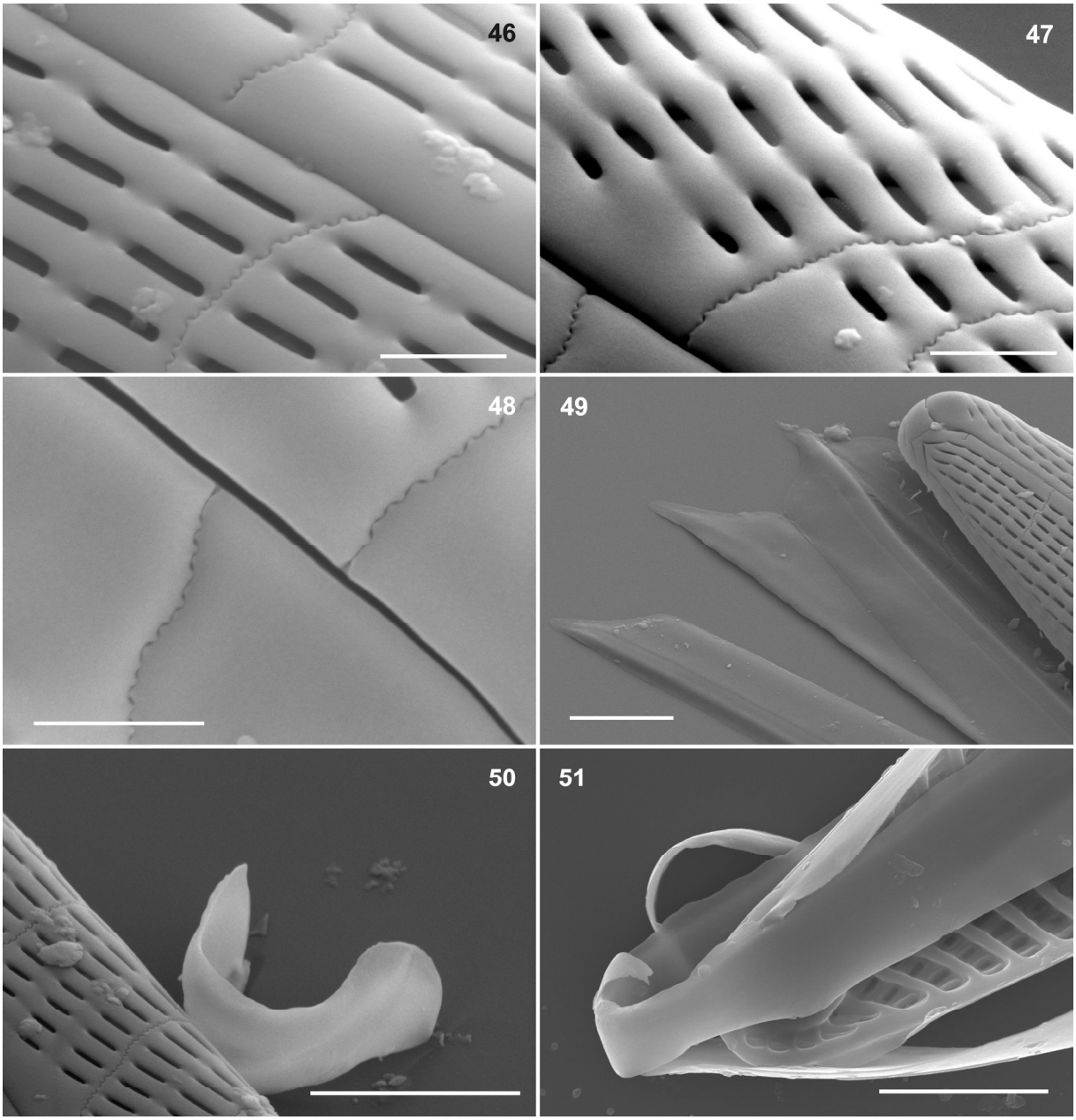
Figs 34–39. SEM of type material of *Navicula nielsfogedii* from sample CCA2071: (34) external view of complete valve; (35–37) valve exterior showing detail of the central area and proximal raphe endings, note the structure previously known as a silica tongue in Fig. (37); (38–39) valve apices showing distal raphe endings. Scale bar 10 µm (34), 2 µm (35, 38, 39), 1 µm (36), 0.5 µm (37).

the taxon did not provide a clear species concept. As diatom studies advanced, these organisms were documented, again mostly with drawings, but from cleaned material mounted in media with a high refractive index allowing the morphology of the cell walls to be better observed, and such drawings are often open to interpretation. When photo–microscopy became more accessible and commonly used species concepts could be more firmly established. Added to this, the rules for botanical nomenclature changed in 1958 (McNeill et al. 2012) necessitating the description of a species from a single specimen (in the case of diatoms this is a type slide) allowing less room for error in interpretation of the original author's concept by later workers. Drawings of cells are open to interpretation and shifts come about as one author identifies what he assumes to be a previously described taxon and then he in turn illustrates this taxon himself (his own concept). This new illustration may be more readily available to the scientific community than the original, which often dated from the mid–1800s, and so if the concepts of the species are different the newer illustration may take precedence simply because of the availability of the newer literature. This has been in particular true for Africa, as literature, even now with the availability of electronic resources, is difficult to obtain locally. With drawings as illustrations, and even photographs, these subtle shifts can occur several times distancing



Figs 40–45. SEM of type material of *Navicula nielsfogedii* from sample CCA2071: (40) internal view of complete valve; (41–42) valve interior showing detail of the central area and proximal raphe endings, note the unilateral thickening of the central nodule; (43–44) valve apices showing internal distal raphe endings and helictoglossae; (45) detail of the internal occlusion of the areolae by perforated hymenes. Scale bar 10 µm (40), 1 µm (42–44), 0.5 µm (45).

the species far from its original concept and perhaps even from the generic classification (see also in Morales et al. 2013). As mentioned before, with the publication of photographic floras, species concepts became more stable but these stable concepts may still have been based on earlier misinterpretation. There have been several attempts to remedy this problem. Schoeman and Archibald (1976–1980) set about describing in detail the diatom flora (both ubiquitous and endemic) of South Africa by checking the type slide of each species they discussed, collecting together the original drawings, light microscope and electron microscope images. Unfortunately for a number of reasons this work was discontinued. Recently however, there has been a resurgence within the diatom community of the notion that we cannot accurately describe and discuss the ecology of taxa without referring to the type material (e.g. Taylor & Cocquyt 2015; Wetzel et al. 2013; Morales et al. 2013). A very good example of such taxonomic confusion may be found in *Navicula feuerbornii*. This species was originally described from Java by Hustedt (1938) from two discrete populations comprising drawings of valves of each population without stipulating which was the type. From the drawings and from the later photographs of the type (Simonsen 1987) it can be clearly seen that the two populations were



Figs 46–51. SEM of type material of *Navicula nielsfogedii* from sample CCA2071: (46–48) external view of valve showing suture like structures; (49–51) structure of the copulae, long bands shown in Fig. (49) and (51), and complimentary short band in Fig. (50). Scale bar 0.5 µm (46–48), 2 µm (49–51).

comprised of distinct taxa with several distinct valve features (these will not be further discussed as it is not the purpose of the present paper to resolve this issue). In this case the author himself had a broad concept of his own species, making it more difficult for other later workers to define theirs (cf. *Nitzschia epiphyticoides* Hustedt, Cocquyt et al. 2012). Foged in his work on the diatoms of Ghana (Foged 1966) illustrates *N. feuerbornii* and also describes a new forma of this taxon *N. feuerbornii* f. *africana*. (Foged accidentaly listed his taxon as *N. feuerbornii* var. *africana* on page 156 in his 'Index to species'.) However, if we compare the type of *N. feuerbornii* Hustedt, even though the concept is broad, there is still only a small similarity to *N. feuerbornii* as illustrated by Foged. This would not be too problematic but Foged then goes on to describe a forma of this taxon, of which he has his own concept, so the resulting situation is the description of a forma which in reality has nothing to do with the true nominate species *sensu* Hustedt and thus taxonomic confusion reigns. This is especially problematic when the taxon in question is common and widespread through the study region.

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Labeo rosae (Cypriniformes: Cyprinidae) in the Congo basin: a relict distribution or a historical introduction?

M. Van Steenberge*†‡, L. Gajdzik†§, A. Chilala‖, J. Snoeks*† and E. Vreven*†

*KU Leuven, Laboratory of Biodiversity and Evolutionary Genomics, Charles Deberiotstraat 32, 3000 Leuven, Belgium, †Vertebrate section, Ichthyology, Royal Museum for Central Africa, Leuvensesteenweg 13, 3080 Tervuren, Belgium, §Université de Liège, Laboratoire de Morphologie Fonctionnelle et Evolutive, Allée de la Chimie 3, 4000 Liège, Belgium and ‖Department of Fisheries, Ministry of Agriculture and Livestock, Lusaka Provincial Office, P. O. Box 350100, Chilanga, Zambia

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Labeo rosae, a species with a native range in Southern Africa, was discovered in the Congo basin by re-identification of two museum specimens previously identified as *Labeo mesops*. The occurrence of this species in the upper Congo implies a range extension of the species of more than 1000 km. Although the species' distribution is mirrored by that of some other Cypriniformes, its occurrence in the Congo might be due to introduction by humans.

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Key words: Africa; biogeography; *Labeo altivelis*; *Labeo mesops*; reservoir lake; taxonomy.

Labeo Cuvier 1816 is, in Africa, the third most diverse cyprinid genus, after *Barbus* Barbus Cuvier & Cloquet 1816 and *Labeobarbus* Rüppell 1835 (Reid, 1985; Skelton *et al.*, 1991). Species of *Labeo* are herbivores that feed on the algal growth covering the substratum. For this, they possess special adaptations such as an inferior, sucker-like mouth with folded lips and a hardened edge (Reid, 1985; Skelton, 2001). Many species of *Labeo* were once of large commercial importance as their spawning migrations sustained important fisheries (Skelton *et al.*, 1991). Yet, in spite of this and their relatively large size the taxonomy of *Labeo* remains problematic and species identification difficult.

African *Labeo* were reviewed by Reid (1985) and, for Congo and Lower Guinea taxa, by Tshibwabwa (1997). In the Congo basin, two groups are distinguished: the plicate and the papillate (Tshibwabwa, 1997). The first, which is by far the most species rich, has rows of transverse plicae on the upper labial fold. The second lacks these plicae and has rows of globular papillae instead. The latter constitutes, at least in the

‡Author to whom correspondence should be addressed. Tel: +32 2 769 56 32; email: maarten.vansteenberge@bio.kuleuven.be

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Congo basin, a monophyletic group (Lowenstein *et al.*, 2011). Five nominal species of papillate *Labeo* are reported from the Congo basin: *Labeo altivelis* Peters 1852, *Labeo mesops* Günther 1868, *Labeo lineatus* Boulenger 1898, *Labeo weeksii* Boulenger 1909 and *Labeo maleboensis* Tshibwabwa 1997.

Prior to Reid's (1985) revision, *L. mesops* was considered endemic to the Lake Malawi basin. Reid (1985), however, extended this species' range considerably. Foremost, he placed two species from the Horn of Africa: *Labeo grammipleura* Vinciguerra 1927 and *Labeo gracilis* Boulenger 1916 into synonymy with *L. mesops*. Secondly, he listed *L. mesops* as occurring in the Tana River in Kenya. Finally, he mentioned a 'doubtful' presence of *L. mesops* in the Lufira (upper Congo). Reid (1985) referred to Goorts *et al.* (1961) as a reference hereof. Yet, no mention of *L. mesops* nor of any species of *Labeo* other than *L. cylindricus* Peters, 1852 is made in that document. Tshibwabwa (1997) contested the first range extension and revalidated *L. grammipleura* and *L. gracilis*. The second range extension was refuted by Seegers *et al.* (2003), who listed the Tana population as of uncertain taxonomical status. Tshibwabwa (1997) did, however, confirm the presence of *L. mesops* in the upper Congo.

The presence of *L. mesops* in the Congo basin was based on only two museum specimens: MRAC 182678-79 (Fig. 1), from the Lufira River, downstream of a small reservoir lake named Koni (10° 42′ S; 27° 15′ E). These specimens were collected by Magis who identified them as *Labeo* sp. (aff. *mesops*) with the local name 'Mushila'. Magis (1961) further mentioned 18 additional specimens from Lake Koni proper, but these could not be retrieved. Tshibwabwa (1997) examined the two Lufira specimens and confirmed their distinctness from all other Congolese species of *Labeo*. Following direct comparison with the holotype of *L. mesops* (BMNH 1864.1.9:64, Lake Malombe), he confirmed their identification as *L. mesops*. The holotype of *L. mesops* is a left-hand side dried skin. Hence, not all measurements and counts (*e.g.* vertebral counts) could be obtained for the latter. Moreover, Tshibwabwa (1997) did not compare the Lufira specimens with additional specimens of *L. mesops* from Lake Malawi, or with the literature. As such, it is not clear whether the differences between the Lufira specimens and the holotype of *L. mesops* (Tshibwabwa, 1997) fall within the range of what can be considered intraspecific variation.

In this study, the two Lufira specimens were compared with the holotype as well as with four additional specimens of *L. mesops* (MRAC 191845, 101298, 101302 and 99-41-P-9), all from Lake Malawi. This revealed that the two Lufira specimens differ from *L. mesops* in the number of scales around the caudal peduncle counted at the narrowest point (S_{CP}: 20 *v.* 16 in *L. mesops*), the number of scales between the dorsal and the caudal-fin (S_{DC}: 20 *v.* 14–18 in *L. mesops*) and the number of branched dorsal rays (R_{DB}: 11 + 1 *v.* 10 + 1 in *L. mesops*, where +1 denotes that the posterior-most ray was divided at its base, although it was counted as one).

Other specimens examined in this study were *L. altivelis* (45), *L. lineatus* (57), *L. weeksii* (72) and *L. maleboensis* (10) from the Congo, the Zambezi and the Rio dos Bons Sinais basins, including the complete type series of these species. All of these show 16 S_{CP}, except for one specimen of *L. altivelis*, which had 17 S_{CP}. As such, the circumpeduncular scale count of 20 also allows the two Lufira specimens to be distinguished from all known papillate species of *Labeo* from the Congo basin. Skelton (2001) mentioned 16–18 S_{CP} for *L. altivelis* and Tshibwabwa (1997) also listed some variation in this characteristic for the Congolese species. Although Skelton



FIG. 1. Specimens MRAC 182678-79: Lufira River, downstream of Koni. Although previously listed as *Labeo mesops*, these were shown to be *Labeo rosae*. Scale bar: 10 cm.

(2001) did not specify how he counted the S_{CP}, Tshibwabwa (1997) made this count starting at the first scale, posterior to the anal-fin base. As such, he followed a method different from the one used here where S_{CP} were counted at the narrowest point of the caudal peduncle.

According to Reid (1985), the only other African species of papillate *Labeo* with 20 S_{CP} are *Labeo niloticus* (Forsskål 1775), currently valid as *Labeo vulgaris* Heckel 1847 (Fricke, 2008), *Labeo horie* Heckel 1847, *Labeo ruddi* Boulenger 1907 and *Labeo rosae* Steindachner 1894. The first three species differ from the Lufira specimens in the number of branched dorsal rays ($14–16+1$ in *L. vulgaris,* $12–14+1$ in *L. horie* and $9–10+1$ in *L. ruddi v.* $11+1$ in the Lufira specimens). Using the revision of Reid (1985), the combination of S_{CP} (20), R_{DB} ($11+1$), S_{LL} ($38+2$, lateral line scales) and V (34, vertebrae, excluding the Weberian apparatus and including the pre-ural centre) indicates that the Lufira specimens are *L. rosae*. The hototype of *L. rosae* (NMW 55584) was examined in this study and the following meristics were counted: S_{CP} (20), R_{DB} ($12+1$), S_{LL} ($36+3$) and V (33). Although the values of the Lufira specimens are somewhat different from those of the holotype, they lie within the range given by Reid (1985). As such, the Lufira specimens are re-identified as *L. rosae*. Given their size (225 and 242 mm standard length, L_S), these specimens have a relatively small dorsal-fin of about the size of the head, which also separates them from similar-sized *L. altivelis* (Marshall, 2011).

Although this re-identification confirms the status of *L. mesops* as an endemic species to the Lake Malawi basin (Snoeks, 2004), it implies a considerable range extension for *L. rosae*. This species' known native range, which contains parts of the Limpopo, the Incomati and the Phongolo Rivers, lies over 1000 km south of the Lufira. Yet, the conditions in these subtropical rivers could be somewhat similar to the relatively cool rivers of the upper Congo plateaus. Two other large papillate *Labeo* also have disjunct distributions (Skelton, 2001). *Labeo altivelis* occurs in middle and lower Zambezi and in some eastward flowing rivers as well as in the Bangweulu–Mweru ecoregion. This species is, however, absent from the intermediate basins of the Kafue, the upper Zambezi and from the upper reaches of the Luangwa (Skelton, 2001). *Labeo ruddi* has an even larger distribution gap as it occurs in the warmer sections of the eastward flowing Incomati and Limpopo systems as well as in the westward draining Cunene (Skelton, 2001). These two distribution zones lie over 1000 km apart. The current distributions of these species of *Labeo* can be explained as being relicts of a once larger distribution in which local extinctions occurred. Given the severe changes in drainage patterns and climate in southern Africa (Cotterill & de Wit, 2011), local extinctions of large species of *Labeo*, which are adapted to large rivers, are not unlikely. A similar scenario was put forward for *Hydrocynus vittatus* Castelau 1861 for which the absence in the Kafue was explained as resulting from a period of local aridity (Cotterill & de Wit, 2011). Two species of *Barbus*, have similar distributions as *L. rosae*. *Barbus motebensis* Steindachner 1894 is only known from the Limpopo headwaters and from the upper Congo drainages of the Lufira and the neighbouring Luapula. *Barbus mattozi* Guimarães 1884 has a somewhat larger distribution. This species occurs in the Limpopo, the upper Congo, the Cunene and the neighbouring Curoca. Remarkably, two records are known from the upper Zambezi, where it appears to be rare (Skelton, 2001). As *B. mattozi* has habitat preferences similar to *L. rosae* (Skelton, 2001; Marshall, 2011), it cannot be excluded that the latter species remains to be discovered in parts of the upper Zambezi basin as well. Both *B. motebensis* and *B. mattozi* probably consist of several closely related species (Skelton, 2001; C. Manda, pers. comm.).

It is, however, remarkable that *L. rosae* was only collected once in the Congo basin and within and downstream of an artificial lake 10 years after it was constructed (Magis, 1961). This raises the question whether the species is native to the basin. *Labeo rosae* is a popular angling species and it was repeatedly introduced into artificial lakes north of its native range (Marshall, 2011). Due to these introductions, it could have colonized the upper Runde in Zimbabwe (Skelton, 2001; Marshall, 2011). Hence, it is possible that *L. rosae* was introduced into or near Lake Koni as well, although the whereabouts of this introduction are unknown. In the Lufira reservoirs, different attempts were made to introduce herbivorous fishes for weed control (Damas *et al.*, 1959). Besides translocations of the local ichthyofauna, this also led to the introduction of non-native species. Moreover, Lake Koni lies at less than 100 km from Kipopo (11° 34′ S; 27° 21′ E), at the time the largest aquaculture research centre in the Democratic Republic of the Congo. Here, the potential of native and alien fish species for aquaculture was tested. Some of the fishes introduced in Kipopo even originated from neighbouring Zambia. Yet, there were no reports of *L. rosae* being introduced into Zambia. The sole species of *Labeo* that was translocated in this country was *L. altivelis* (Thys van den Audenaerde, 1994). Nevertheless, *L. rosae* might have been introduced as well, either deliberately or accidentally. A similar scenario of an accidental introduction could have happened in

Lake Kivu, where *Lamprichthys tanganicanus* (Boulenger 1898) was possibly introduced together with the Lake Tanganyika sardine *Limnothrissa miodon* (Boulenger 1906) (Nshombo & Lushombo, 2010). Given that *L. rosae* is less dependent on rocky substrata than some of its congeners (Marshall, 2011), it could thrive in artificial lakes such as Lake Koni. Yet, as with all species of *Labeo*, it requires flowing water to spawn and there are no references indicating that spawning has ever occurred here.

At the time of this study, it could not be established whether *L. rosae* is native or introduced to the Congo basin. If *L. rosae* is indeed native, it is remarkable that no other specimens were ever recorded. Yet, as many of the larger rivers in the southern part of the Congo system have remained virtually unsampled, new collections could possibly provide more specimens. Moreover, species of *Labeo* are very susceptible to be affected by the construction of dams (Skelton *et al.*, 1991), so they might have become locally extinct prior to further collection efforts. Although this study showed that the two Lufira specimens correspond to the description of *L. rosae*, it cannot be excluded that they belong to an undescribed species. Yet, only two specimens were at hand and species of *Labeo* are known for their large distribution areas and large intraspecific variation (Reid, 1985). The two Lufira specimens were fixed in formalin and are thus unsuitable for genetic analysis. If additional specimens are discovered, population genetics could reveal the true origin of the Lufira population, or the possible presence of a new species. Until then, the presence *L. rosae* in the Lufira is one of the many ichthyological mysteries still present in the Congo basin.

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LINGERING TAXONOMIC CONFUSION IN *LABEO* (ACTINOPTERYGII: CYPRINIFORMES: CYPRINIDAE): CORRECTING THE RECORDS AND BASIS OF TYPE DESIGNATIONS FOR SEVEN CONGOLESE SPECIES

Maarten VAN STEENBERGE[1, 2, 3*], Jos SNOEKS[1, 2], and Emmanuel VREVEN[1, 2]

[1] *Ichthyology Group, Vertebrates Section, Royal Museum for Central Africa, Tervuren, Belgium*

[2] *Laboratory of Biodiversity and Evolutionary Genomics, KU Leuven, Leuven, Belgium*

[3] *Institute of Zoology, University of Graz, Graz, Austria*

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Background. The concept of name-bearing specimens is pivotal for nomenclatural stability. Hence, correctly listing the type specimens and designating lecto- and neotypes are crucial elements in taxonomic revisions. While reviewing the *Labeo* Cuvier, 1816 taxa of the Congo basin, we encountered several differences between the lists of type specimens provided in the various revisions of the genus. The majority of problems were encountered with six nominal species of *Labeo* that were described by Boulenger in 1898: *Labeo barbatus* Boulenger, 1898; *Labeo falcifer* Boulenger, 1898 (later replaced by *Labeo falcipinnis* Boulenger, 1903); *Labeo lineatus* Boulenger, 1898; *Labeo longipinnis* Boulenger, 1898; *Labeo macrostoma* Boulenger, 1898; and *Labeo velifer* Boulenger, 1898. For four of these species: *L. barbatus*, *L. lineatus*, *L. macrostoma*, and *L. velifer*, different specimens appear in the literature as name-bearing types. Also for *Labeo lividus* Roberts et Stewart, 1975, previously a junior synonym of *L. barbatus*, and for *Labeo altivelis* Peters, 1852, a species described from the lower Zambezi but also present in the Congo basin, alternative lists of type specimens were mentioned in the literature.
Material and methods. The correct list of type specimens was compiled based on museum archives and on an examination of the type specimens. The validity of the lectotype designations was checked. When a lectotype designation proved to be ambiguous, the actions of the subsequent revisers were investigated.
Results. We found that the correct lectotypes of *L. barbatus*, *L. falcipinnis*, *L. lineatus*, *L. longipinnis*, *L. macrostoma*, and *L. velifer* are: BMNH 1898.12.28:7, MRAC 17, BMNH 1897.9.30:27, MRAC 113, MRAC 35, and BMNH 1898.12.28:1, respectively. Also for *L. lividus* and *L. altivelis*, confusion existed on the type series and a corrected list of type specimens is presented.
Conclusion. Especially in older species descriptions, a detailed list of studied specimens is often lacking. In such cases, providing a correct list of type specimens is often not a trivial task. This study shows that inaccurate lists of type specimens can lead to invalid taxonomic acts, which could have nomenclatural implications. We hope that increased efforts in the dissemination of basic taxonomic information will reduce such errors in the future.

Keywords: taxonomy, Africa, lectotype designation, type specimen, nomenclature

INTRODUCTION

With over a million nominal species of animals described (Costello et al. 2013), stability in zoological nomenclature is more important than ever. One of the most efficient tools available for achieving this stability is the designation of name-bearing types. Although species names can change as synonymies are proposed and generic rearrangements emerge from novel insights of relatedness, type specimens are fixed, regardless of further studies or changing points of view. Given this importance, it is of no surprise that the necessity of an explicitly listed holotype or syntype series for a valid species description is included in the latest version of the International Code of Zoological Nomenclature (Anonymous 1999: Article 72.3). This was, however, not required for species described prior to 2000. Especially in older descriptions, explicit or even implicit reference to the type specimens is sometimes lacking. This has, in some cases, led to confusion. Before holo- and paratypes were given a separate status, all specimens used for the description of a taxon were viewed as having the same value as syntypes, except when some were explicitly listed as not belonging to the type series or when one

* Correspondence: Dr. Maarten Van Steenberge, Ichtyologie, Dienst Vertebraten, Koninklijk Museum voor Midden Afrika, Leuvensesteenweg 13, 3080 Tervuren, België, phone: +32-2-769-5632, e-mail: (MVS) *maarten.vansteenberge@bio.kuleuven.be*, (JS) *jos.snoeks@africamuseum.be*, (EV) *emmanuel.vreven@africamuseum.be*.

specimen was explicitly mentioned as being "the type" (Anonymous 1999: Article 73.1.1). Given the advantage of having unique name-bearing specimens, lectotypes are often designated in taxonomic revisions of fishes. The ICZN lists a series of recommendations (Anonymous 1999: 74A–G) for lectotype designations that, when followed, enhance stability. Yet, the only real prerequisite for a lectotype designation to be valid is that the selected specimen is part of the type series (Anonymous 1999: Article 74.2).

Although relatively well studied, the taxonomy of species of the cyprinid genus *Labeo* Cuvier, 1816 from the Congo basin remains problematic. Congolese *Labeo* were reviewed by Reid (1985) (for all African species), Tshibwabwa and Teugels (1995) (for species from the Lower Congo), and by Tshibwabwa (1997) (for species from Lower Guinea and the Congo Basin). In his revision of African *Labeo*, Reid (1985) designated three neotypes and 43 lectotypes. For some of these acts, he was criticised by Thys van den Audenaerde (1987). In subsequent revisions, deviating lists of para-, syn-, or lectotypes were presented (Tshibwabwa and Teugels 1995, Tshibwabwa 1997). Sometimes, a detailed explanation was given that settled the case. This was the case for *Labeo greenii* Boulenger, 1902 and *Labeo degeni* Boulenger, 1920. For the former species, Tshibwabwa and Teugels (1995) demonstrated that the original description was based on a single specimen: MRAC 1190, by definition the holotype. This rendered Reid's (1985) lectotype designation, of the same specimen, invalid. For the latter species, Reid (1985) listed BMNH 1919.9.10:192 as the holotype. As Boulenger (1920) mentioned that this species was described based on two syntypes, this act was also considered invalid, and Tshibwabwa and Teugels (1995) designated the other syntype: MRAC 7111, as the lectotype for *L. degeni* instead.

More problematic were the *Labeo* species present in the collection made by Lts. Wilverth, Wagenaar, and De Bauw, which was commissioned by the government of the Congo Free State. This fish collection was on display in the colonial exhibit that was part of the Brussels world fair of 1897. Later, it would become part of the collections of the Congo Museum (currently the Royal Museum for Central Africa, RMCA) in Tervuren. When visiting this exhibition in 1897, Boulenger realised that the majority of the fishes on display were new to science and he requested permission to study them. As Boulenger, at the time, was employed at the British Museum of Natural History (BMNH, currently the Natural History Museum, NHM), these specimens were sent to London under the prerequisite that "types" needed to be sent back to Tervuren although "doubles" could be kept in the collections of the NHM (Thys van den Audenaerde 1987). Boulenger (1898) used this collection for the description of six nominal species of *Labeo*: *Labeo barbatus* Boulenger, 1898; *Labeo falcipinnis* Boulenger, 1903, which was originally described as *Labeo falcifer* Boulenger, 1898, a name which was preoccupied by *Labeo falcifer* Valenciennes, 1842 (Boulenger 1903); *Labeo lineatus* Boulenger, 1898; *Labeo longipinnis* Boulenger, 1898; *Labeo macrostoma* Boulenger, 1898; and *Labeo velifer* Boulenger, 1898. For all of these species, inconsistencies with regard to the listed types were found in the subsequent revisions of the genus (Table 1). These will be discussed below and the correct status and identification of the types will be presented. A note is also included on *Labeo lividus* Roberts et Stewart, 1976, a species described from the lower Congo rapids, and previously a junior synonym of *L. barbatus*. Additional inconsistencies were further encountered within the type series of *Labeo altivelis* Peters, 1852, a species described from the lower Zambezi but also present in the upper reaches of the Congo basin.

MATERIALS AND METHODS

An overview of the type specimens listed in the "check-list of the freshwater fishes of Africa" (CLOFFA, Lévêque and Daget 1984) and in the subsequent revisions on African (Reid 1985) and Congolese *Labeo* species (Tshibwabwa and Teugels 1995, Tshibwabwa 1997) is provided for *L. barbatus*, *L. falcipinnis*, *L. lineatus*, *L. longipinnis*, *L. macrostoma*, and *L. velifer*, and for *L. lividus* and *L. altivelis* (Table 1). For the former six species, the correct list of type specimens was established by analysing the correspondence between Boulenger and the RMCA. These letters are kept in the archives of the Ichthyology lab of the RMCA, where they can be consulted. For each of the revisions (Reid 1985, Tshibwabwa and Teugels 1995, Tshibwabwa 1997), the validity of the lectotype designations was verified. In some cases, two specimens were de facto designated as the lectotype for one and the same species in a single publication. As a lectotype needs to be a unique, unambiguously selected specimen (Anonymous 1999: Article 74.1), these designations are conflicting nomenclatural acts. Therefore, the actions of subsequent authors had to be taken into account. Type specimens were examined, museum archives and catalogues were consulted and labels were checked. Several paralectotypes were re-identified. Institutional abbreviations for collections follow Eschmeyer and Fricke (2015). This implies that for the collections, the RMCA and the NHM will be referred to as MRAC and BMNH, respectively.

RESULTS

***Labeo* specimens sent to G.A. Boulenger in London.** For the six species of *Labeo*—described in Boulenger's (1898) publication: *L. barbatus, L. falcipinnis* (originally described as *L. falcifer*), *L. lineatus*, *L. longipinnis*, *L. macrostoma*, and *L. velifer*—no collection numbers of the specimens used for the original descriptions were provided. Yet, for species described before 2000, any evidence, published or unpublished, may be taken into account to determine what specimens constitute the type series (Anonymous 1999: Article 72.4.1.1). In this case, a list of 147 specimens that were shipped from the Congo Museum to the BMNH was included in the correspondence between E. Coart, secretary and future director of the Congo Museum and G.A. Boulenger (6 June 1898). More importantly, Boulenger added a detailed list

Table 1

Overview of the type specimens of the seven Congolese *Labeo* species discussed in this study

Species	SOURCE					
	Boulenger (unpublished)	Lévêque and Daget 1984	Reid 1985	Tshibwabwa and Teugels 1995	Tshibwabwa 1997	Presently reported study
L. barbatus Boulenger, 1898	BMNH 1898.12.28:7, MRAC 37, 59, 93	ST: BMNH 1898.12.28:7, MRAC 37, 59, 93	**LT: BMNH 1898.12.28:7^{R}** PLT: "in [MRAC] ? missing"	**LT: BMNH 1898.12.28:7^{R}** PLT: MRAC 37, 59, 93	**LT: MRAC 37^{T}** **LT: BMNH 1898.12.28:7$^{?}$** PLT: BMNH 1898.12.28:7*, MRAC 37*, 59, 93	**LT: BMNH 1898.12.28:7^{R}** PLT: MRAC 37, 59, 93
L. falcipinnis Boulenger, 1903	BMNH 1898.12.28:2, MRAC 17, 65, 69	ST: BMNH 1898.12.28:7, MRAC 17, 65, 69	**LT: BMNH 1898.12.28:2^{R}** PLT: [MRAC] 18 (**MRAC 17$^{R, IL}$**)	**LT: MRAC 17$^{T\&T}$** PLT: BMNH 1898.12.28:2, MRAC 65, 69	**LT: MRAC 17$^{T\&T}$** PLT: BMNH 1898.12.28:2 MRAC 65, 69	**LT: MRAC 17^{R}** PLT: BMNH 1898.12.28:2, MRAC 65, 69
L. lineatus Boulenger, 1898	BMNH 1898.12.28:3-4(2), MRAC 12, 19, 91, 138	ST: BMNH 1897.9.30:27, 1898.12.28:3-4(2), MRAC 12, 19, 91, 138, 863, 864(2)	**LT: BMNH 1897.9.30:27^{R}** PLT: BMNH 1898.12.28:3-4(2) "? other types in [MRAC]" (**MRAC 138$^{R, IL}$**)	**LT: BMNH 1897.9.30:27^{R}** PLT: MRAC 12, 138, *L. degeni*: *BMNH 1898.12.28:4, L. weeksii*: *BMNH 1898.12.28:3, MRAC 19, 91*	**LT: MRAC 138^{T}** PLT: BMNH 1897.9.30:27, MRAC 12, 138*, *L. degeni*: *BMNH1898.12.28:4,L.weeksii*: *BMNH 1898.12.28:3, MRAC 19, 91*	**LT: BMNH 1897.9.30:27^{R}** PLT: BMNH 1898.12.28:4, MRAC 138, *L. weeksii*: *BMNH 1898.12.28:3, MRAC 12, 19, 91*
L. longipinnis Boulenger, 1898	MRAC 113	ST: BMNH 1898.12.28:1, MRAC 113	**HT: [MRAC] 113**	**HT: MRAC 113**	**HT: MRAC 113**	**HT: MRAC 113**
L. macrostoma Boulenger, 1898	BMNH 1898.12.28:5-6(2), MRAC 35, 60	ST: BMNH 1898.12.28:5, MRAC 35, 60, 866	**LT: [MRAC] 866^{R}** PLT: BMNH 1898.12.28:5 "? others in [MRAC]"	**LT: MRAC 35$^{T\&T}$** PLT: BMNH 1898.12.28:5, MRAC 60	**LT: MRAC 35$^{T\&T}$** PLT: BMNH 1898.12.28:5, MRAC 60	**LT: MRAC 35$^{T\&T}$** PLT: BMNH 1898.12.28:5, MRAC 60, *L. nasus*: *BMNH 1898.12.28:6*
L. velifer Boulenger, 1898	BMNH 1898.12.28:1, MRAC 22, 82, 157	ST: MRAC 22, 82, 157	**LT: BMNH 1898.12.28:1^{R}** [MRAC] 22, 82, 157 (**MRAC 82$^{R, IL}$**)	ST: BMNH 1898.12.28:1, MRAC 22, 82, 157	**LT: BMNH 1898.12.28:1^{R}** PLT: MRAC 22, 82, 157	**LT: BMNH 1898.12.28:1^{R}** PLT: MRAC 22, 82, 157
L. lividus Roberts et Stewart, 1976		**HT: MCZ 50524** PT: MCZ 50232(10), 50287(5), 50371(1), 50452(4), 50525(5)	**HT: MCZ 50524** PT: BMNH 1976.5.21:16-17(2), MCZ 5025(5), 50232(8), 50287(5), 50371(1), 50452(4)	only partially listed: PT: BMNH 1976.5.21:16-17(2), MCZ 50232(6), 50287(5), MRAC 76-17-P-4	only partially listed: PT: BMNH 1976.5.21:16-17(2), MCZ 50232(6), 50287(5), MRAC 76-17-P-4	**HT: MCZ 50524** PLT: BMNH 1976.5.22.16-17(2), MCZ 50232(6), 50287(5), 50371(1), 50452(4), 50525(4), MRAC 76-17-P-4, USNM 216370(2)
L. altivelis Peters, 1852		ST: [ZMB] 3283, 3284, 3285, 3286, 3287 (6 in total)	**LT: ZMB 3284^{R}** PLT: ZMB 3283, 3285, 3286, 3287, NMW 16097	not listed	**LT: [ZMB] 3284^{R}** PLT: [ZMB] 3283, 3287	**LT: ZMB 3284^{R}** PLT: ZMB 3283, 3285, 3286, NMW 16097

Presumed unique name-bearing specimens (holo- and lectotypes), whether erroneous or valid, are presented in bold; valid (including ambiguous) lectotype designations and selections made by the first reviser are underlined; specimens listed as belonging to a different species are in italics and specimens between brackets are not listed by the authors but referred to in an illustration; HT = holotype, LT = lectotype, ST = syntypes, PT = paratype(s), PLT = paralectotype(s); Superscripts denote lectotype designation claimed to have been made by: Reid 1985 (R), Tshibwabwa & Teugels 1995 (T&T), Tshibwabwa 1997 (T), not specified (?), designation made through an illustration (IL), * = specimens that are listed twice and with a different status, institutional abbreviations between brackets: [MRAC] and [ZMB] were listed by the authors as RGMC and ZMHU, respectively, except when indicated with '( )'; all collection numbers contain only one specimen.

of identifications to the collections when these were sent back. Here, 23 specimens were mentioned that served for the description of these six *Labeo* species. Hence, these specimens should all be considered types. As this list was preserved, the collection numbers of these specimens could be traced.

As many specimens from the original collection of the Congo exhibition stayed in London and therefore were no longer part of the RMCA collection, by the end of the 1940s, a new numbering system was put in place. Yet, the link with the original collection numbers was kept as the new collection numbers were added to the original registers. Hence, we were able to identify the *Labeo* types in the RMCA belonging to this collection. In Boulenger's list of identifications, the original collection numbers of seven specimens of *L. barbatus*, *L. falcipinnis*, *L. lineatus*, *L. macrostoma*, and *L. velifer* were put between brackets. These specimens apparently stayed in the NHM. When consulting the registers of the NHM online, we noticed that these specimens were registered under the collection numbers BMNH 1898.12.28:1 through 7 (Table 1).

For each species, one collection number was underlined in Boulenger's list of identifications. This was also the only number that was originally listed in the registers of the RMCA as the 'type', together with the abbreviation SN (spécimen nominal). We studied the specimens corresponding to the underlined collection numbers and these proved to be the illustrated specimens presented in the original species descriptions (Boulenger 1898). These specimens are: MRAC 37 for *L. barbatus*, MRAC 17 for *L. falcifer* Boulenger, 1898 (later replaced by *L. falcipinnis* Boulenger, 1903), MRAC 138 for *L. lineatus*, MRAC 113 for *L. longipinnis*, MRAC 35 for *L. macrostoma*, and MRAC 82 for *L. velifer.* However, except for providing illustrations of them, Boulenger (1898) did not treat these specimens differently in the original descriptions. Hence, except for MRAC 113, the sole type specimen and therefore the holotype of *L. longipinnis*, all 23 *Labeo* specimens sent to Boulenger had, at the time, equal status as syntypes.

Confusion on the type series of the *Labeo* species from the Congo exhibition. In spite of the detailed list of identifications provided by Boulenger, different authors provided different lists of type specimens (Table 1). Partially, this could be explained by the fact that the information present in Boulenger's correspondence has never been published. Yet, based on this correspondence, we obtained the complete list of specimens sent from the Congo exhibition to the NHM, which contained the type specimens of the six species (Table 1). Here, we clarify some of the confusion concerning the type series of *L. barbatus*, *L. falcipinnis*, *L. lineatus*, and *L. macrostoma*.

In the description of *L. barbatus*, Boulenger (1898) stated that he used specimens from Boma and Matadi. Therefore, specimen MRAC 93, from Manyanga, could, at first sight, not be part of the type series. However, Boulenger (1898) mentioned that he had, next to a large specimen (MRAC 37, from Matadi), several juvenile specimens from Matadi at his disposal. Boulenger (1909) only mentioned the presence of one type in the NHM (corresponding to BMNH 1898.12.28:7, from Boma). Hence, all Matadi specimens must be in the collections of the RMCA. As no other *L. barbatus* were present in the collections at the time, these juveniles must correspond with MRAC 59 (from Matadi) and MRAC 93 (from Manyanga). Most probably, Boulenger (1898) made a mistake in referring to the Manyanga specimen as also originating from Matadi. Reid (1985) mentioned that besides BMNH 1898.12.28:7, all other type specimens of *L. barbatus* were probably missing. They were, however, present in the RMCA collections.

As *L. falcipinnis* was published as a replacement name of *L. falcifer* Boulenger, 1898, which was preoccupied by *L. falcifer* Valenciennes, 1842 (currently *Lobocheilos falcifer*, from Southeast Asia), both species have the same name-bearing types (Anonymous 1999: Article 72:7). For *L. falcipinnis*, Reid (1985) erroneously listed MRAC 18 (using the old abbreviation RGMC 18) as the sole paralectotype. Although this specimen was also part of the original collection of the Congo exhibition, it is not a *Labeo*, but a *Synodontis* Cuvier, 1816 catfish. Boulenger (1899) used this specimen for the description of *Synodontis acanthomias* Boulenger, 1899, for which it is the lectotype (designated by Poll 1971).

The specimen that Reid (1985) listed as the lectotype of *L. lineatus:* BMNH 1897.9.30:27 (Table 1), was not one of the six types listed in Boulenger's correspondence. Yet, in the description of *L. lineatus*, Boulenger (1898) mentioned that a specimen was "also" collected by Bentley at Stanley Falls. This implies that, next to the specimens present in the collection of the Congo exhibition, which were collected by Wilverth, Wagenaar, and De Bauw, an additional specimen was available. Hence, BMNH 1897.9.30:27 represents the seventh type specimen of *L. lineatus*. This specimen was originally identified as *Labeo brachypoma* Günther, 1868. Lévêque and Daget (1984) listed additional types of *L. lineatus* and included MRAC 863 and MRAC 864 (2) in the list of syntypes. Yet, Tshibwabwa and Teugels (1995) showed this to be wrong as Delhez—a naturalist and artist who worked as a collector in service of the government of the Congo Free State—collected these specimens between the 11 and the 18 of February 1899. Hence, Boulenger could not have used them for the description of *L. lineatus*, which was published in December 1898.

Although the BMNH registers and the correspondence between Boulenger and the RMCA showed that BMNH 1898.12.28:6 was a syntype of *L. macrostoma*, this specimen was not listed as such in any of the revisions (Reid 1985, Tshibwabwa and Teugels 1995, Tshibwabwa 1997) nor in the "Check-list of the fresh water fishes of Africa" (Lévêque and Daget 1984) (Table 1). This specimen subsequently served, together with MRAC 867, as a syntype for the description of *Labeo nasus* Boulenger, 1899. Reid (1985) later designated MRAC 867 as the lectotype of this species. As BMNH 1898.12.28:6 was also a type specimen of *L. nasus*, it was probably overlooked by subsequent authors as a member of the type series of *L. macrostoma*.

Conflicting and erroneous lectotype designations. In his revision of African *Labeo,* Reid (1985) designated lectotypes for all of the six species present in the collection of the Congo exhibition except for *L. longipinnis*. The following numbers were provided (Reid 1985): BMNH 1898.12.28:7 for *L. barbatus*; BMNH 1898.12.28:2 for *L. falcipinnis*; BMNH 1897.9.30:27 for *L. lineatus*; MRAC 866 for *L. macrostoma*; and BMNH 1898.12.28:1 for *L. velifer* (Table 1). For this, he was criticised by Thys van den Audenaerde (1987), who referred to a convention between Boulenger and the RMCA that only doubles were to be kept in the collections of the BMNH and that unique name-bearing types were registered in the RMCA. Boulenger indeed acted according to this convention by returning the illustrated specimens to the RMCA. Only this specimen was listed as "type" in the register of the RMCA. For the other specimens, a note stating that they were syntypes was added later. Thys van den Audenaerde (1987) argued that Boulenger (1909) also followed this convention in his "Catalogue of the fresh-water Fishes of Africa in the British Museum". Here, all syntypes that remained in the NHM were listed as being "one of the types". Those that were returned to the RMCA were listed as "types" and the illustrated specimen was referred to as the "type". Boulenger (1909) did, however, use the word "types" in his catalogue to refer to the type specimens of species for which the complete type series was housed in the NHM (e.g., for *Labeo brachypoma* Günther, 1868 and for *Labeo walkeri* Günther, 1903). Although this could suggest that Boulenger already had a notion of holo- and paratypes in mind, this distinction was not yet clearly made at the end of the 19th century. Hence, all type specimens of *L. barbatus*, *L. falcipinnis*, *L. lineatus*, *L. macrostoma*, and *L. velifer* had equal status as syntypes. The convention referred to by Thys van den Audenaerde (1987) does not challenge the validity of the lectotype designations made by Reid (1985). Hence, the fact that for some of these species, other specimens than those put forward by Boulenger (1898) were designated as lectotypes seems irrelevant. Yet, there is more to the story.

For all six *Labeo* species described by Boulenger (1898), Reid (1985) provided the same illustrations as Boulenger (1898). For *L. falcipinnis*, *L. lineatus*, and *L. velifer*, but not for *L. barbatus* and *L. macrostoma*, Reid (1985) referred to these figures as representing the species' lectotype. A lectotype designation by means of an illustration is to be treated as a designation of the specimen illustrated (Anonymous 1999: Article 74.4). Hence, Reid (1985) implicitly designated MRAC 17, MRAC 138, and MRAC 82 as the lectotypes of *L. falcipinnis*, *L. lineatus*, and *L. velifer*, respectively. This implies that he provided two separate lectotype designations for these three species, one by means of an illustrated specimen (MRAC 17, MRAC 138, and MRAC 82 for *L. falcipinnis*, *L. lineatus*, and *L. velifer*, respectively) and one by means of a collection number (BMNH 1898.12.28:2, BMNH 1897.9.30:27, and BMNH 1898.12.28:1 for *L. falcipinnis*, *L. lineatus*, and *L. velifer*, respectively). As there is no page priority in nomenclatural acts, none of these double designations has priority over the other. Hence, following the principle of the first reviser (Anonymous 1999: Article 24.2), the first author that selects one of these acts needs to be followed.

Also for *L. barbatus*, the illustrated specimen does not correspond to the number of the lectotype given by Reid (1985). Yet, no reference is given about the type status of the illustrated specimen. Therefore, the designation of BMNH 1898.12.28:7 as the lectotype of *L. barbatus* is valid, notwithstanding institutional conventions or the fact that recommendations 74 B, E, and F (Anonymous 1999) were ignored. This implies that the subsequent lectotype designations for this species done by Tshibwabwa (1997), which were ambiguous (Table 1), can be ignored. Finally, Tshibwabwa and Teugels (1995) demonstrated that the specimen Reid (1895) designated as the lectotype of *L. macrostoma* (MRAC 866) was not part of the original type series, as it was collected after the species was described. Further, it was not listed in Boulenger's correspondence either. Therefore, Tshibwabwa and Teugels (1995) designated MRAC 35 as the lectotype of *L. macrostoma* instead.

As Reid's (1985) lectotype designations for *L. falcipinnis*, *L. lineatus*, and *L. velifer* were ambiguous, the actions of further reviewers needed to be taken into account. Remarkably, Tshibwabwa and Teugels (1995) and Tshibwabwa (1997) differed largely in accepting the different lectotype designations made by Reid (1985), without providing an explanation for this. In several cases, they ignored Reid's (1985) actions and designated lectotypes themselves. Such a 'designation' should, however, be interpreted as a selection by the first reviser if the specimen is one of Reid's (1985) ambiguously defined 'lectotypes'. For *L. falcipinnis*, Tshibwabwa and Teugels (1995) claimed to designate MRAC 17, the illustrated specimen from the original description (Boulenger 1898), as the lectotype, hence implicitly rejecting Reid's designation of BMNH 1898.12.28:2 as the lectotype. For *L. lineatus*, they accepted BMNH 1897.9.30:27 as the lectotype, as designated by Reid (1985), also without further comments. For *L. velifer*, however, Tshibwabwa and Teugels (1995) ignored Reid's lectotype designations and listed all specimens as syntypes. Tshibwabwa (1997) followed Tshibwabwa and Teugels (1995) for *L. falcipinnis*, in listing MRAC 17 as the lectotype. For *L. lineatus,* he disagreed with Tshibwabwa and Teugels (1995) and claimed to designate MRAC 138 as the lectotype instead. For this, he referred to the convention between G.A. Boulenger and the RMCA, which was discussed above. Tshibwabwa (1997) did, however, list MRAC 138 both as the lectotype and as a paralectotype of *L. lineatus*. For *L. velifer*, Tshibwabwa (1997) referred to BMNH 1898.12.28:1 as the lectotype as designated by Reid (1985) (Table 1).

Given the ambiguity of three of Reid's (1985) lectotype designations (see above), Tshibwabwa and Teugels (1995) are to be considered the first revisers that selected MRAC 17 as the lectotype of *L. falcipinnis* and BMNH 1897.9.30:27 as the lectotype of *L. lineatus*. Tshibwabwa (1997) acted

as the first reviser in selecting BMNH 1898.12.28:1 as the lectotype of *L. velifer*. Reid (1985) remains, however, the author of these lectotype designations (see the example at Anonymous 1999: Article 24.2).

The composite nature of the type series of *L. macrostoma* and *L. lineatus*. For two of the six species in the collection of the Congo exhibition, the type series proved to be polyspecific. The first case concerns the type series of *L. macrostoma*, for which we already mentioned above that BMNH.12.28:6 is a paralectotype of both *L. macrostoma* and *L. nasus*.

Tshibwabwa and Teugels (1995) showed that the type series of *L. lineatus* is polyspecific as well. They transferred BMNH 1898.12.28:4 to *L. degeni* and BMNH 1898.12.28:3, MRAC 19 and 91 to *Labeo weeksii* Boulenger, 1909. We verified these identifications and confirmed the re-identifications except for BMNH 1898.12.28:4, which belongs to *L. lineatus* and not to *L. degeni*. In addition, MRAC 12 also proved to be a *L. weeksii*. Therefore, of the six paralectotypes of *L. lineatus*: only two (BMNH 1898.12.28:4 and MRAC 138) are conspecific with the lectotype (Table 1). Also for *L. barbatus*, it was suggested that the type series is polyspecific as one paralectotype has been re-identified as *L. lividus*. This will be discussed in the following section.

The revalidation of *L. lividus* and its affinity with *L. barbatus*. In a study on the ichthyofauna of the rapids of the lower Congo River (Roberts and Stewart 1976), *L. lividus* was described, a species claimed to be very similar to *L. barbatus*. Although this was not explicitly mentioned in the description of the species, Roberts re-identified MRAC 93 (at that time a syntype of *L. barbatus*) as *L. lividus*. Given their similarity, Reid (1985) synonymised *L. lividus* with *L. barbatus*, albeit with hesitation. This synonymy was further confirmed by Tshibwabwa and Teugels (1995) and by Tshibwabwa (1997).

In a DNA barcoding study, however, Lowenstein et al. (2011) found molecular support for a separate specific status of *L. lividus*. They revalidated *L. lividus* and confirmed the distinguishing characters listed by Roberts and Stewart (1976) that differentiate the two species: shorter, paler barbels and larger, more heavily keratinised lip papillae in *L. lividus* than in *L. barbatus*. Roberts and Stewart (1976) further mentioned that in *L. barbatus*, both barbels are of almost equal length whereas in *L. lividus* the anterior barbels are shorter than the posterior. We examined specimen MRAC 93 and compared it with a paratype of *L. lividus* housed in the RMCA (MRAC 76.17.P.4) and with the description of the species. Although this specimen has the characteristic large keratinised papillae of *L. lividus* on its lower lips, its anterior and posterior barbels are of almost equal length, as in *L. barbatus*. A morphometric study including type specimens of both species, however, confirmed the status of MRAC 93 as *L. barbatus*. It should be noted that the morphological differences between *L. barbatus* and *L. lividus* need to be examined in more detail, which will be part of a forthcoming study. The type series of *L. lividus*, containing 26 specimens was originally housed in the MCZ. Afterwards, some of the paratypes have been transferred to three additional museums, i.e., the MCZ, NHM, and RMCA (Table 1).

Note on the type series of *L. altivelis*. Some problems were also encountered with the type series of *L. altivelis* Peters, 1852. In the original description, Peters (1852) mentioned that the specimens originated from Tete, without mentioning how many were used for the description. Yet, as Peters (1852) listed variation in scale counts, there must be more than one type specimen. Reid (1985) designated a lectotype, both by referring to an illustration (taken from Peters 1868) and by listing collection number ZMB 3284. After examination of the type series, we found that the illustrated specimen indeed corresponded to ZMB 3284. For the paralectotypes, Reid (1985) listed: ZMB 3283, 3285, 3286, 3287, and NMW 16097 whereas Tshibwabwa (1997) only mentioned ZMB 3283 and 3287 (both used the abbreviation for the Berlin museum in use at the time: ZMHU). Both authors erroneously referred to five and three type specimens (lecto- and paralectotypes) housed in the Berlin museum respectively, hence ignoring that two specimens were catalogued under ZMB 3287. These two specimens, however, did not originate from Tete, but from Quellimane (on the Rio de Bons Sinais), a locality mentioned in a redescription of the species (Peters 1868), together with the Licuare River (a tributary of the Rio de Bons Sinais basin, near Quellimane) and Sena (on the Zambezi), but not in the original description. Therefore, these specimens (ZMB 3287) are not types. Specimens ZMB 3283, 3285, and 3286 are all from the type locality and were caught at the same time by the same collector. Moreover, specimen ZMB 3286 was even kept in the same jar as the lectotype. Hence, there is no reason not to consider these specimens as paralectotypes. As for the specimen in the Vienna museum, NMW 16097, it could only be confirmed that this specimen originates from Mozambique and came in the possession of the Vienna museum in 1854. This specimen was acquired through an exchange with Peters, so it cannot be excluded that this is also a type. Moreover, it was exchanged for 24 specimens, which indicates its high value (Wellendorf H. 2014, personal communication). Therefore, until further clarification, NMW 16097 ought to be considered the fourth paralectotype of *L. altivelis*.

DISCUSSION

This study exemplifies that correctly listing the types of a given species is often not a trivial task. Indeed, it has potentially profound consequences as nomenclatural stability depends heavily on name-bearing types. Fortunately, the taxonomic issues presented here did not have any nomenclatural consequences. Yet, given the composite nature of the type series of *L. lineatus* and *L. macrostoma*, nomenclatural confusion could have arisen easily. For *L. lineatus*, more than half of the type specimens are *L. weeksii*. Luckily, the different authors referred to the same biological species when they discussed *L. lineatus*. Indeed, the true (BMNH 1897.9.30:27) and the 'erroneous' lectotype (MRAC 138) do belong to the same species. Yet, the importance of a careful designation

of a lectotype is perhaps best illustrated by the cases of *L. macrostoma* and *L. nasus*. Until Reid's (1985) revision, specimen BMNH 1898.12.28:6 was a syntype, and therefore a name-bearing specimen of both nominal species. Hence, if a different choice of lectotypes had been made, these species would have become synonymies even though two biological species do exist. Given the important nomenclatorial consequences of lectotype designations, the ICZN specifies that they need to be done individually (Anonymous 1999: Article 74.3) and should be done as part of a revisionary work (Anonymous 1999: Recommendation 74G). The current study should therefore be viewed as part of an ongoing endeavour to revise the *Labeo* of the Congo basin (Van Steenberge et al. 2014).

Especially in older species descriptions, it was not common practice to list the number of specimens used, let alone to mention their collection numbers. This problem is recognised by the ICZN as the code explicitly allows the usage of all kinds of evidence to determine what specimens constitute the type series (Anonymous 1999: Article 72.4.1.1). Hence, correctly listing the types may require studying the original literature, the museum registers and databases, investigating specimens and museum labels and, potentially, speculating on the intentions of the author or the reviewer.

Yet, incomplete knowledge of a species' type series could unintentionally lead to violations of the rules of the international code of zoological nomenclature and to nomenclatural instability. In this regard, we can only applaud initiatives such as the Catalogue of Fishes (Eschmeyer and Fricke 2015), which disseminates crucial basic taxonomic information, including lists of type specimens. Moreover, the increasing effort put in the digitalisation of natural history collections (Baird 2010), e.g., in FishBase (Froese and Pauly 2016), will hopefully reduce the number of taxonomic inconsistencies in the future.

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The African hexaploid Torini (Cypriniformes: Cyprinidae): review of a tumultuous history

EMMANUEL J. W. M. N. VREVEN[1,2]*, TOBIAS MUSSCHOOT[1], JOS SNOEKS[1,2] and ULRICH K. SCHLIEWEN[3]

[1]*Vertebrate section, Ichthyology, Royal Museum for Central Africa, RMCA, Leuvensesteenweg 13, Tervuren B-3080, Belgium*
[2]*KU Leuven, Laboratory of Biodiversity and Evolutionary Genomics, Charles Deberiotstraat 32, B-3000 Leuven, Belgium*
[3]*SNSB Bavarian Natural History Collections, Bavarian State Collection of Zoology, Department of Ichthyology, Münchhausenstrasse 21, 81247 München, Germany*

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A review of the tumultuous history of the alpha- and genus-level taxonomy of the hexaploid African Torini, i.e. *Labeobarbus s.l.* (synonym: *Varicorhinus*), as well as of the closely related monospecific genera *Acapoeta* and *Sanagia*, is provided. The main purpose of the present paper is to provide a continental framework for multidisciplinary research on this megadiverse vertebrate group. Based on the inspection of almost all relevant type specimens, a complete and fully annotated checklist of all valid species and junior synonyms is provided for each of the four nominal genera. It comprises ~275 nominal taxa, 125 of which are valid African *Labeobarbus* spp. and three of which are formally named hybrid phenotypes. Particular attention is drawn to the diversity and taxon-specific distribution of mouth phenotypes, which previously served as generic identification characters, i.e. 'rubberlips' with strongly developed, sometimes hypertrophied lips and a mental lobe, and 'chiselmouths' (former *Varicorhinus* spp.) with a cornified sharp cutting edge on their lower jaw. Interestingly, many species are polymorphic and include a large array of intermediate mouth phenotypes. The recurrent occurrence of similar mouth phenotype diversity within, but also far beyond, the borders of *Labeobarbus s.l.* is highlighted, raising numerous evolutionary questions.

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ADDITIONAL KEYWORDS: *Acapoeta* – hexaploidy – hybridization – intraspecific variation – *Labeobarbus* – mouth phenotype – mouth polymorphism – *Sanagia* – *Varicorhinus*.

'. . . one of the most difficult with which it has ever been my lot to deal.' (Boulenger, 1902f: 422)

INTRODUCTION

This paper is aimed to provide an exhaustive review of the systematics and taxonomy of all ~275 described African, large-sized, hexaploid, torine taxa (i.e. Cyprininae: see Yang *et al.*, 2015). These are members of the genus *Labeobarbus* Rüppell, 1835 *s.l.* ('rubberlips'), including its recent junior synonym *Varicorhinus* Rüppell, 1835 ('chiselmouths') (see Tsigenopoulos, Kasapidis & Berrebi, 2010; Berrebi *et al.*, 2014 and Yang *et al.*, 2015), as well as of the two monospecific genera *Acapoeta* Cockerell, 1910 and *Sanagia* Holly, 1926 [i.e. ~275(+2)]. Whereas both of the latter monospecific genera are highly restricted in distribution, i.e. endemic to Lake Tanganyika[1] and the Sanaga River in Cameroon, respectively, the genus *Labeobarbus s.l.* is wide-

*Corresponding author. E-mail: emmanuel.vreven@africamuseum.be

[1]*Acapoeta tanganicae* has recently been collected from the Songwe River (Lake Rukwa basin) and reveals that the species is not endemic to the Lake Tanganyika and its immediately proximate sections of connected rivers (Genner *et al.*, 2015).

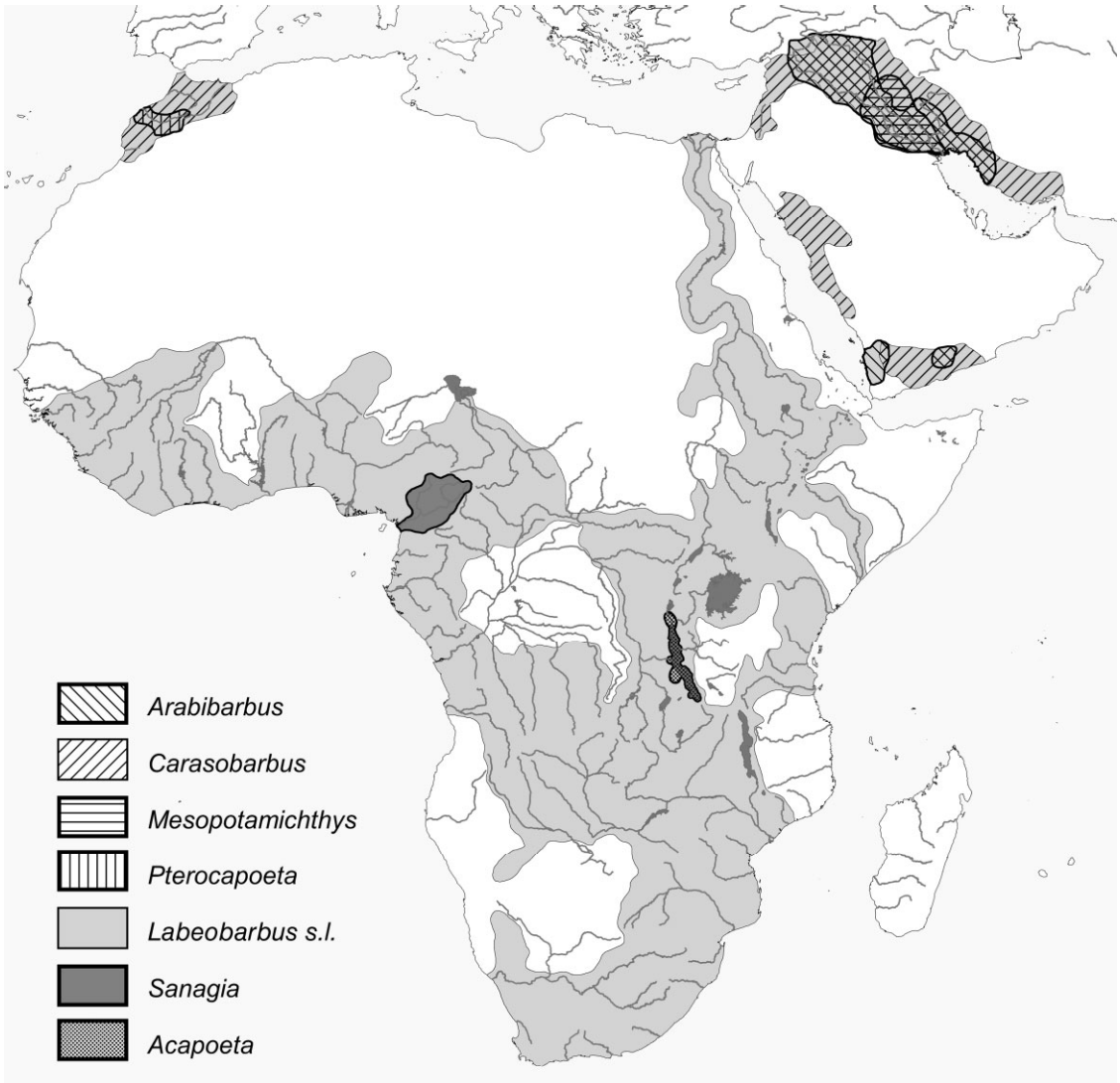


Figure 1. Distribution map of *Labeobarbus s.l. fide* Tsigenopoulos *et al*. (2010) and Berrebi *et al*. (2014), and of *Arabibarbus*, *Carasobarbus*, *Mesopotamichthys*, and *Pterocapoeta* as currently delimited by Borkenhagen (2014) and Borkenhagen & Krupp (2013). As a matter of completeness, the distribution of the monospecific African genera *Acapoeta* and *Sanagia* has been added as well.

spread and present in each of the ten African ichthyofaunal provinces as currently defined (see Snoeks, Harrison & Stiassny, 2011: fig. 3.1; modified from Roberts, 1975) (Fig. 1).

Their complicated history cannot be reviewed adequately without referring to closely related genera from the Arabian Peninsula and Asia, and without referring to three peculiarities of these Torini: (1) their chromosomal ploidy level; (2) their mouth phenotype variation; (3) the repeatedly observed occurrence of their mouth polymorphism. Whereas to date the ploidy level of *Acapoeta* and *Sanagia* remains unknown, the hexaploidy of rubberlips and chiselmouths has been established for numerous species (see Oellermann & Skelton, 1990; Krysanov, Golubtsov & Alexeev, 1991; Golubtsov & Krysanov, 1993; Krysanov & Golubtsov, 1996; Guégan *et al*., 1995; and Arai, 2011). The current compilation lists a total of 275 nominal African species to be included in *Labeobarbus s.l*. Of these, only 125 nominal species are still considered valid African *Labeobarbus* spp., whereas the majority, i.e. 147, are junior synonyms (including one suppressed name), and three others are formally named hybrid phenotypes. The present situation testifies already to the tumultuous taxonomic history of the group as a whole.

Originally, African chiselmouths (former *Varicorhinus* spp.) were diagnostically differentiated from rubberlips (former *Labeobarbus s.s*.) by a cornified sharp cutting edge on their lower jaw, as compared with the typically strongly developed, sometimes even hyper-

trophied lips and a mental lobe. The subsequent discovery of numerous species with several intermediate mouth phenotypes (see below), as well as the occurrence of *Labeobarbus*-like and *Varicorhinus*-like mouth phenotypes outside of Africa, have blurred that systematic pattern, however.

Apart from this African *Labeobarbus–Varicorhinus* (*Lab.–Var.*) species complex, similar patterns of polyploidy and mouth phenotype polymorphism have evolved outside of Africa and in different genera: (1) in the Middle East genus *Arabibarbus* Borkenhagen, 2014 [in *Arabibarbus grypus* (Gray, 1834) (see Roberts & Khaironizam, 2008: fig. 8; Borkenhagen, 2014)]; (2) in the Middle East and North African (sub)genus *Carasobarbus* Karaman, 1971 (synonym: *Kosswigobarbus* Karaman, 1971) [in *Carasobarbus fritschii* (Günther, 1874) see Borkenhagen & Krupp, 2013: fig. 3]; (3) as well as in the Southeast Asian genus *Neolissochilus* Rainboth, 1985 [in *Neolissochilus soroides* (Duncker, 1904) (see Roberts & Khaironizam, 2008: figs 3–6; and Khaironizam, Zakaria-Ismail & Armbruster, 2015: fig. 6B–D)]. All these genera appear closely related to *Labeobarbus* in the wider sense (see Tsigenopoulos *et al*., 2010: fig. 1; Borkenhagen *et al*., 2011: fig. 2; Borkenhagen, 2014: fig. 8; Yang *et al*., 2015: figs 2, 4). Whereas *Carasobarbus* seems to be composed of hexaploid species [*Carasobarbus canis* (Valenciennes, 1842) in Cuvier & Valenciennes, 1842 (see Gorshkova, Gorshkov & Golani, 2002; Arai, 2011)], the ploidy level of *Arabibarbus* remains unknown, but several species of the very closely related southern and Southeast Asian genera *Tor* Gray, 1834 and *Neolissochilus* are all tetraploid [including the type species *Tor tor* (Hamilton, 1822), see Suzuki & Taki, 1981; Khuda-Bukhsh, 1982; Khuda-Bukhsh, Chanda & Barat, 1986; Rishi & Shashikala, 1994; Khuda-Bukhsh *et al*., 1995; Sahoo, Nanda & Barat, 2007; Mani *et al*., 2009; Arai, 2011; the polyploidy level of type species *Neolissochilus sumatranus* (Weber & de Beaufort, 1916) is unknown].

Interestingly, the Caucasian and western Asian (see Bănărescu, 1999) genus *Capoeta* Valenciennes, 1842 (in Cuvier & Valenciennes, 1842) is also composed of hexaploids [including the type species *Capoeta capoeta* (Gueldenstaedt, 1773); see Krysanov 1999; Safar *et al*., 2000; Kiliç Demirok & Ünlü, 2001; Gorshkova *et al*. 2002; Arai, 2011)]. *Capoeta* is member of a distantly related mitochondrial lineage [defined as the *Barbus s.s.* lineage by Berrebi *et al*. (2014); and Cyprininae (Tribe: Barbini) in Yang *et al*. (2015: table 3)], also comprising the European, Middle Eastern, and north-west African (see Tsigenopoulos *et al*., 2003) genus *Luciobarbus* Heckel, 1843, the European and West-Asian (see Kottelat & Freyhof, 2007) genus *Barbus s.s.*, and the Bosnia-Herzegovinan and Croatian endemic (see Kottelat & Freyhof, 2007) and monospecific genus *Aulopyge* Heckel, 1841. In contrast to *Capoeta*, all latter genera are tetraploid [including both the type species, i.e. *Barbus barbus* (Linnaeus, 1758) and *Aulopyge huegelii* Heckel, 1843; see Collares-Pereira & Madeira, 1990; Collares-Pereira & Moreira da Costa, 1999; Arai, 2011; the polyploidy level of the type species *Luciobarbus esocinus* Heckel, 1843 is unknown]. Whereas *Capoeta* species invariably exhibit a typical chiselmouth phenotype, with a characteristic cutting edge covered by a horny sheet on the lower jaw (see Bănărescu, 1999), *Luciobarbus*, *Barbus* (see Kottelat & Freyhof, 2007: fig. 31), and *Aulopyge* instead have a rubbermouth-like or intermediate mouth phenotype. As such, and in contrast to the polymorphic African *Labeobarbus*, the mouth phenotype polymorphism in those other genera appears to covary with mitochondrial lineages.

Even for unrelated central Asian cyprinid genera, Roberts & Khaironizam (2008) pointed to the occurrence of similar mouth phenotype polymorphism in *Schizothorax* Heckel, 1838 [Cyprininae (Tribe: Schizothoracini): see Yang *et al*. (2015: table 3)], which is endemic to the Qinghai–Tibetan Plateau (Roberts & Khaironizam, 2008; see Qi *et al*., 2012: 7, fig. 4). Although its polymorphism is slightly different (see Roberts & Khaironizam, 2008: fig. 9), it is nevertheless clearly reminiscent of the *Lab.–Var.* type and, interestingly, *Schizothorax* is composed of both tetraploid as well as hexaploid species (the polyploidy level of the type species *Schizothorax esocinus* Heckel, 1838 is unknown; for other species, see Zan, Song & Liu, 1986; Collares-Pereira, 1994; and Arai, 2011).

In combination, all these observations suggest a recurrent and possibly correlated pattern of polyploidy and mouth phenotype polymorphism in these cyprinid lineages. The processes that generate these patterns remain completely unexplored. Their origin may be of relevance for evolutionary biology, because the geographically widespread occurrence of this pattern across major cyprinid lineages, in combination with the enormous alpha-level diversity that has evolved in these lineages, points to a universal causal link between ploidy and phenotypic diversity, possibly expressed in one of the most famous examples for lacustrine species flocks, i.e. the *Labeobarbus* species flock of Lake Tana, Ethiopia.

Unfortunately, inter- and intrageneric phylogenetic relationships are poorly known, mainly because of the lack of phylogenetically informative morphological characters, incomplete taxon sampling, and, last but not least, because of the use of mainly mitochondrial DNA (mtDNA) as phylogenetic markers. mtDNA is a single non-recombining locus that is maternally inherited alone, and therefore only allows an incomplete view on species phylogenies to be inferred, an approach that cannot detect hybridization events with confidence, for example (Avise, 2004). With regard to the African *Labeobarbus*

and *Varicorhinus*, Levin *et al*. (2013) reported the mitochondrial paraphyly of *Varicorhinus beso* Rüppell, 1835, type species of the genus, and a chiselmouth-like taxon, *Varicorhinus jubae* Banister, 1984. Based on this observation, they concluded that the *Varicorhinus* mouth phenotype has evolved twice independently, and identified it as a case of parallel evolution. Analogously, the fact that the two hexaploid genera *Capoeta* and *Labeobarbus* are members of two non-monophyletic mitochondrial DNA lineages has been considered evidence for the independent evolution of hexaploidy (i.e. *sensu* Tsigenopoulos *et al*., 2010 and Berrebi *et al*., 2014). Although the single gene (*RAG1*) nuclear DNA data of Yang *et al*. (2015: fig. 4) confirm, at least partially, this hypothesis [i.e. with (1) *Cyprinion* Heckel, 1843 as paternal source (2n) for both genera, and (2) *Luciobarbus* and Torini as maternal source (4n) for *Capoeta* and *Labeobarbus*, respectively], a nuclear multigene approach will undoubtedly be needed to provide sufficient genomic coverage and statistical support to criticially evaluate this hypothesis. In the same line of thought, Qi *et al*. (2012) interpreted the occurrence of similar mouth phenotypes in non-monophyletic mitochondrial lineages of Central Asian Schizothoracinae as evidence for convergent mouth phenotype evolution; however, these interpretations were not verified with recombining nuclear genetic loci, and therefore the presumed convergent occurrence of such peculiar and highly specialized mouth phenotypes in predominantly hexaploid taxa might equally be related to genomic processes, the history of which cannot be deciphered with mtDNA alone.

Incorporating our present knowledge about ploidy, mouth polymorphism, and phylogenetic relationships, this review first presents a detailed overall historic overview of our changing understanding of both these African genera, i.e. *Labeobarbus* and *Varicorhinus*, as well as both the putatively related genera *Acapoeta* and *Sanagia*. Second, a narrative history of our changing interpretation of what intraspecific variation seemingly ought to be in both these genera of hexaploid, large size, Torini is presented. Next, as a result of our almost complete inspection of type specimens, a compilation of the current species diversity in *Labeobarbus s.l*., as well as in *Acapoeta* and *Sanagia*, is provided, with particular attention paid to the level of observed and/or recognized intraspecific mouth phenotype variation for each. Finally, in the discussion, the magnitude of the problems with regard to our current understanding of species diversity in both these genera on a pan-African scale, and beyond, are further exemplified. By providing these baseline data for the first time, we hope to smooth the path for an exhaustive exploration of the megadiversity of this fascinating group of freshwater teleosts as well as answering the challenging questions regarding their evolutionary dynamics.

MATERIAL AND METHODS

The taxonomy, systematics, phylogeny, and intergeneric hybridization of *Labeobarbus* and related genera are reviewed here, based upon an extensive survey of the literature. This was especially needed to identify which of the many African *Barbus*, 'large barbs', and/or *Labeobarbus* species, should be reallocated to or removed from *Labeobarbus*. In this respect, the following pragmatic choices were made. First, the synonymy of *Varicorhinus* with *Labeobarbus*, as proposed by Tsigenopoulos *et al*. (2010), and recently applied by Berrebi *et al*. (2014) in their review paper on *Barbus s.l*., has been fully accepted. Although Yang *et al*. (2015) still retained *Varicorhinus* as a valid genus in their mitochondrial and nuclear DNA publication on cyprinine phylogeny and classification, at least their mtDNA data also support Berrebi *et al*.'s (2014) decision. We refer to this delimitation of the genus as *Labeobarbus s.l*., and to that pre-dating the synonymy as *Labeobarbus s.s*. We have refrained from following the recent revalidation, redefinition, and description of those genera, which would render *Labeobarbus s.l*., in the mitochondrial DNA phylogeny of Tsigenopoulos *et al*. (2010), Berrebi *et al*. (2014), and Yang *et al*. (2015), paraphyletic. This applies to the redefinition of *Varicorhinus* as a monospecific genus by Levin *et al*. (2013), the confirmation and revalidation of *Carasobarbus* (for the first revalidation, see Bănărescu, 1997), and both monospecific genera *Mesopotamichthys* Karaman, 1971 (for the first revalidation, see Bănărescu, 1997) and *Pterocapoeta* Günther, 1902 (for *Carasobarbus*, see Borkenhagen *et al*., 2011 and Borkenhagen & Krupp, 2013; for both *Mesopotamichthys* and *Pterocapoeta*, see Borkenhagen, 2014; for *Pterocapoeta* only, see Geiger *et al*., 2014), and the original description of *Arabibarbus* by Borkenhagen (2014). As a result, all are here enclosed in *Labeobarbus s.l*. (i.e. the *Labeobarbus* clade of Yang *et al*. 2015: fig. 2). This decision also enabled us to avoid the use of the generic appellation '*Labeobarbus*' for species that, following this recent increase of genus names used, are still of uncertain generic affiliation [see Borkenhagen, 2014 (still as '*Barbus*'); Yang *et al*., 2015]. Species recognition, however, and the synonymization of taxa classified as African *Carasobarbus* species by Borkenhagen & Krupp (2013), as proposed by these authors themselves, has been followed here. Furthermore, within *Labeobarbus s.l*., we have concentrated our efforts on compiling African species diversity for which, because of the largely persistent indiscriminate use of the generic appellation *Barbus* (see below), a proper overall delimitation of the *Labeobarbus s.l*. lineage is still lacking. We have, however, refrained from mapping *Labeobarbus s.l*. species diversity from outside Africa, as the recent work

of Borkenhagen *et al*. (2011), Borkenhagen & Krupp (2013), and Borkenhagen (2014) includes a good overview of species diversity from that region for the genera *Arabibarbus*, *Carasobarbus*, and *Mesopotamichthys*. The species list provided has further been completed with a tabulation of the observed mouth phenotype variation after the re-examination of almost all type specimens of all valid species, and, whenever available, mouth phenotype variation as currently perceived in each of the African species, the latter based on: (1) major revisions and/or faunal guides; and (2) wherever possible, a selected re-examination of type specimens of junior synonyms, in such a way as to cover the intraspecific mouth phenotype variation reported in the literature. A list of all examined (type) specimens is provided in the Appendix. Despite the actual synonymy of *Varicorhinus* with *Labeobarbus*, we have indiscriminately used the genus name *Varicorhinus*, if we: (1) refer to the genus as formerly recognized; or (2) refer to it as the highly specialized cutting-edge mouth phenotype ('exposed cornified mandibular cutting edge': see Howes, 1982: 131), and hereby discriminate it from others such as the *Labeobarbus* or intermediate mouth phenotypes (see below). We have referred to a *Varicorhinus*-like mouth phenotype in two particular cases: (1) when referring to comparable mouth phenotype(s) as found in other African *Labeobarbus* species, i.e. with a real cutting edge, but displaying a more extended lip development on the lateral sides of the lower jaw than observed in *V. beso*, where the lips are only poorly developed (for more details, see legend and notes to Table 1); (2) whenever referring to comparable mouth phenotype(s), i.e. with a real cutting edge, as found in other cyprinid genera from outside Africa (e.g. *Chondrostoma* Agassiz, 1832), irrespective of other similarities or differences.

Although it is currently well established that all African large (now *Labeobarbus*) and small *Barbus* [recently transferred to the revalidated genus *Enteromius* Cope, 1867 (see Yang *et al*., 2015) and a few other small genera] do not belong to the genus *Barbus s.s*., i.e. the barbine lineage of tetraploid species from European and Maghreb region of north-west Africa (see Kottelat & Freyhof, 2007; Skelton, 2001; Berrebi *et al*., 2014; Yang *et al*., 2015), we have refrained from using '*Barbus*' (see Farm, 2000; Bamba, Vreven & Snoeks, 2011; Banyankimbona, Vreven & Snoeks, 2012b) to discriminate them from the latter lineage. This decision is motivated by the fact that the current discrimination cannot be extrapolated unequivocally into a historical context pre-dating these insights. Therefore, as a matter of convenience and when used in a historical context, all African *Barbus* (i.e. now *Labeobarbus* and *Enteromius*, and a few smaller genera) have indiscriminately been referred to as *Barbus* and not '*Barbus*' throughout the text.

Institutional abbreviations follow Fricke & Eschmeyer (2015): i.e. collections from the Natural History Museum (NHM), London, UK, and the Royal Museum for Central Africa (RMCA), Tervuren, Belgium, will be referred to as BMNH and MRAC, respectively, i.e. their well-estabilished historical acronyms. Other abbreviations: FL, fork length; SL, standard length; TL, total length.

Compilation of the annotated checklists

As a reference for further research, a full list of all valid African *Labeobarbus s.l*. species (*sensu* Tsigenopoulos *et al*., 2010; Berrebi *et al*., 2014; Yang *et al*., 2015) is provided as an annotated checklist. The list is ordered alphabetically by species or subspecies name, as given in the original description. The genus name and the author(s) name(s), as given in the original description, are also provided. Museum collection abbreviations follow Fricke & Eschmeyer (2015).

The full type series, as currently identified, is listed and the number of type specimens in a record is given between brackets after the collection number, except for holo-, lecto-, and neotypes, which are single specimens by definition. Type series are based on data from the original publication and/or collection data, and have not been verified in all cases. Differences between the number of type specimens identified and the number of type specimens in the original description, if given, are mentioned in the 'Notes' section.

Type locality data have been provided in English, i.e. they may have been translated from the original sources of the original description and/or the museum label(s). Type localities only include those of the name-bearing type(s), as only those are of nomenclatural importance (ICZN, 1999: articles 61 and 72.1.2.–1.3). Whenever available, locality data have been arranged from the smallest to largest geographical units (locality, river, basin, and country), and have been complemented with other information (e.g. elevation) as provided in the original description or on the museum label(s). Unless otherwise stated, all information derives from the original description or from the museum label(s). Additional information on water bodies, drainage basin, and/or country is provided in parenthesis; country names have been adjusted to current use. Coordinates are also provided for the type locality/ies, and are based on the information as provided in the original description on the museum label(s), or as obtained from other sources. In the latter case '±' has been added to the coordinates and their reference source has also been provided. Coordinates always follow the geographical unit to which they refer.

Under the heading 'Current status', the status and current full species name, as to be used, is provided, with reference to the first generic placement of the

species in *Labeobarbus*. The list of African *Labeobarbus* species as provided by Skelton & Bills (2008) was revealed to be incomplete, beyond the fact that *Varicorhinus* spp. were not included because they were not considered a junior synonym at that time. Therefore, allocation of species to *Labeobarbus* by Skelton & Bills (2008) has been backed up with another reference or reference to the present paper. In the case of replacement names and revalidated synonyms, as provided and reinstated in the current paper, reference is made, respectively, to: (1) the allocation of the junior homonym to *Labeobarbus*; (2) the allocation of the previous senior synonym of the revalidated species to *Labeobarbus*. In both cases reference to the current paper has been included. For recent revalidations, such as that of *Labeobarbus pojeri* (Poll, 1944), reference to both the allocation of the previous senior synonym of the revalidated species to *Labeobarbus* and its allocation to *Labeobarbus* within the paper revalidating it have been provided.

Under 'synonyms', actual synonyms with their original names, including author and year of description, and with reference to the authors who first proposed the synonymization, are provided. The senior synonym of the proposed synonymization is also given, except if it is the currently valid species. In the cases concerned, the reference(s) for revalidation and/or resynonymization are also provided. Synonyms are provided in alphabetic order. Nominal species placed in synonymy but recognized as a valid subspecies are also included in the synonyms list, as are subspecies that are not considered valid. A single reference after several synonyms refers to all preceding synonyms.

Under 'Distribution', the currently known distribution with the respective literature sources is given. The distribution of valid subspecies is given after their name, author, and year of description, together covering the total species distribution. Distribution data are merely a compilation of available information, without evaluation of the quality or correctness of the literature reports. Lévêque & Daget (1984) has been used as the main source for the basic distribution information, updated with recent literature sources whenever available; however, distribution information is based on the original sources when the distribution as given in Lévêque & Daget (1984) differs too much from what is given in these original sources. Distribution references are ordered chronologically.

Finally, a 'Notes' section is included for any additional information and discussion referring to data listed under previous headings. As we made extensive use of the CLOFFA (Lévêque & Daget, 1984) and the Catalog of Fishes (Eschmeyer, 2015), especially during the early stages of checklist compilation, the 'Notes' section also contains information on any discrepancies between both sources and between both and other sources used. These notes should be considered an update of the information available in the CLOFFA and in the Catalog of Fishes, which, without doubt, remain baseline sources for any taxonomical study, for which they are hereby acknowledged.

Nominal species that have been identified as hybrid phenotypes are also listed in annotated checklist 1, but without further data. Full data, however, are provided in annotated checklist 2. The species of *Acapoeta* and *Sanagia* are treated as discussed above, and are listed in annotated checklist 3.

Considering the importance of the priority rule (see ICZN, 1999: article 23), whenever possible, multiple references of the same author published during the same year have also been ordered chronologically, rather than according to appearance in the text. References that we have been unable to chronologically situate have consistently been added at the end of the authors' publications for that year (i.e. Boulenger, 1907c and Pellegrin, 1935d). The printed date on a publication itself, unfortunately, does not always correspond to the actual date of publication. In those cases, as a matter of completeness, we have added (in parenthesis) the reference used to identify the latter date after the reference itself (i.e. Duncan, 1937, Banister, 1973, Roux, 1976, Bauer, Günther & Klipfel, 1995, Evenhuis, 2003, Low & Evenhuis, 2014 or Eschmeyer, 2015).

Compilation of the list of examined (type) specimens

The list provides an overview of all (type) specimens examined within the framework of the present paper. As for the compilation of the annotated checklist(s), the list is ordered alphabetically by species or subspecies name, as given in the original description, except for species for which a substitute name has been proposed and are then to be found under the latter name. Junior synonyms have been listed alphabetically under their current senior synonym, i.e. valid species name, as presented in the annotated checklists. Note that only type specimens of junior synonyms relevant to the tabulation of mouth phenotype variation within a given species, provided in Table 1, have been listed here (see Notes to Table 1). To ease the subsequent verification and/or retraction of information, the current list has been subdivided into annotated checklists: i.e. listing the African *Labeobarbus s.l.* species (annotated checklist 1); the African *Labeobarbus s.l.* species identified as hybrid phenotypes (annotated checklist 2); and the species of both the monospecific genera *Acapoeta* and *Sanagia* (annotated checklist 3), respectively. African species identified as not belonging to *Labeobarbus s.l.* have been listed separately under the heading 'non *Labeobarbus* species'.

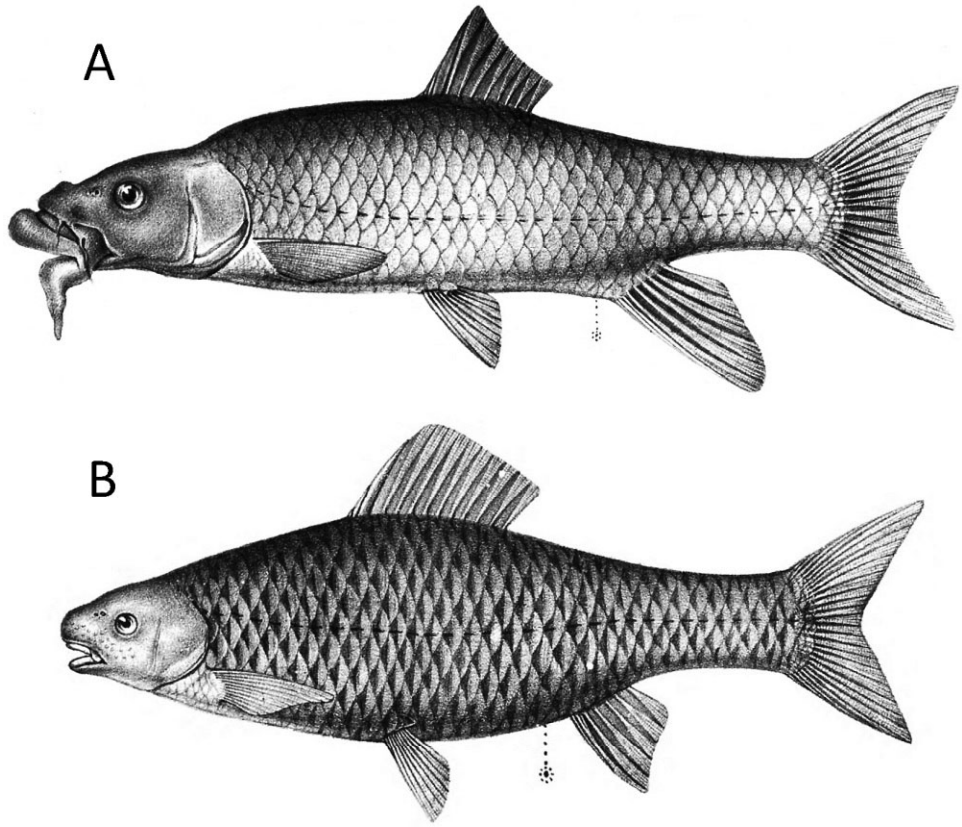


Figure 2. Reproduction of the original illustration of: A, *Labeobarbus nedgia* (from Rüppell, 1835: plate 2, fig. 3); B, *Varicorhinus beso* (from Rüppell, 1835: plate 3, fig. 2) type species of *Labeobarbus* and *Varicorhinus*, respectively (both drawings flipped horizontally).

It has not always been possible to physically re-examine the type specimen(s) ourselves, either because the current loan policies of some of the relevant fish collections prevent sending primary types on loan, or because of budgetary and time constraints to visit each of the remaining fish collections, sometimes just for a single or only a few types. Therefore, some types have only been re-examined using photographs kindly sent to us by the curator(s) in charge, which are indicated by an asterisk (*) after the collection number(s) concerned. Note that for the Lake Tana species, except for the nominal types for which the mouth phenotype data are based on photographs, extensive use has been made of the mouth phenotype data as already provided by Nagelkerke & Sibbing (1997, 2000) (see also Notes to Table 1).

RESULTS

Persistent generic problems: morphological, cytogenetic, and molecular approaches

Labeobarbus and *Varicorhinus* were both originally described from Lake Tana, 'Zana See' (Ethiopia) by Rüppell (1835). The genus name *Labeobarbus*, with *Labeobarbus nedgia* Rüppell, 1835 as the type species, was chosen in reference to a barb [Latin: *barbus* (masculine)] with large fleshy lips [Latin: *labeo* (masculine)], i.e. with 'fleischige Lippen', and also possessing a mental lobe, i.e. the 'ziemlich lange fleischige Bartzaser' (see Rüppell, 1835: 14) (see Fig. 2A). The genus name *Varicorhinus*, with *Varicorhinus beso* Rüppell, 1835 as the type species, was chosen in reference to the many small tubercles, i.e. 'kleine Knorpelwarzen' [Greek: *varix* (feminine or masculine)], on the snout [Greek: *rhis* (feminine)], and the description also indicated the horny lips, i.e. 'häutigen Lippen' (see Rüppell, 1835: 20–21) (see Fig. 2B). Although there is no explicit reference to a clear cutting edge on the horny lower lip in Rüppell's (1835) original description of the genus *Varicorhinus*, this is the main diagnostic character that has subsequently been used for differentiating *Varicorhinus* from *Labeobarbus* (see below). Based on the unambiguous original description, both genera seemed well differentiable and distantly related at that time, a situation that would drastically change over time when more specimens and species became available (see below).

The history of both genera can be roughly subdivided into three major time periods. First, the 'period of discovery', here delimited from 1775 to approximately 1930, but well overlapping with the next period. This period is dominated by the discovery and typological naming of the overwhelming diversity in mouth phenotypes (see An overview of species diversity in African *Labeobarbus*: current state of the art). Second, the 'period of skepsis', starting from 1929 and lasting up to about 1987. This period is characterized by recurrent doubts with regard to the distinct generic status of *Varicorhinus*, as well as doubts about the species-level relevance of the observed mouth phenotype diversity. The latter doubts resulted in a wave of species-level synonymizations (see Phenotypic variation and/or 'intergeneric' hybridization). The existing generic-level classification remained unchanged, however. Finally, the 1990s marked the onset of the 'period of multidisciplinarity', characterized by the application of new research tools that enabled the study of morphology-independent characters, i.e. enzymes, chromosomes, DNA sequences, and parasites. The potential of these methods rejuvenated the attention paid to the long-standing questions of generic delimitation and phylogenetic affinities, and facilitated the continuing step-by-step advance in their elucidation.

The period of discovery

Since Günther's (1868) synonymization, the genus *Labeobarbus* has been a junior synonym of the genus *Barbus s.l.* Cuvier & Cloquet, 1816 (in Cuvier, 1816) – a genus that later was aptly called a 'monstrous aggregation' (Myers, 1960: 213) – for most of its subsequent history. The first and most striking feature that Rüppell (1835) presented in the original description of *Labeobarbus* to differentiate it from other cyprinids was the presence of the large fleshy lips (see above). Günther (1894), however, reported that large specimens of *Barbus* (now *Labeobarbus*) *intermedius* Rüppell, 1835 have more developed lips. As such, he implicitly identified lip development as: (1) an intraspecific variable; and (2) a size-related character. Boulenger (1902f), however, refuted Günther's (1894) interpretation of the observed mouth phenotype variation (our wording) as the holotype of *B. intermedius*, which lacks a mental lobe, is larger than the largest specimens seen by Günther (1894), which instead have a well-developed lower lip with a rounded median lobe [now syntypes of *Barbus gregorii* Boulenger, 1902: a junior synonym of *L. intermedius* (see annotated checklist 1)]. He also argued that the mouth phenotype characters presented by Rüppell (1835) in his original description of the genus *Labeobarbus* were insufficient to grant it generic separation from *Barbus* (now mainly *Enteromius* and a few smaller genera), although they are of greatest value for species distinction (see below), and are independent of sex and age (Boulenger, 1902f).

Even so, the observed mouth phenotype variation in large barbs (now *Labeobarbus*) were revealed to be an intricate issue. Boulenger (1902a: 125), for example, had described two Moroccan species with 'a perfectly [*Varicorhinus*-like] trenchant edge to the lower jaw' as *Capoeta waldoi* Boulenger, 1902 and *Capoeta atlantica* Boulenger, 1902, both of which he later transferred to *Barbus* (Boulenger, 1905b: 44), now *Labeobarbus* [both are currently junior synonyms of *Labeobarbus fritschii* (Günther, 1874), see Lévêque & Daget (1984) and Borkenhagen & Krupp (2013)]. A few months later, Boulenger (1902d) described two more large *Barbus* species (now *Labeobarbus*), i.e. *Barbus* (*Capoeta*) *perplexicans* Boulenger, 1902 and *Barbus* (*Labeobarbus*) *labiatus* Boulenger, 1902, and hereby considered *Capoeta* as a junior synonym of *Barbus*. Although he had apparently based the attribution of *B. perplexicans* to the subgenus *Capoeta* on the presence of 'a strong transverse, horny cutting-edge' on the lower jaw, the attribution of *B. labiatus* to the subgenus *Labeobarbus* apparently was based on the 'extremely developed' lips, 'each produced into a long triangular flap' (see Boulenger, 1902d: 223). Boulenger's struggle to come to terms with mouth phenotype variation is further exemplified by the fact that he had already abandoned his previous subgeneric classification in 1905 (see Boulenger, 1905b), but nevertheless retained species with a *Capoeta*, i.e. *Varicorhinus*-like, mouth phenotype, as large barbs (now *Labeobarbus*) within the huge genus *Barbus*, without questioning the status of *Varicorhinus* as a valid genus.

Ever since, *Varicorhinus* has never been formally synonymized, except for some early authors such as Heckel (1843), Cuvier & Valenciennes (1844), and Günther (1868), despite that its status has been much debated [e.g. by Groenewald (1958) (see below)]. Only Keilhack (1908) had described a species with a cornified real cutting edge on its lower jaw from Lake Malawi as *Barbus njassae* Keilhack, 1908 [currently a junior synonym of *Labeobarbus johnstonii* (Boulenger, 1907) (see Banister & Clarke, 1980)] although he was perfectly aware of its *Varicorhinus*-like mouth phenotype. He explicitly rejected the validity of *Varicorhinus*, and in that context referred to Boulenger (1902b) (see Keilhack, 1908: 165), who had previously attributed species with a well-identifiable *Varicorhinus* mouth ('*Varicorhinus*-Kiefer') to *Barbus* (see above). As a result of Keilhack's (1908) statement, Pappenheim (in Pappenheim & Boulenger, 1914) used *Varicorhinus* as a subgenus of *Barbus*.

Notwithstanding Keilhack's (1908) argument, the influence of Boulenger's famous *Catalogue of African Fresh-Water Fishes in the British Museum (Natural History)* (Boulenger, 1909, 1911a, 1916a) has resulted, until recently, in a general acceptance of Boulenger's

genus-level classification, with *Labeobarbus* as a junior synonym of *Barbus*, and with *Varicorhinus* as a valid genus. Boulenger's catalogue was also followed with regard to diagnostic morphological characters, which he used for separating all 'large barbs' (now *Labeobarbus*) from the 'small barbs' (now *Enteromius* and a few smaller genera). Both groups were mainly differentiated from each other based on the exposed surface of the scales having radiating striae in small *Barbus* (see Boulenger, 1911a: 12), in contrast to parallel or even convergent striae in large *Barbus* (see Boulenger, 1911a). In addition, Boulenger (1911a) differentiated the two groups by overlapping, branched dorsal-fin ray counts, i.e. between six and eight (rarely nine) in small *Barbus*, and between eight and 11 in large *Barbus*. Earlier, Boulenger had also provided, to some degree, the main character for diagnosing *Varicorhinus*, i.e. the lower jaw being 'completely exposed and showing a sharp cutting-edge covered with a horny layer [sheath]' (Boulenger, 1907c: 190; and 1909: 299 & 352).

Although Boulenger (1911a) did not recognize *Labeobarbus* as a separate genus or subgenus in his catalogue, he later again considered it as a subgenus of *Barbus* (see Boulenger, 1919). This classification was not adopted by subsequent authors, however, apart from a few noteworthy exceptions: Pellegrin (1921) retained it as a subgenus of *Barbus*, including the species with scales with numerous more or less parallel striae, thereby following Boulenger's (1911a) division. As a novelty, Pellegrin (1921) differentiated between several other additional subgenera, i.e. *Barbus*, *Enteromius*, and *Puntius* Hamilton, 1822 (Pellegrin, 1921, 1939), because he felt that this was necessary from a practical point of view for species identification. He further listed *Varicorhinus* as different from *Barbus*, mainly based on the absence of lips and the presence of a cornified real cutting sheath on the lower jaw. Nevertheless, Pellegrin (1926) himself described *Barbus* (*Capoeta*) *babaulti* Pellegrin, 1926 [currently a junior synonym of *Labeobarbus oxyrhynchus* (Pfeffer, 1889), see annotated checklist 1] as a new large *Barbus* (now *Labeobarbus*), although he himself reported a cornified real cutting edge on the lower jaw (our rewording) for this taxon. This also explains why he assigned this new species to the subgenus *Capoeta*, and it illustrates well his problems of coming to terms with the observed mouth phenotype variation.

The period of skepsis

The generic problems encountered would not fade away, although different authors would adopt, substantiate and promote different views. Daget (1954) also used the difference in the striation pattern of the scales and the adult size as the first dividing character in his key to the West African species of the genus *Barbus*, but he did not recognize any (sub)division of the genus into different genera or subgenera. Instead, he explicitly stipulated the artificial nature of such a division, which, according to him, would by no means reflect the systematic arrangement. As the genus *Varicorhinus* had not been reported from West Africa at that time (see however Daget, 1962: 72), Daget (1954) did not discuss it (see below).

The subdivision of small versus large barbs as proposed by Boulenger (1911a) was also adopted by Banister (1987), who explicitely quantified the difference in adult size between the two groups, i.e. mostly much smaller than 200 mm SL for the 'small *Barbus*' (now *Enteromius* and a few smaller genera), and mostly much larger than 200 mm SL for the 'large *Barbus*' (now *Labeobarbus*) (Banister, 1987). Banister (1987) also clearly stipulated that this was a pragmatic segregation without any implication for the monophyly of either group. He further reported exceptions to the rule, such as for the large sized *Labeobarbus somereni* (Boulenger, 1911) from the Ruwenzori area, as well as the Ruzizi and Upper Malagarazi rivers (see Banister, 1973: fig. 95; Banyankimbona *et al*., 2012a), for which he illustrated a more radiate striation pattern on the scales (Banister, 1973: fig. 93).

Berrebi (1981) was the first author to again use *Labeobarbus* as a subgenus of *Barbus* since Boulenger (1919) and Pellegrin (1921). Based on a study of three Moroccan large *Barbus*, he argued that according to his observations, and referring erroneously to Boulenger (1911a), who did not adopt a subgeneric classification of the genus *Barbus*, that in *Labeobarbus* the last dorsal spine is never serrated, whereas in the subgenus *Barbus* it always is. Along with earlier authors, he added the difference in striation pattern of the scales as a second diagnostic character. Beyond Pellegrin's (1921) subgeneric division, the absence of serrations on the last unbranched dorsal spine is, however, not fully diagnostic, as in many species of both *Labeobarbus* and *Barbus* this last unbranched ray is not spiny. If present, it lacks serrations in all large *Barbus*, now *Labeobarbus*, as well as several small *Barbus* species (see for instance in Skelton, 2001; Lévêque, 2003; De Weirdt & Teugels, 2007). As it was unsatisfactory for many, Berrebi's suggestion was therefore not followed, and because of the lack of alternative supporting evidence, the classification with *Labeobarbus* as a junior synonym of *Barbus* remained.

The same holds true for the status of *Varicorhinus*. The problematic status of *Varicorhinus* as a separate genus became particularly apparent through the research of South African ichthyologists such as Groenewald, Crass, and Jubb. Groenewald (1958) also used the striation pattern of the scales in his key to the *Barbus* and *Varicorhinus* species of Transvaal (South Africa) to differentiate the *Varicorhinus* and large *Barbus*

(now *Labeobarbus*) – usually referred to as yellowfishes in Southern Africa, because of their golden yellowish overall colour – from the small *Barbus* (Groenewald, 1958: 268). Groenewald was also the first after Keilhack (1908) who explicitly questioned the status of *Varicorhinus* as a valid genus, because the only external difference to other yellowfishes (now *Labeobarbus*) would be the 'square-shaped lower jaw, with its thick muscular covering and conspicuous horny cutting ridge' (Groenewald, 1958: 273). Interestingly, he also noticed that a cornified real cutting ridge is not restricted to *Varicorhinus*, but that a weakly developed one is also present in the 'sector' form of another yellowfish, *Barbus brucii* Boulenger, 1907 [now *Labeobarbus marequensis* (Smith, 1841), following Jubb, 1963].

In a key for the cyprinid genera from Natal, two entries for *Barbus*, i.e. one with 'Mouth terminal' (small *Barbus*, now *Enteromius*) and one with 'Mouth inferior' were published by Crass (1964: 47). His second entry includes under the character state 'lips undeveloped; lower jaw with exposed cutting edge' not only *Barbus* (i.e. large *Barbus*, now *Labeobarbus*) but also *Varicorhinus*; however, he diagnosed *Varicorhinus* not by the presence of an exposed cutting edge, but by barbels being absent or reduced to a single small pair (i.e. less than half the eye diameter) versus always at least one pair of longer barbels (i.e. longer than two-thirds of the eye diameter) in *Barbus* (now *Labeobarbus*). Crass (1964) obviously considered an exposed *Varicorhinus* cutting edge alone not sufficient evidence for identifying a species as belonging to *Varicorhinus*. As a consequence, he retained only a single species in that genus, i.e. *Varicorhinus nelspruitensis* Gilchrist & Thompson, 1911, and stipulated: 'The only difference in the jaw appears to be that the characteristic shape [i.e. exposed *Varicorhinus* cutting edge] is to be found in very young *Varicorhinus* whereas *Barbus* [now *Labeobarbus*] of less than 2 inches [i.e. 1 inch, ~25.4 mm; unclear whether TL, FL, or SL; see Crass, 1964: 17] do not have a wide jaw.' (Crass, 1964: 70).

Three years later, Jubb (1967: 115–116) adopted and enriched Crass' (1964) definition of *Varicorhinus*: 'the wide square lower jaw with sharp cutting edge must be evident in the juvenile stage and not a post-juvenile adaptation. Furthermore, this form of mouth must be uniform for the species throughout its life history'. Another year later, Jubb (1968) added that the character of the lower jaw, which Boulenger (1909) described as being without lips and having a sharp cutting edge covered with a horny sheath, is of doubtful value for systematics, because this *Varicorhinus* form of mouth is also found in five large *Barbus* (now *Labeobarbus*) species (Jubb, 1968; see illustration Jubb, 1967: fig. 21). Finally, Jubb (1967, 1968) accepted the genus *Varicorhinus* only for species with the definition given above, i.e. he only recognized *Varicorhinus nasutus* Gilchrist & Thompson, 1911, *Varicorhinus nelspruitensis*, and *Varicorhinus pungweensis* Jubb, 1959 as *Varicorhinus* species among the Southern African Cyprinidae, and he excluded *B. marequensis* and *B. natalensis* de Castelnau, 1861 (now both *Labeobarbus*) specimens with a *Varicorhinus*-like mouth, because in these polymorphic mouth phenotype species, mouth phenotype differentiation would be apparent only at a minimum size of 100–150 mm FL. Instead, all specimens of less than 60 mm FL would be undifferentiated, i.e. have normal thin lips (Jubb, 1968; see also Jubb, 1967); however, Poll (1967) reported the occurrence of very similar size-related mouth phenotype changes for two *Varicorhinus* species: *Varicorhinus ansorgii* Boulenger, 1906 and *Varicorhinus macrolepidotus* Pellegrin, 1928. He found the lower lip of small (about ≤125 mm TL) *V. macrolepidotus* covered by a horny cover but without a clear cutting edge, in contrast to larger specimens, which have both. Similarly for *V. ansorgii*, Poll (1967: 333) reported a bevel ('bissaux'), i.e. the cornified real cutting edge, only for adults (i.e. large specimens). More recently, Tweddle & Skelton (1998: 372) reported in the original description of *Varicorhinus* (now *Labeobarbus*) *dimidiatus* Tweddle & Skelton, 1998 that although adult specimens indiscriminately have a 'keratinized scraping edge', the mouth of juveniles is crescent-shaped, and becomes straight with round edges in larger individuals. This illustrates that in 'true' *Varicorhinus* mouth phenotype species, ontogenetic changes in mouth morphology also occur. As a consequence, *Varicorhinus* retained its valid status, despite the many efforts to identify stringently diagnostic characters separating *Varicorhinus* from large *Barbus* (now *Labeobarbus*).

The period of multidisciplinarity

In the 1990s molecular and chromosomal characters started to be used to investigate African *Barbus* systematics. Two studies by Agnèse *et al*. (1990) and Berrebi *et al*. (1990) examined enzyme polymorphism in several small and large *Barbus* species from West Africa. Both inferred that the large *Barbus* species studied (i.e. *Labeobarbus* as a subgenus of *Barbus* for Berrebi *et al*., 1990) were tetraploid, like the European *Barbus*, whereas the small African *Barbus* (i.e. now *Enteromius*) were diploid. As such, they independently confirmed the classical morphology-based division into two major groups (e.g. see Boulenger, 1911a), i.e. small versus large African *Barbus*.

Oellermann & Skelton (1990) indirectly questioned the presumed tetraploidy of the large West African barbs, based on the direct observation of hexaploid chromosome numbers of 2n = 148–150 in six of the seven South African large *Barbus* (now *Labeobarbus*). In addition, they revealed the hexaploidy of *Varicorhinus*

nelspruitensis (Oellermann, 1989 *fide* Oellermann & Skelton, 1990) (2n ~ 150). Based on their shared hexaploidy, they thereby presented the first karyological evidence for a closer phylogenetic relationship of the large African barbs with *Varicorhinus*, rather than with *Barbus s.l.* This evidence was complemented by karyological studies of Krysanov *et al.* (1991), Golubtsov & Krysanov (1993), and Krysanov & Golubtsov (1996), who further found hexaploidy (2n = 150) in some riverine Ethiopian large *Barbus* (now *Labeobarbus*) and six different *L.* cf. *intermedius* 'eco- and morphotypes' from the Lake Tana Basin (later described as full species by Nagelkerke & Sibbing 1997: table 5.4), as well as for the type species of *Varicorhinus*, *V. beso*.

Golubtsov & Krysanov (1993) were also the first to claim that further investigations would reveal the hexaploidy of all African 'large *Barbus*' and *Varicorhinus* species. According to them either one unique polyploidization event or two independent events might account for the origin of hexaploidy in both genera. They deemed the latter theory to agree better with the classification into two different genera, but they also acknowledged that a close morphological resemblance of some African *Varicorhinus* and large *Barbus* (now *Labeobarbus*) species had already been stressed by Banister (1976a), referring to Banister (1972), Banister & Clarke (1980), and Howes (1987: statement not found). Therefore, divergence of the hexaploid lineage into a large *Barbus* (now *Labeobarbus*) and into a *Varicorhinus* lineage might well have followed a single hexaploidization event. Within a few years, Guégan *et al.* (1995) also finally confirmed hexaploidy for three West African representatives of the large *Barbus* using karyology, and by doing so, revised the previous tetraploidy hypothesis and supported Golubtsov & Krysanov's (1993) pan-African hexaploidy hypothesis.

Genus-level consequences of the new multidisciplinary studies: the revalidation of Labeobarbus

In line with Boulenger (1919), Pellegrin (1939), Berrebi (1981), and Berrebi *et al.* (1990), and based on isoenzyme as well as monogenean Dactylogyridae parasite data for some Moroccan species, El Gharbi, Lambert & Berrebi (1993) and El Gharbi, Birgi & Lambert (1994) re-used *Labeobarbus* as a subgenus of *Barbus*. This decision was also followed by Berrebi (1995: fig. 1), in his allozyme study, including some North and West African large *Barbus* species.

After more than 125 years since Günther's (1868) first synonymization, however, Doadrio (1994) was the first to reinstate the use of *Labeobarbus* as a full generic name and, at the same time, retained *Varicorhinus* as a valid genus, including *Varicorhinus maroccanus* (Günther, 1902), the sole North African species placed within the latter genus. Despite the shared hexaploidy of the large *Barbus*, identified as *Labeobarbus* by Doadrio (1994), with *Varicorhinus* (see Oellermann & Skelton, 1990; Golubtsov & Krysanov, 1993), he considered the hexaploidy of the former sufficient evidence to warrant it full generic status (Doadrio, 1994).

The decision to reinstate *Labeobarbus* as a subgenus or even a full genus was not followed immediately by others. Poll & Gosse (1995), for example, retained the traditional classification in their key to the African freshwater fish genera. Furthermore, Nagelkerke & Sibbing (1997), referring to Berrebi (1995) and Berrebi *et al.* (1996), who used *Labeobarbus* as a subgenus of *Barbus*, proposed the re-elevation of the subgenus *Labeobarbus* to full generic rank, but they still described their new species in the genus *Barbus*, and continued to use the genus name *Barbus* in their subsequent papers (see Nagelkerke & Sibbing, 1998: 3–7, 2000). They argued in support of Berrebi's (1995) suggestion to re-elevate *Labeobarbus* to genus level only if further and more complete evidence would confirm the monophyly of the African hexaploid barbs.

Finally, Skelton (2001) fully reinstated the genus *Labeobarbus* as a valid genus in the second edition of his *Guide to the Freshwater fishes of Southern Africa*. He hereby implemented this nomenclatural change for the first time in a major African ichthyofaunal work. As a consequence, all South African taxa that had previously been retained in *Barbus* were attributed either to *Barbus*, i.e. the African small *Barbus* (now *Enteromius*), or to *Labeobarbus*, i.e. the African large *Barbus*. Of the former diagnostic differences recognized by Boulenger (1911a), only the difference in striation pattern of the scales was retained by Skelton (2001) to differentiate both genera. Skelton (2001) also recognized the genus *Varicorhinus* (chiselmouths), mainly on the presence of the wide, straight, and inferior mouth, with a sharp and horny lower lip. He also noted that the scales in *Varicorhinus* are longitudinally or parallel striated (Skelton, 2001), as in *Labeobarbus*, and different from *Barbus*, in which they are radiately striated. Skelton's (2001) decision marked the turning point with regard to the general acceptance of *Labeobarbus* as a full genus, and subsequently several authors have accepted it and started to transfer the large African *Barbus* species, bit by bit, and depending on the geographical region of their interest, to the genus *Labeobarbus*. For example, De Weirdt & Teugels (2007) for Lower Guinea, Getahun (2007a) for Ethiopia, Banyankimbona *et al.* (2012a) for Burundi, Kullander & Roberts (2012) for the Lukuga (Democratic Republic of Congo), and Van Steenberge, Vreven & Snoeks (2014) for the Upper Luapula and adjacent regions (for full details, see annotated checklist 1) adopted *Labeobarbus* for the large *Barbus* for the region or basin under their specific attention. Although Skelton & Bills (2008) compiled a first attempt to list all African

Labeobarbus, prior to the proposed synonymy of *Varicorhinus*, their work has remained more or less unnoticed (see An overview of species diversity in African *Labeobarbus*: current state of the art).

Genus-level consequences of the new multidisciplinary studies: the synonymization of Varicorhinus

Even though *Barbus wurtzi* Pellegrin, 1908 was considered a *Varicorhinus* species at some point (see Daget, 1962: 72; Lévêque & Daget, 1984), due to the presence of a real cutting edge in some specimens at least (see Daget, 1962); Lévêque & Guégan (1990), based on a monogenean Dactylogyridae parasite study, reidentified it as a large *Barbus* and placed it back into *Barbus*, now *Labeobarbus*. As a result, the genus *Varicorhinus* has ever since been considered absent from West Africa (see Lévêque, 1990, 2003), despite the occurrence of *Varicorhinus* mouth phenotype specimens, as identified by Daget (1962; for more details, see The present overview: what is to be learned?).

Berrebi & Valiushok (1998) discussed the status of the Lake Tana (Ethiopia) *V. beso*, the type species of the genus *Varicorhinus*, in relation to *B.* (now *L.*) *intermedius*. As they found seven diagnostic loci (on 31 presumptive allozymic loci) differentiating the two taxa they considered them to be two separate species; however, with regard to the status of the genus *Varicorhinus* they referred to their own results and to the study of Nagelkerke & Sibbing (1996), which had identified 'intergeneric' hybrids and hence pointed to *Varicorhinus* as being close or even congeneric with *Barbus* (now *Labeobarbus*). Before making taxonomic changes, they asked for a large phylogenetic analysis with sufficient taxon sampling.

Machordom & Doadrio (2001) first showed on the mtDNA level that the included African hexaploid species – i.e. large *Barbus* (now *Labeobarbus*) and *V. maroccanus* (Günther, 1902), as the single included *Varicorhinus* species – form a well-supported monophyletic clade, and consequently suggested a common hexaploid ancestor for both (see Machordom & Doadrio, 2001: fig. 1). As their phylogenetic analysis was based on mitochondrial DNA only, and therefore only reflects a matrilineal phylogeny, they explicitly stated that their data are not sufficient to differentiate between different evolutionary scenarios, i.e. between dichotomous speciation by divergence or instead by introgression and/or hybridization. This remark seemed especially valid because clear morphological differences distinguish some of the species included in their mtDNA phylogeny, but which appeared as undifferentiated, according to haplotypes from the mtDNA data, a fact that might reflect either hybridization or introgression among these taxa, or incomplete mtDNA lineage sorting after very rapid dichotomous speciation.

Tsigenopoulos *et al.* (2002) also established the monophyly of large barb (now *Labeobarbus*) mtDNA haplotypes, a result based on taxon sampling of hexaploid African 'large *Barbus*' from geographically distant parts of Africa, as well as of two basally diverging taxa, the Moroccan *Barbus reinii* Günther, 1874 and the Middle Eastern *Barbus luteus* (Heckel, 1843), both with currently undetermined ploidy levels. They interpreted this monophyly as the result of a single recent origin (see below), but unfortunately, no *Varicorhinus* species had been included in this study. They were not able to assign any particular diploid or tetraploid African lineage as an African or non-African precursor lineage of these hexaploid large *Barbus* (now *Labeobarbus*). Machordom & Doadrio (2001: fig. 1) previously identified the Palaearctic tetraploids as a sister group, however, and not the African diploids and tetraploids, nor the Asian diploids, thereby clearly suggesting a non-African origin.

Durand *et al.* (2002: fig. 1) obtained highly comparable mtDNA results, supporting the monophyly of the hexaploid African large barbs (i.e. the *Carasobarbus–Varicorhinus* clade; now *Labeobarbus s.l.*), and including *V. beso*, the type species of the genus *Varicorhinus*. As such, they were the first to present genetic evidence for the possible synonymy of both genera. In addition, they identified *B. reinii* [now a *Labeobarbus* species (*s.l.* following our nomenclature), following Tsigenopoulos *et al.* (2010)] from Morocco as well as *Barbus* (*Tor*) *grypus* [now an *Arabibarbus* species, following Borkenhagen (2014), and a *Labeobarbus* (*s.l.*) species following Tsigenopoulos *et al.* (2010)] from Iraq, as early diverging lineages from that *Labeobarbus–Varicorhinus* clade, albeit with low statistical support. Their results also identified a poorly supported sister-group clade composed of the monospecific genus *Kosswigobarbus*, several *Carasobarbus* spp., as well as the Moroccan *Barbus paytoni* Boulenger, 1911 [now also a *Carasobarbus* species according to Borkenhagen & Krupp (2013), and a *Labeobarbus* (*s.l.* following our nomenclature) species following Tsigenopoulos *et al.* (2010)]. As such, they also provided evidence for a *Labeobarbus s.l.* clade, as later identified with greater taxon sampling by Tsigenopoulos *et al.* (2010), Berrebi *et al.* (2014), and Yang *et al.* (2015) (see below).

Naran, Skelton & Villet (2007) studied the karyology of three South African yellowfish [i.e. *Labeobarbus capensis* (Smith, 1841), now *Labeobarbus seeberi* (Gilchrist & Thompson, 1913), following E. Vreven, E.R. Swartz & P.H. Skelton, unpubl. data; *L. marequensis*; and *Labeobarbus polylepis* (Boulenger, 1907)] targeting, against others, the question of their hypothetical non-African origin (see Durand *et al.*, 2002). Although these were found to be hexaploid, they underscored that the species of the Asian genus *Tor* are tetraploid, a fact that, together with their biogeo-

graphical distinctiveness, in their opinion supported the taxonomic restoration of *Labeobarbus* to full generic status. mtDNA-based studies indicate, however, that *Capoeta* as another hexaploid genus (see above; see also Arai, 2011: 49), is phylogenetically nested within the tetraploid genus *Luciobarbus* (see Tsigenopoulos *et al*., 2003: fig. 1; see also Tsigenopoulos *et al*., 2010: fig. 1). Therefore, according to mtDNA results the evolutionary transition from tetraploidy to hexaploidy might be frequent, and hexaploid *Labeobarbus* may have arisen easily from within tetraploid *Tor*. Hence, Naran *et al*. (2007) underscored the need for a molecular phylogenetic study including both *Tor* and *Labeobarbus* to resolve their taxonomic status. The need for such a study has recently been met by Yang *et al*. (2015), who identified *Tor* (including the type species *T. tor*), at least on the mtDNA level, as the well-supported sister group of the *Labeobabarbus* clade, i.e. *Labeobarbus s.l*., comprising the *Labeobarbus*, *Carasobarbus*, and *Pterocapoeta* lineage (for distribution of different *Labeobarbus s.l*. lineages, see Fig. 1).

Tsigenopoulos *et al*. (2010: fig. 1) substantially extended the previous taxon sampling of mtDNA-based phylogenetic analyses and provided the currently most comprehensive phylogenetic data set. Their results showed that: (1) all hexaploid taxa analysed from Africa and the Middle East constitute a clear monophyletic mtDNA clade, with the tetraploid (2n ~ 100) Southeast Asian *Neolissochilus heterostomus* Chen & Yang, 1999 (in Chen, Yang & Chen, 1999) as its sister group, but excluding the hexaploid (2n ~ 150) Middle East genus *Capoeta* as well as tetraploid (2n ~ 100) *Luciobarbus* (cited karyological data, see Arai, 2011); (2) in the hexaploid *Labeobarbus* mtDNA clades several well-supported geographic subclades are identified; (3) *Varicorhinus*, now represented by six species, including the type species *Varicorhinus beso*, is polyphyletic. They concluded that the genus *Varicorhinus* should be considered a junior synonym of *Labeobarbus* as far as it concerns hexaploid species. In addition to their own mtDNA results, they supported this conclusion by mentioning that *Varicorhinus* is a poorly diagnosed genus, based on a limited number of morphological characters (mouth shape and lips structure), and that it had for quite a while been suspected of belonging to the genus *Labeobarbus* because of its genetic similarity (Berrebi, 1995; Durand *et al*., 2002) and hexaploidy (Krysanov & Golubtsov, 1996). Tsigenopoulos *et al*. (2010: fig. 1) had not included the type species of *Labeobarbus* (*L. nedgia*) in their analysis, however, and their suggested synonymization was not followed.

Levin *et al*. (2013) showed that Ethiopian *Varicorhinus* are polyphyletic with respect to mtDNA clades. They interpreted their results as sufficient evidence for a homoplasious origin of the specialized scraping mouth phenotype, which would have evolved not only in *V. beso*, the type species of the genus *Varicorhinus*, but also convergently in *V. jubae*. They further concluded that: (1) the genus *Varicorhinus* should be restricted to the type species *V. beso*, and should therefore be monospecific; whereas (2) *V. jubae*, identified as the mtDNA sister group of the *Labeobarbus gananensis* (Vinciguerra, 1895) complex, and jointly as the sister group of the *L. intermedius* complex and *Labeobarbus ethiopicus* (Zolezzi, 1939) together, should be transferred to *Labeobarbus*.

Most recently, Berrebi *et al*. (2014: fig. 1) fully incorporated the repercussions of the study of Tsigenopoulos *et al*. (2010) in their classification of *Barbus s.l*., and considered *V. beso*, type species of the genus *Varicorhinus*, as well as several other African *Varicorhinus*, to be *Labeobarbus* species. As such, Berrebi *et al*. (2014) effectively implemented the synonymy of *Varicorhinus* with *Labeobarbus*. Although Yang *et al*. (2015: fig. 2) still referred to species with a *Varicorhinus* mouth phenotype as *Varicorhinus* spp., all sub-Saharian Torini species with either a *Labeobarbus* or a *Varicorhinus* mouth phenotype were attributed to the *Labeobarbus* lineage, which together with the *Carasobarbus* and the *Pterocapoeta* lineages make up the *Labeobarbus* clade, here referred to as *Labeobarbus s.l*.

The status of Acapoeta tanganicae *and* Sanagia velifera

Since its original description as a monospecific subgenus of *Varicorhinus* by Cockerell (1910), and its synonymization with the latter genus by Boulenger (1916a), *Acapoeta* had never been considered a valid genus. Then, Fowler (1976), in his *Catalog of World Fishes (XXV)*, elevated *Acapoeta* to full generic level without any justification. This was followed by Lévêque & Daget (1984) in a taxon checklist of African freshwater fishes (CLOFFA). Poll & Gosse (1995) also followed this act and distinguished the only member of *Acapoeta*, *Acapoeta tanganicae* (Boulenger, 1900), from *Varicorhinus*, mainly based on the fact that it has 60 or more lateral line scales and the eyes are in lateral position, whereas *Varicorhinus* species have less than 50 scales and the eyes are generally in superolateral position. Therefore, the status of *Acapoeta* as a genus different from *Varicorhinus*, now within *Labeobarbus s.l*., certainly warrants further attention and confirmation. Unfortunately, karyological and/or genetic data, which would be useful to confirm its affinities within *Labeobarbus s.l*., are currently still lacking.

The same undoubtedly holds true for the genus *Sanagia*, with *Sanagia velifera* Holly, 1926 as the single species of this monospecific genus, which was described from and is endemic to the Sanaga Basin (Cameroon). *Sanagia* has been diagnosed by Holly (1926) mainly by its unique pharyngeal teeth formula (2, 4

and 4, 2), with only two rows of teeth on each pharyngeal jaw (for an illustration, see Banister & Thys van den Audenaerde, 1973: fig. 4), versus three rows of pharyngeal teeth in *Varicorhinus* (see Banister & Poll, 1973), and by the presence of a small cornified real cutting edge on the lower jaw (for an illustration, see Banister & Thys van den Audenaerde, 1973: fig. 2). Poll (1957) found *Sanagia* to be highly similar to *Varicorhinus*, despite the two aforementioned diagnostic characters; however, although the cornified real cutting edge on the lower jaw is similar to that in *Varicorhinus*, it is clearly narrower (for an illustration, see Banister & Thys van den Audenaerde, 1973: fig. 4). Furthermore, *S. velifera* has well-developed lips on the lateral sides of the lower jaw (for an illustration, see Banister & Thys van den Audenaerde, 1973: fig. 4), a peculiar character state that is absent in the typical *Varicorhinus* mouth phenotype. Recently, Yang *et al*. (2015: 112; table 3) assessed the position of *Sanagia* based on a mitochondrial (cytochrome *c* oxidase subunit I, *COI*) fragment of *S. velifera* (GenBank accession HM418112), and found it to be a member of the '*Labeobarbus* lineage', i.e. *Labeobarbus s.s*. As the phylogenetic analysis itself has not yet been presented, however, inclusion of *Sanagia* within *Labeobarbus s.l.* is here considered pending.

The numerous problems encountered on the generic level have never been totally unrelated to the major problems encountered on the α-taxonomic level. Undoubtedly important is the topic of intraspecific versus interspecific variation, and the repeated occurrence of 'intergeneric' hybridization. An introduction to both these topics is given below.

Phenotypic variation and/or 'intergeneric' hybridization

The history of the problem of the genus-level categorization of the large barbs, now Torini, is intimately entwined with the efforts to find a biologically meaningful interpretation of their impressive mouth phenotype variation. Therefore, a historical overview of the gradually but profoundly changing perception of what is intra- versus interspecific mouth phenotype variation is provided here, together with the potential role and consequences of repeatedly postulated 'intergeneric' hybridization in the group.

In the second volume of his *Catalogue of the freshwater fishes of Africa in the British Museum* the major diagnostic characters used by Boulenger (1911a) to subdivide the 90 valid large *Barbus* species (now *Labeobarbus*) were: (1) the ossification of the last simple dorsal fin ray; (2) the position of the base of the ventral fins relative to the dorsal fin; and, last but not least, (3) the mouth phenotype. With regards to the mouth phenotype, he explicitly referred to: (1) the lips of the lower jaw being either 'continuous' or 'interrupted' across the chin, which generally means with or without a mental lobe; (2) the lower jaw having a 'rounded or blunt' or instead a 'sharp' edge, with the sharp edge reminiscent of a *Varicorhinus*-like mouth phenotype. Although not apparent in his key, for ten of the valid species described in his catalogue he documented some kind of intraspecific variation of their lip phenotype. For all of these (e.g. *Barbus bynni* Forsskål, 1775; see also Boulenger, 1907c: 204) except one, this intraspecific variation fell within the boundaries of his two discrete character states, i.e. 'continuous' versus 'interrupted'. In the case of *Barbus hindii* Boulenger, 1902 (a junior synonym of *L. oxyrhynchus*, following Banister, 1973: 94), however, he noted '. . . lips moderately developed; lower continuous across chin (*rarely* interrupted); . . .' (emphasis ours). In this respect, *B. hindii* is an exception in crossing the boundaries between what Boulenger (1911a) generally seems to have regarded as intra- versus interspecific variation.

Based on ten newly collected large *Barbus* specimens from Lake Victoria, all of which he identified as *Barbus radcliffii* Boulenger, 1903, Norman (1928) considered *Barbus lobogenys* Boulenger, 1906, described from 'Bunkako, Buganga, Lake Victoria', a junior synonym of *B. radcliffii*, also described from 'Lake Victoria'. As such, he introduced the synonymy of two nominal large *Barbus* (now *Labeobarbus*) species, despite their very different lip morphologies, originally used by Boulenger as evidence to justify their recognition as valid species (for further details, see below). Worthington (1929), referring to Norman (1928), and based on an even larger series of specimens, redescribed *B. radcliffii*. According to Worthington (1929), the four different phenotypes all belong to one and the same species, which he referred to as *B. radcliffii* (now *Labeobarbus altianalis radcliffii*; see Worthington, 1932; and Banister, 1973): (1) specimens with a discontinuous lower lip across the chin [originally described as *Labeobarbus bayoni* (Boulenger, 1911)]; (2) specimens with continuous lower lip but without the development of a lobe (originally described as *L. radcliffii*); (3) specimens with continuous lower lip and a small lobe (identified as an intermediate form by Worthington, 1929); and finally (4) specimens with continuous and highly developed lips, i.e. including a large lobe (originally described as *L. lobogenys*; see Fig. 3; see also Worthington, 1929: legend to fig. 3). As males, females, and immature fish with large lips were found, Worthington (1929) explicitly excluded sexual dimorphism as a possible explanation for this lip polymorphism. His reinterpretation of the observed lip polymorphism in large *Barbus* (now *Labeobarbus*) also meant a radical conceptual change. Indeed, whereas before the difference between a 'continuous' versus an 'interrupted' lip morphology had generally been inter-

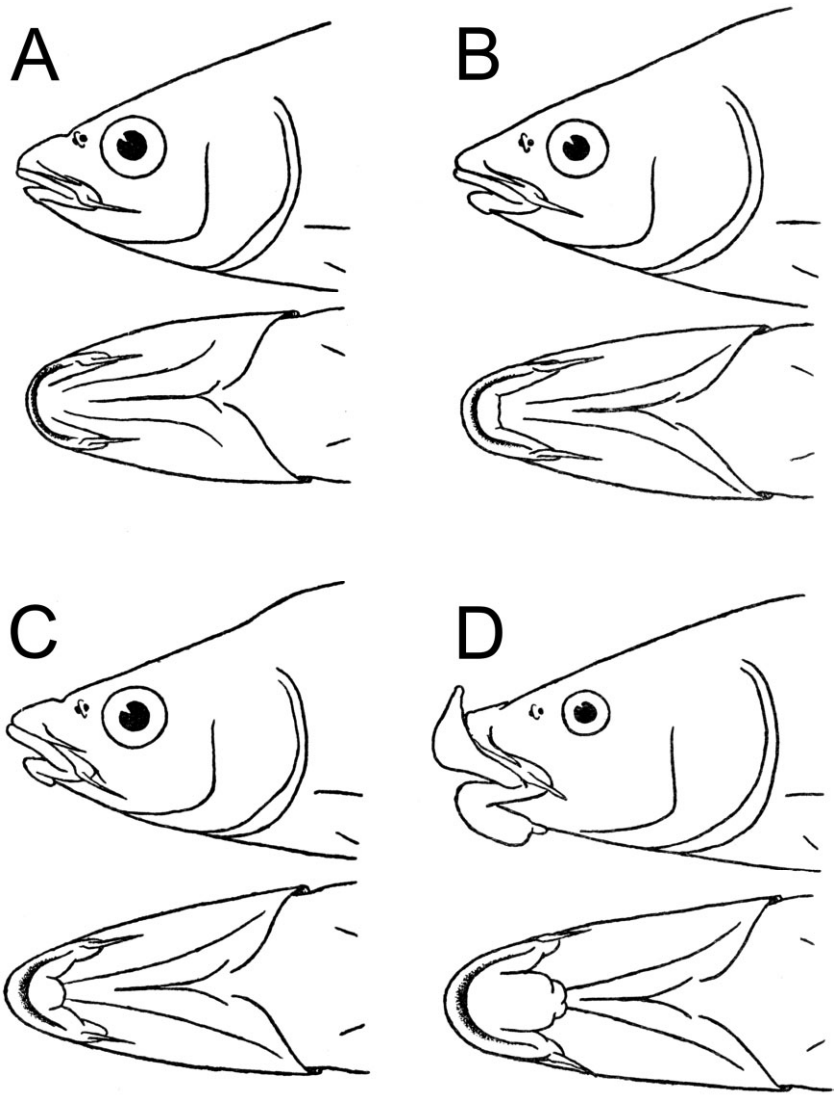


Figure 3. Illustration of four different, intraspecific, head phenotypes – in lateral and ventral view for each – as documented for *Labeobarbus radcliffii*, actually *Labeobarbus altianalis*, by Worthington (1929: 432, text to fig. 3): A, *Labeobarbus bayoni*; B, *L. radcliffii*; C, 'intermediate' phenotype; and D, *Labeobarbus lobogenys* (lateral views flipped horizontally). Reproduced from Worthington (1929), with permission from John Wiley and Sons.

preted as a species-specific difference (see above: Boulenger, 1911a), in the case of *B. radcliffii*, Worthington (1929) reinterpreted most if not all of it as intraspecific variation.

Later, Worthington (1932: fig. 1) illustrated and documented the same for *Labeobarbus altianalis eduardianus* (Boulenger, 1901), i.e. the Lake Edward, Lake George, and Kazinga Channel subspecies of *Labeobarbus altianalis* (Boulenger, 1900), for which he also illustrated three different mouth phenotypes similar to the ones described above for *B. radcliffii*. As an aside, he speculated that once enough specimens became available, the morphologically different endemic large barbs species from Lake Tana (Ethiopia) and the Upper Blue Nile (now *Labeobarbus*), i.e. *Barbus leptosoma* Boulenger, 1902 (actually a junior synonym of *L. intermedius*; see Banister, 1973), *Barbus degeni* Boulenger, 1902 (actually a junior synonym of *B. nedgia*; see Nagelkerke & Sibbing, 2000), and *Barbus nedgia*, might ultimately be found to be one and the same species, thereby illustrating the possible further implications of his findings.

Pellegrin (1935b), referring to Worthington (1932), reported that he found in sympatry even greater lip ('buccal': mouth) phenotype variation within *L. altianialis* from the Kivu and eastern tributaries of the Congo. Based on that, he recognized five different 'varieties', i.e. subspecies (see ICZN, 1999: article 45.6.4.), within *L. altianalis* from this region. In contrast to the Worthington (1932) case, Pellegrin also documented

non-overlapping scale counts between his subspecies. Nowadays, two of those, i.e. *Labeobarbus altianalis paucisquamatus* (Pellegrin, 1935) and *Labeobarbus altianalis longifilis* (Pellegrin, 1935), are considered full species, whereas *Labeobarbus altianalis labiosa* (Pellegrin, 1933) has been considered as a junior synonym of *L. altianalis* and *Labeobarbus altianalis lobogenysoides* (Pellegrin, 1935) has been considered as a junior synonym of *Labeobarbus paucisquamatus* (see Banister, 1972). Pellegrin's (1935b) case clearly illustrates the struggle to provide a biologically meaningful interpretation to the observed lip phenotype variation, as well as the difficulties in inferring what can and what should indeed be considered intra- versus interspecific lip phenotype variation.

A few years later, Worthington & Ricardo (1937) briefly reported that in *Labeobarbus tropidolepis* (Boulenger, 1900), endemic to the Lake Tanganyika drainage, specimens with 'interrupted' and 'continuous' lips across the chin can also be found. As a consequence, Worthington (1929, 1932) and Worthington & Ricardo (1937) concluded that lip morphology and therefore also Boulenger's key (1911a: 3, 7, 11) are of doubtful (diagnostic) value, at least for *L. a. radcliffii*, *L. a. eduardianus*, and *L. tropidolepis*. Also, Barnard (1943), referring to Worthington (1929), came to the same conclusion, i.e. that the description of new species solely on the presence of enlarged fleshy lips and labial lobes appears unwarranted. Barnard (1943: fig. 166) illustrated a similar case for *B. capensis* (now *L. seeberi*; E. Vreven, E.R. Swartz & P.H. Skelton, unpubl. data), in which both, the normal as well as the 'rubber-lip' 'varieties' are found.

Groenewald (1958: 273) further broadened the problem as he questioned the recognition of *Varicorhinus* as a valid genus different from *Barbus* (now *Labeobarbus*), by referring to the single difference between them being the 'square-shaped lower jaw, with its thick muscular covering and conspicuous horny cutting ridge'. As such, Groenewald (1958) described three different mouth phenotypes ('varieties' in his words) for *B. brucii*, which he also compared with the sympatric *Varicorhinus brucii* Boulenger, 1907: (1) a mouth phenotype without a mental lobe and with a sharp-edged lower jaw, but without a cornified real cutting edge, as found in *V. brucii* = *B. brucii* forma *sector* (= *Barbus sector* Boulenger, 1907c); (2) a mouth phenotype with a mental lobe = *B. brucii* forma *typica* (= *B. brucii*); (3) a rubberlip mouth phenotype, characterized by extremely thickened lips = *B. brucii* forma *gunningi* (= *Barbus gunningi* Gilchrist & Thompson, 1913). Groenewald (1958) also stressed that *B. brucii* as well as *V. brucii* are hardly distinguishable when seen from the side. *Barbus brucii*, *B. sector*, *B. gunningi*, as well as *V. brucii* are currently considered junior synonyms of *L. marequensis*, following Jubb (1963; for details on synonymy, see Jubb, 1968), with *L. marequensis* currently known from along the tropical east coast of South Africa (see Skelton, 2001: 9, 172, figs). So, although Groenewald (1958) questioned the validity of the genus *Varicorhinus*, he explicitly (see above) did not include the typical *Varicorhinus* mouth phenotype, i.e. *V. brucii*, with its cornified real cutting edge, within the intraspecific variation of *B. brucii*.

Groenewald (1958) not only documented the extreme lip polymorphism of *B. brucii* and *Barbus holubi* Steindachner, 1894 [actually a junior synonym of *Labeobarbus aeneus* (Burchell, 1822)], both with thin, normal, and thick-lipped (rubberlip) specimens, but he also provided an interesting observation for the peculiar phenomenon of lip polymorphism. He reported the case of a rubberlip specimen of *B. holubi* (now *L. aeneus*), the lips of which, after the specimen had been kept in a small pond with standing water and muddy bottom, gradually became thinner, and after a period of nearly 1 year could hardly be distinguished from the normal or thin-lipped form of the species. He concluded from this observation that no taxonomic importance can be attached to this extreme variation in lip development, because it appeared to him '. . . that the degree of lip development is connected with the feeding habits and method of feeding . . .' (Groenewald, 1958).

In addition to *L. marequensis*, Crass (1964: fig. 7) also reported and nicely illustrated (see Fig. 4) a similar *Varicorhinus*- and *Labeobarbus*-like intraspecific mouth phenotype polymorphism ('morphs') for what is now *L. natalensis* from the tropical east coast of South Africa, i.e. KwaZulu-Natal (see Skelton, 2001). Very different from Groenewald (1958), he also regarded the typical *Varicorhinus* mouth phenotype with its clear cutting edge as part of intraspecific lip phenotype variation. Crass (1964) thereby further completed the conceptual extension of Groenewald (1958) and Jubb (1963, 1967, 1968) by including the *Varicorhinus* mouth phenotype within a single large polymorphic *Barbus* (now *Labeobarbus*) species. Considering this reinterpretation of the observed mouth phenotype polymorphism by these authors, two points should be noted: (1) their interpretation of observed strong mouth phenotype polytypy as being intraspecific variation appears to be the consequence of an apparent lack of correlation with any covarying characters that would support a species status; (2) the logical consequence of the inclusion of the typical *Varicorhinus* mouth phenotypes – i.e. with their real cutting edge – within the variation found in large *Barbus* species (now *Labeobarbus*) questioned the validity of the genus *Varicorhinus* as a distinct genus.

Gaigher (1975: 162), referring to Jubb (1967), also considered the typical *Varicorhinus* mouth phenotype as part of intraspecific variation, and speculated that

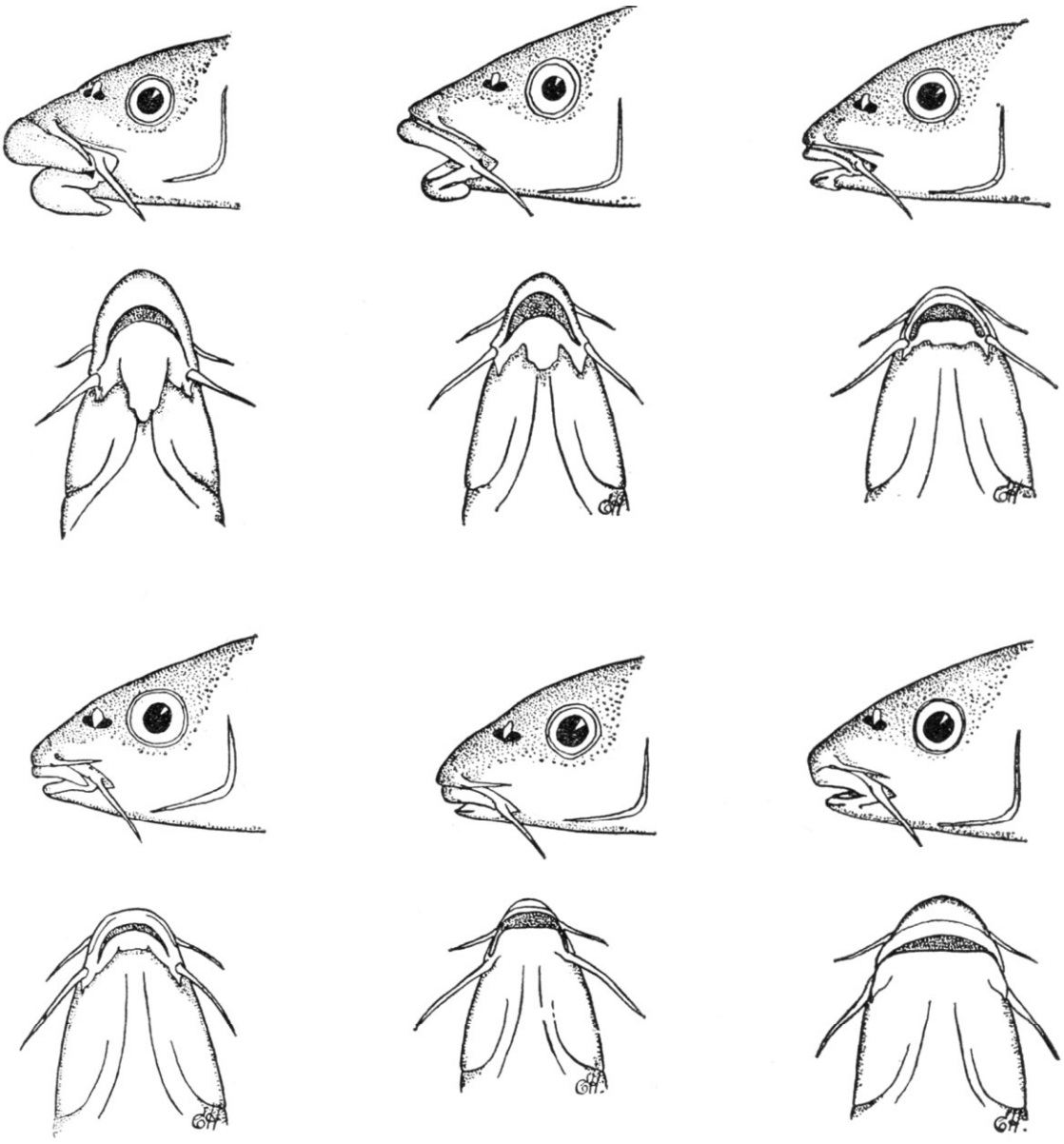


Figure 4. Illustration of six different, intraspecific, head phenotypes – in lateral and ventral view for each – as documented for *Labeobarbus natalensis* by Crass (1964: 53, fig. 7). Reproduced from Crass (1964), with permission from Shuter & Shooter Publishers (Pty) Ltd.

'*Varicorhinus*' and 'rubberlip' mouth forms are not genetically determined but might develop as trophic adaptations to different types of substrate. He interpreted the '*Varicorhinus*' mouth as a phenotypically plastic response to scraping epilithic algae and other food particles, whereas he considered the 'rubberlip' as a response to feeding between stones and pebbles (for the food regime, see du Plessis, 1956; Matthes, 1963). According to him, the ability to develop extreme mouth forms within a single species would be present not only in *L. marequensis*, *L. natalensis*, and *L. aeneus* (as *B. holubi* in his paper), but in the polymorphic *L. polylepis* of the upper Elands River (Southern Africa) as well. In contrast to the Lower Elands River, *L. polylepis* in the isolated stretches are not sympatric with *L. marequensis*. Still, according to Gaigher (1975), in these stretches reduced interspecific competition could have led to the development of polymorphic mouths, because of increased ecological opportunites for *L. polylepis* as a result of little interspecific competition. Gaigher (1975) therefore was the first to explicitily correlate mouth phenotype polytypy in a *Lab.–Var.*

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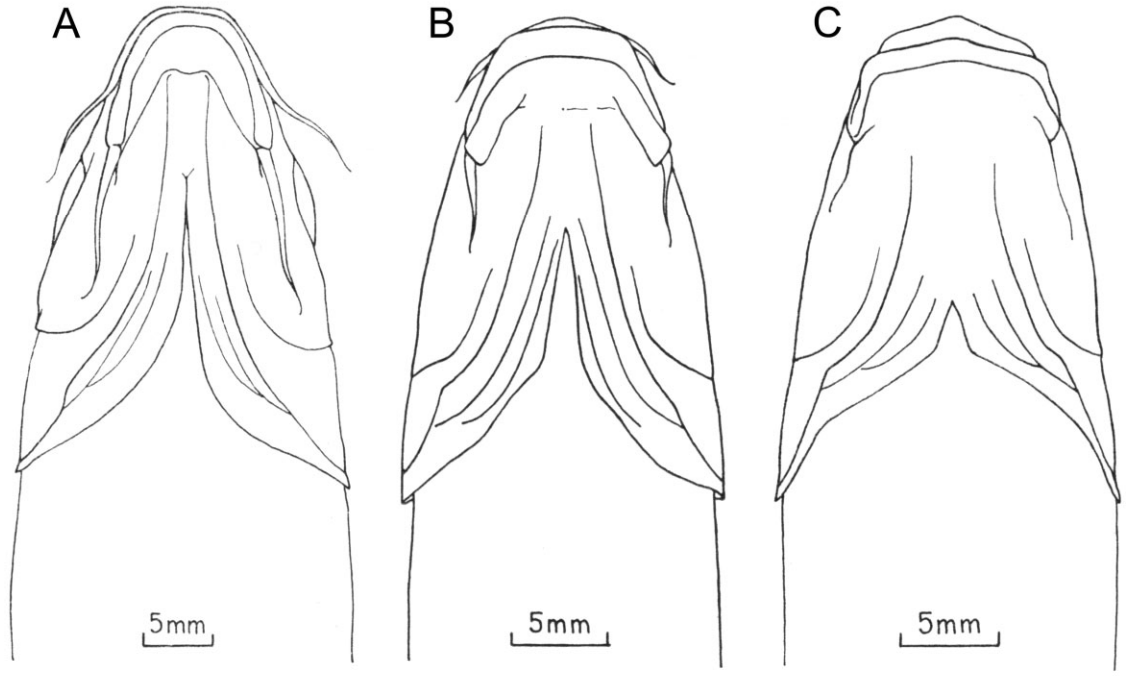


Figure 5. Schematic illustration of the ventral side of the head of: A, *Labeobarbus somereni*; B, *Labeobarbus alluaudi* – tentatively identified as an intergeneric hybrid between *L. somereni* and *Varicorhinus ruwenzorii* (now *Labeobarbus ruwenzorii*); C, *L. ruwenzorii* (from Banister, 1972: figs 2, 10, 12).

species complex with ecological opportunity, and intraspecific competition as a factor driving divergent trophic adaptation.

Polymorphism as a result of interspecific and/or intergeneric hybridization?

Boulenger (1902f) considered the large *Barbus* (now *Labeobarbus*) – referred to by him as the *Barbus bynni* Forsskål, 1775 group – as one of the most difficult he ever dealt with; however, he was confident that he had correctly estimated ontogenetic changes, because he had compared numerous specimens of all sizes, and therefore had not unduly multiplied the number of species. Nevertheless, he explicitely referred to the possibility that some of his species might be founded on hybrids (Boulenger, 1902f). It took decades before this idea would materialize in a concrete hypothesis or in case studies.

Almaça (1970) referred to the Moroccan *Barbus issenensis* Pellegrin, 1922 as a possible intergeneric hybrid between *Barbus massaensis* Pellegrin, 1922 [currently placed in the tetraploid *Luciobarbus* (see Arai, 2011), following Machordom & Doadrio (2001)] and *V. maroccanus*. By doing so, he was the first to suggest intergeneric hybridization between *Varicorhinus* and *Barbus* as an explanation for the occurence of intermediate mouth phenotypes.

Banister (1972), however, was the first to thoroughly and convincingly document a first possible case of intergeneric hybridization between large *Barbus* (now *Labeobarbus*) and *Varicorhinus* morphologically. He considered *Labeobarbus alluaudi* (Pellegrin, 1909) a possible intergeneric hybrid between two valid species, i.e. *Varicorhinus ruwenzorii* (Pellegrin, 1909) and *L. somereni*, both described from the eastern flanks of the Ruwenzori/Rwenzori mountain range (affluents of Lake George; Nile Basin) (Banister, 1972): both parental species not only differ in the typical *Labeobarbus* and *Varicorhinus* mouth phenotype characters, with an intermediate mouth phenotype found in his presumed 'intergeneric' hybrids (Banister, 1972: figs 2, 9, 15) (see Fig. 5), but also in selected meristics (i.e. number of lateral line scales and number of gill rakers on the first gill arch), osteological characters (e.g. the degree of development of a premaxilla ascending process; Banister, 1972: figs 6, 12, 19), and distance measurements (e.g. head length and anterior and posterior barbel lengths). It is important to point out here the differences in lateral line scales as these meristic characters are independent from the different trophic adaptations, and that this consilient but independent evidence strongly favours hybridization rather than intraspecific variation as an explanation for morphological intermediacy.

Before publishing his second case of possible intergeneric hybridization, Banister (1973) published his revision of the East and Central African large *Barbus*

(now *Labeobarbus*). In contrast to his previous publication (i.e. Banister, 1972), in this work he interpreted the observed high similarities, except in mouth phenotypes, between some large *Barbus* (now *Labeobarbus*) and *Varicorhinus* species drastically differently. For the entire Congo River Basin *s.l.* (i.e. including Lake Tanganyika and the Malagarazi Basin) he reported three large *Barbus*, now Torini species, to have specimens with both a *Labeobarbus* form (= *Labeobarbus* mouth phenotype) or a 'sector' form (= *Varicorhinus* mouth phenotype), i.e. he considered meristically and morphologically similar *Labeobarbus* and *Varicorhinus* mouth phenotypes in the Congo Basin as members of only three polymorphic species. He justified synonymizations explicitly in the first of the three cases by stating that there is '. . . sufficient awareness of the variability of *Barbus* [now *Labeobarbus*] species mouth parts to suggest that it is not unlikely that some African *Varicorhinus* species are highly modified individuals of various polymorphic *Barbus* [i.e. *Labeobarbus*] species' (Banister, 1972: 37). His first valid species was *Labeobarbus caudovittatus* (Boulenger, 1902) (referred to as a large *Barbus* in Banister, 1973), with *Varicorhinus stappersii* Boulenger, 1917 as a junior synonym of it. The second valid species would be *Labeobarbus trachypterus* (Boulenger, 1915), with *Varicorhinus bredoi* Poll, 1948 as a junior synonym. Interestingly in this case, Banister (1973: 120) reported for *L. trachypterus*: 'In small fishes a fleshy lower lip, often with a small mental lobe, is present, but in larger fishes the tendency is for the lower jaw to have a flat, cutting anterior margin.' By making this statement, he implicitly interpreted observed mouth phenotype variation in *L. trachypterus* as a size-related phenomenon (see *The period of discovery*). The third valid species would be *L. tropidolepis*, with *Varicorhinus chapini* Nichols & La Monte, 1950 as a full junior synonym (previously tentatively identified as a junior synonym of *L. tropidolepis* by Poll, 1953), for which he mentioned the slightly unusual mouth of the unique, small-sized holotype, and also reported the measurements as not differing from equally sized *L. tropidolepis* specimens. For the latter synonymy, however, Banister (1973) mistakenly referred to Poll (1952) instead of Poll (1953), and did not report having seen the holotype of the junior synonym himself, which does not possess a cornified cutting edge (Poll, 1953), and therefore cannot be a *Varicorhinus* mouth phenotype. In addition, in the same paper, Banister (1973) introduced two additional cases from outside the Congo Basin *s.l.*, where one or several nominal *Varicorhinus* mouth phenotype species (originally described in *Capoeta* at the genus or subgenus level) were formally synonymized with a large *Barbus* (now *Labeobarbus*) species by Banister (1973) himself (see An overview of species diversity in African *Labeobarbus*: current state of the art).

A few years later, Banister (1976a) presented his second case of possible intergeneric hybridization, this time between *V. tanganicae* (currently *Acapoeta*; see above) and *L. tropidolepis*, from Lake Tanganyika at Lunkungwe (Tanzania). Going back to his original concept of two different species (Banister, 1972), he considered these two taxa being two distinct parental species. As in the previous case (Banister, 1972), these not only differ in the typical *Labeobarbus* and *Varicorhinus* mouth phenotype characters, with an intermediate mouth phenotype found in presumed intergeneric hybrids, but also in meristics (i.e. number of lateral line scales, number of circumpeduncular scales, and number of gill rakers on the first gill arch), as well as in some distance measurements (e.g. head length, and anterior and posterior barbel lengths; %SL). As in the first case (Banister, 1972), he compared hybrids with specimens of both putative parental species, and found them not only intermediate with regards to trophic and related head morphology characters, but also in meristic characters independent of, or unrelated to, head morphology and related feeding behaviour. Banister might have justified the decision to synonymize (Banister, 1973), or not (Banister, 1972, 1976a), *Varicorhinus* with *Labeobarbus*-like taxa by his ability or disability to document any non-mouth phenotype-dependent differences: in the Congo-Basin cases (Banister, 1973), he did not find such differences, whereas he found them in the Ruwenzori and Tanganyika Basin cases (Banister, 1972, 1976a).

Banister (1976a: 184) considered Groenewald's (1958) adaptation hypothesis (our wording) unlikely, because of the apparently non-adaptive meristic differences between some sympatric *Labeobarbus* and *Varicorhinus* mouth phenotypes reported by him (see Banister, 1972, 1976a), and because of the differences in external mouth morphology between the *Labeobarbus* versus *Varicorhinus* mouth phenotypes, which he found to be correlated with structural osteological differences (for details, see Banister, 1972: figs 6–7, 12, 13).

Later, Banister & Clarke (1980) and also Banister (1984) reported two more cases of strong overall similarity between *Labeobarbus* and *Varicorhinus* forms, all from outside the Congo River Basin.

1. *Labeobarbus johnstonii* proved to be a variable species with a continuum between the *Varicorhinus* mouth phenotype (originally described as *Varicorhinus nyasensis* Worthington, 1933), with a broad ventral mouth, a lower lip with a sharp, cornified real cutting edge and short barbels, and the *Labeobarbus* mouth phenotype, with a narrower and subterminal mouth, a soft lower lip, and longer barbels (Banister & Clarke, 1980; see also Tweddle, 1996). Banister & Clarke (1980: fig. 22) published a histogram of mouth widths for

L. johnstonii specimens from Lake Malawi showing a bimodal distribution of values for a wide-mouthed form with a cornified real cutting edge to the lower lip and a narrower-mouthed form with soft lips. Banister & Clarke (1980: plate 5) further noted that in *L. johnstonii* a horny sheath is usually associated with the broad ventral mouth, but not invariably so. A horny sheath, developed to various degrees, is present in narrow-mouthed individuals (approximately two-thirds of their sample), but may be absent from specimens with an otherwise *Varicorhinus* mouth phenotype (approximately one-third of their sample). They interpreted the bimodal distribution with comparatively few intermediates as tentative evidence for disruptive selection acting through a trophic advantage of either form for epilithic feeding or 'adventitious' feeding (Banister & Clarke, 1980). They also reported a higher frequency of wide-mouthed forms in rocky areas as compared with the equal occurrence of the generalized form in all habitats. Unfortunately, they provided no details on the size (SL) of the examined specimens, which renders it impossible to test for (probably existent) positive, allometric growth of the mouth width within *L. johnstonii*. Interestingly, in the same paper, Banister & Clarke (1980) also identified a single possible hybrid specimen between two *Labeobarbus* species, i.e. *L. johnstonii* and *L. eurystomus* (Keilhack, 1908) [= *L. brevicauda* (Keilhack, 1908), following Seegers (1995); here = *L. latirostris* (see below)].

2. The second case refers to a *Varicorhinus* mouth phenotype being described as a taxonomically valid species, *V. jubae*, and to a *Labeobarbus* mouth phenotype, *L. gananensis*, both from the Juba River in Ethiopia. That the *Varicorhinus* mouth phenotype was described by Banister (1984) as a new species seems to contradict somewhat his previous decisions on synonymizations, as no obvious meristics differences were documented between the *Labeobarbus* and the *Varicorhinus* species from the Juba. According to Banister (1984) both differ only in their *Labeobarbus* versus *Varicorhinus* mouth phenotype-related head characters, i.e. gill raker counts, pharyngeal bone size, and barbel length. He even reported that both species resemble each other more closely than their congenerics. He further added that the more conspicuous differences are related to feeding, and, surprisingly, added that he had '. . . no immediate explanation for this . . .' phenomenon of 'intergeneric' siblings. Our interpretation of Banister's seemingly erratic concept is that his decision for the Juba case might well have been motivated by the absence of intermediate mouth phenotypes, which he had detected in his previous cases, in which he had synonymized meristically similar and sympatric *Labeobarbus* and *Varicorhinus* phenotypes. Although Banister (1976a, 1984) repeatedly mentioned the need for it, and his personal work in progress, a full revision of the genus *Varicorhinus* by Banister has unfortunately never been published. Nevertheless, the synonymization of nominal species with very different mouth phenotypes has continued after Banister's work, when Lévêque & Daget (1984) synonymized two Moroccan *Capoeta* species, i.e. *C. atlantica* and *C. waldoi*, with the Moroccan *Barbus fritschii fritschii* (now *Carasobarbus*, following Borkenhagen & Krupp, 2013), both without explicit motivation, however.

Nagelkerke & Sibbing (1996) identified in their Lake Tana collections four potential hybrid specimens between large *Barbus* (now *Labeobarbus*) and *Varicorhinus* (i.e. *V. beso*, the single species known from the basin). They morphologically inferred the hybrid status of these specimens based on the simultanous presence of a horny edged, shovel-shaped lower jaw, unknown in Lake Tana *Labeobarbus*, and the presence of well-developed barbels, unknown in *V. beso*. These authors stipulated that the occurrence of hybridization does not compromise the existence of real species in Lake Tana, and that even persistent hybridization at low levels does not necessarily threaten the genetic integrity of two parent groups; however, they did not consider the possibility of a true polymorphism.

Finally, Tweddle & Skelton (1998) reported two *Varicorhinus* mouth phenotype specimens of uncertain taxonomic identity from the Ruo River, Upper Zambezi, in Malawi. Both were identified as potential hybrids between *V. dimidiatus* and *Varicorhinus xyrocheilus* (Tweddle & Skelton, 1998), although according to the authors one of them could possibly be an aberrant specimen of *V. dimidiatus*. This interesting observation strongly suggests that hybridization might not be restricted to *Labeobarbus* and *Varicorhinus* mouth phenotypes, but might also occur among similar mouth phenotype species. Indeed, this was already indirectly suggested by Banister & Clarke (1980) when they identified a large *Barbus* (now *Labeobarbus*) specimen from Lake Malawi as a hybrid between *L. eurystomus* (= *L. latirostris*, see below) and *L. johnstonii*.

Except for Banister's (1972, 1976a) two classical and groundbreaking cases, no further thorough explorations on the occurrence of this phenomenon within this group of African Torini have been made since (for a tentative identification of hybrid specimens, however, see Banister & Clarke, 1980; Nagelkerke & Sibbing, 1996; Tweddle & Skelton, 1998). The repercussions of this inattention on the possible under- and/or over-recognition of species diversity within this group are manifold. To the present day a biologically sound reso-

lution of the alpha-level taxonomy of the *Lab.–Var.* species complexes is hampered by an obvious lack of elucidation of the evolutionary origin(s) and/or dynamics that have generated and maintained mouth phenotype diversity. This, however, is a prerequisite for a stable alpha- as well as the generic level taxonomy.

An overview of species diversity in African *Labeobarbus*: current state of the art

In light of all the aforementioned new developments in the field (see Tsigenopoulos *et al.*, 2010; Borkenhagen & Krupp, 2013; Levin *et al.*, 2013; Berrebi *et al.*, 2014; Borkenhagen, 2014), the need for a comprehensive list of all species-level taxa to be included in or excluded from *Labeobarbus s.l.* has become an important prerequisite for any further studies of the African large hexaploid Torini, in particular, and the African and Middle East ichthyofauna in general. Therefore, a table listing all currently valid African *Labeobarbus s.l.*, *Acapoeta*, and *Sanagia* species has been compiled (see Table 1). For details with regards to how data have been compiled, see the Material and methods section and the legend to Table 1.

The compilation of the present overview: what, how, and why?

Our current compilation lists a total of 125(+2) valid African *Labeobarbus s.l.* species known to date (Table 1). Mouth phenotype diversity has been tabulated with the two major hypotheses in mind: on one hand, the two morphologically well-documented cases of 'intergeneric' hybridization cited above, where Banister (1972, 1976a) illustrated intermediate mouth phenotype(s) to represent hybrid specimens; and on the other hand, the decision by Banister (1972) and others (Groenewald, 1958; Jubb, 1963, 1967, 1968; Crass, 1964) to interpret the huge observed mouth phenotype variation, encompassing both the *Labeobarbus* as well as the *Varicorhinus* mouth phenotypes, as intraspecific variation. The latter way of looking at the observed mouth phenotype diversity has also resulted in this aspect becoming somehow largely invisible, as it is presumed to be largely uninformative or even irrelevant for species diagnosis. Therefore, the current tabulation should be seen as a first effort in mapping the terrain, and has been undertaken to give a first glimpse of the current problems, questions raised, and further research needed.

The present overview: details on the taxonomic decisions made

To fully understand the extent and taxonomic conclusions of the provided list, the following points are explained in greater detail: (1) species previously explicitely allocated to *Labeobarbus* but which have been removed; (2) the identification of implicit synonymies according to the literature; (3) a small *Barbus* revealed to be a *Labeobarbus* species; (4) *Labeobarbus* spp. identified as possible hybrid phenotypes in the literature; (5) the neotype designation for *L. beso*, previously *V. beso*, the type species of the genus *Varicorhinus*; (6) substitute names (ICZN, 1999: article 60) following the synonymization of *Varicorhinus* with *Labeobarbus*; (7) the lectotype designation for *Labeobarbus sandersi* (Boulenger, 1912), previously a *Varicorhinus* species; (8) generic level synonyms of *Labeobarbus* for Africa.

1. Six Ethiopian species reported by Getahun (2007a: 94) as belonging to the genus *Labeobarbus* were, in fact, attributed to it in error and should have remained in *Barbus* (now *Enteromius*; see Yang *et al.*, 2015). These are: *Barbus anema* Boulenger, 1903; *Barbus arambourgi* Pellegrin, 1935; *Barbus kerstenii* Peters, 1868; *Barbus neglectus* Boulenger, 1903; *Barbus stigmatopygus* Boulenger, 1903; and *Barbus werneri* Boulenger, 1905 (actually a junior synonym of *B. stigmatopygus*: see annotated checklist 1). Indeed, all these are small *Barbus*, now *Enteromius*, with radiately striated scales and a dorsal fin formula of III7–8. One of them, i.e. *B. kerstenii*, even has a spiny, serrated, last unbranched dorsal fin ray, a character state never found in *Labeobarbus*. Also, the attribution of *Barbus litamba* Keilhack, 1908 to *Labeobarbus* by Snoeks (2004) is in error. Although this is indeed a large *Barbus* (maximum size, 315 mm SL; see Lévêque & Daget, 1984), this species also has a spiny, serrated, last unbranched dorsal fin ray, which, as stated above, is a character state that has never been documented for any of the karyotyped, hexaploid, *Labeobarbus*. Instead, the few karyotyped large African *Barbus* with a spiny, serrated, last unbranched dorsal fin ray were all shown to be diploid, as for *Barbus mattozi* Pereira Guimarães, 1884, or tetraploid, as for *B. capensis* (under *Barbus andrewi* Barnard, 1937) and *Barbus serra* Peters, 1864 (see Tsigenopoulos *et al.*, 2002). The same holds true for *Barbus rapax* Steindachner, 1894 (actually a junior synonym of *B. mattozi* following Jubb, 1963, although questioned by Skelton, 2001: 161), a sawfin *Barbus* (see Skelton, 2001) with radiating striae that has also erroneously been identified as a yellowfish (now *Labeobarbus*) by Groenewald (1958) in the past. Furthermore, following E. Vreven, E.R. Swartz & P.H. Skelton (unpubl. data), *B. capensis* has to be removed from *Labeobarbus* as a re-examination of the holotype revealed it to be a senior synonym of *B. andrewi* and hence not a *Labeobarbus* species. Therefore, in the present list, the name of *L. seeberi*, previously a junior synonym of *B. capensis*, has been used for the southern African clanwilliam yellowfish.

Table 1. Overview of all African (i.e. all species present on the African continent as delimited in Roberts, 1975: 266), large barbs, now Torini species, placed in *Labeobarbus s.l.*

			Mouth phenotype(s)					
Current name		Author & date	*Labeobarbus*	Intermediate	*Varicorhinus*	Notes	Max. size	References
1	*Labeobarbus acuticeps*	(Matthes, 1959)		a & ± **a**$_h$			403 TL	DeV&TvdA, 1990: 18
	Labeobarbus acutirostris	(Bini, 1940)		**a**$_h$, r & n		N1	411 FL	Na&Si, 1997: 130
	Labeobarbus aeneus[N2]	(Burchell, 1822)	h & f	x	rce	N3	500 FL	Sk, 2001: 169
	Labeobarbus altianalis	(Boulenger, 1900)	f	± **a**$_l$, r & n		N4	540 SL	Ec, 1992: 44
	Labeobarbus altipinnis	(Banister & Poll, 1973)		**n**$_h$	rce	N5	338 SL	Ba&Po, 1973: 82; Lé&Da, 1984: 336
	Labeobarbus ansorgii	(Boulenger, 1906)		**n**$_h$			300 TL	Bo, 1906a: 111; Lé&Da, 1984: 337
	Labeobarbus aspius	(Boulenger, 1912)		**n**$_s$			420 TL	Bo, 1912: 14; Lé&Da, 1984: 228; DeW&Te, 2007: 516–517
	Labeobarbus axelrodi	(Getahun, Stiassny & Teugels, 2004)			**rce**$_h$		160 SL	Ge *et al*., 2004: 160–161; Ge, 2007b: 542–543
	Labeobarbus batesii	(Boulenger, 1903)		± **a**$_h$ & n		N6	435 TL	Lé&Da, 1984: 230; Le, 2003: 367–368; DeW&Te, 2007: 520–521
10	*Labeobarbus beso*	(Rüppell, 1835)			**rce**$_n$		360 TL	Lé&Da, 1984: 337
	Labeobarbus boulengeri[N7]	current paper			**rce**$_l$		160 TL	Bo, 1910: 548; Lé&Da, 1984: 338
	Labeobarbus brauni	(Pellegrin, 1935)		**n**$_s$?	**rce**$_s$	N8	195 SL; 245 TL	Pe,1935: 402
	Labeobarbus brevicephalus	(Nagelkerke & Sibbing, 1997)		**n**$_h$		N9	317 FL	Na&Si, 1997: 131; Na&Si, 2000: 192
	Labeobarbus brevispinis	(Holly, 1927)		**a**$_s$ & **n**$_s$			230 TL	Lé&Da, 1984: 232; DeW&Te, 2007: 520–521
	Labeobarbus bynni	(Forsskål, 1775)	f, ± **f**$_n$	a & n		N10	820 TL	Lé&Da, 1984: 234
	Labeobarbus cardozoi	(Boulenger, 1912)	**f**$_s$				530 TL	Bo, 1912: 12; Lé&Da, 1984: 238; DeW&Te, 2007: 526–527
	Labeobarbus caudovittatus	(Boulenger, 1902)	f	**a**$_s$ & n	rce (1 *V.* syn)	N11	800 TL	Lé&Da, 1984: 239; DeW&Te, 2007: 524 & 527; Ec, 1992: 45
	Labeobarbus clarkeae	(Banister, 1984)		**pap**$_h$			161 SL	Ba, 1984: 277
	Labeobarbus claudinae	(De Vos & Thys van den Audenaerde, 1990)	f	**a**$_h$			234 SL; 300 TL	DeV&TvdA, 1990: 7
20	*Labeobarbus codringtonii*	(Boulenger, 1908)	h & f	**n**$_h$		N12	390 TL	Sk, 2001: 173
	Labeobarbus compiniei	(Sauvage, 1879)	h & **f**$_h$			N13	730 TL	Sa, 1879: 102; Lé&Da, 1984: 241; DeW&Te, 2007: 528–529
	Labeobarbus crassibarbis	(Nagelkerke & Sibbing, 1997)		a, **r**$_h$ & n		N14	505 FL	Na&Si, 1997: 132; Na&Si, 2000: 193
	Labeobarbus dainellii	(Bini, 1940)	f	a, **r**$_h$ & n		N15	490 FL	Na&Si, 1997: 133
	Labeobarbus dartevellei	(Poll, 1945)		**n**$_h$			117 TL	Po, 1945: 299–300; Lé&Da, 1984: 241
	Labeobarbus dimidiatus	(Tweddle & Skelton, 1998)			**rce**$_h$	N16	245 SL	Tw&Sk, 1998: 371; Sk, 2001: 174
	Labeobarbus ensifer	(Boulenger, 1910)		**pap**$_s$		N17	195 TL	Bo, 1910: 546; Lé&Da, 1984: 337
	Labeobarbus ensis	(Boulenger, 1910)	**f**$_s$	**a**$_s$, **r**$_s$ & **n**$_s$			140 TL	Bo, 1910: 550; Lé&Da, 1984: 243
	Labeobarbus ethiopicus	(Zolezzi, 1939)		a & n		N18	258 SL	Ba, 1973: 41; Lé&Da, 1984: 243
	Labeobarbus fasolt	Pappenheim (in Pappenheim & Boulenger, 1914)	**f**$_h$				500 TL	Pa&Bo, 1914: 241; Lé&Da, 1984: 245

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30	*Labeobarbus fimbriatus*	(Holly, 1926)		**n**$_s$			213 TL	Ho, 1926: 157; Lé&Da, 1984: 338
	Labeobarbus fritschii	(Günther, 1874)		**n**$_s$	rce (2 *V.* syn)	N19	180 TL	Lé&Da, 1984: 246
	Labeobarbus gananensis	(Vinciguerra, 1895)	**f**$_h$	a & n			176 SL	Ba, 1973: 45; Lé&Da, 1984: 247
	Labeobarbus gestetneri	(Banister & Bailey, 1979)		**n**$_h$			249 TL	Ba&Bai, 1979: 218; Lé&Da, 1984: 247
	Labeobarbus girardi	(Boulenger, 1910)		**n**$_s$			300 TL	Bo, 1910: 551; Lé&Da: 1984: 247
	Labeobarbus gorgorensis	(Bini, 1940)		a, ± **a**$_h$, r & n		N20	618 FL	Na&Si, 1997: 134
	Labeobarbus gorguari	(Rüppell, 1835)		± **a**$_h$, r & n		N21	532 FL	Na&Si, 1997: 135
	Labeobarbus gruveli	(Pellegrin, 1911)		**n**$_h$			280 TL	Pe, 1911: 185; Lé&Da, 1984: 247; Lé&Gu, 1990: 52–54; Le, 1990: 308–309
	Labeobarbus gulielmi	(Boulenger, 1910)		**a**$_s$ & **n**$_s$			150 TL	Bo, 1910: 551; Lé&Da, 1984: 248
	Labeobarbus habereri	(Steindachner, 1912)	**f**$_h$				162 TL	Ho, 1927: 141; Lé&Da, 1984: 249; DeW&Te, 2007: 518–519
40	*Labeobarbus harterti*	(Günther, 1901)		**n**$_s$			285 TL	Lé&Da, 1984: 246
	Labeobarbus huloti	(Banister, 1976)	f	a & **n**$_h$			282 SL	Ba, 1976b: 192; Lé&Da, 1984: 250
	Labeobarbus humphri	(Banister, 1976)	f	**a**$_h$			214 SL	Ba, 1976b: 198; Lé&Da, 1984: 250
	Labeobarbus intermedius	(Rüppell, 1835)	h & f	a, **r**?$_h$ & n	rce (1 *V.* syn)	N22	489 SL	Lé&Da, 1984: 255
	Labeobarbus iphthimostoma	(Banister & Poll, 1973)			**rce**$_h$		181 SL	Ba&Po, 1973: 87; Lé&Da, 1984: 338
	Labeobarbus iturii	(Holly, 1929)	**f**?$_h$			N23	365 TL	Ho, 1929: 35; Lé&Da, 1984: 255
	Labeobarbus jaegeri	(Holly, 1930)			**rce**$_h$		188 TL	Ho, 1930: 199; Lé&Da, 1984: 338; Ge, 2007b: 541–542
	Labeobarbus johnstonii	(Boulenger, 1907)	f	a & **n**$_h$	rce (1 *V.* syn)	N24	320 SL	Ba&Cl, 1980: 505–506; Lé&Da, 1984: 256; Ec, 1992: 45
	Labeobarbus jubae	(Banister, 1984)			**rce**$_h$	N25	135 SL	Ba, 1984: 273
	Labeobarbus jubbi	(Poll, 1967)	f			N26	166 SL; 225 TL	Po, 1967: 177; Lé&Da, 1984: 257
50	*Labeobarbus kimberleyensis*	(Gilchrist & Thompson, 1913)		**a**$_h$			825 FL	Sk, 2001:168
	Labeobarbus lagensis	(Günther, 1868)		**n**$_h$			230 SL/255 TL	Lé&Da, 1984: 259; Lé&Gu, 1990: 49; Le, 1990: 304; Le, 2003: 363–364
	Labeobarbus latirostris[N27]	(Keilhack, 1908)		**n**$_l$			370 SL	Ke, 1908: 166
	Labeobarbus leleupanus	(Matthes, 1959)			**rce**$_h$		220 SL	Lé&Da, 1984: 338
	Labeobarbus longidorsalis	(Pellegrin, 1935)			**rce**$_h$		260 SL; 320 TL	Pe, 1935: 403; Lé&Da, 1984: 338
	Labeobarbus longifilis	(Pellegrin, 1935)	f	**a**$_l$			247 mm SL	Ba, 1973: 76
	Labeobarbus longissimus	(Nagelkerke & Sibbing, 1997)		r & **n**$_h$		N28	548 FL	Na&Si, 1997: 135
	Labeobarbus lucius	(Boulenger, 1910)		**n**$_s$			230 TL	Bo, 1910: 553; Lé&Da, 1984: 262; DeW&Te, 2007: 514–515
	Labeobarbus lufupensis	(Banister & Bailey, 1979)		**n**$_h$			194 SL	Ba&Bai, 1979: 227; Lé&Da, 1984: 338
	Labeobarbus macroceps	(Fowler, 1936)		**n**$_h$			320 TL	Fo, 1936: 283; Lé&Da, 1984: 263
60	*Labeobarbus macrolepidotus*	(Pellegrin, 1928)		**n**$_s$	rce	N29	540 TL	Lé&Da, 1984: 339
	Labeobarbus macrolepis	(Pfeffer, 1889		**n**$_l$			400 SL	Ec, 1992: 46
	Labeobarbus macrophthalmus	(Bini, 1940)		a, **r**$_h$, & n		N30	425 FL	Na&Si, 1997: 136
	Labeobarbus malacanthus	(Pappenheim, 1911)		± **a**$_h$			150 TL	Pa, 1911: 517; Lé&Da, 1984: 264; DeW&Te, 2007: 521–522

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Table 1. *Continued*

	Current name	Author & date	Mouth phenotype(s)			Notes	Max. size	References
			Labeobarbus	Intermediate	*Varicorhinus*			
	Labeobarbus marequensis	(Smith, 1841)	h & f	**a**?$_h$, r & n	rce (2 *V.* syn)	N31	470 TL	Sk, 2001: 172
	Labeobarbus mariae	(Holly, 1926)		**n**?$_s$	**rce**$_s$ (*V.*-like)	N32	300 SL	Ho, 1926: 156; Lé&Da, 1984: 339; Ge, 2007b: 543–544
	Labeobarbus maroccanus	(Günther, 1902)			**rce**$_s$		400 TL	Lé&Da, 1984: 339
	Labeobarbus matris	(Holly, 1928)		**a**$_h$			322 TL	Ho, 1928: 4
	Labeobarbus mawambi	Pappenheim (in Pappenheim & Boulenger, 1914)		**n**$_h$			150 TL	Lé&Da, 1984: 268
	Labeobarbus mawambiensis	(Steindachner, 1911)	**f**$_s$	± **a**$_s$ & **a**$_s$			150 TL	Lé&Da, 1984: 268
70	*Labeobarbus mbami*	(Holly, 1927)		**n**$_h$			230 SL	DeW&Te, 2007: 524–525
	Labeobarbus megastoma	(Nagelkerke & Sibbing, 1997)		**a**$_h$, r & n		N33	824 FL	Na&Si, 1997: 137; Na&Si, 2000: 197
	Labeobarbus micronema	(Boulenger, 1904)		**n**$_s$			340 TL	Lé&Da, 1984: 268; DeW&Te, 2007: 518–519
	Labeobarbus mirabilis	Pappenheim (in Pappenheim & Boulenger, 1914)		**r**$_h$			353 SL	Ba, 1973: 92; Lé&Da, 1984: 270
	Labeobarbus mungoensis	(Trewavas, 1974)	f	**a**$_h$ & n			179 SL	Lé&Da, 1984: 271; DeW&Te, 2007: 522–523
	Labeobarbus nanningsi	de Beaufort, 1933	**f**$_h$				320 TL	Po, 1967: 157; Lé&Da, 1984: 271
	Labeobarbus natalensis[N34]	(de Castelnau, 1861)	h & f	a & n	rce	N35	638 TL	Sk, 2001: 170
	Labeobarbus nedgia	Rüppell, 1835	± **h**$_h$ & f			N36	707 FL	Na&Si, 1997: 138
	Labeobarbus nelspruitensis	(Gilchrist & Thompson, 1911)			**rce**$_s$	N37	320 TL	Sk, 2001: 175
	Labeobarbus nthuwa	Tweddle & Skelton, 2008		**a**$_h$ & n	rce	N38	236 SL	Tw&Sk, 2008: 29
80	*Labeobarbus osseensis*	(Nagelkerke & Sibbing, 2000)	f	**n**$_h$		N39	264 SL	Na&Si, 2000: 190
	Labeobarbus oxyrhynchus	(Pfeffer, 1889)	h & f	a, r & **n**$_h$	rce (1 *V.* & 1 *V.*-like syn)	N40	400 SL	Ec, 1992: 46
	Labeobarbus pagenstecheri	(Fischer, 1884)		± **a**$_l$	rce (*V.*-like)	N41	350 SL	Ec, 1992: 45
	Labeobarbus parawaldroni	(Lévêque, Thys van den Audenaerde & Traore, 1987)	**h**$_h$ & f			N42	230 SL	Lé *et al.*, 1987: 347; Lé&Gu, 1990: 48; Lé, 1990: 303; Lé, 2003: 363–364
	Labeobarbus paucisquamatus	(Pellegrin, 1935)	h	**a**$_l$ & r		N43	248 SL	Ba, 1973: 106; Lé&Da, 1984: 279
	Labeobarbus pellegrini	(Bertin & Estève, 1948)			**rce**?$_h$	N44	212 TL	Pe, 1932: 959; Lé&Da, 1984: 339
	Labeobarbus petitjeani	(Daget, 1962)		**a**$_s$, **r**$_s$ & **n**$_s$		N45	175 SL	Lé&Gu, 1990: 53
	Labeobarbus platydorsus	(Nagelkerke & Sibbing, 1997)		a, r, ± **r**$_h$ & n		N46	635 FL	Na&Si, 1997: 139; Na&Si, 2000: 199
	Labeobarbus platyrhinus	(Boulenger, 1900)		**r**$_h$			400 SL	Ec, 1992: 45
	Labeobarbus platystomus	Pappenheim (in Pappenheim & Boulenger, 1914)			**rce**$_s$		210 TL	Lé&Da, 1984: 339
90	*Labeobarbus pojeri*	(Poll, 1944)		**a**$_h$ & n			180 TL	Po, 1944: 6
	Labeobarbus polylepis	(Boulenger, 1907)	x?	**a**$_h$	rce?	N47	585 TL	Lé&Da, 1984: 282
	Labeobarbus progenys	(Boulenger, 1903)		**n**$_h$			180 TL	Lé&Da, 1984: 282; Le, 2003: 366–367; DeW&Te, 2007: 516–517
	Labeobarbus pungweensis	(Jubb, 1959)			**rce**$_h$	N48	180 SL	Sk, 2001: 176
	Labeobarbus reinii	(Günther, 1874)	**f**$_s$	**a**$_s$			555 TL	Pe, 1921: 136; Lé&Da, 1984: 285
	Labeobarbus rhinoceros[N49]	Copley, 1938		**n**$_l$			295 TL	Ba, 1973: 83
	Labeobarbus rhinophorus	(Boulenger, 1910)		**n**$_s$			150 TL	Lé&Da, 1984: 285
	Labeobarbus robertsi	(Banister, 1984)		**pap**$_h$			220 SL	WaLu, 2010: 142

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	Labeobarbus rocadasi	(Boulenger, 1910)		± $\mathbf{a}_s$, $\mathbf{r}_s$ & $\mathbf{n}_s$			350 TL	Lé&Da, 1984: 285; DeW&Te, 2007: 526–527
	Labeobarbus rosae	(Boulenger, 1910)		$\mathbf{n}?_s$	$\mathbf{rce}_s$ (*V.*-like)	N50	95 TL	Lé&Da, 1984: 286
100	*Labeobarbus roylii*	(Boulenger, 1912)	$\mathbf{h}_s$ & $\mathbf{f}_s$				550 TL	DeW&Te, 2007: 523–524
	Labeobarbus ruandae	Pappenheim (in Pappenheim & Boulenger, 1914)			$\mathbf{rce}_h$		150 TL	Lé&Da, 1984: 340
	Labeobarbus ruasae	Pappenheim (in Pappenheim & Boulenger, 1914)	± $\mathbf{f}_s$	± $\mathbf{a}_s$			430 SL; 495 TL	DeV&TvdA, 1990: 19
	Labeobarbus ruwenzorii	(Pellegrin, 1909)			$\mathbf{rce}_s$	N51	231 TL	Lé&Da, 1984: 340
	Labeobarbus sacratus	(Daget, 1963)	f	a, $\mathbf{r}_s$ & $\mathbf{n}_s$		N52	256 SL	Lé&Gu, 1990: 56; Le, 1990: 307; Le, 2003: 367–368
	Labeobarbus sandersi	(Boulenger, 1912)		n	$\mathbf{rce}_l$	N53	370 TL	Bo, 1912: 12; Lé&Da, 1984: 340
	Labeobarbus seeberi[N54]	(Gilchrist & Thompson, 1913)	h & f	$\mathbf{a}_s$, $\mathbf{r}_s$ & $\mathbf{n}_s$	rce (*V.*-like)	N55	987 TL	Sk, 2001: 171
	Labeobarbus semireticulatus	(Pellegrin, 1924)		$\mathbf{n}?_s$	$\mathbf{rce}_s$	N56	128 SL; 158 TL	Pe, 1924: 287; Lé&Da, 1984: 340; Ge, 2007b: 545–546
	Labeobarbus somereni	(Boulenger, 1911)		± $\mathbf{n}_h$			400 SL	Ec, 1992: 46
	Labeobarbus stappersii	(Boulenger, 1915)	f	**a** & $\mathbf{n}_h$		N57	594 SL	Ba, 1973: 119; Lé&Da, 1984: 288
110	*Labeobarbus steindachneri*	(Boulenger, 1910)			$\mathbf{rce}_s$		330 SL	Ge, 2007b: 546–547
	Labeobarbus stenostoma	(Boulenger, 1910)			$\mathbf{rce}_h$		105 TL	Bo, 1910: 546; Lé&Da, 1984: 341
	Labeobarbus surkis	(Rüppell, 1835)		r & $\mathbf{n}_h$		N58	430 FL	Na&Si, 1997: 140
	Labeobarbus tornieri	(Steindachner, 1906)			$\mathbf{rce}_h$		184 SL	Ge, 2007b: 547–548
	Labeobarbus trachypterus	(Boulenger, 1915)		$\mathbf{n}_h$	rce (1 *V.* syn)	N59	239 SL	Ba, 1973: 120; Lé&Da, 1984: 291–292
	Labeobarbus tropidolepis	(Boulenger, 1900)		a, $\mathbf{r}_l$ & n		N60	850 SL	Ec, 1992: 44
	Labeobarbus truttiformis	(Nagelkerke & Sibbing, 1997)		$\mathbf{n}_h$		N61	442 FL	Na&Si, 1997: 141; Na&Si, 2000: 200
	Labeobarbus tsanensis	(Nagelkerke & Sibbing, 1997)		$\mathbf{a}_h$, r & n		N62	394 FL	Na&Si, 1997: 142; Na&Si, 2000: 202
	Labeobarbus upembensis	(Banister & Bailey, 1979)			$\mathbf{rce}_h$		203 SL	Ba&Bai, 1979: 229; Lé&Da, 1984: 341
	Labeobarbus urotaenia[N63]	(Boulenger, 1913)	$\mathbf{f}_s$	$\mathbf{a}_s$			50 TL	Lé&Da, 1984: 295
120	*Labeobarbus varicostoma*	(Boulenger, 1910)		$\mathbf{pap}_h$			170 TL	Bo, 1910: 547; Lé&Da, 1984: 341
	Labeobarbus versluysii	(Holly, 1929)		$\mathbf{a}_s$		N64	111 TL	Ho, 1929: 33; DeW&Te, 2007: 528–529
	Labeobarbus werneri	(Holly, 1929)			$\mathbf{rce}?_s$	N65	145 TL	Ge, 2007b: 548–549
	Labeobarbus wittei	(Banister & Poll, 1973)		$\mathbf{n}_h$	rce	N66	375 TL	Ba&Po, 1973: 91; Lé&Da, 1984: 341
	Labeobarbus wurtzi	(Pellegrin, 1908)		$\mathbf{n}_h$	rce	N67	285 SL	Lé&Gu, 1990: 61
125	*Labeobarbus xyrocheilus*	(Tweddle & Skelton, 1998)			$\mathbf{rce}_h$	N68	178 SL	Tw&Sk, 1998: 379; Sk, 2001: 370
	Hybrids and possible hybrids							
	Labeobarbus alluaudi	(Pellegrin, 1909)		$\mathbf{n}_s$			198 SL	Ba, 1972: 276; Ba, 1973: 9; Lé&Da, 1984
	Labeobarbus microbarbis	(David & Poll, 1937)		$\mathbf{n}_h$			230 SL; 270 TL	DeV&TvdA, 1990: 20–21
	Labeobarbus microterolepis	(Boulenger, 1902)		± $\mathbf{n}_h$			118 SL	Ba, 1973: 89; Lé&Da, 1984: 268
	Others							
	Acapoeta tanganicae	(Boulenger, 1900)			$\mathbf{rce}_l$	N69	610 TL	Lé&Da, 1984: 217
	Sanagia velifera	Holly, 1926			$\mathbf{rce}_s$ (*V.*-like)	N70	158 SL; 190 T	Lé&Da, 1984: 336; DeW, 2007: 536–537

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Nominal species representing hybrid phenotypes, or suspected to be doing so, according to the literature cited, have been listed separately at the end of the table. Also both *Acapoeta* and *Sanagia* species have been added at the end as a matter of completeness (i.e. total +2). Unjustified revalidations, however, i.e. the reuse of formally synonymized species names without any explicit justification with regards to this decision, have not been followed. The same holds true for unsubstantiated proposals of possible new synonyms/ies. All species names are organized in alphabetic order.

For each species the following information is provided: current species name (Current name); author(s) and date of the original description (Author & date); maximum size (Max. size) reported (FL, fork length; SL, standard length; TL, total length; all in mm); documented mouth phenotype(s) [Mouth phenotype(s)], and as such possible mouth polymorphism; and references (References) used for the current compilation of the tabulated information with regards to the maximum reported size. Additional information has been provided under Notes, referred to in the table with superscript N, and followed by the number of the particular note. The notes themselves are to be found below the table.

Mouth phenotype(s) as well as possible mouth phenotype polymorphism have been tabulated for each of the valid species to get a first pan-African overview of this peculiar phenomenon. For this, the observed mouth phenotype diversity has been subdivided into three major categories, i.e. the *Labeobarbus* versus *Varicorhinus* mouth phenotype as the two extreme mouth phenotype categories and with a third, the intermediate category, in between these. The typical *Labeobarbus* mouth phenotype has been defined as characterized by the presence of, at least, a free mental lobe, and has been further subdivided according to the presence/absence of hypertrophied lips (i.e. the anteromedian part of the upper lip forming a flap-like extension clearly covering the anteromedian part of the snout). The typical *Varicorhinus* mouth phenotype has been restricted to species with the presence of a clear cornified cutting edge on the lower jaw. Finally, specimens with an attached or rudimentary (i.e. no real lobe, but only a discontinuous suture line demarcating a lobe-like structure) mental lobe, or without a mental lobe, have been tabulated as intermediates. Priority has been given to tabulate the mouth phenotype(s) of the type(s) with special attention in differentiating the mouth phenotype(s) as found in the name-bearing type(s), which is/are the only one(s) with nomenclatural standing (see ICZN, 1999: articles 72.1.2. and 72.1.3.). Whenever available, and based on sound, reliable, revisionary evidence [see Nagelkerke & Sibbing (1997, 2000) for the Lake Tana (Ethiopia) species and Skelton (2001) for the Southern African species], mouth phenotype polymorphism as reported for the species as a whole, i.e. including non-types, has been included.

The abbreviations used to characterize these mouth phenotypes are as follows: a, attached [equals continuous lower lip development (LLD) of Nagelkerke & Sibbing (2000: fig. 2)]; f, free; h, hypertrophied; n, no [equals interrupted LLD of Nagelkerke & Sibbing (2000: fig. 2)]; and r, rudimentary mental lobe. A few *Labeobarbus* species have pappilae (pap) on the lower as well as the upper jaw instead. These have explicitely been reported as such under the intermediate mouth phenotype, although, in these cases, this does not imply the intermediacy of this character state. A question mark (?) has been used when, unfortunately: (1) the detailed mouth phenotype remains undocumented [i.e. not stipulated in the literature and the name-bearing/nominal type(s) are lost or have not been examined]; (2) the present character state is doubtful, this most often because of damage or due to the actual state of preservation of the type(s) (see Notes). Whenever personal observation of the mouth phenotype character state on the name-bearing/nominal type(s) of the valid species has been possible, reference is made to these results. The character state as found in the name-bearing/nominal type(s) is made explicit by using bold font for the mouth phenotype(s). Details on the nominal type(s) have been added in subscript: h, holotype; l, lectotype; s, syntypes. An 'x' has been used when the literature refers to the existence of one of the three major mouth phenotypes without, however, providing the necessary details to add more information. As a general rule, we have refrained from explicitely tabulating mouth phenotype(s) as found in junior synonym(s); however, when former *Varicorhinus* species have been synonymized with a valid *Labeobarbus* mouth phenotype species, this has explicitly been stipulated as follows: *V.* syn or *V.*-like syn, *Varicorhinus* or *Varicorhinus*-like mouth phenotype synonym(s), preceded by the number of such synonyms. A *Varicorhinus*-like mouth phenotype refers to the fact that it has a typical real cutting edge (rce), but nevertheless differs in other respects from the typical *Varicorhinus* mouth phenotype, which has been documented in the notes. Indeed, in the typical *Varicorhinus* mouth phenotype, the fleshy lips on the lateral sides of the lower jaw are poorly developed (see Boulenger, 1909: plate 33 for *V. beso*), i.e. covering approximately only half of the lateral sides of the lower jaw, but very often much less (one-third or even only one-quarter, as in *V. beso* itself; with important intraspecific variation in some species as currently delimited). Instead, in the *Varicorhinus*-like mouth phenotype, the fleshly lips on the lateral sides of the lower jaw are obviously more developed, i.e. covering approximetely two-thirds or more of the lateral sides of the lower jaw (e.g. *L. rosae*; *Sanagia velifera*). Nevertheless, considering: (1) the very poor development of the lips in *V. beso* (see above); (2) the important inter- and sometimes even intraspecific variation, this character is certainly in need of further attention (see text). In addition, whenever relevant with regards to the tabulated overall intraspecific mouth phenotype variation, reference is also made to the mouth phenotype of the name-bearing/nominal type(s) of other junior synonyms (i.e. non *Varicorhinus* nominal species) in the notes. The junior synonyms examined have been listed in the notes, grouped according to their mouth phenotype, and listed from the *Labeobarbus* through the intermediate to the *Varicorhinus* mouth phenotype.

Abbreviations of the author names for the references cited are as follows: Ba, Banister; Ba&Cl, Banister & Clarke; Ba&Bai, Banister & Bailey; Ba&Po, Banister & Poll; Bo, Boulenger; DeV&TvdA, De Vos & Thys van den Audernaerde; DeW, De Weirdt; DeW&Te, De Weirdt & Teugels; Fo, Fowler; Ec, Eccles; Ge, Getahun; Ho, Holly; Ke, Keilhack; Lé, Lévêque; Lé&Da, Lévêque & Daget; Lé&Gu, Lévêque & Guegan; Na&Si, Nagelkerke & Sibbing; Pa, Pappenheim; Pe, Pellegrin; Po, Poll; Sa, Sauvage; Sk, Skelton; Tw&Sk, Tweddle & Skelton; and WaLu, Wamuini Lunkayilakio.

N1. Intermediate mouth phenotype polymorphism [interrupted (i.e. no lobe) or continuous (i.e. attached lobe) (see Nagelkerke & Sibbing, 1997: table 5.5, see also page 130)]. **N2.** No type (Lévêque & Daget, 1984: 220). Burchell (1822: 280) most probably had several specimens before him when describing the species, as he provided some variation for the number of pectoral fin rays. **N3.** *Labeobarbus* to *Varicorhinus* mouth phenotype specimens present (see Skelton, 2001: 169). Synonyms: holotype of *Labeobarbus gilchristi* (Boulenger, 1911) (BMNH 1909.12.8.1: 149 mm SL) with a typical *Labeobarbus* hypertrophied mouth phenotype (h) and holotype of *Labeobarbus mentalis* (Gilchrist & Thompson, 1913) (SAIAB 134770: 257 mm SL) with a typical *Labeobarbus* free mental lobe (f). **N4.** *Labeobarbus* + intermediate mouth phenotype polymorphism (see Worthington, 1929: 432, fig. 3; 1932: 126; fig. 1). Synonyms: one of both syntypes of *Labeobarbus lobogenys* (Boulenger, 1906) [BMNH 1906.5.30.117–221 (two instead of five syntypes): 218–223 mm SL] with a typical *Labeobarbus* free mental lobe (f); at least some of the syntypes of *Labeobarbus kiogae* (Worthington, 1929) [BMNH 1929.1.24.105–108 (seven instead of four syntypes): 185–417 mm SL] with an intermediate mouth phenotype (r); and holotype of *Labeobarbus eduardianus* (Boulenger, 1901) (BMNH 1906.9.7.41: 379 mm SL) and syntypes of *Labeobarbus fergusonii* (Boulenger, 1901) (BMNH 1906.9.7.42–43: 223–224 mm SL) with an intermediate mouth phenotype (n). **N5.** Although reported by Banister & Poll (1973: 84), the holotype (MRAC 179729: 266 mm SL) lacks an rce (see also illustration in Banister & Poll, 1973: 85, fig. 2); however, two paratypes have one (MRAC 179730, 97 mm SL; MRAC 179735, 338 mm SL). **N6.** Intermediate mouth phenotype polymorphism [interrupted (i.e. no lobe) or continuous (i.e. attached lobe) (see Thys van den Audenaerde, 1967: 35, fig. 12)]. **N7.** Replacement name for *Varicorhinus latirostris* Boulenger, 1910 (now *Labeobarbus*) (see text and annotated checklist 1). **N8.** MNHN 1935-0066 syntype (171 mm SL) with an rce; however, MRAC 42933 syntype (195 mm SL) actually without an rce, possibly lost over time. **N9.** Intermediate mouth phenotype [interrupted (i.e. no lobe) (see Nagelkerke & Sibbing, 1997: table 5.5., see also page 131)]. **N10.** Synonyms: holotype of *Labeobarbus seguensis* (Pellegrin, 1925) (MNHN 1925-0193, 495 mm SL) with a typical *Labeobarbus* free mental lobe (f); holotype of *Labeobarbus occidentalis* (Boulenger, 1911) (BMNH 1909.3.3.14, 74 mm SL) with an intermediate mouth phenotype (a); and holotype of *Labeobarbus meneliki* (Pellegrin, 1905) (MNHN 1905-0275, 191 mm SL) with an intermediate mouth phenotype (n). **N11.** Synonyms: holotype of *Labeobarbus euchilus* (Boulenger, 1920) (BMNH 1919.7.24.7, 66 mm SL) with a typical *Labeobarbus* free mental lobe (f); syntypes of *Labeobarbus miochilus* (Boulenger, 1920) (BMNH 1919.4.24.8–9, 70–71 mm SL; MRAC 6992, 61 mm SL) with an intermediate mouth phenotype (n); and syntypes of *Varicorhinus stappersii* Boulenger, 1917 (now *Labeobarbus*) [BMNH 1920.5.25.36–37, 186–244 SL; MRAC 14197, 250 mm SL; and 14222, 175–172 mm SL (two instead of one syntype)], with a typical *Varicorhinus* mouth phenotype rce. **N12.** Variable *Labeobarbus* mouth phenotype polymorphism present, with frequently thickened lips (rubber lips) (see Skelton, 2001: 173). Synonyms: holotype of *Labeobarbus altidorsalis* (Boulenger, 1908) (BMNH 1908.11.6.26, 3212 mm SL) and syntypes of *Labeobarbus chilotes* (Boulenger, 1908) (BMNH 1908.11.6.24–25, 125–199 mm SL), with a typical *Labeobarbus* free mental lobe (f). **N13.** *Labeobarbus* mouth phenotype polymorphism. Synonyms: holotype of *Labeobarbus labiatomimus* (Pellegrin, 1914) (MNHN 1886-0395, 240 mm

SL), with a typical *Labeobarbus* hypertrophied mouth phenotype (h). **N14.** Intermediate mouth phenotype polymorphism [interrupted (i.e. no lobe) or continuous (i.e. attached lobe) (see Nagelkerke & Sibbing, 1997, table 5.5., see also page 132)]. **N15.** Intermediate + *Labeobarbus* mouth phenotype polymorphism [interrupted (i.e. no lobe) or continuous (i.e. attached lobe), or small lobe (see Nagelkerke & Sibbing, 1997: table 5.5., see also page 133)]. **N16.** See also Tweddle & Skelton (1998: 372). **N17.** Boulenger (1910: 546, 1916a: 214) reports a 'cutting-edge' for (some?) syntypes, but there is none. **N18.** Holotype (MCZR) not seen. Based on three BMNH, non-type, specimens (BMNH 1971.7.12.1–3, 240–258 mm SL; see Banister, 1973: 41). **N19.** Synonyms: syntypes of *Labeobarbus rothschildi* (Günther, 1901) (BMNH 1901.4.26.6–7, 127–134 mm SL), *Labeobarbus riggenbachi* (Günther, 1902) (BMNH 1902.7.28.19, 115 mm SL and 1902.7.28:20–21, 136–137 mm SL) and *Labeobarbus paytonii* (Boulenger, 1911) [BMNH 1903.10.29.17–20, 50–116 mm SL (seven instead of four syntypes)], all with intermediate mouth phenotypes (n); syntypes of *Capoeta atlantica* Boulenger, 1902 (BMNH 1902.1.4.18–19, 99–112 mm SL) and one of both syntypes of *Capoeta walldoi* Boulenger, 1902 [BMNH 1902.1.4.16–17: 129–140 mm SL (probably lost over time in the largest of both syntypes)] have a *Varicorhinus* mouth phenotype with a typical rce; however, the rce is very fine, does not cover the entire anterior width of the lower jaw, and the fleshy lips of the sides of the lower jaw are better developed than in the typical *Varicorhinus* mouth phenotype. **N20.** Intermediate mouth phenotype polymorphism [interrupted (i.e. no lobe) or continuous (i.e. attached lobe) (see Nagelkerke & Sibbing, 1997: table 5.5., see also page 134)]. **N21.** Intermediate mouth phenotype polymorphism [interrupted (i.e. no lobe) or continuous (i.e. attached lobe) (see Nagelkerke & Sibbing, 1997: table 5.5., see also page 135)]. **N22.** Synonyms: most of the syntypes of *Labeobarbus gudaricus* (Boulenger, 1906) [BMNH 1908.1.20.131–132, 140–218 mm SL (six instead of two specimens) except BMNH 1908.1.20.133, 234 mm SL, which has an intermediate mouth phenotype (a)], with a typical *Labeobarbus* free mental lobe (f) and sometimes even a hypertrophied mouth phenotype (h); as reported by Nagelkerke & Sibbing (1997: 121), two syntypes of *Labeobarbus leptosoma* (Boulenger, 1902) (BMNH 1902.12.13.300–302), two of the syntypes have a continuous lower lip (f; 138 and 205 mm SL), whereas it is indeed interrupted in the third one (r, 208 mm SL) as in *Barbus intermedius*; although both have originally been described as *Barbus* species, at least one of the syntypes of *Labeobarbus macmillani* (Boulenger, 1906) [BMNH 1908.1.20.103–106, 141–192 mm SL (i.e. the largest syntype; cover seems lost over time in the other syntypes) and 1937.4.20.68, 176 mm SL] and most of the syntypes of *Labeobarbus plagiostomus* (Boulenger, 1902) [BMNH 1902.12.13.271–272, 176–193 mm SL (i.e. the smallest of both syntypes; cover seems lost over time in the other syntype) and 1902.12.13.273, 176 mm SL], with a typical *Varicorhinus*-like rce, except for one of the syntypes of the latter species, where it seem lost through time; and holotype of *Capoeta bingeri* Pellegrin, 1905 (MNHN 1905-0252), also with a typical *Varicorhinus* mouth phenotype, i.e. an rce. **N23.** Holotype (NMW) apparently lost. **N24.** Intermediate and *Varicorhinus* mouth phenotype polymorphism present (see Banister & Clarke, 1980: 500–504). Synonyms: at least one of the syntypes of *Labeobarbus globiceps* (Worthington, 1933) (BMNH 1932.11.15.275–282, 235 mm SL) with a typical *Labeobarbus* free mental lobe (f); and one of the two syntypes of *Labeobarbus nyassensis* (Worthington, 1933) (BMNH 1932.11.15.387–392, 207 mm SL; and BMNH 1932.11.15.393–395, 202–298 mm SL) – the latter a former *Varicorhinus* species – with a typical *Varicorhinus* rce. **N25.** See also Levin *et al.* (2013: 401, fig. 1b). **N26.** Holotype (Museo do Dundo: MD 1078) not seen. Statement based on a re-examination of paratypes (MRAC 161065 and 161066, 63 and 85 mm SL, respectively) only. **N27.** Previously identified as a junior synonym of *Labeobarbus johnstonii* (Boulenger, 1907), but to be considered a valid species. Species revalidated in the present paper (see text and annotated checklist 1). **N28.** Intermediate mouth phenotype polymorphism [interrupted (i.e. no lobe) or ± continuous (i.e. rudimentary lobe) (see Nagelkerke & Sibbing, 1997: table 5.5., see also page 135)]. **N29.** Whereas all syntypes of *Labeobarbus macrolepidotus* lack an rce and therefore have an intermediate mouth phenotype (n), the specimens of the Inkisi Basin all have a typical *Varicorhinus* mouth phenotype, i.e. an rce. **N30.** Intermediate mouth phenotype polymorphism [interrupted (i.e. no lobe) or continuous (i.e. attached lobe) (see Nagelkerke & Sibbing, 1997: table 5.5., see also page 136)]. **N31.** Synonyms: two syntypes of *Labeobarbus zambezensis* Peters, 1852 (ZMB 3246, 80 and 93 mm SL), with a typical *Labeobarbus* mouth phenotype (f & h, respectively) and another (NMW 49730: 77 mm SL) with an intermediate mouth phenotype (n); holotype of *Labeobarbus brucii* (Boulenger, 1907) (BMNH 1907.3.15.34, 131 mm SL) with a typical *Labeobarbus* free mental lobe (f); at least one of the syntypes of *Labeobarbus inermis* (Peters, 1852) (ZMB 4736, syntype of 58 mm SL) with an intermediate mouth phenotype (r); holotype of *Varicorhinus brucii* Boulenger, 1907 (now *Labeobarbus*) (BMNH 1907.3.15.37, 149 mm SL) [with replacement name *Barbus oliphanti* Keilhack, 1910 (now *Labeobarbus*)] and holotype of *Varicorhinus nasutus* Gilchrist & Thompson, 1911 (now *Labeobarbus*) (SAIAB 134736, ±367 mm SL), both with a typical *Varicorhinus* rce; and holotype of *Labeobarbus sector* (Boulenger, 1907) also with a typical *Varicorhinus* rce (BMNH 1907.3.15.35, 116 mm SL). **N32.** Originally described as a *Varicorhinus* species by Holly (1926); however, without special reference to the rce in the original description. In two of the three syntypes the cornified cover is entirely absent (NMW 7221, 128 mm SL, and NMW 7222-223, 88 mm SL), and most probably lost over time. Only in the third syntype (NMW 7222-223, 107 mm SL) does a faint rce seem to remain; however, all syntypes have well-developed fleshy lips on the lateral sides of the lower jaw, not at all resembling the almost entire absence of lips as found in the typical *Varicorhinus* mouth phenotype. **N33.** Intermediate mouth phenotype polymorphism [interrupted (i.e. no lobe) or continuous (i.e. attached lobe) (see Nagelkerke & Sibbing, 1997: table 5.5., see also page 137)]. **N34.** Apparently no type (see Levêque & Daget, 1984: 271). **N35.** *Labeobarbus* and *Varicorhinus* mouth phenotype polymorphism present (see Crass, 1964: 53, fig. 7; Skelton, 2001: 170). Synonyms: holotype of *Labeobarbus lobochilus* (Boulenger, 1911) (BMNH 1908.12.28.96, 143 mm SL) with a hypertrophied typical *Labeobarbus* free mental lobe (h); holotype of *Labeobarbus mfongosi* (Gilchrist & Thompson, 1913) (SAIAB 135057, 200 mm SL) with a typical *Labeobarbus* free mental lobe (f); syntypes of *Labeobarbus bowkeri* (Boulenger, 1902) (BMNH 1874.3.5.1–2, 124–128 mm SL, BMNH 1894.7.10.4, 101 mm SL) with an intermediate mouth phenotype (a or n) or a typical, although small, *Labeobarbus* free mental lobe (f); holotype of *Labeobarbus robinsoni* (Gilchrist & Thompson, 1913) (SAIAB 135055, 164 mm SL) with an intermediate mouth phenotype (a); and holotype of *Labeobarbus zuluensis* (Gilchrist & Thompson, 1913) (SAIAB 134939, 325 mm SL) with an intermediate mouth phenotype (n). **N36.** *Labeobarbus* mouth polymorphism [lobe: small or large (see Nagelkerke & Sibbing, 1997: table 5.5, see also page 138)]. **N37.** Groenewald (1958: 277) and others. **N38.** Intermediate and *Varicorhinus*-like mouth phenotype polymorphism present (see Tweddle & Skelton, 2008: 30). Indeed, one of the paratypes (SAIAB 51928, 94 mm SL) has an rce. **N39.** Intermediate + *Labeobarbus* mouth phenotype polymorphism [interrupted (i.e. no lobe) or continuous (i.e. attached lobe), or small lobe (see Nagelkerke & Sibbing, 2000: table 3)]. **N40.** Synonyms: holotype of *Labeobarbus labiatus* (Boulenger, 1902) (BMNH 1902.5.26.37, 236 mm SL) with a hypertrophied lobe mouth phenotype (h); at least some of the syntypes of *Labeobarbus tanensis* (Günther, 1894) (BMNH 1893.12.2.24–29, 191–286 mm SL) with a typical, although small, *Labeobarbus* free mental lobe (f); syntypes of *Labeobarbus hindii* (Boulenger, 1902) (BMNH 1902.5.26.25–28, 84–202 mm SL) with an intermediate mouth phenotype (a, r, or n); and holotype of *Barbus* (*Capoeta*) *babaulti* Pellegrin, 1926 (MNHN 1926-0285, 290 mm SL) and both syntypes of *Barbus* (*Capoeta*) *perplexicans* Boulenger, 1902 (BMNH 1902.5.26.35–36, 115–144 mm SL), both with a *Varicorhinus*-like mouth phenotype, i.e. with well-developed fleshy lips on the lateral sides of the lower jaw (and even more so in the latter of both nominal species), not at all resembling the almost entire absence of lips as found in the typical *Varicorhinus* mouth phenotype. **N41.** We follow Ladiges *et al.* (1958) with regards to the lectotype designation for *Labeobarbus pagenstecheri* (see annnoated checklist 1): 'The mouth is sub-terminal with a sharp [*Varicorhinus* rce] edge to the lower jaw in the paralectotype [ZMH H342, 210 mm SL] but rubber lips are developed in the . . . lectotype [ZMH H341, 319 mm SL]' (see Banister, 1973: 101; see also Seegers, 2008: 155); however, the paralectotype has well-developed fleshy lips on the lateral sides of the lower jaw, not at all resembling the almost entire absence of lips as found in the typical *Varicorhinus* mouth phenotype. **N42.** *Labeobarbus* mouth polymorphism (see Lévêque *et al.*, 1987: 348, fig. 1). **N43.** Holotype of *Labeobarbus altianalis lobogenysoides* (Pellegrin, 1935) (MNHN 1935-0154) with a hypertrophied lobe mouth phenotype (h). **N44.** Lower jaw not bearing a cornified cover anymore; the cover seems to have fallen off. As such, the presence of an rce cannot be fully confirmed for this species. No details provided by Pellegrin (1932) in the original description. **N45.** see also Daget (1962: 78, fig. 16). **N46.** Intermediate mouth phenotype polymorphism [interrupted (i.e. no lobe) or continuous (i.e. attached lobe) (see Nagelkerke & Sibbing, 1997: table 5.5., see also page 139)]. **N47.** Gaigher (1975: 162) reports the presence of *Varicorhinus* mouth phenotype in *Labeobarbus polylepis*. Skelton (2001: 170) reported that the lips are variable, but without any further details. **N48.** See also Jubb (1959: 308). **N49.** Substitute name for *Barbus mariae* Holly, 1929, now *Labeobarbus mariae* (Holly, 1929) (see text and annotated checklist 1). With the kind help of Helmut Wellendorf (2013), former curator of fishes at the NMW, the holotype of *Barbus matris* (NMW 8000, 243 mm SL) as well as both the lectotype [NMW 96652 (ex. 6562), 225 mm SL] and paralectotype of *Barbus mariae* [NMW 96653 (ex. 6562), 206 mm SL], the latter two designated but not seen by Banister (1973: 93–94), have all been traced (see text and annotated checklist 1). **N50.** An rce present in the two largest syntypes (79 and 80 mm SL).

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Nevertheless, absent in the smallest syntype (44 mm SL), but possibly lost over time. Despite the presence of a typical *Varicorhinus* rce in both the largest syntypes, they both also have well-developed fleshy lips on the lateral sides of the lower jaw, not at all resembling the almost entire absence of lips as found in the typical *Varicorhinus* mouth phenotype species. **N51.** All syntypes have an rce (MNHN 1909-0583-0585, 63–68 mm SL). According to Banister (1972: 121), present with increasing size, and absent in a specimen of 40 mm SL only (no further details provided). Note, however, that Banister (1972: 271) himself states that he studied specimens from 57 to 231 mm SL only. **N52.** *Labeobarbus* and intermediate mouth phenotype polymorphism [see Lévêque & Gruégan, 1990: 54, fig. 14 (syntypes) & 55, fig. 15 (specimen: from Daget, 1962: 76, fig. 15 as *Barbus gruveli*)]. **N53.** An rce is present in the lectotype but absent in two paralectotypes, i.e. the smallest of the BMNH 1912.4.1.334–336 paralectotypes (149 mm SL), as well as in the MRAC 1526 paralectotype (173 mm SL). **N54.** As demonstrated by E. Vreven, P.H. Skelton & E.R. Schwartz (unpubl. data), *Barbus capensis* is not a *Labeobarbus* but instead a senior synonym of *Barbus andrewi*. Therefore, *Barbus seeberi*, previously a junior synonym of *Barbus capensis*, and indeed a yellowfish or *Labeobarbus*, becomes the valid species name for the clanwilliam yellowfish (see also text and annotated checklist 1). **N55.** *Labeobarbus* mouth phenotype polymorphism (see Skelton, 2001: 171). In addition, specimens with an rce have been found (SAIAB 54113, 134 mm SL; SAIAB 54688, 176 mm SL); however, both have well-developed fleshy lips on the lateral sides of the lower jaw, not at all resembling the almost entire absence of lips as found in the typical *Varicorhinus* mouth phenotype. In addition, specimens with a typical *Labeobarbus* free mental lobe (f) (SAIAB 58362: 179 mm SL) and hypertrophied lips have also been found (h) (SAIAB 58418, 141 mm SL; SAIAB 65536, 101 mm SL). **N56.** One syntype with (MNHN 1924-0052, 56 mm SL) and one actually without (MNHN 1924-0052, 117 mm SL) an rce; however, the rce was most probably lost over time in the latter syntype. **N57.** Synonyms: holotype of *Labeobarbus moeruensis* (Pellegrin, 1922) (MRAC 14765, 578 mm SL) and holotype of *Labeobarbus oxycephalus* (Boulenger, 1915) (MRAC 14233, 266 mm SL), both with a typical *Labeobarubs* free mental lobe (f); holotype of *Labeobarbus curtus* (Boulenger, 1915) (MRAC 17172, 233 mm SL) with an intermediate mouth phenotype (a). **N58.** Intermediate mouth phenotype polymorphism [interrupted (i.e. no lobe) or ± continuous (i.e. attached lobe) (see Nagelkerke & Sibbing, 1997: table 5.5., see also page 132)]. **N59.** Synonyms: *Varicorhinus bredoi* Poll, 1948 (now *Labeobarbus*) [holotype IRSNB 76(1), 130 mm SL; paratypes IRSNB 77(2), 60–67 mm SL], with an rce (see Poll, 1948: 9–10, fig. 1–2), although, as mentioned by Poll (1948) himself, clearly absent in the smallest of both paratypes, which has an intermediate mouth phenotype (n). **N60.** Synonyms: *Varicorhinus chapini* Nichols & La Monte, 1950 (now *Labeobarbus*) [holotype: AMNH 18785] with '. . . a narrower, less specialized mouth than is usual in the genus [*Varicorhinus*] . . .' (Nichols & La Monte, 1950: 175). Indeed, holotype with '. . . lower jaw with a sharp, firm but not cartilaginous, edge, . . .' (Nichols & La Monte, 1950: 175), i.e. without the typical *Varicorhinus* mouth cutting edge, but with an attached lobe (a), and therefore with an intermediate mouth phenotype instead. **N61.** Intermediate mouth phenotype [interrupted (i.e. no lobe) (see Nagelkerke & Sibbing, 1997: table 5.5., see also page 141)]. **N62.** Intermediate mouth phenotype polymorphism [interrupted (i.e. no lobe) or continuous (i.e. attached lobe) (see Nagelkerke & Sibbing, 1997: table 5.5., see also page 142)]. **N63.** Based on pers. observ. (E. Vreven 2013, pers. observ.) of the syntypes (BMNH 1912.12.6.5, 38 mm SL; MRAC 1191, 36 mm SL; MRAC 1192, 41 mm SL), clearly described based on three juvenile *Labeobarbus* syntype specimens: i.e. the one MRAC syntype with a typical *Labeobarbus* free mental lobe (f) (MRAC 1192) and the BMNH and the other MRAC syntype with an intermediate mouth phenotype (a). **N64.** See also Holly (1930: plate 1, fig. 8a), as illustrated for one of the syntypes. **N65.** Syntype (NMW 13948, 59 mm SL) seemingly without an rce. Most probably lost over time as Holly (1929: 32), in his original description of the species, refers to an rce: 'Der unterkiefer trägt eine dünne Hornschneide; . . .'. Additional syntype (NMW 13949) not found (H. Wellendorf, pers. comm., 2000; E. Vreven, pers. observ., 2013). **N66.** Banister & Poll (1973: 91) reported the presence of a cornified cover, but not the presence of an rce. Indeed, the holotype lacks the rce, which conforms to the illustration provided by Banister & Poll (1973: 93, fig. 10); however, one paratype clearly has one (MRAC 179742, 385 mm SL). **N67.** The holotype of *Labeobarbus wurtzi* lacks an rce. Instead, a cornified cover, although damaged, and without an rce, is still present on the lower jaw; however, specimens with an rce have been found in collections (MNHN and MRAC) and, most probably, represent a species new to science (see text). **N68.** See Tweddle & Skelton (1998: 377). **N69.** According to Banister (1976a: 177), the anterior edge of the lower jaw is gently curved and a cornified cover is altogether lacking in fishes less than 60 mm SL. **N70.** Although an rce is present, it covers, approximately, only half of the anterior width of the lower jaw (see also Banister & Thys van den Audenaerde, 1973: 184, fig. 2) instead of almost the entire anterior width, resulting in a spoon-like rather than a spade-like cutting edge, as found in *Labeobarbus* spp. with a *Varicorhinus*, or a *Varicorhinus*-like, mouth phenotype. In addition, both syntypes have well-developed fleshy lips on the lateral sides of the lower jaw, not at all resembling the almost entire absence of lips found in the typical *Varicorhinus* mouth phenotype.

Furthermore, *Varicorhinus capoetoides* Pellegrin, 1938 has not been retained in our list either. Two characters, and especially the first one, make the placement of this species in the genus *Varicorhinus*, now *Labeobarbus s.l.*, untenable: (1) the last dorsal fin ray is a long, bony, and serrated spine (see also Pellegrin, 1938) not known to occur in any other African *Varicorhinus*, now *Labeobarbus s.l.*, species; (2) the high number of lateral line scales, i.e. 87 (see Pellegrin, 1938), a number only comparable with that found in *Acapoeta tanganicae*. Furthermore, as stipulated by Pellegrin (1938; see also Blache, 1964), the type locality is highly doubtful. In addition, the species is only known from its holotype and has not been found in the Chad Basin since then. As such, the holotype, which is in poor condition, is here identified as a mislabelled *Capoeta* species, possibly *Capoeta trutta* (Heckel, 1843) (J. Freyhof, pers. comm., 2014). Indeed, although Pellegrin (1938: 373–374) did not explicitely mention a typical real cutting edge, and the specimen currently lacks one, this might have been fallen of, i.e. lost over time.

2. Two Southern African species have not been listed as valid species, i.e. *Labeobarbus altidorsalis* (Boulenger, 1908) and *Labeobarbus elephantis* (Boulenger, 1907). Both are here considered implicit synonyms (P.H. Skelton, pers. comm., 2015), respectively, of *Labeobarbus codringtonii* (Boulenger, 1908) and *Labeobarbus polylepis*, following Skelton's (1993, 2001) work (see annotated checklist 1).
3. Listing of all African species to be included into *Labeobarbus* is mainly hampered by the fact that during this study, at least one *Barbus* species previously identified as a small barb, i.e. *Barbus urotaenia* Boulenger, 1913 (see Table 1), was revealed to be a *Labeobarbus* species. Although described from small specimens, based on the presence of a free mental lobe and nine branched dorsal fin rays it is readily identifiable as a *Labeobarbus* species.
4. In addition, three nominal species, i.e. *L. alluaudi*, *Labeobarbus microbarbis* (David & Poll, 1937), and *Labeobarbus microterolepis* (Boulenger, 1902), have been listed as hybrids or possible hybrids already. For *L. alluaudi*, the case has been convincingly documented by Banister (1972), and is followed here. For *L. microbarbis*, the case has been first suggested by Banister (1973) and further documented by De Vos & Thys van den Audenaerde (1990), who confirmed Banister's (1973) suggestion; however, for *L. microterolepis*, the case has only been suggested by Banister (1973) and subsequently confirmed by Golubtsov, Dgebuadze & Mina (2002), but without providing new supporting evidence. The status of *L. microterolepis* as a possible hybrid thus certainly needs further attention. As hybrid nominal species are never formally synonymized, these nominal species already previously identified as hybrids or possible hybrids have been listed separately (see Table 1 and annotated checklist 2), as these names should not be used as valid names (see ICZN, 1999: article 23.8).
5. *Varicorhinus beso* was described from Lake Tana (Ethiopia) by Rüppell in 1835 based on a single specimen, the holotype; however, as reported by Eschmeyer (2015) the SMF holotype cannot be found, a statement further confirmed by Mr T. Alperman (pers. comm., 2013), curator at the SMF, Frankfurt, Germany. As a result the holotype is here considered lost. The present situation is highly unsatisfactory for several reasons. *Varicorhinus beso* is the type species of the genus *Varicorhinus* and, although the genus *Varicorhinus* has recently been synonymized with *Labeobarbus* (see Berrebi *et al.*, 2014), its status and delimitation as a valid genus has been (see Levin *et al.*, 2013) and most probably will be further debated. Therefore, a neotype is needed (see ICZN, 1999: article 75), and is designated here, following the qualifying conditions provided in ICZN (1999: article 75.3). The NHM, London, UK, houses an unpublished, and as such unavailable, neotype (BMNH 1968.7.24.18; 156.8 mm SL), collected by Sandhurst in 1964, originating from Bahardar (Baherdar ±11°37′N, 37°24′E), also on Lake Tsana (i.e. Tana, Ethiopia), and most probably selected by the late K.E. Banister (NHM); however, we have refrained from identifying this specimen as the neotype of *V. beso*. Instead, BMNH 1902.12.13.365 (290.6 mm SL), also originating from Bahardar, Lake Tsana (i.e. Tana, Ethiopia), and collected by E. Degen (1 June 1902), is here designated as the neotype of *V. beso*. The latter specimen has been illustrated by Boulenger (1907c: plate 33) in his *Fishes of the Nile*, and the illustration has been further reproduced in his monumental *Catalogue of the Fresh-Water Fishes of Africa* (Boulenger, 1909: fig. 268). Boulenger (1907c: plate 33) illustrates a specimen of ± 293 mm SL (± 205 mm SL on the drawing, scale 7/10), which indeed can be identified as BMNH 1902.12.13.365 based on its size (SL) and exact disposition of the tubercles on the right-hand side of the head that perfectly match between the illustration and those on the NHM specimen. A detailed description of *V. beso* and a key to the species of the genus *Varicorhinus* as recognized at the time can be found in Boulenger (1907c, 1909). As such, the neotype exemplifies and corresponds with what has since been identified as *V. beso*. In addition, considering (1) the subsequent importance of the presence of a cornified, clear cutting edge as a diagnostic character for *Varicorhinus*, and (2) the general importance of the mouth

phenotype and its polymorphism in these hexaploid Torini, the mouth of the neotype is in excellent condition and exemplifies a well-developed, cornified, clear cutting edge. This is especially important considering the fact that Rüppell (1835) himself did not mention the presence of a cornified, clear cutting edge (see Persistent generic problems: morphology, cytogenetic, and molecular approaches) as a diagnostic character for either the genus *Varicorhinus* or the species *V. beso*. Furthermore, the illustration of *V. beso* as provided by Rüppell (1835: plate 3; fig. 2) shows a fish without a clear cutting edge on the lower jaw, and in that respect looks more reminiscent of intermediate mouth phenotypes. Nevertheless, to stabilize the current nomenclature the neotype has been identified to match with what has been considered *V. beso* since Boulenger (1902f, 1906c; and more explicitly 1907c: plate 33).

6. As a result of the proposed (Tsigenopoulos *et al*., 2010) and recently applied (Berrebi *et al*., 2014) synonymy of *Varicorhinus* with *Labeobarbus*, and in concordance with other discoveries (see below), two valid species names become secondary junior homonyms, and are in need of a substitute name (see ICZN, 1999: article 60). A third case of secondary homonymy is also discussed, although a replacement name is not needed in this specific case. Finally, a fourth case of possible future secondary homonymy is also highlighted.

   With regards to *L. brevicauda*, the right pharyngeal bone illustrated by Banister & Clarke (1980: fig. 5; 335 mm SL), and which according to the legend to the figure belongs to the holotype (ZMB 18175: 351 mm SL), does not in fact belong to this species. Indeed, the holotype has the left instead of the right pharyngeal bone dissected. This pharyngeal bone clearly has five teeth on row I and not four teeth, as in the illustrated pharyngeal bone. In addition, it has no molariform teeth at all. The drawing, however, perfectly matches the right pharyngeal bone, with four teeth on row I, of one of the syntypes of *Labeobarbus latirostris* Keilhack, 1908 (i.e. the whole specimen here designated as the lectotype, ZMB 18174, but not the head-only specimen, which here becomes the paralectotype, ZMB 34766; see below). The lectotype of *L. latirostris* also matches the size provided by Banister & Clarke (1980: 485; 335 mm SL) of the holotype of *L. brevicauda*. In addition, the barbels of the lectotype of *L. latirostris* are long, and therefore match the diagnosis given for *Labeobarbus eurystomus* by Banister & Clarke (1980: 489) rather than that for *L. johnstonii* [anterior barbels (Ab), mean 5.7% SL, and posterior barbels (Pb), mean 5.6% SL, in *L. eurystomus*, following Banister & Clarke (1980) = *L. brevicauda*, following Seegers, 1995 (see below), versus Ab 2.5% SL and Pb 3.5% SL in *L. johnstonii*]. Banister & Clarke (1980) also referred to some typical differences in colour pattern, but we have been unable to confirm this as, at least in these specimens, the colour pattern is entirely faded. These discoveries strongly suggest that Banister & Clarke (1980) unfortunately seem to have switched parts of their synonymizations, as *L. brevicauda* was identified as a junior synonym of *L. eurystomus* (= *L. brevicauda*, following Seegers, 1995), although it has five non-molariform pharyngeal teeth on the first row, as in *L. johnstonii*, whereas *L. latirostris* was identified as a junior synonym of *L. johnstonii*, although it has four molariform teeth on the first row, unlike in the latter species. As a result, the nomenclatural consequences of these findings are twofold: (1) *L. brevicauda* becomes a junior synonym of *L. johnstonii*; and (2) *L. latirostris* becomes the valid species name for what Banister & Clarke (1980) incorrectly referred to as *L. eurystomus*, and had been renamed by Seegers (1995) as *L. brevicauda*, following the invalid lectotype designation of Banister & Clarke (1980) for *L. eurystomus* (for more details, see annotated checklist 1 for *L. johnstonii* under the 'Notes' section), but unfortunately overseeing the additional errors of the latter authors (see above). The complete syntype of *L. latirostris* is here identified as the lectotype, as the second specimen (a head only), the paralectotype (now ZMB 34766), belongs to *L. johnstonii* (five teeth on row I and none of them molariform). As a result, a new replacement name (*nomen novum*; see ICZN, 1999: article 60.3) is needed for *Varicorhinus latirostris* Boulenger, 1910, which also becomes a *Labeobarbus* and has no synonyms. *Labeobarbus boulengeri* is here proposed in acknowledgement of George Boulenger's extensive work on Angolan large *Barbus* and *Varicorhinus*, both now *Labeobarbus*. For full details on the types see annotated checklist 1. The largest of the syntypes is here designated as the lectotype of the species (BMNH 1911.6.1.6: 136.6 mm SL), whereas the others become paralectotypes [ANSP 37905(1) (not seen), BMNH 1911.6.1.7–10(4), NMW 48865(1), and ZMB 18211(1)]. Indeed, following the recommendation of the ICZN (1999: recommendation 74B), the illustrated specimen should, by preference, be identified as the lectotype. The illustrated specimen in Boulenger (1916a: fig. 139) is about 95.0 mm SL. The scale provided is 2/5, which would give ~237.5 mm SL. As the largest of the syntypes does not even come close to this size, we suspect the scale is in error and should most probably read 2/3 instead, as this would give an SL of approximately 142.5 mm, which is much closer to the 136.6 mm SL of the largest of the BMNH syntypes.

Varicorhinus mariae Holly, 1926 has become *L. mariae*. As a result, *Barbus* (now *Labeobarbus*) *mariae* Holly, 1929 described from the Kitui River in Kenya needs a substitute name (see ICZN, 1999: article 60). Banister (1973: 283) stated that although the original description of both *L. matris* (Holly, 1928) and *L. mariae* (Holly, 1929) were very similar, he refrained from putting them in synonymy without having seen the types; however, several authors (Lévêque & Daget, 1984; Seegers, De Vos & Okeyo, 2003) have since considered *B. matris* a synonym of *B. mariae*, but with hesitation, without further justification, and without respecting the proper priority of names. The types of both species, previously considered lost, have been located in the NMW, and both nominal species are clearly distinguishable from each other, rejecting any claims regarding their possible synonymization. Amongst other character states, both nominal species differ from each other in the number of gill rakers [15 + 1 + 3 (=19) in *B. matris* versus 9 + 1 + 2 (=12) or 10 + 1 + 2 (= 13), in *B. mariae*], the number of scales below the lateral line (3.5 versus 4.5), and the length of the unsegmented dorsal spine (18.8 versus 26.3–29.0% SL; E. Vreven, pers. observ., 2013). Therefore, both are here retained as valid species. Furthermore, *Barbus* (now *Labeobarbus*) *rhinoceros* Copley, 1938 has been identified as a junior synonym of *B. mariae* by Banister (1973: 83). As a result, and according to ICZN (1999: article 60.2), it becomes the valid replacement name for the latter taxon. Contrary to Seegers *et al*. (2003), *L. rhinoceros* is not considered a *nomen nudum* (see ICZN, 1999: glossary) as, according to ICZN (1999: article 13): (1) the name is accompanied by a brief description making reference to a 'pronounced horn' [see Copley (1938: 191); for an illustration see Banister (1973: fig. 68 for *B. mariae*)], distinguishing it from all other East African *Barbus* (now *Labeobarbus*); (2) although Copley (1938) seems to have had a single specimen (holotype) presented by Playford before him, a name bearing type designation only became mandatory for species descriptions after 1999 (see ICZN, 1999: article 72.3). No type(s) is/are known (see Lévêque & Daget, 1984; Eschmeyer, 2015); however, in the past, one and four additional specimens, all originating from the Athi River and presented to the NHM by Copley (BMNH 1936.12.22.35 and 1936.12.36–39, all currently in the same jar) have been labelled as the holotype and paratypes of *B. rhinoceros*, respectively, but this has been subsequently amended. Therefore, and considering that (1) the specimens were deposited at the NHM in 1936, i.e. well before the actual description of the species in 1938, and (2) that Copley (1938) refers to a single specimen, the type status of these specimens remains doubtful and in need of further research. Nevertheless, those are the specimens used by Banister (1973) for his detailed redescription of *B. mariae* now *B. rhinoceros*. For full details on the types of *B. matris* and *B. mariae*, also see annotated checklist 1. The suggested possible synonymy of both *B. matris* and *B. mariae* (now *L. rhinoceros*) with *L. oxyrhynchus* (see Seegers *et al*., 2003: 32) is not followed, as no evidence was provided by the authors.

Labeobarbus macrolepis (Pfeffer, 1889), a new combination first proposed by Skelton & Bills (2008), and later confirmed (see Banyankimbona *et al*., 2012a; present paper), is preoccupied by *Labeobarbus macrolepis* Heckel, 1838, currently a junior synonym of *Tor putitora* (Hamilton, 1822) (Kottelat, 2013). The junior secondary homonym *L. macrolepis* (Pfeffer, 1889) does not need a replacement name as: (1) 'the junior species-group name has not been replaced'; and (2) 'the relevant taxa are no longer considered congeneric' (ICZN, 1999: article 59.2).

Following Banister (1973), *Varicorhinus stappersii* Boulenger, 1917 is to be considered a junior synonym of *L. caudovittatus* (see annotated checklist 1); however, the syntypes of *V. stappersii* Boulenger, 1917 all have a *Varicorhinus* mouth phenotype, whereas both syntypes of *L. caudovittatus* have an intermediate mouth phenotype (see Table 1). Therefore, its current status as a junior synonym needs further attention. If, contrary to Banister's (1973) opinion, further research reveals *V. stappersii* to be a valid species instead, a replacement name will be needed for the former as it is preoccupied by *Barbus* (now *Labeobarbus*) *stappersii* Boulenger, 1915. Being currently a junior synonym, however, it is not (yet) to be replaced, as the replacement name would be unavailable (see ICZN, 1999: articles 11.5 and 15.1).

7. The syntype series of *L. sandersi*, originally described as *Varicorhinus sandersi* by Boulenger (1912), is revealed to be polymorphic, including specimens with a real *Varicorhinus* cornified cutting edge, such as the one illustrated by Boulenger (1912: fig. 1, plate 19; see Table 1) in the original description (size of the drawn specimen: ±315 mm SL), and specimens with an intermediate mouth phenotype, i.e. having a horny cover but lacking the real *Varicorhinus* cutting edge or *Labeobarbus*-like mental lobe (see notes to Table 1). Following ICZN (1999: recommendation 74B), the illustrated specimen (BMNH 1912.4.1.333; i.e. the largest of the syntypes, 316 mm SL), is here identified as the lectotype of the species. In addition, for reasons of nomenclatural stability, we have preferred to identify a specimen with a *Varicorhinus* mouth phenotype as the name-bearing type of *L. sandersi* rather than an

intermediate one that might, in analogy to Banister's (1972, 1976a) documented cases of hybridization, subsequently reveal to represent a hybrid mouth phenotype rather than a valid species.

8. The past recognition of *Barbus* as a 'monstrous aggregation' (Myers, 1960: 213) has resulted in the synonymization of numerous genera. Following the recognition of *Labeobarbus* as a separate genus, several of these junior synonyms have to be reallocated to *Labeobarbus* instead of *Barbus*.
   As such, in addition to the synonymization of *Varicorhinus* with *Labeobarbus* (see Berrebi *et al*., 2014), and taking into account all African *Labeobarbus* species identified in the present paper, at least three African junior synonyms are also to be listed under *Labeobarbus*: (1) *Barbellion* Whitley, 1931; (2) *Barynotus* Günther, 1868 (*sensu* Jordan, 1919), both with *Barynotus lagensis* Günther, 1868 (Nigeria) as the type species; and (3) *Lanceabarbus* Fowler, 1936 (originally described as a subgenus of *Barbus*), with *Barbus tanensis* Günther 1894 (Kenya; a junior synonym of *L. oxyrhynchus*; see annotated checklist 1) as the type species. Further details on these junior synonyms are provided in the notes to Table 1 and in annotated checklist 1. As *B. capensis* is in fact not a *Labeobarbus* (see E. Vreven, E.R. Swartz & P.H. Skelton, unpubl. data) and the name *L. seeberi* should be used for this southern African species instead, *Cheilobarbus* Smith, 1841 (originally described as a subgenus of *Barbus*) is not to be included as a junior synonym of *Labeobarbus*.
   Although *Pseudotor* Karaman, 1971, with *B. fritschii* (Morocco) as the type species, has been identified as a junior synonym of *Carasobarbus* by Borkenhagen *et al*. (2011) and Borkenhagen & Krupp (2013), and the monospecific genus *Pterocapoeta*, with *Pterocapoeta maroccana* Günther, 1902 (Morocco) as the type species, has recently been revalidated by Borkenhagen *et al*. (2011), both are here included in *Labeobarbus s.l*. pending further research. Furthermore, when considering *Labeobarbus s.l*. several non-African genera are to be included as well. First, both *Carasobarbus*, with *Systomus luteus* Heckel, 1843 (Syria) as the type species, and *Kosswigobarbus*, with *Cyclocheilichthys kosswigi* Ladiges, 1960 (Turkey) as the type species, are included here. Although both were referred to as subgenera in Tsigenopoulos *et al*. (2010), this subgeneric nomenclature was not adopted in their tree, despite both type species being included in their analyses. Furthermore, Berrebi *et al*. (2014) did not retain any subgeneric nomenclature either. Therefore, pending further research, both are included here in *Labeobarbus s.l*. As stipulated elsewhere, we have refrained from following the revalidation of *Carasobarbus* by Bănărescu (1997), as recently confirmed by Borkenhagen *et al*. (2011) and Borkenhagen & Krupp (2013), as this would render *Labeobarbus s.l*. paraphyletic according to the results of Tsigenopoulos *et al*. (2010) and Berrebi *et al*. (2014). The same holds true for the monospecific genus *Mesopotamichthys*, with *Barbus sharpeyi* Günther, 1874 as the type type species, originally revalidated by Bănărescu (1997), as recently confirmed by Borkenhagen (2014), and the recently described genus *Arabibarbus*, with *Arabibarbus hadhrami* Borkenhagen 2014 (Yemen) as the type species. Indeed, these three nominal genera are here all included in *Labeobarbus s.l*. pending further research. Finally, although Tsigenopoulos *et al*. (2010) also included the (sub)genus *Tor*, we have refrained from doing so as the type species itself, *Cyprinus tor* Hamilton, 1822, was not included in the analysis of Tsigenopoulos *et al*. (2010), nor in the compilation of Berrebi *et al*. (2014). Indeed, *Tor*, including the type species *T. tor*, has been inferred to be: (1) a valid genus, and to represent (2) the sister group to the *Labeobarbus* clade, i.e. here referred to as *Labeobarbus s.l*., by Yang *et al*. (2015: fig. 2) based on their mitochondrial DNA data set.

The present overview: what is to be learned?

Based on the tabulated overview (see Table 1), some revealing details with regard to the observed mouth phenotype diversity and its distribution are discussed: (1) valid African *Labeobarbus* spp. with one or several *Varicorhinus* or *Capoeta* genus or subgenus synonyms; (2) the generic history of *L. wurtzi*; (3) other African species originally described based on one or several specimens with an intermediate mouth phenotype; (4) African *Labeobarbus* spp. with a prognathous mouth; (5) species originally described as *Varicorhinus* spp. but lacking the typical cornified cutting edge; (6) African *Labeobarbus* spp., originally described as *Varicorhinus* spp. but with papillated lips instead of the typical cornified cutting edge; (7) details on the continental distribution of both the *Labeobarbus* and *Varicorhinus* mouth phenotypes.

1. Eight of the listed African *Labeobarbus s.l*. species currently have a *Varicorhinus* junior synonym or originally a *Capoeta* genus or subgenus one. These are: *L. caudovittatus* (one *Varicorhinus* synonym), *L. fritschii* (two *Capoeta* synonyms = *Varicorhinus*; E. Vreven, pers. observ., 2013), *L. intermedius* (one *Capoeta* synonym = *Varicorhinus*; E. Vreven, pers. observ., 2013), *L. johnstonii* (one *Varicorhinus* synonym), *L. marequensis* (two *Varicorhinus* synonyms), *L. oxyrhynchus* [two *Capoeta* (subgenus) synonyms = *Varicorhinus*; E. Vreven, pers. observ., 2013],

L. trachypterus (one *Varicorhinus* synonym), and *L. tropidolepis* (one *Varicorhinus* synonym) (for more details, see notes to Table 1). Although *Varicorhinus chapini* Nichols & La Monte, 1950 (currently a junior synonym of *L. tropidolepis*, following Banister, 1973) was described as a *Varicorhinus* species, it lacks a cornified real cutting edge on the anterior edge of the lower jaw (for more details, see Table 1). This means that seven (i.e. 5.6%) valid African *Labeobarbus* species have, at least, one nominal junior synonym for which the lower jaw, somehow, bears a cornified real cutting edge as found in *V. beso* (see Boulenger, 1907c: plate 33; Levin, 2012: fig. 2), now *L. beso*. In addition, three additional *Labeobarbus* species, i.e. *L. aeneus*, *L. natalensis*, and *Labeobarbus nthuwa* Tweddle & Skelton, 2008 have been reported to include *Labeobarbus* as well as *Varicorhinus* mouth phenotype specimens (see Skelton, 2001 and Tweddle & Skelton, 2008), which according to Gaigher (1975) also holds true for *L. polylepis* (four species, i.e. about 3%); however, the cutting edge is not always as well developed as in *V. beso*, as it may clearly not cover the entire width of the anterior edge of the lower jaw and may also lack the ventral cover of the lower jaw (here referred to as the plastron). This is the case, for instance, in the two largest of the BMNH syntypes (BMNH 1911.6.1.39–41: 79.3 and 80.1 mm SL) of *Labeobarbus rosae* (Boulenger, 1910). In addition, in these specimens the lateral sides of the lower jaw bear well-developed fleshy lips, illustrating additional intermediate mouth phenotype variation in need of further attention.

2. *Labeobarbus wurtzi*, although originally described as a large *Barbus* species (see Pellegrin, 1908), was transferred to the genus *Varicorhinus* by Daget (1962: fig. 14), although not stated explicitly, based on the fact that, as stipulated in his description of the Guinean *V. wurtzi* specimens he studied, the lower jaw has a horny cover with a striated surface forming a cutting edge (i.e. 'bord trenchant' see Daget, 1962: 72). Lévêque & Guégan (1990), however, based on both morphological as well as parasitological criteria (i.e. monogenea Dactylogyridae fauna), transferred it back to the group of the large *Barbus*, i.e. now *Labeobarbus*. Indeed, although Lévêque & Guégan (1990) reported the wide mouth with the presence of a horny covering ('étui corné') on the lower lip, they did not mention the presence of the typical *Varicorhinus* cutting edge that Daget (1962), instead, had explicitly reported. Re-examination of the holotype of *L. wurtzi* (MNHN 1908–0097; 112.0 mm SL) shows that, although damaged, the holotype still partially bears a horny cover on the lower jaw, but nevertheless lacks the typical *Varicorhinus* clear cutting edge and, in that respect, as well as by the presence of fleshy lips on the lateral sides of the lower jaw, has a typical intermediate mouth phenotype. The smallest of both the *L. wurtzi* specimens from Kaba (Guinea) examined by Daget (1962) (MNHN 1959–0153; two specimens of 127.5–132.5 mm SL) has a real cornified *Varicorhinus* cutting edge on the lower jaw, however (see Table 1). In addition, verification of the MRAC and MNHN specimen holdings for *L. wurtzi* showed that other specimens also have a typical *Varicorhinus* mouth phenotype [MRAC 1986-13-P-114 (124.2 mm SL), MNHN 1959–0153(2) (smallest of both: 127.5 mm SL), MNHN 1987–0689(3) (116.6–165.5 mm SL), MNHN 1988–1955(3) (both largest specimens: 172.5 and 175.6 mm SL), and MNHN 1991–0519(1) (180.3 mm SL)]. These specimens illustrate that specimens with the typical *Varicorhinus* mouth phenotype are – although far less abundant compared with the numerous *L. wurtzi* intermediate mouth morphotype specimens in the MNHN and MRAC collections – not absent from this part of Africa, and might well represent an undescribed *Labeobarbus* species with a *Varicorhinus* mouth phenotype.
3. The fact that, as for *L. wurtzi*, a *Labeobarbus* species has originally been described on a single specimen or several specimens with one or several intermediate mouth phenotypes (see Table 1) is certainly not unique for the latter species. Indeed, this is, for instance, also the case for both *L. micronema* syntypes (BMNH 1904.2.29.37–38) originating from the Kribi River Basin (Cameroon), which both also lack the presence of a mental lobe, typical for the *Labeobarbus*-like phenotype (see Boulenger, 1911a: fig. 57) (see Table 1). As for the *L. wurtzi* holotype, however, both syntypes have well-developed lips on the lateral sides of the lower jaw (see Boulenger, 1911a: fig. 57). Furthermore, although both these specimens lack the typical real cornified cutting edge, they have a broad mouth like the typical broad mouth found in the *Varicorhinus*-like mouth phenotype, and in that respect clearly differ from the narrow mouth of the typical *Labeobarbus*-like mouth phenotypes. This kind of mouth phenotype is also reminiscent of the mouth phenotype illustrated for the hybrid specimens identified by Banister (1972: fig. 15; 1976a: plate 2).
4. It is to be noted, however, that all species with a prognathous lower jaw also lack the presence of a mental lobe [see *Labeobarbus aspius* (Boulenger, 1912), *Labeobarbus macroceps* (Fowler, 1936), *L. mariae*, *L. matris*, and *Labeobarbus progenys* (Boulenger, 1903), as riverine species; see also de Graaf *et al*. (2010: fig. 1) for the prognathous, i.e. piscivorous, Lake Tana *Labeobarbus* spp.: *Labeobarbus acutirostris* (Bini, 1940), *Labeobarbus*

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longissimus (Nagelkerke & Sibbing, 1997), *Labeobarbus macrophthalmus* (Bini, 1940), *Labeobarbus megastoma* (Nagelkerke & Sibbing, 1997), *Labeobarbus truttiformis* (Nagelkerke & Sibbing, 1997), and to a lesser extent *Labeobarbus gorguari* (see Nagelkerke & Sibbing, 1997, 2000)]. Two additional Lake Tana *Labeobarbus* species, i.e. *Labeobarbus dainellii* (Bini, 1940) and *Labeobarbus platydorsus* (Nagelkerke & Sibbing, 1997), without a prognathous lower jaw, are also known to be piscivorous (see Nagelkerke & Sibbing, 1997; de Graaf *et al*., 2010) and at least some specimens of *L. dainellii* are known to have a small lobe. As such, the lack of a mental lobe does not seem to unequivocally point towards an intermediate, possibly hybrid, status of the nominal species concerned; however, a hybrid origin is not to be excluded, and is certainly worth further detailed investigation.

5. In six currently valid African *Labeobarbus* species (i.e. about 5%), all originally described within *Varicorhinus*, i.e. *Varicorhinus altipinnis* Banister & Poll, 1973 (Lufira River Basin, DRC), *V. ansorgii* (Quango River Basin, Angola), *Varicorhinus fimbriatus* Holly, 1926 (Sanaga River Basin, Cameroon), *Varicorhinus lufupensis* Banister & Bailey, 1979 (Lufupa River Basin, DRC), *V. macrolepidotus* (Kasai River system, DRC), and *Varicorhinus wittei* Banister & Poll, 1973 (Lufira River Basin, DRC), the name-bearing type(s) (i.e. the holotype or all examined syntypes) lack the typical cornified, real cutting edge on the lower jaw (see Table 1). In that respect, these name-bearing types also resemble some of the intermediates, i.e. hybrid phenotypes, as described and illustrated by Banister (1972: fig. 15; 1976a: plate 2). As a result, in analogy with the cases documented by Banister (1972, 1976a), these might well represent hybrid phenotypes instead of valid species.
6. Results also show that four currently valid *Labeobarbus* species (i.e. about 3%), namely *Labeobarbus robertsi* (Banister, 1984) (Inkisi River Basin, DRC) (see Banister, 1984: fig. 9), *Labeobarbus clarkeae* (Banister, 1984) (Quanza River Basin, Angola), *Labeobarbus ensifer* (Boulenger, 1910) (Lucalla River Basin, Angola), and *Labeobarbus varicostoma* (Boulenger, 1910) (Lucalla River Basin, Angola), all have papillae towards the anterior outer edge of the upper as well as the lower jaw (see Table 1). As all were originally described within the genus *Varicorhinus*, the existence of this additional, very distinct, mouth phenotype has largely been overlooked. Furthermore, this mouth phenotype seems, based on the current evidence, highly localized, as it has only been reported from the Quanza and Lucalla rivers in Angola and the Inkisi River, Lower Congo, in the DRC.
7. Based on the currently available data resulting from the compilation provided, *Labeobarbus* as well as *Varicorhinus* mouth phenotype(s) and/or species seem to be widespread in Africa, as they both apparently occur in each of the ten ichthyofaunal provinces (see Snoeks *et al*., 2011) recognized today (see Fig. 6 and associated table). The spacial distribution of *Varicorhinus* mouth phenotype species seems to have been particularly obscured. Several reasons can be put forward for this: (1) numerous synonymizations of nominal *Varicorhinus* mouth phenotype(s) species with similar sympatric *Labeobarbus* species (see above under no. 1); (2) unnamed *Varicorhinus* mouth phenotypes, within the current context of the accepted high intraspecific mouth phenotype polymorphism within *Labeobarbus* (see above under no. 1); and (3) apparently entirely overlooked *Varicorhinus* mouth phenotypes in other cases (see above under no. 2, *L. wurtzi*).

In addition, the overall continental distribution shows the *Labeobarbus* mouth phenotype(s) and/or species to be, apparently, more widespread compared with the *Varicorhinus* mouth phenotype and/or species. For instance, the *Varicorhinus* mouth phenotype is currently unknown from within the *L. bynni* region – i.e. large parts of the Nilo Sudanic ichthyofaunal province – including the Upper Senegal, Volta, and Niger, including the coastal rivers between both, as well as the Chad and the 'Lower' Nile Basin. The latter mouth phenotype is not entirely absent from the Nilo Sudanic ichthyofaunal province, however, and is expressed, among other taxa, in *L. jubae*, originally described as a *Varicorhinus* species, from the Juba River (see Banister, 1984; Levin *et al*., 2013; Table 1). In addition, the current state of knowledge should be taken cautiously. Indeed, as illustrated, the so-called established absence of the *Varicorhinus* mouth phenotype in the Upper Guinea ichthyofaunal province (*sensu* Lévêque, 1997: fig. 2.1.) is here refuted (see above under no. 2 for *L. wurtzi*). Although rare, the re-examination of existing collections revealed it to be present in the area and confirms Daget's (1962) statement in this respect (see above under no. 2, *L. wurtzi*).

The present compilation has also enabled us to illustrate the taxonomic evolution of the African *Labeobarbus s.l.* species numbers since the original description of the genus in 1835 (see Fig. 7). Several major trends can be observed. First, the highest number of described species per decade is between *c*. 1891 and *c*. 1940, which corresponds well with the period of intensive activity of G.A. Boulenger (1858–1937; NHM) and, later on, J. Pellegrin (1873–1944; MNHN) (see also Paugy, 2010: fig. 4; Skelton & Swartz, 2011). This activity curve is perfectly in tune with the expansion of exploration accompanying the colonial 'scramble for

Africa' (see also Skelton & Swartz, 2011). Furthermore, although the cumulative number of valid species largely follows the trend of the cumulative number of nominal taxa up to *c*. 1960s, thereafter a period of intensive synonymization up to *c*. 1990 brought a strong decrease in the number of valid species. The conceptual shift in the interpretation of mouth phenotype variation also accounts for the peculiar history of the African valid *Labeobarbus s.l*. species numbers, which contrasts with the overall numbers of African fish species descriptions, and exemplifies the huge impact of the conceptualization of species on the practice of their recognition and delimitation. African fish species descriptions have been steadily increasing since the *c*. 1880s (see Paugy, 2010: fig. 5; Skelton & Swartz, 2011: fig. 1), but very few *Labeobarbus s.l*. species descriptions occurred after the active period of G.A. Boulenger and J. Pellegrin, *c*. 1930s. Also, revalidations have been rare except for the 1990s when numerous Lake Tana (Ethiopia) endemic species were revalidated by Nagelkerke & Sibbing (1997) after the extensive over-synonymization with *B. intermedius*, now *Labeobarbus*, by Banister (1973).

DISCUSSION

General repercusions and perspectives on an African scale

For a long time, the large, hexaploid barbs have been considered part of the genus *Barbus*, a 'monstrous aggregation' (Myers, 1960: 213). Recognition of the former as a separate lineage, now referred to as *Labeobarbus*, has at least enabled the delineation of a more easily surveyed entity; however, partially as a result of the recent synonymy of *Varicorhinus*, *Labeobarbus s.l*. currently still contains approximately 125(+2) valid African species, and as such still represents one of the largest genera of African freshwater fishes. With its numerous particularities, including the striking mouth phenotype polymorphism and the apparent, although still largely underestimated, propensity to hybridize, the alpha-taxonomy is still in a poor state of resolution and the tasks ahead, therefore, remain overwhelming.

The current taxonomic situation of the African Torini can be briefly summarized as follows. On the one hand, valid species with a *Labeobarbus*, *Varicorhinus*, or intermediate mouth phenotype are often considered monomorphic with regards to mouth phenotype, and these are often the more poorly investigated taxa. On the other hand, however, quite a few valid species are now regarded as being highly polymorphic, and they are often the seemingly better known species (see Table 1). These cases mostly include taxa with the extreme *Labeobarbus* and *Varicorhinus* mouth phenotypes, as well as with intermediate mouth phenotypes, all assembled under the taxonomic umbrella of one valid species. These two rather opposing taxonomic manifestations of mouth phenotype diversity within *Labeobarbus s.l*. are historically rooted, and these roots still have their repercussions on the present-day taxonomic situation. Whereas Boulenger (1909, 1916a) and other early authors described most mouth phenotypes as discrete species, the gradual conceptual change in interpreting mouth phenotype polymorphism in some of the more extensively documented species, for example, the Southern African *L. marequensis*, led gradually to the present-day taxonomic solution that these species are taxa representing extreme cases of intraspecific polymorphism (see Skelton, 1993, 2001; Marshall, 2011). It is not unlikely that the systematics and taxonomy of *Labeobarbus s.l*. will remain in this schizophrenic and unsatisfactory state, because we seem as yet unable to fully grasp the nature of species in these African hexaploid Torini, and hence there is the need for integrated comprehensive and interdisciplinary studies to provide convincing and testable hypotheses. Although detailed morphological descriptions with a focus on documenting sympatric mouth polymorphism and ontogenetic development would provide the necessary detailed phenotypic data for many cases, mitochondrial and nuclear DNA-based phylogenetics as well as genomics including cross-breeding experiments would be indispensable to gain a better understanding of the evolutionary dynamics underlying the observed mouth phenotype megadiversity across Africa and the Middle East. The current review paper hopefully provides a first framework to taxonomically and phenotypically allocate results and implications of upcoming case studies, such as the one currently undertaken on the polymorphic species complexes in two Congo subdrainages, i.e. the Inkisi Basin (E. Vreven, S. Wamuini Lunkayilakio, T. Musschoot, E. Decru, J. Snoeks & U. Schliewen, unpubl. data) and the Epulu Basin (DRC; E. Decru, A. Walanga, J. Snoeks & E. Vreven, unpubl. data).

Similarities beyond the African continent?

The occurrence of a highly specialized *Varicorhinus*-like mouth phenotype is not unique to the African species of the genus *Labeobarbus s.l*. Instead, a tentative compilation of its distribution illustrates that it is a recurrent phenomenon in several cyprinid genera with very different levels of ploidy, i.e. diploids, tetraploids, and again hexaploids. Indeed, altogether, one Leuciscinae genus (*sensu* Gaubert, Denys & Oberdorff, 2009) and eight Cyprininae genera (*sensu* Yang *et al*., 2015) are composed entirely of species with the lower jaw bearing a horny cover with a sharp cutting edge on its anterior edge and often fleshy on the mouth

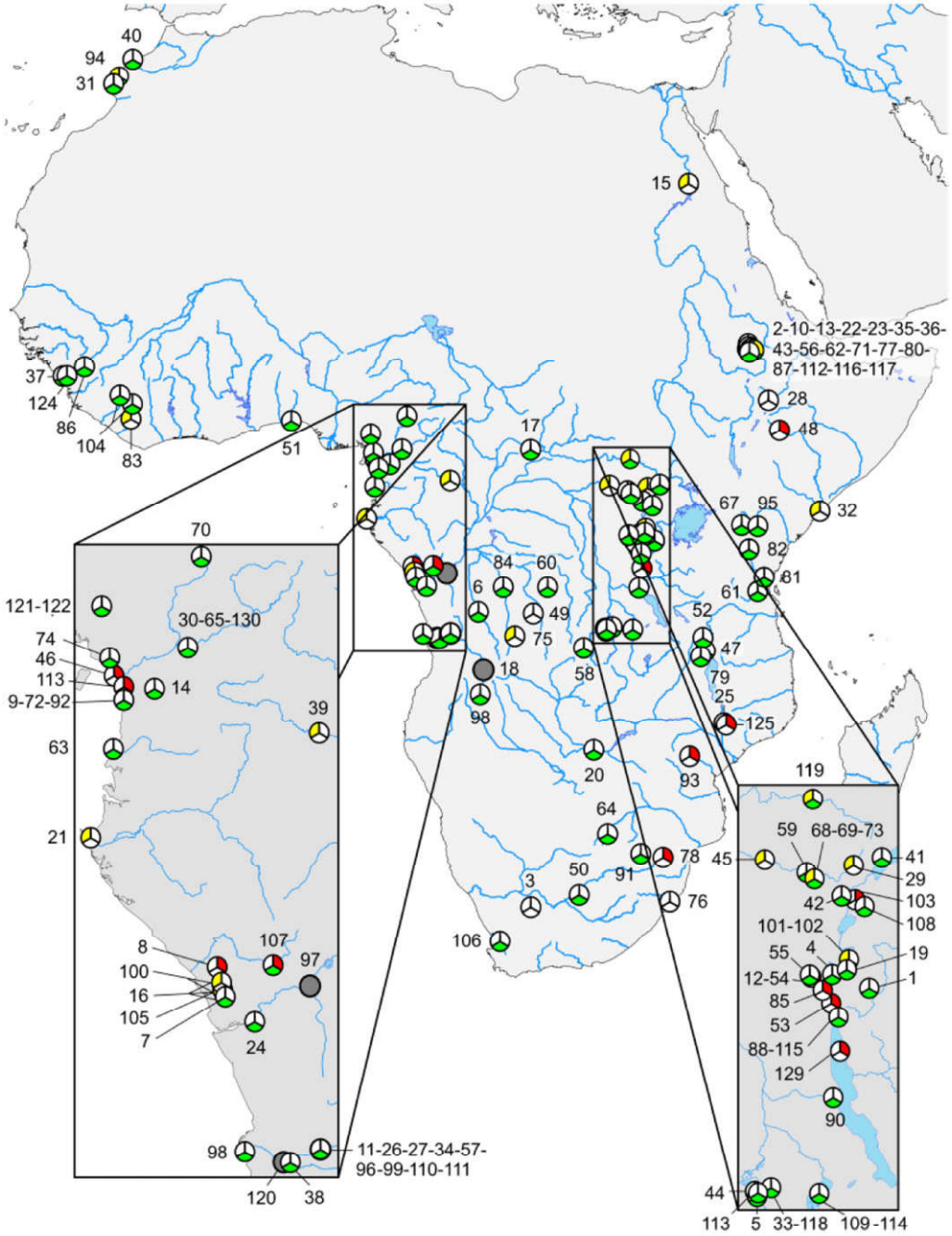


Figure 6. Map of Africa illustrating the overall distribution of the different mouth phenotypes over the continent. Mouth phenotype(s) and mouth phenotype polymorphism have been mapped according to: (1) the three major mouth phenotypes, i.e. *Labeobarbus*, intermediate, or *Varicorhinus* mouth phenotype, and the papillated mouth phenotype as retained in Table 1; (2) using the coordinates of the type localities of the valid species only. For the few cases where coordinates could not be attributed to the type locality, the type region has been used to map the data. Mouth phenotype polymorphism as illustrated on the map refers to that found in the nominal type specimen(s) (i.e. holotype, lectotype, neotype, or syntypes) of each valid species only. In addition, mouth phenotype polymorphism as documented for both – i.e. (1) the nominal type specimen(s) of each valid species only, and (2) all other type(s) and non-type specimens of each valid species – has been visualized on the right side of the map. For each of the valid species under consideration: ◔ refers to the occurrence of the *Labeobarbus* mouth phenotype; ◕ refers to the occurrence of the *Varicorhinus* mouth phenotype; ◔ refers to the occurrence of the intermediate mouth phenotype; and a fully filled grey type locality dot refers to the occurrence of the papillated mouth phenotype (never in combination with any of the other phenotypes).



Figure 6. *Continued*

corners only (see Table 2). In addition, four more Cyprininae genera (*sensu* Yang *et al*., 2015) are known to contain at least some species with a *Varicorhinus*-like mouth phenotype, and the same holds true for another Leuciscinae genus (*sensu* Gaubert *et al*., 2009) (see Table 2). Furthermore, the occurrence of comparable *Labeobarbus*/*Varicorhinus*-like mouth phenotype polymorphism is not restricted to the African species of the genus *Labeobarbus s.l.* (see Table 2), because a similar *Labeobarbus*/*Varicorhinus* mouth phenotype polymorphism has been documented for *B. grypus* (see Roberts & Khaironizam, 2008: 47–48, 50), known from the Tigris–Euphrates Basin and rivers from southern Iran (Borkenhagen, 2014); now an *Arabibarbus*, following Borkenhagen (2014), and part of *Labeobarbus s.l.* (see Tsigenopoulos *et al*., 2010; Berrebi *et al*., 2014; Yang *et al*., 2015). In addition, it has also been documented outside *Labeobarbus s.l.* (see Table 2). First, Roberts & Khaironizam (2008) observed and documented a comparable mouth phenotype variation and polymorphism in *Neolissochilus*. Within *Neolissochilus soroides* (Duncker, 1904) from the Sungai Gombak, a small tributary of the Sungai Kelang River (Malaysia), they documented the occurrence of specimens with a generalized, normal, or *Neolissochilus*-like, i.e. an intermediate, mouth phenotype, besides specimens with a *Lissochilus*- or *Acrossocheilus*-like, i.e. *Varicorhinus*-like, mouth phenotype and specimens with a *Tor*-like, i.e. *Labeobarbus*-like, mouth phenotype. Recently, Khaironizam *et al*. (2015) confirmed the occurrence of three mouth phenotypes in *N. soroides* that are very similar to the *Labeobarbus*, intermediate, and *Varicorhinus* mouth phenotypes documented herein. Their meristic analyses identified no differences between

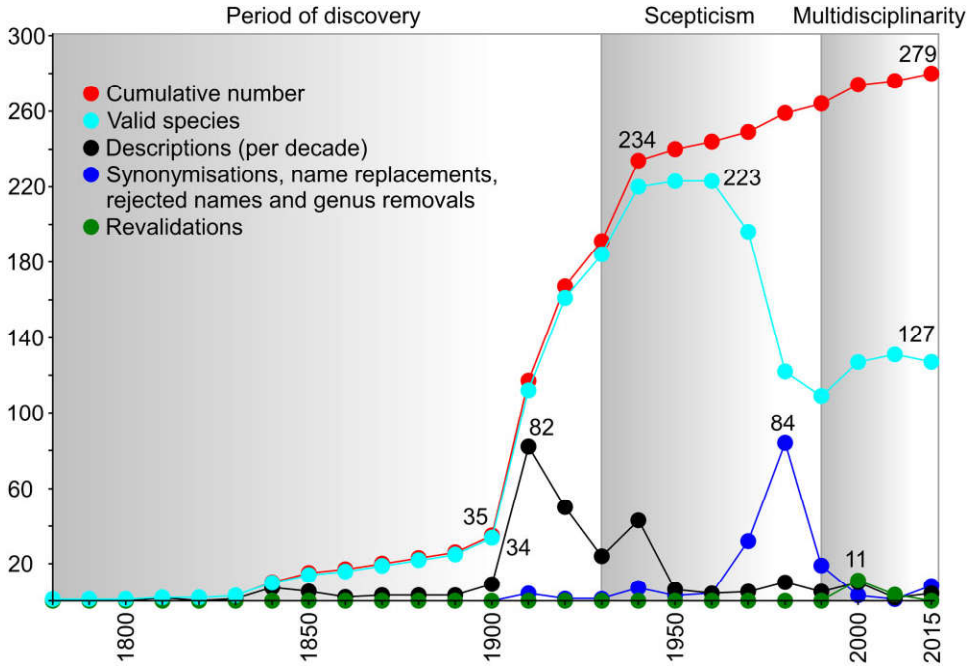


Figure 7. Diagram illustrating chronological changes in species numbers for *Labeobarbus*, *sensu* Tsigenopoulos *et al*. (2010) and Berrebi *et al*. (2014), in Africa. All numbers are provided per decade.
For this diagram, all species have been attributed to *Labeobarbus* although many of them have originally been described in other genera in use at the time of their original description. The two species of the monospecific genera *Acapoeta* and *Sanagia* have been included in these counts. *Nomina nuda* are not included in the counts, as they are unavailable for nomenclatural purposes. A preoccupied name is counted as +1 by its original description and replaced (–1) by its replacement name (+1) at the date of replacement. No distinction has been made between species or subspecies descriptions, and as such both have been counted as +1 at the time of their original description. Giving both equal weighting gives a better overview of overall taxonomic activity in the genus as currently recognized. As nowadays none of the valid species, except for the West African and Nilotic *Labeobarbus bynni* and the East African *Labeobarbus intermedius* (see annotated checklist 2), is considered to contain subspecies, all previously described subspecies have been considered as formally synonymized, which translates into –1 for each, at the date of the first publication formally rejecting or instead clearly neglecting them. As such, of course, a change from subspecies to species level is not visible, and neither is the reverse; however, what has been made visible is the synonymization of subspecies with other subspecies or with the valid species.

the mouth phenotypes, but morphometrics revealed selected mouth phenotype-related differences in lower jaw length and lower jaw width. Khaironizam *et al*. (2015) concur with Roberts & Khaironizam (2008) in interpreting the observed mouth phenotypes as trophic polymorphism, but they also stipulated the need to further examine the question with genomic techniques.

In addition, comparable mouth phenotype variation and intergradation has also been documented within the genus *Schizothorax*. Indeed, although according to Roberts & Khaironizam (2008: fig. 9) the genus *Schizothorax* (however, see Qi *et al*., 2012) is composed of species having a *Labeobarbus*- or *Tor*-like mouth phenotype, the genus *Oreinus* McClelland, 1838 is composed of species with a *Varicorhinus*-, *Lissochilus*-, or *Acrossocheilus*-like mouth phenotype and a variety of intermediate mouth phenotypes bridging the mouth phenotypic gap between both these genera. Hora (1934 *fide* Roberts & Khaironizam, 2008: fig. 9) illustrated the mouth phenotype intergradation in between *Schizothorax labiatus* (McClelland, 1842) and *Oreinus sinuatus* var. *griffithi* McClelland, 1842 (both *Schizothorax sinuatus* Heckel, 1838 and *Oreinus griffithi* are now junior synonyms of *Schizothorax plagiostomus* Heckel, 1838 *fide* Coad, 1981) from the mountainous area of Chitral in north-eastern Afghanistan (see Roberts & Khaironizam, 2008). Interestingly, some of the illustrated intermediate mouth phenotypes are characterized by the presence of numerous papillae, on the lower jaw. This is remarkable, as some of the *Labeobarbus s.l.* species do not have a *Labeobarbus*, *Varicorhinus*, or a fully intermediate mouth phenotype, but instead bear numerous papillae on their lower

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Table 2. Overview of the occurrence of comparable *Varicorhinus* mouth phenotype, and comparable *Labeobarbus/Varicorhinus* mouth phenotype polymorphism, in other Cyprinid genera.

Genus	Author & date	Subfamily	No. of species	Occurrence	Ploidy level (2n)	References
Occurrence of comparable *Varicorhinus* mouth phenotype						
Acrocheilus	Agassiz, 1855	Leuciscinae	1	all spp.	50*	Robe, 1998: 132; Gau *et al*., 2009: 669, fig. 13; Ar, 2011: 65
Capoeta	Valenciennes, 1842	Cyprininae (Torini)	22	all spp.	148 & 150*	Ar, 2011: 48–49; Le *et al*., 2013: 400; Ya *et al*., 2015: 99, table 3
Cyprinion	Heckel, 1843	Cyprininae (Barbini)	9	all spp.	50*	Ar, 2011: 49; Le *et al*., 2013: 400; Ya *et al*., 2015: 99, table 3
Herzensteinia	Chu, 1935	Cyprininae (Schizothoracini)	1	all spp.	unknown	Gau *et al*., 2009: 664, fig. 8; Qi *et al*., 2012: 3, fig. 1; Ya *et al*., 2015: 99, table 3
Onychostoma	Günther, 1896	Cyprininae (Acrossocheilini)	21	all spp.	50	Ar, 2011: 50; Le *et al*., 2013: 400; Ta, 1975: 143; Ya *et al*., 2015: 99, table 3
Platypharodon	Herzenstein, 1891	Cyprininae (Schizothoracini)	1	all spp.	90*	Ar, 2011: 70; Qi *et al*., 2012: 3, fig. 1; Ya *et al*., 2015: 99, table 3
Scaphiodonichthys	Vinciguerra, 1890	Cyprininae (Barbini)	4	all spp.	50	Do, Ma&Ra, 2012: 439; Le *et al*., 2013: 400; Ta, 1975: 143; Ya *et al*., 2015: 99, table 3
Scaphognathops	Smith, 1945	Cyprininae (Poropuntiini)	3	all spp.	unknown	Ta, 1975: 143; Ra, 1996: 100; Ya *et al*., 2015: 99, table 3
Semiplotus	Bleeker, 1860	Cyprininae (Barbini)	3	all spp.	50	Sa, Na&Ba, 2009: 501; Le *et al*., 2013: 400
Chondrostoma s.l.	Agassiz, 1832	Leuciscinae	20 *s.s.* / 37 *s.l.*	some spp.	50*	Ar, 2011: 62; Gan *et al*., 2004; Gau *et al*., 2009: 670, fig. 14; Ko&Fr, 2007: 150; Roba *et al*., 2007
Diptychus	Steindachner, 1866	Cyprininae (Schizopygopsini)	2	some spp.	98 & 100*	Ar, 2011: 70; Qi *et al*., 2012: 3, fig. 1; Ya *et al*., 2015: 99, table 3
Poropuntius	Smith, 1931	Cyprininae (Poropuntiini)	40	some spp.	50	Ar, 2011: 50; Le *et al*., 2013: 400; Ya *et al*., 2015: 99, table 3
Schizocypris	Regan, 1914	Cyprininae (Barbini)	3	some spp.	92	Ar, 2011: 70; Le *et al*., 2013: 400; Ya *et al*., 2015: 99, table 3
Schizopygopsis	Steindachner, 1866	Cyprininae (Schizopygopsini)	8	some spp.	90 & 92	Ar, 2011: 70; Qi *et al*., 2012: 3, fig. 1; Ya *et al*., 2015: 99, table 3
Occurrence of comparable *Labeobarbus/Varicorhinus* mouth phenotype polymorphism						
Neolissochilus	Rainboth, 1985	Cyprininae (Torini)	23	at least one sp.	98 & 100	Ar, 2011: 49–50; Robe&Ka, 2008: 32–34; Ya *et al*. 2015: 99, table 3
Schizothorax	Heckel, 1838	Cyprininae (Schizothoracini)	62	at least one sp.	90–150	Ar, 2011: 71; Robe & Ka, 2008: 50–51, fig. 9; Ya *et al*., 2015: 99, table 3

For more details, see the main text. Subfamily-level classification according to Gaubert *et al*. (2009) and Arai (2011), further updated following Yang *et al*. (2015) for the Cyprininae, with the addition of the Tribe (between brackets) for members of the latter subfamily. Species numbers as provided per genus follow FishBase (Froese & Pauly, 2015). Abbreviations of the author names for the references cited are as follows: Ar, Arai; Do, Ma&Ra, Donsakul, Magtoon & Rangsiruji; Gan, Gante; Gau, Gaubert; Ko&Fr, Kottelat & Freyhof; Le, Levin; Ra, Rainboth; Sa, Na&Ba, Sahoo, Nanda & Barat; Ta, Taki; Roba, Robalo; Robe&Ka, Roberts & Khaironizam; Ya, Yang.
*Ploidy level (2n) as found in the type species of the genus.

as well as upper jaw. Within *Labeobarbus s.l.* this mouth phenotype is currently only reported from the Lower Congo, i.e. the Inkisi River Basin (DRC) and the Quanza River Basin (Angola) (see above), and seems to imply the recurrent occurrence of other, less widespread, mouth phenotypes as well.

The recurrent occurrence of *Varicorhinus*-like mouth phenotypes has already received particular attention in Schizothoracinae (*sensu* Qi *et al*., 2012) as well as *Chondrostoma s.l.* (*sensu* Gante, Collares-Pereira & Coelho, 2004) (see below). Qi *et al*. (2012: fig. 3A) interpreted the paraphyletic occurrence of specimens with a *Varicorhinus*-like 'sharp outer horny sheath' on the lower jaw in their cytochrome *b* mtDNA phylogenetic tree of the Schizothoracinae, as evidence for convergent and parallel evolution. Although the recurrent occurrence of such highly similar and specialized mouth phenotypes, such as that with the *Varicorhinus*-like 'sharp outer horny sheath', might indeed result from convergent as well as parallel de novo evolution of character states, other possibly equally or more parsimonious explanations appear possible. Similar to *Labeobarbus s.l.* (Tsigenopoulos *et al*., 2010: fig. 1), there is mtDNA-based phylogenetic signal for a paraphyly of the highly specialized cutting edge mouth phenotype in Schizothoracinae. Although the homology of these highly similar *Varicorhinus*-like mouth phenotypes has yet to be demonstrated, this recurrent and highly similar expression of a specific mouth phenotype allows us to hypothesize that the cutting edge phenotype might be encoded by ancient homologous Cyprinidae genes that are sometimes but not always expressed, e.g. possibly after hybridization events.

The same hypothesis might hold explanatory potential for the similar situation in *Chondrostoma s.l.* Here again, the paraphyletic occurrence of a ventral rasping mouth reinforced by a horny layer that forms a cutting edge on the lower lip, i.e. a cutting-edge phenotype, in their combined mitochondrial and nuclear DNA-based phylogeny, led Robalo *et al*. (2007) to hypothesize that this phenotype evolved several times through convergent evolution. Based on this, they proposed five morphologically poorly diagnosed (see Kottelat & Freyhof, 2007) new genera within the *Chondrostoma s.l.* lineage: two without a horny layer on the lower lip, *Achondrostoma* Robalo *et al*., 2007 and *Iberochondrostoma* Robalo *et al*., 2007, and three with a horny layer on the lower lip, i.e. *Parachondrostoma* Robalo *et al*., 2007, *Pseudochondrostoma* Robalo *et al*., 2007, and *Protochondrostoma* Robalo *et al*., 2007. Although Robalo *et al*. (2007) mentioned the possibility of introgression/hybridization to explain the observed pattern, they argued against a causative role despite extensive evidence for hybridization within *Chondrostoma s.l.* (Gante *et al*., 2004; I. Doadrio, unpubl. data, *fide* Robalo *et al*., 2007). Furthermore, Perea *et al*. (2010: fig. 4) revealed *Achondrostoma* as defined by Robalo *et al*. (2007) as paraphyletic in their mtDNA results, and they identified additional basal inconsistencies in the *Chondrostoma* phylogeny (see Perea *et al*., 2010: figs 4, 6). This contradictory phylogenetic evidence might allow us to speculate that also within *Chondrostoma s.l.*, the mosaic pattern of occurrence of the specialized *Chondrostoma* mouth is at least partially based on complex patterns of past and current introgression/hybridization (see Gante *et al*., 2004). Here, too, the genomic processes governing the expression of the specialized *Chondrostoma* mouth must be elucidated before a better understanding of its first apparition and re-apparition will be possible.

Finally, Roberts (1998) also reported the occurrence of a 'discrete trophic polymorphism' in two species of *Poropuntius* Smith, 1931, i.e. *Poropuntius bolovenensis* Roberts, 1998 from Laos and *Poropuntius genyognathus* Roberts, 1998 from Burma. The former case in particular was well documented by Roberts (1998), when he recognized 'four distinct forms or morphs' in this species with 'pronounced trophic polymorphism' (see Roberts, 1998: 124). He gave them subspecific rank: (1) *Poropuntius bolovenensis bolovenensis* Roberts, 1998, with the lips and horny jaw sheaths moderately developed and the margin of the lower horny jaw sheath rounded, with no trenchant cutting edge; (2) *Poropuntius bolovenensis acuticeps* Roberts, 1998, with hypertrophied lips but, however, with the lower lip not continuous and lacking the typical mental lobe of the *Labeobarbus* mouth phenotype; (3) *Poropuntius bolovenensis glaridostoma* Roberts, 1998, with the lower horny jaw sheath greatly thickened and broad, and with a sharp transverse cutting margin reminiscent of the *Varicorhinus* mouth phenotype; and finally (4) *Poropuntius bolovenensis laticeps* Roberts, 1998, with a mouth nearly as broad as that of *P. b. glaridostoma*, but without the extreme reduction of the lower lip or the development of a lower horny jaw sheath with a trenchant margin. In contrast to Roberts (1998), Kottelat (2000) interpreted this mouth phenotype variation very differently, i.e. he considered each of Roberts' (1998) subspecies as full species. As Roberts' infrasubspecific morph names are taxonomically unavailable (see Kottelat, 2000; see also Eschmeyer, 2015, although with different argumentation) he consequently provided new names for two of Roberts' morphs: *Poropuntius consternans* Kottelat, 2000, for *P. b. acuticeps*, and *Poropuntius lobocheiloides*, Kottelat, 2000, for *P. b. glaridostoma*, whereas *P. b. laticeps* was tentatively identified as conspecific with *P. lobocheiloides* (see Kottelat, 2013), and he even identified a fourth species for the Xe Nam Noi or Xe Nam Noy Basin, which he named *Poropuntius solitus* Kottelat, 2000. Kottelat (2000) based his interpretation on evidence from additional non-feeding-related, meristic

differences in these taxa, which had already been reported by Roberts (1998), and he complemented Roberts' (1998) data with his own additional observations on differences in meristics, habitus, and the relative position of the fins. Surprisingly, Kottelat (2000) did not consider interspecific hybridization as a hypothesis, which could at least explain part of the mouth phenotype variation in these taxa (see above for *Labeobarbus*). As for the *Labeobarbus*/*Varicorhinus* mouth phenotype polymorphism, here also the radically different interpretation of the mouth phenotype variation clearly points to the dissatisfactory state of our current understanding of this phenomenon across multiple cyprinid lineages.

Interestingly, Roberts (1998) already hypothesized that a genetic basis for such polymorphism could have already been present in proto-Cyprinidae Ostariophysi (our wording). He further pointed to the observation that these apparently ancient and genetically controlled phenotypic differences are repeatedly expressed as intraspecific mouth phenotype variation, mainly in species inhabiting lakes or streams isolated from neighbouring water bodies by barriers such as waterfalls, as is the case for *P. bolovenensis*, for example. Isolation from species-rich fish communities would enhance ecological opportunities for specialized mouth phenotypes, and thereby favour the rapid re-expression of ancient genomic potentialities, which only seemingly appear to be 'evolutionary novelties' (see Roberts, 1998: 132). Species not expressing alternative phenotypes would nevertheless silently carry all genomic information necessary to generate an array of discrete phenotypes, with their expression depending on ecological conditions (Roberts, 1998). Even if Kottelat (2000) classified the observed mouth phenotype polymorphism in *Poropuntius* drastically differently, the idea of ancient genomic processes governing – in combination with environmental factors – the expression of the highly specialized potentialities for alternative mouth phenotypes across multiple cyprinid lineages remains an appealing hypothesis to explain for its widespread occurrence.

Is the combination of hexaploidy and recurrent hybridization promoting phenotypic diversity?

All these numerous cases of a well-defined polymorphism within *Labeobarbus s.l.* and across genera raise numerous questions with regards to the origin of both the hexaploidy as well as the observed mouth phenotype characters, and their polymorphic variation. For example, both *Labeobarbus* and *Capoeta* are hexaploid, and mitochondrial DNA results (see Tsigenopoulos *et al*., 2010; Yang *et al*., 2015: fig. 2) support their reciprocal monophyly, i.e. an independent origin of both haplotype lineages. Both these mitochondrial DNA phylogenies therefore support a primarly independent, possibly allopatric, origin of these lineages. Furthermore, Yang *et al*.'s (2015: fig. 4) analysis of a single nuclear gene (*RAG1*) suggests that these hexaploid lineages might have derived from two independent hybridization events between their respective tetraploid ancestors: i.e. tetraploid Torini and *Luciobarbus*, a member of the barbins, respectively (maternal source), and *Cyprinion* (paternal source). Considering that the data presented are based on a single nuclear gene, and that many nodes are weakly supported, however, this hypothesis of hybridization cannot be tested against the one of incomplete lineage sorting without additional (genomic) data. Interestingly, the extreme *Labeobarbus*/*Varicorhinus* mouth polymorphism as found in *Labeobarbus s.l.* is altogether lacking, for example, in *Capoeta*, where all species are reported to have a clear cutting edge on the lower jaw (*fide* Bănărescu, 1999), as found in the *Varicorhinus* mouth phenotype of *Labeobarbus s.l.* Mouth phenotype variation is not entirely lacking in *Capoeta* (see Karaman, 1969), however, and instead has been reported for: (1) the overall shape of the mouth (horsehoe-shaped versus rectangular); (2) the development of a horny cover; and (3) the sharpness of the real cutting edge, which even seems to be entirely absent in some specimens (E. Vreven, pers. observ., 2015). In addition, Küçük *et al*. (2009) reported the presence of flesly lips for *Capoeta pestai* (Pietschmann, 1933) and *Capoeta mauriccii* Küçük *et al*., 2009, as well as an overall superficial resemblance of both to the species of the genus *Luciobarbus* with regard to their pointed heads and general shape. Therefore, two major mouth phenotypes of *Labeobarbus s.l.* might well be, under a hybridization scenario, the product of introgression of *Capoeta*-type cutting edge genes into a fleshy lip proto-*Labeobarbus* genome, for example. A similar scenario may also be envisaged for the more distantly related cyprinine genus *Schizothorax*, which comprises tetraploid and hexaploid species alike (see Arai, 2011), and for which comparable mouth phenotype variation has also been reported (see above). Considering that for many cyprinine species and even genera the ploidy level remains unknown, additional cases of mixed tetraploidy and hexaploidy within the cyprinine genera, coupled with additional cases of a *Varicorhinus*-like mouth phenotype in sometimes polymorphic genera, cannot entirely be excluded. Indeed, Van de Peer, Maere & Meyer (2009) suggested that although descendants of whole-genome duplications (WGDs) often do not survive, they can be very successful if they do survive. Morphologically, however, most *Capoeta* species have the last unbranched dorsal fin ray denticulated (see Karaman, 1969; Bănărescu, 1999), at least in juveniles and subadults (but not in all, e.g. *Capoeta caelestis* Schöter,

Özuluğ & Freyhof, 2009), whereas such denticulations, although present in *Luciobarbus* (see Bănărescu & Bogutskaya, 2003), never occur in *Labeobarbus s.l.*

An alternative hypothesis for the recurrent origin of mouth phenotypes not only across genera but within *Labeobarbus s.l.* has been suggested by Levin *et al.* (2013) with regards to the *V. beso* and *Varicorhinus*-like mouth phenotypes. According to the mtDNA evidence of these authors, a cutting edge might have evolved multiple times de novo in Ethiopia, a hypothesis that these authors took as justification for the recognition of *Varicorhinus* as a monospecific genus; however, the occurrence of monomorphic phenotypes may camouflage a heterozygotic genome encoding a silent polymorphism (see Roberts, 1998), which under certain environmental circumstances and/or after hybridization events, for example, might become re-expressed and again produce polymorphic phenotypes, especially in polyploid taxa (Otto, 2007).

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ANNOTATED CHECKLIST 1: AFRICAN *LABEOBARBUS S.L.* SPECIES

acuticeps, *Barbus* Matthes, 1959. River Nyawarongo [Mbuye] (±2°26′S, 30°21′E, D.F.E. Thys van den Audenaerde, unpubl. data), Rwanda. Holotype: MRAC 130313. Paratypes: MRAC 130310 (1), 130311-312 (2), 130314 (1). Current status: valid as *Labeobarbus acuticeps* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Nyabarongo, Akanyaru, upper Akagera (including some lakes in the Bugusera depression) and middle Akagera (below Rusumo Falls) basins (De Vos & Thys van den Audenaerde, 1990). Notes: (1) paratype MRAC 130310 is also a paratype of *Barbus* (now *Labeobarbus*) *claudinae* De Vos & Thys van den Audenaerde, 1990; (2) considered to be 'dubiously distinct from *B. altianalis*' by Banister (1973: 8), but De Vos & Thys van den Audenaerde (1990) mention clear differences in the number of gill rakers and lateral line scales, and the barbel length, to distinguish both species.

acutirostris, *Barbus brunelli* Bini, 1940. Debre Mariam (11°38′N, 37°24′E, Nagelkerke & Sibbing, 1997) (locality 1), Lake Tana, Ethiopia. Neotype: RMNH 32870 (designated by Nagelkerke & Sibbing, 1997). Current status: valid as *Labeobarbus acutirostris* (placed in *Labeobarbus* in Getahun, 2007a). Synonym of *Barbus intermedius* in Banister (1973); raised to species level and revalidated as *Barbus acutirostris* by Nagelkerke & Sibbing (1997). Synonyms: none. Distribution: Endemic to Lake Tana, Ethiopia (Nagelkerke & Sibbing, 1997). Notes: originally described as a subspecies of *Barbus* (now *Labeobarbus*) *brunellii*, a junior synonym of *L. intermedius*, following Banister (1973).

aeneus, *Cyprinus* Burchell, 1822. Zak River (±29°39′S, 21°11′E, D.F.E. Thys van den Audenaerde, unpubl. data) [trib. of Orange River, South Africa]. No types known (Hocutt & Skelton, 1983). Current status: valid as *Labeobarbus aeneus* (placed in *Labeobarbus* in Skelton, 2001). Synonyms: *Barbus gilchristi* Boulenger, 1911 (synonymy in Jubb, 1963); *Barbus holubi* Steindachner, 1894 (synonymy in Barnard, 1943); *Barbus mentalis* Gilchrist & Thompson, 1913 (synonymy in Jubb, 1963). Distribution: natural range, Orange–Vaal system (Skelton, 2001). Various introductions in South Africa and Zimbabwe (Skelton, 2001; Marshall, 2011).

alluaudi, *Barbus* Pellegrin, 1909. A hybrid; see annotated checklist 2.

altianalis, *Barbus* Boulenger, 1900. Lake Kivu (±2°0′S, 29°10′E, USBGN, 1964b), north-east of Lake Tanganyika. Lectotype: BMNH 1906.9.6.13 (designated by Banister, 1973). Paralectotypes: BMNH 1906.9.6.14 (1), 1906.9.6.15 (1). Current status: valid as *Labeobarbus altianalis* (placed in *Labeobarbus* in Skelton & Bills, 2008; Banyankimbona *et al*., 2012a). Synonyms: *Barbus altianalis labiosa* Pellegrin, 1933 (synonymy in Banister, 1973); *Barbus bayoni* Boulenger, 1911; *Barbus eduardianus* Boulenger, 1901; *Barbus fergusonii* Boulenger, 1901 (synonymy in Worthington, 1932); *Barbus hollyi* Lohberger, 1929 (synonymy in Greenwood, 1966); *Barbus kiogae* Worthington, 1929; *Barbus kivuensis* Pappenheim, 1914 in Pappenheim & Boulenger, 1914 (synonymy in Banister, 1973); *Barbus lobogenys* Boulenger, 1906 (synonymy in Worthington, 1932); *Barbus longirostris* Worthington, 1929; *Barbus obesus* Worthington, 1929 (synonymy in Banister, 1973); *Barbus pietschmanni* Lohberger, 1929 (synonymy in Greenwood, 1966); *Barbus radcliffii* Boulenger, 1903 (synonymy in Norman, 1925); *Labeo rueppellii* Pfeffer, 1896 (suppressed name; see Reid, 1980). Distribution: Lake Kivu and its affluents, Ruzizi, middle Akagera (below Rusumo Falls), and lakes Edward, Albert, Victoria, and Kioga (De Vos & Thys van den Audenaerde, 1990). Also in the Victoria Nile and Lake George (Lévêque & Daget, 1984). Notes: based on colour differences, Banister (1973) distinguishes three subspecies (*B. a. altianalis*, *B. a. eduardianus* and *B. a. radcliffii*), but these are not retained by De Vos & Thys van den Audenaerde (1990).

altipinnis, *Varicorhinus* Banister & Poll, 1973. Kilwezi (±09°06′S, 26°46′E, collection database), right bank affluent of the Lufira, DRC. Holotype: MRAC 179729. Paratypes: BMNH 1972.10.2.1 (1), MRAC 179730 (1), 179731 (1), 179732-733 (2), 179734 (1), 179735 (1), IRSNB 643 (1). Current status: valid as *Labeobarbus altipinnis* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Lufira River system (Lévêque & Daget, 1984). Notes: NMBA 3983 (1), 3985 (1), and 3988-89 (2) given in Eschmeyer (2015) as paratypes, are verified as syntypes of *V.* (now *Labeobarbus*) *macrolepidotus*.

anema, *Barbus* Boulenger, 1903. Not a *Labeobarbus* species (see present paper).

ansorgii, *Varicorhinus* Boulenger, 1906. Fort Don Carlos [= Tembo Aluma] (±7°42′S, 17°17′E, D.F.E. Thys van den Audenaerde, unpubl. data), at junction of Cambo and Kwango rivers, Loanda, Angola. Holotype: BMNH 1904.5.2.161. Current status: valid as *Labeobarbus ansorgii* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Cuango River, Angola (Lévêque & Daget, 1984).

arambourgi, *Barbus* Pellegrin, 1935. Not a *Labeobarbus* species (see present paper).

aspius, *Barbus* Boulenger, 1912. Boma Vonde (±5°08′S, 12°39′E, D.F.E. Thys van den Audenaerde, unpubl. data), Lebuzi River [Shiloango Basin, DRC]. Syntypes: BMNH 1912.4.1.354 (1), MRAC 1536 (1). Current status: valid as *Labeobarbus aspius* (placed in *Labeobarbus* in De Weirdt & Teugels, 2007). Synonyms: none. Distribution: Lower Guinea endemic, known from the Lebuzi River (Chiloango Basin) in Cabinda (Angola) and DRC (De Weirdt & Teugels, 2007). Notes: Lévêque & Daget (1984) only mention a holotype, whereas the original description clearly mentions and is based on two syntype specimens.

axelrodi, *Varicorhinus* Getahun, Stiassny & Teugels, 2004. Place called 'Porte du Mayombe' (4°20′S, 12°26′E), Loukoula River, 9 km downstream of Mpounga, Republic of the Congo. Holotype: MRAC 91-68-P-1132. Paratypes: AMNH 232315 (1), 232907 (3), 233184 (1), CUMV 87041 (1), 88131 (1), MRAC 90-057-P-1297-1300 (4), 90-057-P-1314-1327 (14), 99-55-P-246-247 (2), 99-55-P-249 (1), 99-90-P-459-460 (2). Current status: valid as *Labeobarbus axelrodi* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Lower Guinea endemic, known from the Ogowe (Louetsi, Ngounie) River Basin and Nyanga River in southwestern Gabon, and the Kouilou system in Republic of the Congo (Getahun, 2007b). Notes: Getahun *et al*. (2004) and Eschmeyer (2015) incorrectly give MRAC 99-90-P-459-461, with three specimens, as part of the paratypes. The correct number for this record, with only two specimens, is MRAC 99-90-P-459-460.

babaulti, *Varicorhinus* Pellegrin, 1932. Preoccupied by *Barbus* (*Capoeta*) *babaulti* Pellegrin, 1926, junior synonym of *Labeobarbus oxyrhynchus* (Pfeffer, 1889); replaced by *Varicorhinus pellegrini* Bertin & Estève, 1948. Bertin & Estève (1948) transferred *Barbus babaulti* Pellegrin, 1926 to the genus *Varicorhinus*, creating the need for a replacement name for *Varicorhinus babaulti* Pellegrin, 1932.

batesii, *Barbus* Boulenger, 1903. Kribi [Kienke] River (± 2°56′N, 9°54′E, USBGN, 1962b), southern Cameroon. Holotype: BMNH 1902.11.12.128. Current status: valid as *Labeobarbus batesii* (placed in *Labeobarbus* in De Weirdt & Teugels, 2007). Synonyms: *Barbus linnellii* Lönnberg, 1904 (synonymy in Trewavas, 1962). Distribution: widespread throughout Lower Guinea, from the Cross River in Cameroon to the Chiloango Basin; also reported from the Dja (middle Congo Basin) and from Tibesti (northern Chad) (Lévêque, 2003; De Weirdt & Teugels, 2007). Notes: (1) although the species description is based on a single specimen, Lévêque & Daget (1984) give BMNH 1904.2.29.32–36 as paratypes. They are not listed as types in the BMNH collection (J. Maclaine, pers. comm., 2015). Eschmeyer (2015) also explicitely lists these BMNH specimens as non-types. (2) Report of this species from Tibesti (De Weirdt & Teugels, 2007) in northern Chad, far outside the common distribution area of the species, needs confirmation.

beso, *Varicorhinus* Rüppell, 1835. Bahardar (±11°37′N, 37°24′E, USBGN, 1963), Lake Tana, Ethiopia. Neotype: BMNH 1902.12.13.365 (designated in present paper). Current status: valid as *Labeobarbus beso* (placed in *Labeobarbus* in Berrebi *et al*., 2014). Synonyms: *Dillonia abyssinica* Heckel, 1847; *Chondrostoma dillonii* Valenciennes, 1844 in Cuvier & Valenciennes, 1844; *Labeo varicorhinus* Valenciennes, 1844 in Cuvier & Valenciennes, 1844 (synonymy in Boulenger, 1907c). Distribution: Lake Tana, Blue Nile, Awash (Lévêque & Daget, 1984; Levin *et al*., 2013) and Atbara river systems (Levin *et al*., 2013). Notes: (1) the NHM (London, UK) houses an unpublished, and as such unavailable, neotype (BMNH 1968.7.24.18: 156.8 mm SL), collected during the Sandhurst Ethiopian Expedition (1964), originating from Bahardar (Baherdar ±11°37′N, 37°24′E), also on Lake Tsana (i.e. Tana, Ethiopia), and most probably selected by the late K.E. Banister (NHM); however, we have refrained from identifying this specimen as the neotype of *V. beso* (see present paper); (2) the junior synonym *Chondrostoma dillonii* Valenciennes, 1844 in Cuvier & Valenciennes (1844) is the type species of the genus *Dillonia* Heckel, 1847, and as such the latter genus becomes a junior synonym of *Varicorhinus* (see Lévêque & Daget, 1984), now *Labeobarbus*.

boulengeri, *Labeobarbus* (present paper). Lucala (near railway station) (±9°16′23″S, 15°14′42″E, Google Earth), above the falls on the Lucala River, Angola. Lectotype: BMNH 1911.6.1.6 (designated in present paper). Paralectotypes: ANSP 37905 (1), BMNH 1911.6.1.7–10 (4), NMW 48865 (1), ZMB 18211 (1). Current status: valid as *Labeobarbus boulengeri* (placed in *Labeobarbus* in present paper). Synonyms: replacement name for *Varicorhinus latirostris* Boulenger, 1910 (present paper), preoccupied by *Barbus* (currently *Labeobarbus*) *latirostris* Keilhack, 1908. Distribution: Luculla River system (Lévêque & Daget, 1984) and the lower Congo River (Lowenstein *et al*., 2011). Notes: (1) the NMW paralectotype number was until now unknown from literature sources; (2) the total number of type specimens only adds up to eight, whereas the original publication mentions ten syntypes; (3) MRAC collection specimens from the Inkisi River, the major left-bank tributary of the lower Congo, were identified as *V.* cf. *latirostris* in Wamuini Lunkayilakio *et al*. (2010), and probably represent a species new to science.

brauni, *Varicorhinus* Pellegrin, 1935. Near Lukando (±2°05′S, 28°30′E, D.F.E. Thys van den Audenaerde, unpubl. data), Kanséhété River, Luhoho River affluent, DRC. Syntypes: MNHN 1935-0066 (1), MRAC 42933 (1). Current status: valid as *Labeobarbus brauni* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Luhoho River system, Congo River Basin (Lévêque & Daget, 1984).

brevicephalus, *Barbus* Nagelkerke & Sibbing, 1997. Yigashu (11°40′N, 37°25′E, Nagelkerke & Sibbing, 1997) (locality 10), Lake Tana, Ethiopia. Holotype: RMNH 32880. Paratypes: RMNH 32881-32889 (9). Current status: valid as *Labeobarbus brevicephalus* (placed in *Labeobarbus* in Getahun, 2007a). Synonyms: none. Distribution: Lake Tana and tributaries, Ethiopia (Nagelkerke & Sibbing, 1997).

brevispinis, *Barbus ruspolii* Holly, 1927. Lolodorf (±3°14′N, 10°44′E, USBGN, 1962b), Lokundje River and Nachtigal (±4°21′N, 11°38′E, USBGN, 1962b) (rapids), Sanaga River, both in Cameroon. Syntypes: NMW 7315 (1), 7316 (1). Current status: valid as *Labeobarbus brevispinis* (raised to species level in Pellegrin, 1928b; placed in *Labeobarbus* in De Weirdt & Teugels, 2007). Synonyms: *Barbus brevispinis monunensis* Pellegrin, 1928 (synonymy in Lévêque & Daget, 1984). Distribution: Lokundje and Sanaga river systems and Lake Monoun in Cameroon; also in the Faro-Bénué Basin in Cameroon/Nigeria (De Weirdt & Teugels, 2007). Notes: originally described as a subspecies of *Barbus* (now *Labeobarbus*) *ruspolii*, which is currently a junior synonym of *L. bynni*, following Banister (1973).

bynni, *Barbus* Forsskål, 1775. Aswan (±24°05′N, 32°53′E, Banister, 1973), Nile River, Egypt. Neotype: BMNH 1907.12.2.1230 (designated by Banister, 1973). Current status: valid as *Labeobarbus bynni* (placed in *Labeobarbus* in Getahun, 2007a). Synonyms: *Barbus bynni rudolfianus* Worthington, 1932 (synonymy in Lévêque & Daget, 1984); *Barbus foureaui* Pellegrin,

1919 (synonymy in Lévêque, 1990); *Barbus lancrenonensis* Blache & Miton, 1960 (synonymy in Trewavas, 1974); *Cyprinus lepidotus* Geoffroy St. Hilaire, 1809 (synonymy in Lévêque & Daget, 1984); *Barbus meneliki* Pellegrin, 1905 (synonymy in Banister, 1973); *Barbus occidentalis* Boulenger, 1911 (synonymy in Lévêque, 1990); *Barbus ruspolii* Vinciguerra, 1897 (synonymy in Banister, 1973); *Barbus seguensis* Pellegrin, 1925 (synonymy in Daget, 1954); *Barbus waldroni* Norman, 1935 (synonymy in Lévêque, 1990). Distribution: Nilo, Sudan. *Labeobarbus bynni bynni* (Forsskål, 1775): known from the Nile River and associated lakes. *Labeobarbus bynni occidentalis* (Boulenger, 1911): Senegal, Volta, Oueme, Ogun, Niger, and Chad basins. *Labeobarbus bynni waldroni* (Norman, 1935): Ivory Coast and Ghana, to the west of the Volta, including the Sassandra, Bandama, Niouniourou, Comoé, and Tano (Lévêque & Guégan, 1990; Lévêque, 2003). Notes: the subspecies *L. bynni occidentalis* was placed in '*Labeobarbus*' in Yang *et al*. (2015), whereas *L. bynni* specimens are classified in *Labeobarbus*; the classification of *L. b. occidentalis* may be based on a misidentified specimen.

capensis, *Barbus* Smith, 1841. Not a *Labeobarbus* species (E. Vreven, E.R. Swartz & P.H. Skelton, unpubl. data).

capoetoides, *Varicorhinus* Pellegrin, 1938. Not a *Labeobarbus* species (see present paper).

cardozoi, *Barbus* Boulenger, 1912. N'Kutu [= Ncuto, = Necuto] (±4°57′S, 12°35′E, D.F.E. Thys van den Audenaerde, unpubl. data), Loango River [Shiloango Basin, Cabinda, Angola]; Buco Zau [= Bucozan] (±4°45′S, 12°33′E, D.F.E. Thys van den Audenaerde, unpubl. data), Luali River [Shiloango Basin, Cabinda, Angola]; and Boma Vonde (±5°08′S, 12°39′E, D.F.E. Thys van den Audenaerde, unpubl. data), Lebuzi River [Shiloango Basin, DRC]. Syntypes: ANSP 38550–52 (3), BMNH 1912.4.1.343–348 (6), 1912.4.1.349–352 (4), 1912.4.1.353 (1), MRAC 1528–29 (2), 1530–32 (3), NMW 9604–9607 (4), 54014 (1), 79712 (1), ZMB 18810 (6). Current status: valid as *Labeobarbus cardozoi* (placed in *Labeobarbus* in De Weirdt & Teugels, 2007). Synonyms: none. Distribution: Chiloango Basin in Cabinda (Angola) and DRC, and the Dja River (middle Congo River Basin) (De Weirdt & Teugels, 2007). Also reported from the Niari (Daget, 1961) and Loeme (map in De Weirdt & Teugels, 2007) in Republic of the Congo, but this needs confirmation. Notes: (1) the total number of type specimens adds up to 31, whereas the original description mentions 28 syntypes; (2) BMNH 1912.4.1.343–348 contains six specimens (J. Maclaine, pers. comm., 2015), in contrast to Eschmeyer (2015) who only mentions five.

caudovittatus, *Barbus* Boulenger, 1902. Banzyville (±4°18′N, 21°10′E, D.F.E. Thys van den Audenaerde, unpubl. data), Ubangi River, DRC. Syntypes: BMNH 1901.12.26.26 (1), MRAC 1168 (1). Current status: valid as *Labeobarbus caudovittatus* (placed in *Labeobarbus* in De Weirdt & Teugels, 2007). Synonyms: *Barbus chilotes sakaniae* Poll, 1938; *Barbus euchilus* Boulenger, 1920; *Barbus lestradei* David, 1936 (synonymy in Banister, 1973); *Barbus miochilus* Boulenger, 1920 (synonymy in Poll, 1946); *Varicorhinus stappersii* Boulenger, 1917 (synonymy in Banister, 1973). Distribution: Congo River system, including the lower Luapula system (Lévêque & Daget, 1984) and Lake Mweru (Van Steenberge *et al*., 2014), tributaries of Lake Tanganyika, and the Rusisi (Lévêque & Daget, 1984); in Lower Guinea reported from the Ogooué, Nyanga, and Douigni basins in Gabon (De Weirdt & Teugels, 2007). Notes: (1) as the original description is based on two specimens and a holotype designation is lacking, the holotype and paratype status of the type specimens as provided by Lévêque & Daget (1984), for the MRAC and BMNH specimen, respectively, is incorrect (see also ICZN, 1999: article 73.2). The holotype and paratype status in Lévêque & Daget (1984) are not to be considered valid lecto- and paralectotype designations (ICZN, 1999: article 74.6); (2) *Labeobarbus stappersii* (Boulenger, 1915) is currently a valid species. A replacement name for *Varicorhinus stappersii* Boulenger, 1917, currently a junior synonym of *L. caudovittatus*, is not needed following the rules of ICZN (1999: articles 11.5, 11.6, and 15.1; also see the text).

clarkeae, *Varicorhinus* Banister, 1984. Rio Cunje (~12°0′0″S, 17°40′0″E), Cuanza affluent, Ceilunga, Angola. Holotype: MRAC 164456. Paratype: MRAC 164457 (1). Current status: valid as *Labeobarbus clarkeae* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: only known from Cunaza, Ceilunga, Angola (Banister, 1984).

claudinae, *Barbus* De Vos & Thys van den Audenaerde, 1990. Satinsyi River, 7 km upstream of its confluence with the Nyabarongo (1°51′0″S, 29°38′0″E), near Ngororero, Rwanda. Holotype: MRAC 86-01-P-501. Paratypes: MRAC 91755–56 (2), 92214 (1), 130310 (1), 85-44-P-141-144 (4), 85-44-P-269 (1), 85-44-P-281-289 (9), 86-01-P-502-508 (7), 86-09-P-444-451 (8), 87-11-P-1366-372 (7), 87-11-P-1373-384 (12). Current status: valid as *Labeobarbus claudinae* (placed in *Labeobarbus* in Banyankimbona *et al*., 2012a). Synonyms: none. Distribution: Nyabarongo and upper Akagera basins (upstream of Rusumo Falls) (De Vos & Thys van den Audenaerde, 1990). Notes: (1) paratype MRAC 130310 is also a paratype of *Barbus* (now *Labeobarbus*) *acuticeps* Matthes, 1959; (2) the apparently single paratype in record MRAC 86-09-P-444, as given in the original species description, with a range of standard lengths, actually refers to MRAC 86-09-P-444-451, containing eight paratypes.

codringtonii, *Barbus* Boulenger, 1908. Zambesi River above Victoria Falls (±17°55′S, 25°51′E, D.F.E. Thys van

den Audenaerde, unpubl. data) [Zambia]. Holotype: BMNH 1908.11.6.23. Current status: valid as *Labeobarbus codringtonii* (placed in *Labeobarbus* in Skelton, 2001). Synonyms: *Barbus altidorsalis* Boulenger, 1908 (implicit synonymy in Skelton, 1993, 2001; synonymy confirmed by P.H. Skelton, pers. comm., 2014; see also present paper); *Barbus chilotes* Boulenger, 1908 (synonymy in Jubb, 1963); *Barbus hypostomatus* Pellegrin, 1936 (synonymy in Jubb, 1963). Distribution: Okavango and upper Zambezi (Skelton, 2001). Also in the Cunene (Lévêque & Daget, 1984). Notes: including *Barbus altidorsalis* Boulenger, 1908 as a junior synonym. *Labeobarbus altidorsalis* is not reported in Skelton (2001), and as such must be considered an implicit junior synonym of *L. codringtonii*, the only *Labeobarbus* species known from the Kafue River, i.e. the Upper Zambezi. See also Jackson (1961) on the dubious status of this nominal species.

compiniei, *Barynotus* Sauvage, 1879. Ogowe (±0°49′S, 9°0′E, USBGN, 1962a), French Congo [Gabon, Republic of the Congo]. Holotype: MNHN A-2845 (stuffed). Current status: valid as *Labeobarbus compiniei* (placed in *Labeobarbus* in De Weirdt & Teugels, 2007). Synonyms: *Barbus labiatomimus* Pellegrin, 1914 (synonymy in Mahnert & Géry, 1982). Distribution: Lower Guinea endemic, known from the Komo and Ogooué basins in Gabon, up to the Loeme in Republic of the Congo, including the Nyanga and Kouilou-Niari (De Weirdt & Teugels, 2007). Notes: specific epithet often misspelled as *compinei* (see Lévêque & Daget, 1984), a misspelling that can be traced back up to Sauvage (1880; as *Barynotus campiniei* on p. 22 and *B. compinei* on p. 49), who himself described the species only the year before.

crassibarbis, *Barbus* Nagelkerke & Sibbing, 1997. Angara (12°13′N, 37°18′E, Nagelkerke & Sibbing, 1997) (locality 14), Lake Tana, Ethiopia. Holotype: RMNH 32890. Paratypes: 32891–99 (9). Current status: valid as *Labeobarbus crassibarbis* (placed in *Labeobarbus* in Getahun, 2007a). Synonyms: none. Distribution: endemic to Lake Tana, Ethiopia (Nagelkerke & Sibbing, 1997).

dainellii, *Barbus* Bini, 1940. South-Dek (11°52′N, 37°14′E, Nagelkerke & Sibbing, 1997) (locality 27), Lake Tana, Ethiopia. Neotype: RMNH 32900 (designated by Nagelkerke & Sibbing, 1997). Current status: valid as *Labeobarbus dainellii* (placed in *Labeobarbus* in Getahun, 2007a). Synonym of *Barbus intermedius* in Banister (1973); revalidated by Nagelkerke & Sibbing (1997). Synonyms: *Barbus dainellii macrocephalus* Bini, 1940 (synonymy in Nagelkerke & Sibbing, 1997). Distribution: endemic to Lake Tana, Ethiopia (Nagelkerke & Sibbing, 1997).

dartevellei, *Barbus* Poll, 1945. Matadi (±5°49′S, 13°27′E, USBGN, 1964b), Congo River, DRC. Holotype: MRAC 47781. Current status: valid as *Labeobarbus dartevellei* (placed in *Labeobarbus* in Lowenstein *et al*., 2011). Synonyms: none. Distribution: only known from the holotype (Lévêque & Daget, 1984).

dimidiatus, *Varicorhinus* Tweddle & Skelton, 1998. Likabula River (15°56′S, 35°30′E), upper Ruo River, Malawi. Holotype: SAIAB 53080. Paratypes: SAIAB 53079 (15), SAIAB 53083 (21). Current status: valid as *Labeobarbus dimidiatus* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: River Ruo and several of its tributaries above Zoa Falls (Tweddle & Skelton, 1998).

ensifer, *Varicorhinus* Boulenger, 1910. Lucala (near railway station) (±9°16′23′S, 15°14′42′E, Google Earth), above the falls on the Lucala River, Angola. Syntypes: ANSP 37994 (8), BMNH 1910.11.28.134–143 (10), 1910.11.28.144 (1), NMW 48864 (10), ZMB 18213 (8). Current status: valid as *Labeobarbus ensifer* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Lucalla River system (Lévêque & Daget, 1984). Notes: the original record BMNH 1910.11.28.134–44 at present contains only ten (not the expected 11) specimens, and was amended to BMNH 1910.11.28.134–43; there is a note saying a specimen was removed and mounted in the Fish Gallery in November 1931, which would be BMNH 1910.11.28.144, but the specimen has currently not yet been retrieved (J. Maclaine, pers. comm., 2015).

ensis, *Barbus* Boulenger, 1910. Lucala (near railway station) (±9°16′23′S, 15°14′42′E, Google Earth), above the falls on the Lucala River, Angola. Syntypes: ANSP 37902 (2), BMNH 1911.6.1.11–18 (8), NMW 54083 (4), ZMB 18217 (4). USNM 28297, 28373, 29223, 29536 and 29611 are lost (see Eschmeyer, 2015). Current status: valid as *Labeobarbus ensis* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Luculla River, Rio Cunje system, Angola (Lévêque & Daget, 1984). Notes: (1) the total number of type specimens adds up to 17, excluding the USNM specimens that are lost (see Eschmeyer, 2015), whereas the original publication only mentions 15 syntypes; (2) Lévêque & Daget (1984) only give the BMNH and ANSP specimens as types (10); (3) the number of specimens in ZMB 18217 is verified as four, in contrast to the ZMB collection data, but in accordance with Eschmeyer (2015).

ethiopicus, *Barbus* Zolezzi, 1939. Lake Zwai (±8°00′N, 38°48′E, USBGN, 1963), Ethiopia. Holotype: MCZR (number unknown). Current status: valid as *Labeobarbus ethiopicus* (placed in *Labeobarbus* in Getahun, 2007a). Synonyms: none. Distribution: Rift Lakes drainage basin (Getahun, 2007a), including Lake Tana (Tsigenopoulos *et al*., 2002; Borkenhagen, 2014), Lake Zwai (type locality) (Lévêque & Daget, 1984), and its affluent Meki River (Golubtsov & Krysanov, 1993; Levin *et al*., 2013). Notes: (1) BMNH 1971.7.12.1–3 (3), given syntype status in Lévêque & Daget (1984) and Eschmeyer (2015), do not have type status; (2) holotype,

previously housed at the Laboratorio centrale di Idrobiologia (Rome, Italy), and now at the Museo Civico di Zoologia di Roma (MCZR, Rome, Italy) (M. Capula, pers. comm., 2014), not seen; (3) the MCZR has recently (2014) been ravaged by a flood (S. Valdesalici, pers. comm., 2014). Unfortunately, no further details have been provided as to the whereabouts of the holotype after this misfortune.

fasolt, *Barbus* Pappenheim, 1914. Irumu (±1°29′N, 29°51′E, D.F.E. Thys van den Audenaerde, unpubl. data), Ituri River, DRC. Holotype: ZMB 19061. Current status: valid as *Labeobarbus fasolt* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Rivers Aruwimi (Boulenger, 1920a), Ituri (Pappenheim & Boulenger, 1914; Boulenger, 1916a) and Uele (Nichols & Griscom, 1917), DRC. Possibly also at Kisangani (= Stanleyville) (Nichols & Griscom, 1917).

fimbriatus, *Varicorhinus sandersi* Holly, 1926. Nachtigal (±4°21′N, 11°38′E, USBGN, 1962b) (rapids), Sanaga River, Cameroon. Syntypes: NMW 7224–7226 (3), 7227–7233 (7). Current status: valid as *Labeobarbus fimbriatus* (raised to species level in Holly, 1930; placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: a Lower Guinea endemic, found in the Sanaga River Basin, Cameroon (Lévêque & Daget, 1984; Getahun, 2007b). Notes: syntypes NMW 7224–7226 (3) not listed in Eschmeyer (2015), and incorrectly included in NMW 7224–7233 in Lévêque & Daget (1984).

fritschii, *Barbus* Günther, 1874. Oued Ksib [Oued el Ksib] (31°28′59″N, 9°46′3″W, Borkenhagen & Krupp, 2013), Morocco. Syntypes: BMNH 1874.1.30.27–31 (5). Current status: valid as *Labeobarbus* fritschii (placed in *Labeobarbus* in Doadrio, 1994). Synonyms: *Capoeta atlantica* Boulenger, 1902 (synonymy in Lévêque & Daget, 1984); *Barbus paytonii* Boulenger, 1911 (synonymy in Lévêque & Daget, 1984; revalidated by Azeroual *et al*., 2000; again placed in synonymy in Borkenhagen & Krupp, 2013); *Barbus riggenbachi* Günther, 1902; *Barbus rothschildi* Günther, 1901; *Capoeta waldoi* Boulenger, 1902 (synonymy in Lévêque & Daget, 1984). Distribution: widespread and abundant in northern and central Morocco; occurs in the Oued al Maleh, Oued Bou Regreg, Oued Igrounzar, Oued Moulouya, Oued Oum er Rbia, Oued Sebou, and Oued Tennsift drainage systems, and in numerous small coastal rivers; most records are from Morocco, but one specimen is from the Oued Kiss in Algeria (Borkenhagen & Krupp, 2013). Notes: (1) Eschmeyer (2015) mentions that SMF 636 (7) and 952 (8) are not syntypes, in contrast to the SMF online collection database. Borkenhagen & Krupp (2013), who consider this a *Carasobarbus* species, also do not include the SMF specimens in the type series. Unfortunately, the number of type specimens is not mentioned in the original description; (2) a *Carasobarbus* species in Borkenhagen & Krupp (2013) and Yang *et al*. (2015); (3) type species of the genus *Pseudotor* Karaman, 1971. To be considered a junior synonym of the genus *Labeobarbus s.l.*; (4) *Labeobarbus paytonii* (Boulenger, 1911) considered to be a junior synonym of *Labeobarbus fritschii harterti* (Günther, 1901) by Lévêque & Daget (1984), valid by Doadrio (1994), but a junior synonym of *L. fritschii* by Borkenhagen & Krupp (2013).

gananensis, *Barbus* Vinciguerra, 1895. Ganana River (± 0°15′S, 42°38′E, D.F.E. Thys van den Audenaerde, unpubl. data), between Lugh and Bardera [Somalia]. Holotype: MSNG 17525. Paratypes: MSNG 17331 (4). Current status: valid as *Labeobarbus gananensis* (placed in *Labeobarbus* in Getahun, 2007a). Synonyms: none. Distribution: Juba and Awata rivers in Somalia (Lévêque & Daget, 1984). Reported from the Wabi Shebele Basin (Getahun, 2007a), including the Genale River, on the Eastern Plateau in Ethiopia (Levin *et al*., 2013). Notes: (1) Tortonese (1961), in his type catalogue, mentions 'Olotipo deteriorato' (= holotype detoriated); (2) the species description is based on five type specimens, which means four paratypes exist next to the holotype, not five as mentioned in Eschmeyer (2015).

gestetneri, *Barbus* Banister & Bailey, 1979. Above the falls on the Kalumengonga River (8°49′S, 27°13′E) (site 5), Upemba National Park, Shaba, DRC. Holotype: BMNH 1976.10.12.98. Paratypes: BMNH 1976.10.12.86–97 (12). Current status: valid as *Labeobarbus gestetneri* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: Only known from the type locality (Lévêque & Daget, 1984).

girardi, *Barbus* Boulenger, 1910. Lucala (near railway station) (±9°16′23″S, 15°14′42″E, Google Earth), above the falls on the Lucala River, Angola. Syntypes: ANSP 37973 (1), BMNH 1911.6.1.31–36 (6), NMW 54137 (1), ZMB 18215 (1). Current status: valid as *Labeobarbus girardi* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: Lucalla River, Angola (Lévêque & Daget, 1984). Notes: the total number of type specimens only adds up to nine, whereas the original description mentions 11 syntypes.

gorgorensis, *Barbus intermedius* Bini, 1940. Debre Mariam (11°38′N, 37°24′E, Nagelkerke & Sibbing, 1997) (locality 1), Lake Tana, Ethiopia. Neotype: RMNH 32910 (designated by Nagelkerke & Sibbing, 1997). Current status: valid as *Labeobarbus gorgorensis* (placed in *Labeobarbus* in Getahun, 2007a). Synonym of *Barbus intermedius* in Banister (1973); raised to species level and revalidated as *Barbus gorgorensis* by Nagelkerke & Sibbing (1997). Synonyms: none. Distribution: Endemic to Lake Tana, Ethiopia (Nagelkerke & Sibbing, 1997).

gorguari, *Barbus* Rüppell, 1835. Lake Tana (±12°00′N, 37°20′E, USBGN, 1963), possibly at Goraza [probably = Korata] (11°45′N, 37°27′E, Nagelkerke & Sibbing,

1997), Ethiopia. Holotype: SMF 2586 (stuffed). Current status: valid as *Labeobarbus gorguari* (placed in *Labeobarbus* in Getahun, 2007a). Synonym of *Barbus intermedius* in Banister (1973); revalidated by Nagelkerke & Sibbing (1997). Synonyms: none. Distribution: Endemic to Lake Tana, Ethiopia (Nagelkerke & Sibbing, 1997).

gruveli, *Barbus* Pellegrin, 1911. Dubreka (±9°48′N, 13°31′W, D.F.E. Thys van den Audenaerde, unpubl. data; USBGN, 1965b), French Guinea [Guinea]. Holotype: MNHN 1911-0040. Current status: valid as *Labeobarbus gruveli* (placed in *Labeobarbus* in Skelton & Bills, 2008; Berrebi *et al*., 2014). Synonyms: none. Distribution: only known from the holotype (Lévêque & Guégan, 1990; Lévêque, 2003).

gulielmi, *Barbus* Boulenger, 1910. Dondo (±9°38′S, 14°25′E, D.F.E. Thys van den Audenaerde, unpubl. data), Quanza River, Angola. Syntypes: BMNH 1911.6.1.29–30 (2), NMW 54138 (1). Current status: valid as *Labeobarbus gulielmi* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: only known from the type locality (Lévêque & Daget, 1984).

habereri, *Barbus* Steindachner, 1912. Dscha [Dja] River (±2°2′N, 15°12′E, USBGN, 1962b), southern Cameroon. Holotype: NMW 7274. Current status: valid as *Labeobarbus habereri* (placed in *Labeobarbus* in De Weirdt & Teugels, 2007). Synonyms: none. Distribution: Sanaga Basin, Kelle River (Nyong River tributary) and Dja River (middle Congo River Basin) in Cameroon (De Weirdt & Teugels, 2007). Notes: (1) placed in '*Labeobarbus*' in Yang *et al*. (2015); (2) according to Boulenger (1916: 231, 239), Steindachner (1914: plate 3, figs 1, 3) inverted the illustrations of *L. habereri* and *L. mawambiensis*. Indeed, Figure 1 on plate 3 illustrates a fish with a damaged caudal and anal fin, as stipulated in the original description of *L. habereri* by Steindachner (1914: 24) himself. As such, Figure 1 illustrates the holotype of *L. habereri*, whereas Figure 3 instead illustrates a specimen of *L. mawambiensis*. Note, however, that for the latter species the illustrated specimen has 5.5 scales between the lateral line and the dorsal midline, whereas all examined syntypes have 3.5 or 4.5 scales.

harterti, *Barbus* Günther, 1901. Oum Erbiah [Oum er Rbia River] (33°19′40″N, 8°20′2″W, Borkenhagen & Krupp, 2013), Morocco. Syntypes BMNH 1901.7.26.4–5 (2). Current status: valid as *Labeobarbus harterti* (placed in *Labeobarbus* in Doadrio, 1994). Subspecies of *Barbus fritschii* in Lévêque & Daget (1984); revalidated by Doadrio (1994). Synonyms: none. Distribution: rivers of the Oued Oum er Rbia and Tennsift drainage systems in Morocco (Borkenhagen & Krupp, 2013). Notes: (1) a *Carasobarbus* species according to Borkenhagen & Krupp (2013) and Yang *et al*. (2015); (2) misspelled as '*harteti*' in Lévêque & Daget (1984) (as subspecies of *Barbus fritschii*) and Doadrio (1994).

huloti, *Barbus* Banister, 1976. Zega on the Vuda River (1°44′N, 30°45′E), Lake Albert [= Lake Albert Nyanza, = Lake Mobuto Sese Seko] basin, DRC. Holotype: IRSNB 558. Paratypes: BMNH 1975.4.30.1 (1), IRSNB 563 (10). Current status: valid as *Labeobarbus huloti* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: only known from the type locality (Lévêque & Daget, 1984).

humphri, *Barbus* Banister, 1976. Tabie River, about 25 kilometers south of Beni (±0°30′N, 29°28′E, USBGN, 1964b), North Kivu District, DRC. Holotype: IRSNB 559. Paratypes: BMNH 1975.4.30.2 (1), IRSNB 564 (10). Current status: valid as *Labeobarbus humphri* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: only known from the type locality (Lévêque & Daget, 1984).

intermedius, *Barbus* Rüppell, 1835. Lake Tana (± 12°00′N, 37°20′E, USBGN, 1963), possibly at Goraza [probably = Korata] (11°45′N, 37°27′E, Nagelkerke & Sibbing, 1997), Ethiopia. Holotype: SMF 6778 (stuffed). Current status: valid as *Labeobarbus intermedius* (placed in *Labeobarbus* in Getahun, 2007a). Synonyms: *Barbus affinis* Rüppell, 1835; *Barbus alticola* Boulenger, 1906; *Capoeta bingeri* Pellegrin, 1905; *Barbus bottegi* Boulenger, 1906; *Barbus brevibarbis* Boulenger, 1902; *Barbus brunellii* Bini, 1940; *Barbus duchesnii* Boulenger, 1902; *Barbus duchesnii ibridus* Bini, 1940; *Barbus duchesnii maximus* Bini, 1940; *Barbus elongatus* Rüppell, 1835; *Barbus erlangeri* Boulenger, 1904; *Barbus eumystus* Boulenger, 1906; *Barbus gregorii* Boulenger, 1902; *Barbus gudaricus* Boulenger, 1906; *Barbus harringtoni* Boulenger, 1902; *Barbus hursensis* Boulenger, 1902; *Barbus ilgi* Pellegrin, 1905; *Barbus intermedius microstoma* Bini, 1940; *Barbus jarsinus* Boulenger, 1902; *Barbus kassamensis* Boulenger, 1902 (synonymy in Banister, 1973); *Barbus leptosoma* Boulenger, 1902 (synonymy in Bini, 1940); *Barbus macmillani* Boulenger, 1906; *Barbus macronema* Boulenger, 1902; *Barbus macronema parenzani* Zolezzi, 1939; *Barbus margaritae* Boulenger, 1906; *Barbus mento* Boulenger, 1902; *Barbus neuvillei* Pellegrin, 1905; *Barbus oreas* Boulenger, 1902; *Barbus plagiostomus* Boulenger, 1902; *Barbus platystomus* Boulenger, 1902; *Barbus platystomus daga* Bini, 1940; *Barbus platystomus dekkensis* Bini 1940; *Barbus platystomus prognathus* Bini, 1940; *Barbus platystomus vatovae* Zolezzi, 1939; *Barbus procatopus* Boulenger, 1916; *Barbus rueppelli* Boulenger, 1902; *Barbus volpinii* Parenzan, 1940; *Barbus zaphiri* Boulenger, 1906; *Barbus zuaicus* Boulenger, 1906 (synonymy in Banister, 1973). Distribution: *Labeobarbus intermedius intermedius* Rüppell, 1835, widely distributed throughout southern Ethiopia and into northern Kenya, certainly as far as Lake Baringo, but excluding the lake itself; *Labeobarbus intermedius*

australis (Banister, 1973), only known from Lake Baringo (Lévêque & Daget, 1984). Notes: (1) Getahun (2007a) reports *Labeobarbus neuville* (Pellegrin, 1905) [a misspelling for *L. neuvillei* (Pellegrin, 1905)] as a valid species. As Getahun (2007a) did not provide any arguments for this revalidation *L. neuvillei* is here still considered a junior synonym of *L. intermedius* following Banister (1973) and awaiting further research; (2) the same holds true for *L. zaphiri* (Boulenger, 1906), which has been used as a valid species name by Tsigenopoulos *et al*. (2010) and Yang *et al*. (2015), also without any argumentation for this revalidation since Banister's (1973) synonymization.

iphthimostoma, *Varicorhinus* Banister & Poll, 1973. Kateke (±08°56′S, 26°42′E, collection database), alluent of the Muov'we, Lufira system, DRC, 960 m a.s.l. Holotype: MRAC 179736. Paratypes: BMNH 1972.10.2.2 (1), IRSNB 644 (1), MRAC 179737 (1). Current status: valid as *Labeobarbus iphthimostoma* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Muye and Kateke, Lufira system (Banister & Poll, 1973; Poll, 1976). Notes: following ICZN (1999: articles 31.2 and 34.2), the species name *iphthimostoma* [from the Greek adjective *iphthimos* (solid, robust) and the Greek noun *stoma* (mouth); meaning with a solid and robust mouth] does not need to agree in gender with the genus name *Labeobarbus* (masculine).

iturii, *Barbus* Holly, 1929. Ituri River (±1°40′N, 27°01′E, USBGN, 1964b), DRC. Holotype: NMW (lost). Current status: valid as *Labeobarbus iturii* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: only known from the type locality (Lévêque & Daget, 1984). Notes: during a recent search in the NMW collection, the holotype could not be found (H. Wellendorf, pers. comm., 2014) and is thus considered lost.

jaegeri, *Varicorhinus* Holly, 1930. Sanaga (±03°35′N, 9°38′E, USBGN, 1962b), Cameroon. Holotype: NMW 13957. Current status: valid as *Labeobarbus jaegeri* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Lower Guinea endemic, known from the Sanaga River Basin, Cameroon (Getahun, 2007b).

johnstonii, *Barbus* Boulenger, 1907. Between Kondowe [Livingstonia] (±10°36′S, 34°07′E, D.F.E. Thys van den Audenaerde, unpubl. data) and Karonga (± 9°56′S, 33°56′E, D.F.E. Thys van den Audenaerde, unpubl. data), Lake Malawi [Malawi]. Holotype: BMNH 1897.6.9.280. Current status: valid as *Labeobarbus johnstonii* (placed in *Labeobarbus* in Skelton & Bills, 2008; Berrebi *et al*., 2014). Synonyms: *Barbus intermedius brevicauda* Keilhack, 1908 (synonymy in Boulenger, 1911a; revalidated in Seegers, 1995; again placed in synonymy in the present paper); *Barbus intermedius eurystomus* Keilhack, 1908 (synonymy in Seegers, 1995); *Barbus globiceps* Worthington, 1933; *Barbus njassae* Keilhack, 1908; *Varicorhinus nyasensis* Worthington, 1933 (synonymy in Banister & Clarke, 1980). Distribution: Lake Malawi Basin (Lévêque & Daget, 1984). Notes: *Labeobarbus eurystomus* is here considered a junior synonym of *L. johnstonii* (Boulenger, 1907), following Seegers (1995). According to Seegers (1995), the lectotype designation for *Barbus* (now *Labeobarbus*) *intermedius eurystomus* Keilhack, 1908, as undertaken by Banister & Clarke (1980) (ZMB 18175, 125 mm SL), is invalid as it is preceded by a lectotype designation by Keilhack (1910: 103, table 6, specimen no. 15 and plate. 2, fig. 6) himself, who even illustrated what he referred to as the 'typus', making this specimen (ZMB 18175, 255 mm SL from 'Njassa bei Langenbuarg, 77 m Tiefe, 31.8.99') indeed the lectotype of this, now, nominal species (see ICZN, 1999, especially articles 74.4 and 74.5), referred to as *L. eurystomus*. According to Seegers (1995) this specimen corresponds with one of the other three specimens originally belonging to ZMB 18175, and attributed by Banister & Clarke (1980) to *Barbus* (now *Labeobarbus*) *johnstonii* Boulenger, 1907. Given the standard lengths provided by Banister & Clarke (1980) (152, 183, and 245 mm SL, respectively) it seems to refer to the largest of these syntypes. As a result, *L. eurystomus* becomes a junior synonym of *L. johnstonii* and *Barbus* (now *Labeobarbus*) *intermedius brevicauda*, previously a junior synonym of *L. eurystomus sensu* Banister & Clarke (1980), became a valid species, *L. brevicauda*, following Seegers (1995); however, the correct name for *L. eurystomus sensu* Banister & Clarke (1980) and *L. brevicauda sensu* Seegers (1995) is *L. latirostris* (see text).

jubae, *Varicorhinus* Banister, 1984. Juba River, close to Sidam-Bale bridge (5°45′N, 39°37′E), Ethiopia, 1200 m a.s.l. Holotype: BMNH 1976.7.1.13. Paratypes: BMNH 1976.7.1.14–15 (2). Current status: valid as *Labeobarbus jubae* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: known from the Welmel, Genale, and Awata rivers (Juba River system) in Ethiopia (Levin *et al*., 2013).

jubbi, *Barbus* Poll, 1967. Muíta (±7°50′S, 21°22′E, D.F.E. Thys van den Audenaerde, unpubl. data; USBGN, 1956), Luembe River, Angola. Holotype: MD 1078. Paratypes: MRAC 161065 (ex. MD 2299) (1), 161066 (ex. MD 6363) (1), MD 2299 (1), 6363 (1). Current status: valid as *Labeobarbus jubbi* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: various tributaries of the upper Kasai drainage, including the Luachimo and Luembe (middle Congo River Basin) (Poll, 1967; Lévêque & Daget, 1984).

kerstenii, *Barbus* Peters, 1868. Not a *Labeobarbus* species (see present paper).

kimberleyensis, *Barbus* Gilchrist & Thompson, 1913. Kimberley Reservoir (±28°45′S, 24°46′E, D.F.E. Thys van den Audenaerde, unpubl. data), Cape Province, South Africa. Holotype: SAIAB 134771 (ex. SAM 9645).

Current status: valid as *Labeobarbus kimberleyensis* (placed in *Labeobarbus* in Skelton, 2001). Synonyms: *Barbus pienaarii* Fitzsimons, 1949 (synonymy in Jubb, 1963). Distribution: Orange-Vaal River system, South Africa (Skelton, 2001).

lagensis, *Barynotus* Günther, 1868. Lagos (±6°28′N, 3°25′E, D.F.E. Thys van den Audenaerde, unpubl. data), west Africa [Nigeria]. Holotype: BMNH 1866.3.8.12. Current status: valid as *Labeobarbus lagensis* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: only known from the holotype (Lévêque & Guégan, 1990; Lévêque, 2003). Notes: (1) type species of the genus *Barynotus* Günther, 1868. Objectively invalid as preoccupied by *Barynotus* Germar, 1817 (Coleoptera). Replaced by objective junior synonym *Barbellion* Whitley, 1931, which must be considered a junior synonym of the genus *Labeobarbus*; (2) species name misspelled as *lagoensis* by various authors (e.g. Boulenger, 1905b, 1911a; Pellegrin, 1923; Trewavas & Irvine, 1947; Lowe-McConnell, 1972; Lévêque & Daget, 1984; Skelton & Bills, 2008).

latirostris, *Barbus intermedius* Keilhack, 1908. Probably from Lake Malawi or the Kiwira River (±9°37′S, 33°57′E, USBGN, 1965c), possibly the Kiwira River at Langenburg [= Neu Langenburg, = Tukuyu] (±9°15′S, 33°39′E, USBGN, 1965c), Tanzania. Lectotype: ZMB 18174 (designated in present paper). Paralectotype: ZMB 34766 (1). Current status: valid as *Labeobarbus latirostris* [placed in *Labeobarbus* in Skelton & Bills (2008) (using the senior synonym name at that time *Labeobarbus johnstonii*; *L. latirostris* is at present a valid species, cf. infra); present paper]. Synonym of *Barbus johnstonii* in Banister & Clarke (1980); raised to species level and revalidated as *Labeobarbus latirostris* in present paper. Synonyms: none. Distribution: Lake Malawi Basin (Lévêque & Daget, 1984).

latirostris, *Varicorhinus* Boulenger, 1910. Preoccupied by *Barbus latirostris* Keilhack, 1908; replaced by *Labeobarbus boulengeri* (present paper).

leleupanus, *Varicorhinus* Matthes, 1959. Nyamagana River (±02°55′S, 29°08′E, collection database), Burundi. Holotype: MRAC 92213. Paratypes: MRAC 92211 (1), 92212 (1), 130532–33 (2). Current status: valid as *Labeobarbus leleupanus* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Rusizi River (De Vos *et al*., 2001) and Lake Tanganyika Basin (Matthes, 1962; Eccles, 1992). Notes: although the original species description by Matthes (1959b) does not mention the number of type specimens or any collection numbers, Matthes (1962) gives an additional paratype (71.2 mm SL, Luberizi River, collected by G. Marlier, date 13.4.55) without registration number, next to the ones listed above; a corresponding specimen could not be identified in the MRAC collection.

litamba, *Barbus* Keilhack, 1908. Not a *Labeobarbus* species (see present paper).

longidorsalis, *Varicorhinus* Pellegrin, 1935. Near Lukando (±2°05′S, 28°30′E, D.F.E. Thys van den Audenaerde, unpubl. data), Kanséhété River, Luhoho River affluent, DRC. Holotype: MNHN 1935-0065. Current status: valid as *Labeobarbus longidorsalis* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: tributaries of the Congo River in the Kivu region (Lévêque & Daget, 1984).

longifilis, *Barbus altianalis* Pellegrin, 1935. Loama (±2°01′S, 28°27′E, D.F.E. Thys van den Audenaerde, unpubl. data), upper Luhoho Basin, DRC. Lectotype: MNHN 1935-0150 (designated by Banister, 1973). Paralectotypes: MNHN 1935-0145 (1), 1935-0146 (1), 1935-0147 (1), 1935-0148 (1), 1935-0149 (1), 1935-0151 (1), 1935-0152 (1). Current status: valid as *Labeobarbus longifilis* (raised to species level in Banister, 1973; placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: Nya-Barongo and Loama rivers, in Kivu region (Lévêque & Daget, 1984). Notes: Nyabarongo specimen in Pellegrin's type series (MNHN 1935-0075) put under *Barbus* (now *Labeobarbus*) *paucisquamatus* by Banister (1973).

longissimus, *Barbus* Nagelkerke & Sibbing, 1997. Kentefami (11°40′N, 37°23′E, Nagelkerke & Sibbing, 1997) (locality 11), Lake Tana, Ethiopia. Holotype: RMNH 32930. Paratypes: RMNH 32931–39 (9). Current status: valid as *Labeobarbus longissimus* (placed in *Labeobarbus* in Getahun, 2007a). Synonyms: none. Distribution: endemic to Lake Tana, Ethiopia (Nagelkerke & Sibbing, 1997).

lucius, *Barbus* Boulenger, 1910. Lucala (near railway station) (±9°16′23′S, 15°14′42′E, Google Earth), above the falls on the Lucala River, Angola. Syntypes: BMNH 1911.6.1.42–43 (2), NMW 54246 (1). Current status: valid as *Labeobarbus lucius* (placed in *Labeobarbus* in De Weirdt & Teugels, 2007). Synonyms: none. Distribution: Lucalla River in Angola and Niari River in Republic of the Congo (De Weirdt & Teugels, 2007).

lufupensis, *Varicorhinus* Banister & Bailey, 1979. Nasondoye (10°22′S, 25°06′E) (site 2), Lufupa River, Shaba, DRC. Holotype: BMNH 1975.9.5.1. Paratype: BMNH 1975.9.5.2 (1). Current status: valid as *Labeobarbus lufupensis* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Only known from the type locality (Banister, 1984).

macroceps, *Barbus* Fowler, 1936. Epulu River Ferry (±1°15′N, 28°21′E, USBGN, 1964b), Ituri Basin, Kibali-Ituri District, DRC. Holotype: ANSP 65759. Current status: valid as *Labeobarbus macroceps* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: based on current evidence, a local endemic from above the falls on the Epulu River (A. Walanga, pers. comm., 2015).

macrolepidotus, *Varicorhinus* Pellegrin, 1928. Luluabourg [Kananga] (±05°53′S, 22°25′E, collection database) area, Lulua River, Kasai affluent, DRC. Syntypes: MNHN 1928-0011 (1), MRAC 19945 (1), 138767 (1; ex. MNHN 1928-0012), NMB 3983 (1), 3985 (1), 3988 (1), 3989 (1). Current status: valid as *Labeobarbus macrolepidotus* (placed in *Labeobarbus* in present paper). Synonyms: *Barbus callewaerti* Nichols & La Monte, 1933 (synonymy in Poll, 1967). Distribution: Lower Congo River in DRC and the Kasai drainage (middle Congo River Basin) in Angola (Poll, 1967) and DRC (Pellegrin & Roux, 1928). Notes: the total number of type specimens adds up to seven, whereas the original description mentions only five syntypes.

macrolepis, *Barbus* Pfeffer, 1889. Mbusine [Mbussini] (±6°12′S, 38°01′E, D.F.E. Thys van den Audenaerde, unpubl. data), Rukagura Stream, East Africa [Tanzania]. Lectotype: ZMH H330 (ex. 68 79) (designated by Ladiges, von Wahlert & Mohr, 1958). Paralectotypes: BMNH 1909.2.25.8 (ex. ZMH) (1), ZMB 31672 (ex. ZMH) (2), ZMH H331 (ex. 68 79) (2), H332 (ex. 380/7467) (4), H333 (ex. 69 19) (1), H474 (ex. 69 03) (1). Current status: valid as *Labeobarbus macrolepis* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: Katare, Malagarasi swamp, and Wami, Ruaha, and Rufiji rivers (Lévêque & Daget, 1984) [Tanzania]. Notes: (1) lectotype incorrectly referred to as ZMH H331 in Banister (1973: appendix 3), whereas the text itself correctly gives ZMH H330; (2) *Labeobarbus macrolepis* Heckel, 1838 is currently a junior synonym of *Tor putitora* (Hamilton, 1822) (*fide* Kottelat, 2013). A replacement name for *L. macrolepis* (Pfeffer, 1889) is not needed, following the rules of ICZN (1999: article 59.2; see also text).

macrophtalmus, *Barbus gorguarii* Bini, 1940. Bet Menzo (11°45′N, 37°25′E, Nagelkerke & Sibbing, 1997) (locality 4), Lake Tana, Ethiopia. Neotype: RMNH 32940 (designated by Nagelkerke & Sibbing, 1997). Current status: valid as *Labeobarbus macrophtalmus* (placed in *Labeobarbus* by Getahun, 2007a). Synonym of *Barbus intermedius* in Banister (1973); raised to species level and revalidated as *Barbus macrophtalmus* by Nagelkerke & Sibbing (1997). Synonyms: none. Distribution: endemic to Lake Tana, Ethiopia (Nagelkerke & Sibbing, 1997). Notes: although a junior homonym of *Barbus macrophthalmus* Bleeker, 1855 from Java and Indonesia [currently a synonym of *Barbonymus balleroides* (Valenciennes, 1842)], because of its transfer to *Labeobarbus* this species is no longer congeneric and thus does not need replacement (see ICZN, 1999: article 59.2).

malacanthus, *Barbus* Pappenheim, 1911. Uelleburg, Uelle River [= Benito River] (±1°36′N, 9°37′E, USBGN, 1962), Equatorial Guinea. Holotype: ZMB 18392. Current status: valid as *Labeobarbus malacanthus* (placed in *Labeobarbus* in De Weirdt & Teugels, 2007). Synonyms: none. Distribution: equatorial Guinea and Gabon, including the Ogooué Basin and the rivers Nyanga and Douigni (De Weirdt & Teugels, 2007).

marequensis, *Barbus* (*Cheilobarbus*) Smith, 1841. Marico River (±24°12′S, 26°53′E, USBGN, 1954a), near the border of Bechuanaland and the Transvaal, South Africa (Greenwood & Crass, 1959). Holotype: BMNH 1845.7.3.95 (stuffed). Current status: valid as *Labeobarbus marequensis* (placed in *Labeobarbus* in Skelton, 2001). Synonyms: *Barbus brucii* Boulenger, 1907; *Varicorhinus brucii* Boulenger, 1907; *Barbus cookei* Gilchrist & Thompson, 1913; *Barbus dwaarsensis* Gilchrist & Thompson, 1913 (synonymy in Jubb, 1963); *Barbus fairbairnii* Boulenger, 1908; *Barbus gunningi* Gilchrist & Thompson, 1913 (synonymy in Jubb, 1963); *Barbus inermis* Peters, 1852 (synonymy in Lévêque & Daget, 1984); *Varicorhinus nasutus* Gilchrist & Thompson, 1911 (synonymy in Poll, 1976; revalidated in Skelton, 1993; again placed in synonymy in Tweddle & Skelton, 1998); *Barbus oliphanti* Keilhack, 1910; *Barbus rhodesianus* Boulenger, 1902; *Barbus sabiensis* Gilchrist & Thompson, 1913; *Barbus sector* Boulenger, 1907; *Barbus swierstrae* Gilchrist & Thompson, 1913; *Barbus victoriae* Boulenger, 1908; *Labeobarbus zambezensis* Peters, 1852 (synonymy in Jubb, 1963). Distribution: widely distributed from the middle and lower Zambezi south to the Pongolo system (Skelton, 2001). Notes: (1) the junior synonym *Barbus oliphanti* Keilhack, 1910 is a replacement name for *Varicorhinus brucii* Boulenger, 1907, preoccupied by *Barbus brucii* Boulenger, 1907, when Keilhack (1908) placed *Varicorhinus* in synonymy with *Barbus*; (2) the replacement name and junior synonym *Barbus oliphanti* Keilhack, 1910 (see also previous note) is not included in Lévêque & Daget (1984); (3) for more details on the status of BMNH 1845.7.3.95 as the holotype of the species, see Greenwood & Crass (1959).

mariae, *Barbus* Holly, 1929. Preoccupied by *Varicorhinus* (now *Labeobarbus*) *mariae* Holly, 1926; replaced by *Labeobarbus rhinoceros* (present paper).

mariae, *Varicorhinus* Holly, 1926. Nachtigal (±4°21′N, 11°38′E, USBGN, 1962b) (rapids), Sanaga River, Cameroon. Syntypes: NMW-7221 (1), 7222–7223 (2). Current status: valid as *Labeobarbus mariae* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: endemic to Lower Guinea, where it is found in the Sanaga and Wouri river basins (Getahun, 2007b).

maroccana, *Pterocapoëta* Günther, 1902. Oum Erbiah [Oum er Rbia River] (±33°19′N, 8°20′W, USBGN, 1970) and Talmist River, Morocco. Syntypes: BMNH 1902.7.28.37–38 (2), 1902.7.28.39 (1). Current status: valid as *Labeobarbus maroccanus* (placed in *Labeobarbus* in Berrebi *et al*., 2014). Synonyms: none. Distribution: Morocco (Lévêque & Daget, 1984), including Oued Srou and Oued Oum er Rbia (Borkenhagen, 2014). Notes: (1) type species of the genus

Pterocapoëta Gunther, 1902, previously a junior synonym of *Varicorhinus* (see Boulenger, 1905b, 1909; Karaman, 1971; Lévêque & Daget, 1984) and now of *Labeobarbus s.l.*; (2) *Pterocapoëta* correctly spelled as *Pterocapoeta* (see ICZN, 1999: article 32.5.2), as all original names published with an apostrophe should be corrected; (3) to be in agreement with ICZN (1999: articles 31.2 and 34.2), the species name *maroccana* (adjective; meaning from Morocco) must become *maroccanus* to agree in gender with the genus name *Labeobarbus* (masculine); (4) valid as *Pterocapoeta maroccana* (monospecific genus) in Borkenhagen (2014), Geiger *et al*. (2014), and Yang *et al*. (2015).

matris, *Barbus* Holly, 1928. Near Nairobi (±1°17′S, 36°49′E, USBGN, 1964a), Athi River, Kenya. Holotype: NMW 8000. Current status: valid as *Labeobarbus matris* (placed in *Labeobarbus* in present paper). Synonym of *Barbus mariae* in Lévêque & Daget (1984); revalidated in the present paper. Synonyms: none. Distribution: Only known from the type locality (Lévêque & Daget, 1984). Notes: Despite the very similar original descriptions of both *L. matris* (Holly, 1928) and *L. mariae* (Holly, 1929), Banister (1973) refrained to synonymize both without having seen the type specimens. Nevertheless, several authors (Lévêque & Daget, 1984; Seegers *et al*., 2003) considered *L. matris* a senior synonym of *L. mariae* (now *L. rhinoceros*), indeed with hesitation, but without further justification for the synonymy, and not respecting the proper priority of names. Previously considered lost, types of both species were found back in the NMW and both species are clearly diagnosable from each other (see text), rejecting any claims regarding a possible synonymization of both.

mawambi, *Barbus* Pappenheim, 1914. Mawambi (±1°03′N, 28°36′E, D.F.E. Thys van den Audenaerde, unpubl. data), Ituri [DRC]. Holotype: ZMB 19062. Current status: valid as *Labeobarbus mawambi* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: Ituri River, DRC (Lévêque & Daget, 1984). Notes: possibly the same species as *B. mirabilis* (Banister, 1973).

mawambiensis, *Barbus hindii* Steindachner, 1911. Mawambi (±1°03′N, 28°36′E, D.F.E. Thys van den Audenaerde, unpubl. data), Ituri River [DRC]. Syntypes: NMW 54177 (2), 54286 (3), 54287 (2), 54288 (2). Current status: valid as *Labeobarbus mawambiensis* (raised to species level in Steindachner, 1912; placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: rivers Ituri and Dja (middle Congo River Basin) in DRC and Cameroon, respectively (Steindachner, 1914; Boulenger, 1916a; Poll, 1967), although some doubt is cast on the identity of the specimens from the Dja by Trewavas (1974). Notes: (1) originally described as a subspecies of *Barbus* (now *Labeobarbus*) *hindii*, currently a junior synonym of *L. oxyrhynchus* following Banister (1973); (2) the total number of type specimens adds up to nine, whereas the original publication only mentions seven syntypes; (3) according to Boulenger (1916: 231, 239), Steindachner (1914: plate 3, figs 1, 3) inverted the illustrations of *L. habereri* and *L. mawambiensis*. Indeed, figure 1 on plate 3 illustrates a fish with a damaged caudal and anal fin, as stipulated in the original description of *L. habereri* by Steindachner (1914: 24) himself. As such, figure 1 illustrates the holotype of *L. habereri*, whereas figure 3 instead illustrates a specimen of *L. mawambiensis*. Note, however, that for the latter species the illustrated specimen has 5.5 scales between the lateral line and the dorsal midline, whereas all examined syntypes have 3.5 or 4.5 scales.

mbami, *Barbus perplexicans* Holly, 1927. Mbami [Mayo] River (±6°49′N, 12°00′E, USBGN, 1962b), Cameroon. Holotype: NMW 7528. Current status: valid as *Labeobarbus mbami* (raised to species level in Holly, 1930; placed in *Labeobarbus* in De Weirdt & Teugels, 2007). Synonyms: none. Distribution: Lower Guinea endemic, only known from the Sanaga River Basin in Cameroon (De Weirdt & Teugels, 2007). Notes: originally described as a subspecies of *Barbus* (now *Labeobarbus*) *perplexicans*, a junior synonym of *L. oxyrhynchus* following Banister (1973).

megastoma, *Barbus* Nagelkerke & Sibbing, 1997. Rema (11°51′N, 37°28′E, Nagelkerke & Sibbing, 1997) (locality 25), Lake Tana, Ethiopia. Holotype: RMNH 32950. Paratypes: RMNH 32951–59 (9). Current status: valid as *Labeobarbus megastoma* (placed in *Labeobarbus* in Getahun, 2007a). Synonyms: none. Distribution: Lake Tana and its tributaries (Nagelkerke & Sibbing, 1997).

microbarbis, *Barbus* David & Poll, 1937. A hybrid; see annotated checklist 2.

micronema, *Barbus* Boulenger, 1904. Kribi [Kienke] River (±2°56′N, 9°54′E, USBGN, 1962b), southern Cameroon. Syntypes: BMNH 1904.2.29.37–38 (2). Current status: valid as *Labeobarbus micronema* (placed in *Labeobarbus* in De Weirdt & Teugels, 2007). Synonyms: none. Distribution: Lower Guinea endemic, known from the Sanaga, Nyong, Kribi, and Ivindo rivers in Cameroon and Gabon (De Weirdt & Teugels, 2007).

microterolepis, *Barbus* Boulenger, 1902. A hybrid; see annotated checklist 2.

mirabilis, *Barbus* Pappenheim, 1914. Mawambi (±1°03′N, 28°36′E, D.F.E. Thys van den Audenaerde, unpubl. data), Ituri [DRC]. Holotype: ZMB 19059. Current status: valid as *Labeobarbus mirabilis* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: only known from the type locality (Lévêque & Daget, 1984). Notes: possibly a synonym of *B. somereni* (Banister, 1973).

mungoensis, *Barbus* Trewavas, 1974. Wowe River, tributary of Mungo River (±4°04′N, 9°31′E, USBGN, 1962b), Cameroon. Holotype: BMNH 1973.5.14.163.

Paratypes: BMNH 1973.5.14.164–182 (19). Current status: valid as *Labeobarbus mungoensis* (placed in *Labeobarbus* in De Weirdt & Teugels, 2007). Synonyms: none. Distribution: Blackwater, Menge, Mungo, and Sanaga river basins in Cameroon (De Weirdt & Teugels, 2007). Notes: Trewavas (1974) mentions 18 type specimens in the original description, but the total number of type specimens listed adds up to 20. Possibly two specimens in the paratypes series BMNH 1973.5.14.164–182 are not types (J. Maclaine, pers. comm., 2015).

nanningsi, *Labeobarbus* de Beaufort, 1933. Lunda Department (±9°30′S, 20°00′E, USBGN, 1956), Angola. Holotype: ZMA 113010. Current status: valid as *Labeobarbus nanningsi* [described as a *Labeobarbus* species; placed in *Barbus* in Matthes (1964), in *Barbus* (subgenus *Labeobarbus*) in Poll (1967), and again in *Labeobarbus* in the present paper]. Synonyms: none. Distribution: Luachimo River (Kasai drainage, middle Congo River Basin) in Angola (Poll, 1967; Lévêque & Daget, 1984).

natalensis, *Barbus* de Castelnau, 1861. Umvoti Mission, Tugela River (±29°14′S, 31°30′E, USBGN, 1954b) near the boundary between Zulu Land and Natal, South Africa. Syntypes: lost. Current status: valid as *Labeobarbus natalensis* (placed in *Labeobarbus* in Skelton, 2001). Synonyms: *Labeobarbus aureus* Cope, 1867 (synonymy in Crass, 1960); *Barbus bowkeri* Boulenger, 1902; *Barbus dendrotrachelus* Fowler, 1934; *Barbus grouti* Fowler, 1934; *Barbus lobochilus* Boulenger, 1911; *Barbus marleyi* Fowler, 1934; *Barbus mfongosi* Gilchrist & Thompson, 1913; *Barbus robinsoni* Gilchrist & Thompson, 1913; *Barbus stigmaticus* Fowler, 1934; *Barbus tugelensis* Fowler, 1934; *Barbus zuluensis* Gilchrist & Thompson, 1913 (synonymy in Jubb, 1963). Distribution: KwaZulu-Natal, widespread from the Mkuze southwards to the Umtamvuna on the Transkei border; translocated to the Save in Zimbabwe (Skelton, 2001). Notes: a recent search for the syntypes in the de Castelnau's fish collection at the University of Liège (see Loneux, 2005) did not produce any results (P. Skelton, pers. comm., 2014).

nedgia, *Labeobarbus* Rüppell, 1835. Goraza [probably = Korata] (11°45′N, 37°27′E, Nagelkerke & Sibbing, 1997) market, Lake Tana, Ethiopia. Holotype: SMF 2619 (stuffed). Current status: valid as *Labeobarbus nedgia* [described as a *Labeobarbus* species, placed in *Barbus* in Günther (1868), and again in *Labeobarbus* in Getahun (2007a)]. Synonym of *Barbus intermedius* in Banister (1973); revalidated in Nagelkerke & Sibbing (1997). Synonyms: *Barbus degeni* Boulenger, 1902; *Barbus degeni leptorhinus* Bini, 1940 (synonymy in Nagelkerke & Sibbing, 1997). Distribution: Lake Tana and tributaries (Nagelkerke & Sibbing, 1997), extending southwards to Lake Gandjule (Margherita) and the Sagan River (which connects Lake Gandjule with Lake Stephanie), and including the rivers Didessa and Omo (Boulenger, 1907c). Notes: (1) type species of the genus *Labeobarbus*; (2) although the original description mentions several specimens, only a holotype exists.

neglectus, *Barbus* Boulenger, 1903. Not a *Labeobarbus* species (see present paper).

nelspruitensis, *Varicorhinus* Gilchrist & Thompson, 1911. Nelspruit (25°57′S, 30°59′E, collection database), Transvaal [Gauteng], South Africa. Syntypes: SAIAB 134824 (ex. SAM 10518) (1), 135756 (ex. SAM 21698) (1). Current status: valid as *Labeobarbus nelspruitensis* (placed in *Labeobarbus* in Berrebi *et al*., 2014). Synonyms: none. Distribution: escarpment streams of the Incomati and Phongolo systems (Skelton, 2001).

nthuwa, *Labeobarbus* Tweddle & Skelton, 2008. Runyina (11°01′S, 33°47′E), tributary of South Rukuru River, Lake Malawi affluent, Malawi. Holotype: SAIAB 39341. Paratypes: SAIAB 39293 (1), 40787 (6), 51928 (2), 79494 (2). Current status: valid as *Labeobarbus nthuwa* (no generic reallocations). Synonyms: none. Distribution: South Rukuru River, Malawi (Tweddle & Skelton, 2008).

osseensis, *Barbus* Nagelkerke & Sibbing, 2000. Debre Mariam (11°38′N, 37°24′E, Nagelkerke & Sibbing, 1997) (locality 1), Lake Tana, Ethiopia. Holotype: RMNH 33721. Paratypes: RMNH 33722-30 (9). Current status: valid as *Labeobarbus osseensis* (placed in *Labeobarbus* in Getahun, 2007a). Synonyms: none. Distribution: only known from the southern parts of Lake Tana (Nagelkerke & Sibbing, 2000).

oxyrhynchus, *Barbus* Pfeffer, 1889. Korogwe (±5°09′S, 38°29′E, USBGN, 1965c), Rufu River, Tanzania. Lectotype: ZMH H339 (ex. 69 07) (designated by Ladiges *et al*., 1958). Paralectotypes: ZMH H340 (ex. 69 07) (7). Current status: valid as *Labeobarbus oxyrhynchus* (placed in *Labeobarbus* in Skelton & Bills, 2008; Berrebi *et al*., 2014). Synonyms: *Barbus ahlselli* Lönnberg, 1911; *Barbus athi* Hubbs, 1918; *Barbus* (*Capoeta*) *babaulti* Pellegrin, 1926; *Barbus donyensis* Holly, 1929; *Barbus hindii* Boulenger, 1902; *Barbus krapfi* Boulenger, 1911; *Barbus* (*Labeobarbus*) *labiatus* Boulenger, 1902 (synonymy in Banister, 1973); *Barbus mathoiae* Boulenger, 1911 (synonymy in Fowler, 1936); *Barbus nairobi* Holly, 1928; *Barbus* (*Capoeta*) *perplexicans* Boulenger, 1902; *Barbus tanensis* Günther, 1894 (synonymy in Banister, 1973). Distribution: Pangani, Athi-Tana, and Nero-Narok river systems, Lorian swamps in the northern Euasso Nyiro River system (Lévêque & Daget, 1984). Notes: senior synonym of *B. tanensis* Günther, 1894, the type species of the subgenus *Lanceabarbus* Fowler, 1936, to be considered a junior synonym of the genus *Labeobarbus*.

pagenstecheri, *Barbus* Fischer, 1884. Stream flowing from the Kilimandjaro (±3°04′S, 37°22′E, USBGN, 1965c)

[Pangani Basin], Massai-Land, Tanzania. Lectotype: ZMH H341 (ex ZMH 3851) (designated by Ladiges *et al*., 1958). Paralectotype: ZMH H342 (ex. ZMH 3850) (1). Current status: valid as *Labeobarbus pagenstecheri* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: (Upper?) Pangani drainage, Tanzania (Seegers, 2008). Notes: (1) the suggestion of Banister (1973: 103) that 'the 2 specimens referred to *B. pagenstecheri* [i.e. the two type specimens] may represent a local population of *B. oxyrhynchus*' is contested by Seegers (2008), arguing the sympatric and possibly even syntopic occurrence of both nominal species; (2) lectotype designation by Boulenger (1911a), as accepted by Banister (1973), is invalid following ICZN (1999: article 74.5). Boulenger (1911a) apparently did not consider the larger specimen as a (syn)type, and his subsequent use of the term 'type' for the other specimen does not constitute a valid lectotype designation. In contrast to Seegers (2008) we do accept the lectotype designation by Ladiges *et al*. (1958), which is not in contradiction to ICZN (1999: article 74.3, which is the same as ICZN, 1985: article 74(d), cited by Seegers, 2008). Lectotype designations by Ladiges *et al*. (1958) are made on a species-by-species basis (i.e. per nominal taxon), and not collectively by a general statement. As such, and in contrast to Boulenger (1911a) and Banister (1973), the largest of both syntypes is the lectotype.

parawaldroni, *Barbus* Lévêque, Thys van den Audenaerde & Traore, 1987. Toyebli (±6°37′N, 8°29′W, USBGN, 1965a), Cess River, Ivory Coast. Holotype: MRAC 73-10-P-2296. Paratypes: MRAC 73-10-P-2297 (1), 73-10-P-2298-2299 (2), 73-5-P-1936 (1). Current status: valid as *Labeobarbus parawaldroni* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: Lofa, Saint Paul, Nipoué (= Cess) in eastern Liberia, and Cavally and Tabou in western Côte d'Ivoire (Lévêque & Daget, 1984; Lévêque, 2003).

paucisquamata, *Barbus altianalis* Pellegrin, 1935. Kitembo (±5°53′S, 19°08′E, D.F.E. Thys van den Audenaerde, unpubl. data; USBGN, 1964b), Nya-Barongo River [DRC]. Lectotype: MNHN 1935-0076 (designated by Banister, 1973). Paralectotypes: MNHN 1935-0077 (1), 1935-0078 (1), MRAC 42932 (1). Current status: valid as *Labeobarbus paucisquamatus* (raised to species level in Banister, 1973; placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: *Barbus altianalis lobogenysoides* Pellegrin, 1935 (synonymy in Banister, 1973). Distribution: Luhoho River system, including the rivers Loama and Nyabarongo (Upper Congo River Basin), in DRC (Lévêque & Daget, 1984). Notes: (1) to be in agreement with ICZN (1999: articles 31.2 and 34.2), the species name *paucisquamata* [adjective; from the Latin adjective *paucus* (with a small number) and the Latin adjective *squamatus* (covered with scales), meaning the one covered with few scales] must become *paucisquamatus* to agree in gender with the genus name *Labeobarbus* (masculine); (2) the junior synonym *Barbus altianalis lobogenysoides* Pellegrin, 1935 was described in Pellegrin 1935b, as given in Eschmeyer (2015).

pellegrini, *Varicorhinus* Bertin & Estève, 1948. River west of Bukavu (±2°30′S, 28°52′E, USBGN, 1962c), at the extreme south-west of Lake Kivu, DRC. Holotype: MNHN 1932-0181. Current status: valid as *Labeobarbus pellegrini* (placed in *Labeobarbus* in present paper). Synonym of *Barbus oxyrhynchus* in Banister (1973); revalidated by Lévêque & Daget (1984). Synonyms: replacement name for *Varicorhinus babaulti* Pellegrin, 1932 (Bertin & Estève, 1948), preoccupied by *Barbus* (*Capoeta*) *babaulti* Pellegrin, 1926, currently a synonym of *Barbus oxyrhynchus* Pfeffer, 1889. Distribution: only known from the holotype (Lévêque & Daget, 1984), which probably originates from the Lowa River Basin, Kivu Region (Pellegrin, 1932; Marlier, 1954). Notes: the species name *Varicorhinus babaulti* Pellegrin, 1932 attributed to a species originally described from a 'Rivière près de Bukavu, région du Kivu' (MNHN 1932-0181) has been replaced by *V. pellegrini* Bertin & Estève, 1948 (see Bertin & Estève, 1948), as these authors considered *V. babaulti* to be preoccupied by *Barbus babaulti* Pellegrin, 1926, originally described from the 'Région de Nairobi, Kénia' (MNHN 1926-0285), and reallocated into the genus *Varicorhinus* by the same authors (see Bertin & Estève, 1948). In spite of their placement in *Varicorhinus*, Banister (1973) synonymized *V. babaulti* (Pellegrin, 1932), i.e. *V. pellegrini*, as well as *B. babaulti* Pellegrin, 1926, i.e. *V. babaulti*, with *Barbus*, now *Labeobarbus*, *oxyrhynchus*; however, the latter synonymy has not been followed by Lévêque & Daget (1984), who considered *V. pellegrini* [= *V. babaulti* (Pellegrin, 1932)] to be a valid species (see also Eschmeyer, 2015). Their decision concords with the fact that the type locality of *V. pellegrini* falls way out of the currently known East African distribution of *L. oxyrhynchus*, as given by Banister (1973) himself. Therefore, despite Banister's (1973) synonymization, the decision of Lévêque & Daget (1984) is followed here and *V. pellegrini* is retained as a valid species with the *Varicorhinus* mouth phenotype.

petitjeani, *Barbus* Daget, 1962. Ballay (±10°31′N, 11°55′W, USBGN, 1965b), Bafing River, upper Senegal Basin, Guinea. Syntypes: MNHN 1959-0108 (3). Current status: valid as *Labeobarbus petitjeani* (placed in *Labeobarbus* in Skelton & Bills, 2008; Berrebi *et al*., 2014). Synonyms: none. Distribution: Bafing River in the upper Senegal Basin and the upper Niger in Guinea (Lévêque & Guégan, 1990; Lévêque, 2003).

platydorsus, *Barbus* Nagelkerke & Sibbing, 1997. Rema (11°51′N, 37°28′E, Nagelkerke & Sibbing, 1997) (locality 25), Lake Tana, Ethiopia. Holotype:

RMNH 32970. Paratypes: RMNH 32971–79 (9). Current status: valid as *Labeobarbus platydorsus* (placed in *Labeobarbus* in Getahun, 2007a). Synonyms: none. Distribution: endemic to Lake Tana, Ethiopia (Nagelkerke & Sibbing, 1997).

platyrhinus, *Barbus* Boulenger, 1900. South of Usambura [= Usumbura, = Bujumbura] (±3°22′S, 29°22′E, D.F.E. Thys van den Audenaerde, unpubl. data), Lake Tanganyika. Holotype: BMNH 1906.9.6.12. Current status: valid as *Labeobarbus platyrhinus* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: Lake Tanganyika and its affluent the Koki River; probably rare in the lake and more common in the tributaries (Lévêque & Daget, 1984). Notes: Banister (1973) leaves some doubt on the validity of this species, and identifies it as a possible junior synonym of *B. tropidolepis*.

platystomus, *Varicorhinus* Pappenheim, 1914. Wase River, Rwanda, 1800 m a.s.l. Syntypes: ZMB 19051 (1), 34769 (1). Current status: valid as *Labeobarbus platystomus* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Mukungwa River in Rwanda (De Vos *et al*., 2001). Notes: (1) *Barbus platystomus* Boulenger, 1902 is currently a junior synonym of *Labeobarbus intermedius* (Rüppell, 1835). It is clear from the glossary of the ICZN (1999) that the word 'taxon' refers to a valid taxon, in our case a valid species, when used in article 57.3.1. of ICZN (1999) (M. Kottelat, pers. comm., 2015). As such, unless *Barbus platystomus* Boulenger, 1902 becomes a valid *Labeobarbus* species, a replacement name for *Labeobarbus platystomus* (Pappenheim, 1914) is not needed; (2) following ICZN (1999: articles 31.2 and 34.2), the species name *platystomus* [from the Greek adjective *platys* (wide and flat) and the Greek noun *stomus* (mouth); meaning with a wide and flat mouth] does not need to agree in gender with the genus name; (3) misspelled as *Varicorhinus platystoma* in Lévêque & Daget (1984).

pojeri, *Barbus* Poll, 1944. Albertville [Kalemie] (±5°56′S, 29°12′E, USBGN, 1964b) region, Lukuga River, DRC. Holotype: IRSNB 71. Paratype: IRSNB 599 (1). Current status: valid as *Labeobarbus pojeri* (placed in *Labeobarbus* in Kullander & Roberts, 2012). Synonym of *Barbus euchilus* in Poll (1953), which was, together with *Barbus pojeri*, placed in synonymy with *Barbus caudovittatus* in Banister (1973); the latter was transferred to *Labeobarbus* by De Weirdt & Teugels (2007); *Barbus pojeri* revalidated by Kullander & Roberts (2012) as *Labeobarbus pojeri*. Synonyms: none. Distribution: Lukuga River, from near Kalemie (Poll, 1944) up to the Kisimba–Kilia rapids (Kullander & Roberts, 2012) in DRC.

polylepis, *Barbus* Boulenger, 1907. Klein Olifant River (±25°41′S, 29°20′E, D.F.E. Thys van den Audenaerde, unpubl. data), Transvaal [now Gauteng], South Africa. Holotype: BMNH 1907.3.15.33. Current status: valid as *Labeobarbus polylepis* (placed in *Labeobarbus* in Skelton, 2001). Synonyms: *Barbus elephantis* Boulenger, 1907 (implicit synonymy in Skelton, 1993, 2001; synonymy confirmed by P.H. Skelton, pers. comm., 2015; see also present paper); *Barbus lineolatus* Gilchrist & Thompson, 1913 (synonymy in Jubb, 1963). Distribution: restricted to southern tributaries of the Limpopo, and the Incomati and Phongolo systems (Skelton, 2001). Notes: (1) including *Barbus elephantis* Boulenger, 1908 as a junior synonym. See also Jubb (1961, 1967) on the dubious status of this nominal species, as a possible synonym of *B. natalensis* (Jubb, 1961), a possible hybrid, or a synonym of *B. marequensis* (Jubb, 1967); (2) holotype in Eschmeyer (2015) incorrectly referred to as BMNH 1907.3.15.3 (probably a typographical error).

progenys, *Barbus* Boulenger, 1903. Kribi [Kienke] River (±2°56′N, 9°54′E, USBGN, 1962), southern Cameroon. Holotype: BMNH 1902.11.12.127. Current status: valid as *Labeobarbus progenys* (placed in *Labeobarbus* in De Weirdt & Teugels, 2007). Synonyms: none. Distribution: widespread in West–Central Africa, including the basins of the Sanaga, Ntem, Ogooué, Nyanga, and Niari (De Weirdt & Teugels, 2007), and the Cross River in Cameroon (Vivien, 1991; Lévêque, 2003); also known from Dja River and Dundo (Angola) (De Weirdt & Teugels, 2007). Notes: (1) holotype in Eschmeyer (2015) incorrectly referred to as BMNH 1902.11.13.127 (probably a typographical error); (2) Poll (1967) mentions *Barbus* sp. (aff. *progenys* Boulenger) from Dundo (Luachimo River, Angola); it is unclear if the report from Dundo in De Weirdt & Teugels (2007) refers to the same specimen(s) or reference.

pungweensis, *Varicorhinus* Jubb, 1959. Pungwe River (18°24′S, 32°58′E), Inyanga district, Zimbabwe. Holotype: SAIAB 120014 (ex. AMG 850). Paratypes: SAIAB 120015 (ex. AMG 851) (1), 120016 (ex. AMG 852) (1), 120017 (ex. AMG 853) (1), 120018 (ex. AMG 854) (1). Current status: valid as *Labeobarbus pungweensis* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Pungwe and Buzi rivers in Zimbabwe and Mozambique (Skelton, 2001). Notes: number of paratypes in Eschmeyer (2015) incorrectly given as seven instead of four.

reinii, *Barbus* Günther, 1874. Tensift River (±32°02′N, 9°21′W, USBGN, 1970), Morocco. Syntypes: BMNH 1874.1.30.22–24 (3), SMF 579 (4). Current status: valid as *Labeobarbus reinii* (placed in *Labeobarbus* in Doadrio, 1994). Synonyms: none. Distribution: Rivers of Morocco (Lévêque & Daget, 1984), including Oued Tennsift and Oued Ksob (Borkenhagen, 2014). Notes: (1) the original species description mentions only three type specimens, but the total number of type specimens given in Eschmeyer (2015) is seven, including

BMNH 1874.1.30.22–24 (3). The latter may be the true types (J. Maclaine, pers. comm., 2015); (2) placed in '*Labeobarbus*' in Yang *et al*. (2015).

rhinoceros, *Barbus* Copley, 1938. Athi River [Kenya?]. No types known (Eschmeyer, 2015). Holotype and paratype status of BMNH 1936.12.22.35–39 needs further research (see also text). Current status: valid as *Labeobarbus rhinoceros* (placed in *Labeobarbus* in Skelton & Bills, 2008; Berrebi *et al*., 2014). Synonyms: replacement name for *Barbus mariae* Holly, 1929, preoccupied by *Varicorhinus* (now *Labeobarbus*) *mariae* Holly, 1926 (present paper). Distribution: Athi and Tana river systems in Kenya (Lévêque & Daget, 1984). Notes: (1) see note for *Labeobarbus matris*; (2) Lévêque & Daget (1984) erroneously used 1958 instead of 1938 as the year of original description; (3) previously considered lost, the lectotype and paralectotype of *Barbus mariae* Holly, 1929, designated by Banister (1973), were found back at the NMW and are currently registered as NMW 96552 and 96553, respectively.

rhinophorus, *Barbus* Boulenger, 1910. Lucala (near railway station) (±9°16′23″S, 15°14′42″E, Google Earth), above the falls on the Lucala River, Angola. Syntypes: BMNH 1911.6.1.37–38 (2). Current status: valid as *Labeobarbus rhinophorus* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: none. Distribution: only known from the type locality (Lévêque & Daget, 1984).

robertsi, *Varicorhinus* Banister, 1984. Sanga waterfalls (4°50′S, 14°57′E) at the tailwaters of the hydroelectric dam at Sanga on the Inkisi River, Congo Basin, DRC. Holotype: BMNH 1983.3.30.20. Paratypes: BMNH 1983.3.30.21–38 (18). Current status: valid as *Labeobarbus robertsi* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Inkisi River, DRC (Wamuini Lunkayilakio *et al*., 2010).

rocadasi, *Barbus* Boulenger, 1910. Rivers Quanza [= Cuanza] (±13°49′S, 17°26′E, D.F.E. Thys van den Audenaerde, unpubl. data; USBGN, 1956) and Lucalla [= Lucala] (±9°20′S, 13°11′E), Angola. Syntypes: ANSP 37986–91 (6), BMNH 1911.6.1.19–20 (2), 1911.6.1.21–25 (6), 1911.6.1.26 (1, skeleton), 1911.6.1.27–28 (2), NMW 13347–13352 (6), ZMB 18214 (6). Current status: valid as *Labeobarbus rocadasi* (placed in *Labeobarbus* in De Weirdt & Teugels, 2007; Skelton & Bills, 2008). Synonyms: none. Distribution: Nyong River in Cameroon (De Weirdt & Teugels, 2007) and Quanza and Luculla rivers in Angola (Lévêque & Daget, 1984; De Weirdt & Teugels, 2007). Notes: (1) the presence of this species in the Nyong River in Cameroon (De Weirdt & Teugels, 2007) needs confirmation, but seems questionable given the species is otherwise only known from the Quanza Basin in Angola; (2) the number of syntypes in BMNH 1911.6.1.21–25 was verified as six, not the expected five.

rosae, *Barbus* Boulenger, 1910. Lucala (near railway station) (±9°16′23″S, 15°14′42″E, Google Earth), above the falls on the Lucala River, Angola. Syntypes: BMNH 1911.6.1.39–41 (3), NMW 54500 (1), ZMB 18216 (1). Current status: valid as *Labeobarbus rosae* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: only known from the type locality (Lévêque & Daget, 1984). Notes: Lévêque & Daget (1984) incorrectly put the type locality in the Zambezi River system.

roylii, *Barbus* Boulenger, 1912. N′Kutu [Ncuto] (±4°57′S, 12°35′E, D.F.E. Thys van den Audenaerde, unpubl. data), Loango River [Shiloango Basin, Cabinda, Angola] and Buco Zau [= Bucozan] (±4°45′S, 12°33′E, D.F.E. Thys van den Audenaerde, unpubl. data), Luali River [Shiloango Basin, Cabinda, Angola]. Syntypes: ANSP 38548 (1), BMNH 1912.4.1.338–340 (3), 1912.4.1.341–342 (2), MRAC 1533 (1), 1534 (1), 1535 (1), NMW 54501 (1), ZMB 18809 (1). Current status: valid as *Labeobarbus roylii* (placed in *Labeobarbus* in De Weirdt & Teugels, 2007). Synonyms: none. Distribution: Lower Guinea endemic, known from the Kouilou and Chiloango basins in Republic of the Congo and DRC (De Weirdt & Teugels, 2007). Notes: The total number of type specimens adds up to 11, whereas the original description only mentions ten syntypes.

ruandae, *Varicorhinus* Pappenheim, 1914. Mkunga [Mukunga] near Ruasa [Rwasa] (±1°32′S, 29°42′E, D.F.E. Thys van den Audenaerde, unpubl. data), Rwanda. Holotype: ZMB 19050. Current status: valid as *Labeobarbus ruandae* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Rivers of Rwanda (Lévêque & Daget, 1984). Notes: the two paratypes of *Barbus* (now *Labeobarbus*) *microbarbis* have been reidentified as this species (Banister, 1973).

ruasae, *Barbus* Pappenheim, 1914. Mkunga [Mukunga] near Ruasa [Rwasa] (±1°32′S, 29°42′E, D.F.E. Thys van den Audenaerde, unpubl. data), Rwanda. Lectotype: ZMB 19053 (designated by Banister, 1973). Paralectotype: ZMB 22652 (1). Current status: valid as *Labeobarbus ruasae* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: only known from the Mukungwa River, an affluent of the Nyabarongo River in Rwanda (Lévêque & Daget, 1984).

ruwenzorii, *Capoëta* (*Pterocapoëta*) Pellegrin, 1909. Wimi River (±0°23′N, 29°54′E, D.F.E. Thys van den Audenaerde, unpubl. data), Ruwenzori. Syntypes: MNHN 1909-0583 (1), 1909-0584 (1), 1909-0585 (1). Current status: valid as *Labeobarbus ruwenzorii* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Ruwenzori area, Mubuku and Sibwe systems (Lévêque & Daget, 1984).

sacratus, *Barbus* Daget, 1963. Zié (±7°44′N, 8°22′W, USBGN, 1965b), Diougou Basin and Sérédou (±8°23′N, 9°17′W, USBGN, 1965b), Diani River (upper Saint Paul Basin), Guinea. Syntypes: MNHN 1959-0119 (4), 1959-

0139 (1). Current status: valid as *Labeobarbus sacratus* (placed in *Labeobarbus* in Skelton & Bills, 2008; Berrebi *et al*., 2014). Synonyms: none. Distribution: known from the coastal basins of the Guinean ridge, from the Tominé (= Corubal) River in Guinea to the Saint John River in east Liberia (Lévêque & Guégan, 1990; Lévêque, 2003). Notes: in his original description of *Barbus sacratus*, Daget (1963) designated four syntypes and a paratype, which are all to be considered syntypes following ICZN (1999: article 73.2).

sandersi, *Varicorhinus* Boulenger, 1912. N'Kutu [Ncuto] (±4°57′S, 12°35′E, D.F.E. Thys van den Audenaerde, unpubl. data), Loango River [Shiloango Basin, Cabinda, Angola]. Lectotype: BMNH 1912.4.1.333 (designated in present paper). Paralectotypes: BMNH 1912.4.1.334–336 (two instead of three), 1912.4.1.337 (1), MRAC 1525 (1), 1526 (1), 1527 (1), ZMB 18808 (1). Current status: valid as *Labeobarbus sandersi* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Lower Guinea endemic, known from southern Cameroon to the Chiloango River in Cabinda (Angola) (Getahun, 2007b). Notes: the original BMNH syntype series, currently split into a lectotype and paralectotypes, consists of five consecutive numbers, although only four specimens are present; the whereabouts of a potential fifth specimen are unknown. Even if this specimen was to be located, the number of type specimens still only adds up to nine, whereas ten syntypes were reported in the original description.

seeberi, *Barbus* Gilchrist & Thompson, 1913. Olifants River (±32°11′S, 18°54′E, D.F.E. Thys van den Audenaerde, unpubl. data), Cape Province, South Africa. Syntypes: BMNH 1936.8.4.6 (1), SAIAB 134867 (ex. SAM 10672) (2). Current status: valid as *Labeobarbus seeberi* [placed in *Labeobarbus* in Skelton & Bills (2008) (using the at that time senior synonym name *Labeobarbus capensis*; *L. seeberi* is at present the valid species name, cf. infra); present paper]. Synonym of *Barbus capensis* in Barnard (1937); revalidated in the present paper. See also E. Vreven, E.R. Swartz & P.H. Skelton, unpubl. data. Synonyms: none. Distribution: Clanwilliam Olifants system, Western Cape, South Africa (Skelton, 2001). Notes: as demonstrated by E. Vreven, E.R. Swartz & P.H. Skelton (unpubl. data), *Barbus capensis* is not a *Labeobarbus* but is instead a senior synonym of *B. andrewi*. *Barbus seeberi*, previously a junior synonym of *B. capensis* and indeed a yellowfish, or *Labeobarbus*, therefore becomes the valid species name for the clanwilliam yellowfish.

semireticulatus, *Varicorhinus* Pellegrin, 1924. Louvisi River (±4°17′S, 13°57′E, USBGN, 1962c), Kouilou, Republic of the Congo. Syntypes: MNHN 1924-0052 (2). Current status: valid as *Labeobarbus semireticulatus* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: only known from the type locality (Getahun, 2007b).

somereni, *Barbus* Boulenger, 1911. Sebwe River (±0°10′N, 30°12′E, D.F.E. Thys van den Audenaerde, unpubl. data), snow-water stream on Mount Ruwenzori, Uganda, elevation 6000 feet. Holotype: BMNH 1911.7.26.1. Current status: valid as *Labeobarbus somereni* (placed in *Labeobarbus* in Skelton & Bills, 2008; Banyankimbona *et al*., 2012a). Synonyms: *Barbus altianalis urundensis* David, 1937 (synonymy in Banister, 1973). Distribution: rivers Sibwe, Mubuku, Tokwe, and Kirimia in the Ruwenzori area; also found in the rivers Nyawarongo and Akianaru (Rwanda), Mutamphu, Chirangobwe (Lake Kivu Basin), Mwogo (Kagera system), upper Malagarasi (Burundi), Kitenge (Ruzizi), and the Nyamagana and Nyakagunda (Burundi) (Banister, 1973; Lévêque & Daget, 1984). Possibly also in the Kagera and Malagarasi in Tanzania (Eccles, 1992). Notes: possibly 'related' to *Varicorhinus* (now *Labeobarbus*) *ruwenzorii*, with which it may hybridize (Banister, 1973: 114).

stappersii, *Barbus* Boulenger, 1915. Lake Moero [= Mweru] (±9°00′S, 28°45′E, USBGN, 1964b), in front of Lukonzolwa, DRC. Holotype: MRAC 14250. Current status: valid as *Labeobarbus stappersii* (placed in *Labeobarbus* in Skelton & Bills, 2008; Van Steenberge *et al*., 2014). Synonyms: *Barbus curtus* Boulenger, 1915; *Barbus moeruensis* Pellegrin, 1922; *Barbus oxycephalus* Boulenger, 1915 (synonymy in Banister, 1973). Distribution: Lake Mweru (Lévêque & Daget, 1984; Van Steenberge *et al*., 2014), Luapula River (Lévêque & Daget, 1984), and the Bangweulu-Chambesi (Van Steenberge *et al*., 2014).

steindachneri, *Varicorhinus* Boulenger, 1910. Lucala (near railway station) (±9°16′23′S, 15°14′42′E, Google Earth), above the falls on the Lucala River, Angola. Syntypes: ANSP 37992 (2), BMNH 1910.11.28.147–155 (9), 1910.11.28.156 (1), 1910.11.28.157 (1, glycerine), 1910.11.28.158 (1, skeleton), 1911.6.1.1–5 (5), NMW 48867 (3), USNM 86618 (1), ZMB 18212 (3). Current status: valid as *Labeobarbus steindachneri* (placed in *Labeobarbus* in Berrebi *et al*., 2014). Synonyms: none. Distribution: Lower Guinea endemic found from Cameroon to Cabinda (Getahun, 2007b). Notes: (1) the type series of *V. steindachneri* revealed to be polyspecific with one specimen (BMNH 1911.6.1.5: 55.4 mm SL) identifiable as *V. ensifer*; nevertheless, it is clear from Boulenger's original description and the numerous diagnostic characters separating *V. steindachneri* and *V. ensifer* that the latter specimen was not intended to be considered part of the species, as conceived by Boulenger himself; (2) the original syntypes record BMNH 1910.11.28.147–158 contained only nine specimens, hence the collection number was amended to 1910.11.28.147–155. BMNH 1910.11.28.157 is in glycerine and BMNH 1910.11.28.158 is a skeleton. The latter specimen is probably found but there is at present not enough

data to say conclusively. The whereabouts of BMNH 1910.11.28.156 are currently unknown (J. Maclaine, pers. comm., 2015); (3) syntype BMNH 1910.11.28.158 is not mentioned in Lévêque & Daget (1984) or Eschmeyer (2015); (4) Lévêque & Daget (1984) gave BMNH 1910.11 .20.147–157 as the syntype record numbers, which is probably a typographical error for BMNH 1910.11.28.147–157.

stenostoma, *Varicorhinus* Boulenger, 1910. Lucala (near railway station) (±9°16′23′S, 15°14′42′E, Google Earth), above the falls on the Lucala River, Angola. Holotype: BMNH 1910.11.28.145. Current status: valid as *Labeobarbus stenostoma* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: only known from the holotype (Lévêque & Daget, 1984). Notes: identifications from the Inkisi (lower Congo River Basin, DRC) are incorrect (Wamuini Lunkayilakio *et al*., 2010).

stigmatopygus, *Barbus* Boulenger, 1903. Not a *Labeobarbus* species (see present paper).

surkis, *Barbus* Rüppell, 1835. Entos (11°39′N, 37°22′E, Nagelkerke & Sibbing, 1997) (locality 17), Lake Tana, Ethiopia. Neotype: RMNH 32980 (designated by Nagelkerke & Sibbing, 1997). Current status: valid as *Labeobarbus surkis* (placed in *Labeobarbus* in Getahun, 2007a). Synonym of *Barbus intermedius* in Banister (1973); revalidated by Nagelkerke & Sibbing (2000). Synonyms: none. Distribution: Lake Tana; also described from the Didessa River (Nagelkerke & Sibbing, 1997).

tornieri, *Varicorhinus* Steindachner, 1906. Nyong district (±3°17′N, 9°54′E, USBGN, 1962b), German Cameroon [Cameroon]. Holotype: NMW 46053. Current status: valid as *Labeobarbus tornieri* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Lower Guinea endemic, known from the Mungo, Sanaga, Nyong, and Ntem rivers (Cameroon), Rio Benito (Rio Muni), and Ogowe River (Gabon) (Getahun, 2007b). Notes: (1) date in Lévêque & Daget (1984) incorrect as 1907; (2) Eschmeyer (2015) reports the type series as 'syntypes: NMW', without collection number, but the original species description is based on a single specimen.

trachypterus, *Barbus* Boulenger, 1915. Lake Moero [= Mweru] (±9°00′S, 28°45′E, USBGN, 1964b), in front of Lukonzolwa, DRC. Holotype: MRAC 11830. Current status: valid as *Labeobarbus trachypterus* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Synonyms: *Varicorhinus bredoi* Poll, 1948 (synonymy in Banister, 1973). Distribution: Upper Lualaba (David & Poll, 1937), Lake Mweru (Boulenger, 1915) and lower Luapula (below Mumbatuta Falls) (Banister, 1973; Balon & Stewart, 1983).

tropidolepis, *Barbus* Boulenger, 1900. Usambura (Usumbura) [= Bujumbura] (±3°22′S, 29°22′E, D.F.E. Thys van den Audenaerde, unpubl. data), Lake Tanganyika [Burundi]. Lectotype: BMNH 1906.9.6.19 (designated by Banister, 1973). Paralectotypes: BMNH 1906.9.6.20–21 (2), MRAC 327 (1), 328 (1), 329 (1). Current status: valid as *Labeobarbus tropidolepis* (placed in Labeobarbus in Skelton & Bills, 2008; Banyankimbona *et al*., 2012a). Synonyms: *Varicorhinus chapini* Nichols & La Monte, 1950 (synonymy proposed in Poll, 1953; established in Banister, 1973). Distribution: Lake Tanganyika Basin (Lévêque & Daget, 1984).

truttiformis, *Barbus* Nagelkerke & Sibbing, 1997. Wanzaie (11°46′N, 37°43′E, Nagelkerke & Sibbing, 1997) (locality 30), Gumara River (±11°53′N, 37°32′E, USBGN, 1963), tributary of Lake Tana, Ethiopia. Holotype: RMNH 32990. Paratypes: RMNH 32991–99 (9). Current status: valid as *Labeobarbus truttiformis* (placed in *Labeobarbus* in Getahun, 2007a). Synonyms: none. Distribution: endemic to Lake Tana and its tributaries (Nagelkerke & Sibbing, 1997).

tsanensis, *Barbus* Nagelkerke & Sibbing, 1997. Blue Nile (11°37′N, 37°24′E, Nagelkerke & Sibbing, 1997) (locality 13), Lake Tana, Ethiopia. Holotype: RMNH 33000. Paratypes: RMNH 33001–09 (9). Current status: valid as *Labeobarbus tsanensis* (placed in *Labeobarbus* in Getahun, 2007a). Synonyms: none. Distribution: endemic to Lake Tana, Ethiopia (Nagelkerke & Sibbing, 1997).

upembensis, *Varicorhinus* Banister & Bailey, 1979. Above the falls on the Kalumengonga River (8°49′S, 27°13′E) (site 5), Upemba National Park, Shaba, DRC. Holotype: BMNH 1975.9.5.5. Paratypes: BMNH 1975.9.5.6–12 (7), 1975.9.5.13–17 (5). Current status: valid as *Labeobarbus upembensis* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: only known from the type locality (Banister, 1984).

urotaenia, *Barbus* Boulenger, 1913. Dungu (±3°37′N, 28°33′E, D.F.E. Thys van den Audenaerde, unpubl. data), upper Uelé River, DRC. Syntypes: BMNH 1912.12.6.5 (1), MRAC 1791 (1), 1792 (1). Current status: valid as *Labeobarbus urotaenia* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: only known from the type locality (Lévêque & Daget, 1984).

varicostoma, *Varicorhinus* Boulenger, 1910. Lucala (±9°37′S, 14°14′E, USBGN, 1956), above the falls on the Lucala River, Angola. Holotype: BMNH 1910.11.28.146. Current status: valid as *Labeobarbus varicostoma* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: only known from the holotype (Lévêque & Daget, 1984).

versluysii, *Barbus* Holly, 1929. Bakoko area (±5°28′N, 9°18′E, USBGN, 1962b), Cameroon. Syntypes: NMW 13954–13956 (3). Current status: valid as *Labeobarbus versluysii* (placed in *Labeobarbus* in De Weirdt & Teugels, 2007). Synonyms: none. Distribu-

tion: Lower Guinea endemic, present in the Wouri, Sanaga, and Nyong river basins in Cameroon (De Weirdt & Teugels, 2007).

werneri, *Barbus* Boulenger, 1905. Not a *Labeobarbus* species (see present paper). Listed as a valid (*Labeobarbus*) species in Getahun (2007a), but considered a synonym of *Barbus stigmatopygus* in Lévêque (1989), Lévêque, Paugy & Teugels (1991), and Seegers *et al*. (2003).

werneri, *Varicorhinus* Holly, 1929. Bakoko area (±5°28′N, 9°18′E, USBGN, 1962b), Cameroon. Syntypes: NMW 13948 (1), 13949 (1). Current status: valid as *Labeobarbus werneri* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Lower Guinea endemic, known from scattered localities throughout the region, including the Sanaga and Nyong in Cameroon, and the Rembo Nkomi and Nyanga in Gabon (Getahun, 2007b). Notes: only one specimen left according to the NMW collection; originally NMW 13948 and 13949 were in the same jar, but only the former is still present (with a label bearing the number 13948 attached to the specimen and on the jar); NMW 13949 thus seems to have been lost (H. Wellendorf, pers. comm., 2000).

wittei, *Varicorhinus* Banister & Poll, 1973. Muye (±08°59′S, 26°48′E, collection database), right-bank affluent of the Lufira, 800–900 m a.s.l. Holotype: MRAC 179738. Paratypes: BMNH 1972.10.2.3 (1), MRAC 179739–741 (3), 179742 (1), IRSNB 645 (1). Current status: valid as *Labeobarbus wittei* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: Kilwezi and Muye, Lufira system (Banister & Poll, 1973).

wurtzi, *Barbus* Pellegrin, 1908. Near Tabili (±9°50′N, 13°13′W, USBGN, 1965b), Grandes Chûtes (probably near the Grandes Chûtes Dam, ±9°55′N, 13°06′W, USBGN, 1965b) [Samu River, Konkouré Basin], French Guinea [Guinea]. Holotype: MNHN 1908-0097. Current status: valid as *Labeobarbus wurtzi* (placed in *Labeobarbus* in Skelton & Bills, 2008; Berrebi *et al*., 2014). Synonyms: *Barbus barryi* Daget, 1962 (synonymy in Lévêque *et al*., 1989); *Barbus holasi* Daget, 1965 (synonymy in Lévêque, 1983). Distribution: many coastal basins of West Africa, from the Konkouré River in Guinea to at least the Tano, west of the Volta in Ghana (Lévêque & Guégan, 1990; Lévêque, 2003).

xyrocheilus, *Varicorhinus* Tweddle & Skelton, 1998. Border Lujeri Tea Estate/Swazi Tea research station (16°04′S, 35°40′E), upper Ruo River, lower Shire, Zambezi system, Malawi. Holotype: SAIAB 53082. Paratypes: SAIAB 34336 (11). Current status: valid as *Labeobarbus xyrocheilus* (placed in *Labeobarbus* in present paper). Synonyms: none. Distribution: River Ruo above Zoa Falls (Tweddle & Skelton, 1998). Notes: holotype in Eschmeyer (2015) incorrectly referred to as SAIAB 52082 (probably a typographical error).

ANNOTATED CHECKLIST 2: AFRICAN NOMINAL *LABEOBARBUS S.L.* SPECIES IDENTIFIED AS HYBRID PHENOTYPES

alluaudi, *Barbus* Pellegrin, 1909. Wimi River (±0°23′N, 29°54′E, D.F.E. Thys van den Audenaerde, unpubl. data), Ruwenzori (lower zone). Syntypes: MNHN 1909-0586 (1), 1909-0587 (1). Current status: *Labeobarbus alluaudi* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Considered a hybrid in Banister (1972). Distribution: Ruimi (= Wimi), Mubuku and Sibwe rivers on the eastern flank of the Ruwenzori Mountain, Uganda (Lévêque & Daget, 1984). Notes: identified as a possible intergeneric hybrid between *B. somereni* and *V. ruwenzori* (now both *Labeobarbus* species) by Banister (1972).

microbarbis, *Barbus* David & Poll, 1937. Lake Luhondo (±1°30′S, 29°45′E, USBGN, 1964c), Rwanda. Holotype: MRAC 41847. Paratypes: MRAC 41848–49 (2). Current status: *Labeobarbus microbarbis* (placed in *Labeobarbus* in Skelton & Bills, 2008; present paper). Considered a possible hybrid in Banister (1973); considered a hybrid in De Vos & Thys van den Audenaerde (1990). Distribution: only known from the holotype (Lévêque & Daget, 1984). Notes: (1) in Lévêque & Daget (1984: 268) as 'only known from the holotype', but two paratypes exist that are not mentioned in Lévêque & Daget (1984); (2) the two paratypes have been reidentified as *Varicorhinus ruandae* (Banister, 1973); (3) the holotype illustrated in figure 30 in David & Poll (1937; upper specimen) was incorrectly indicated as having an SL of 170 mm, instead of 270 mm; in addition, the second specimen illustrated is a paratype and thus not conspecific (see previous note); (4) identified as a possible intergeneric hybrid between *Barbus* and *Varicorhinus* (now both *Labeobarbus* species; see Banister, 1973), and between *B. ruasae* and *V. ruandae* (now both *Labeobarbus* species; see De Vos & Thys van den Audenaerde, 1990).

microterolepis, *Barbus* Boulenger, 1902. Buggali, Maki River (±8°05′N, 38°50′E, D.F.E. Thys van den Audenaerde, unpubl. data), flowing towards Lake Swai from the eastern slope of the Adami Mountains, Addia country, Ethiopia, elevation about 4000 feet. Holotype: BMNH 1902.12.13.220. Current status: *Labeobarbus microterolepis* (placed in *Labeobarbus* in Getahun, 2007a). Considered a possible hybrid in Banister (1973); considered a hybrid in Golubtsov *et al*. (2002). Distribution: only known from the type locality (Lévêque & Daget, 1984). Notes: according to Banister (1973), an aberrant specimen of *Barbus* (now *Labeobarbus*) *intermedius*, a valid species, or possibly a hybrid between *B. ethiopicus* and *B. intermedius* (now both *Labeobarbus* species), but more specimens are needed to confirm this (Banister, 1973). Golubtsov *et al*. (2002) confirmed the identification of *L. microterolepis* as a hybrid between

L. ethiopicus and *L. intermedius*, although no evidence whatsoever was provided to support this view.

ANNOTATED CHECKLIST 3. ANNOTATED CHECKLIST FOR *ACAPOETA* AND *SANAGIA*

tanganicae, *Capoeta* Boulenger, 1900. North end of Lake Tanganyika. Lectotype: BMNH 1906.9.6.9 (designated by Banister, 1976a). Paralectotypes: BMNH 1906.9.6.10 (1), 1906.9.6.11 (1, skeleton). Current status: valid as *Acapoeta tanganicae* (placed in the new subgenus *Acapoeta* by Cockerell, 1910, raised to genus level by Fowler, 1976). Synonyms: none. Distribution: endemic to Lake Tanganyika (Lévêque & Daget, 1984), both in the lake and the affluent rivers (Poll, 1953; Banister, 1976a). Notes: (1) skeleton type given in the BMNH online collection needs verification. Although the original description by Boulenger (1900a) does not mention the number of specimens, Boulenger (1901b) mentions three specimens from 'the northern end of Lake Tanganyika', further specified in Boulenger (1909) as two types and one skeleton. It remains unclear from Boulenger (1909) if the latter specimen is a type, but a similar listing of the syntypes of *Barbus rocadasi* in Boulenger (1916a) also lacks 'type' for a syntype of this species denoted specifically as a skeleton; (2) type species of the monospecific genus *Acapoeta* Cockerell, 1910; (3) the placement of this species in the subgenus *Acapoeta* by Cockerell (1910) is not followed by subsequent authors (e.g. Boulenger, 1920c; Poll, 1953; Banister, 1976a), who treat it as a *Varicorhinus*, until it was raised to the genus level of *Acapoeta* by Fowler (1976); the few publications after Fowler (1976) that mention the species (e.g. Lévêque & Daget, 1984; Poll & Gosse, 1995) use *Acapoeta* as a valid genus.

velifera, *Sanagia* Holly, 1926. Nachtigal (±4°21′N, 11°38′E, USBGN, 1962b) (rapids), Sanaga River, Cameroon. Syntypes: NMW 7261–7262 (2). Current status: valid as *Sanagia velifera* (no generic reallocations). Synonyms: none. Distribution: Sanaga and Nyong River basins in Cameroon (De Weirdt, 2007). Notes: (1) the two syntypes that were reported as 'apparently lost' by Lévêque & Daget (1984) have been found back in the NMW (H. Wellendorf, pers. comm., 2014); (2) MNHN 1978-0721 and MRAC 174990–97 (8), mentioned as possible types in Eschmeyer (2015), are not types; (3) type species of the monospecific genus *Sanagia* Holly, 1926.

APPENDIX

List of (type) Specimens Examined

African *Labeobarbus s.l.* species: list of examined (type) specimens

acuticeps, *Barbus* Matthes, 1959. Holotype: MRAC 130313. Paratypes: MRAC 130310 (1), 130311–312 (2), 130314 (1). – *acutirostris*, *Barbus brunelli* Bini, 1940. Neotype: RMNH 32870 (*). – *aeneus*, *Cyprinus* Burchell, 1822. No types known. Synonyms: *Barbus gilchristi* Boulenger, 1911. Holotype: BMNH 1909.12.8.1; *Barbus mentalis* Gilchrist & Thompson, 1913. Holotype: SAIAB 134770 (ex SAM 9644). – *altianalis*, *Barbus* Boulenger, 1900. Lectotype: BMNH 1906.9.6.13. Paralectotypes: BMNH 1906.9.6.14 (1), 1906.9.6.15 (1). Synonyms: *Barbus eduardianus* Boulenger, 1901. Holotype: BMNH 1906.9.7.41; *Barbus fergusonii* Boulenger, 1901. Syntypes: BMNH 1906.9.7.42–43 (2); *Barbus kiogae* Worthington, 1929. Syntypes: BMNH 1929.1.24.105–108 (7 instead of 4); *Barbus lobogenys* Boulenger, 1906. Syntypes: 1906.5.30.117–121 (2 instead of 5). – *altipinnis*, *Varicorhinus* Banister & Poll, 1973. Holotype: MRAC 179729. Paratypes: BMNH 1972.10.2.1 (1), MRAC 179730 (1), 179731 (1), 179732-733 (2), 179734 (1), 179735 (1), IRSNB 643 (1). – *ansorgii*, *Varicorhinus* Boulenger, 1906. Holotype: BMNH 1904.5.2.161. – *aspius*, *Barbus* Boulenger, 1912. Syntypes: BMNH 1912.4.1.354 (1), MRAC 1536 (1). – *axelrodi*, *Varicorhinus* Getahun, Stiassny & Teugels, 2004. Holotype: MRAC 91-68-P-1132. Paratypes (partim): MRAC 90-057-P-1297-1300 (4), 90-057-P-1314-1327 (14), 99-55-P-246-247 (2), 99-55-P-249 (1), 99-90-P-459-461 (3). – *batesii*, *Barbus* Boulenger, 1903. Holotype: BMNH 1902.11.12.128. – *beso*, *Varicorhinus* Rüppell, 1835. Neotype: BMNH 1902.12.13.365. Specimens: BMNH 1968.7.24.18. – *boulengeri*, *Labeobarbus* (present paper). Lectotype: BMNH 1911.6.1.6. Paralectotypes (partim): BMNH 1911.6.1.7–10 (4), NMW 48865 (1), ZMB 18211 (1). – *brauni*, *Varicorhinus* Pellegrin, 1935. Syntypes: MNHN 1935-0066 (1), MRAC 42933 (1). – *brevicephalus*, *Barbus* Nagelkerke & Sibbing, 1997. Holotype: RMNH 32880 (*). – *brevispinis*, *Barbus ruspolii* Holly, 1927. Syntypes: NMW 7315 (1), 7316 (1). – *bynni*, *Barbus* Forsskål, 1775. Neotype: BMNH 1907.12.2.1230. Synonyms: *Barbus meneliki* Pellegrin, 1905. Holotype: MNHN 1905-0275; *Barbus occidentalis* Boulenger, 1911. Holotype: BMNH 1909.3.3.14; *Barbus seguensis* Pellegrin, 1925. Holotype: MNHN 1925-0193. – *cardozoi*, *Barbus* Boulenger, 1912. Syntypes (partim): BMNH 1912.4.1.343–348 (6), 1912.4.1.349–352 (4), 1912.4.1.353 (1), MRAC 1528-29 (2), 1530-32 (3), ZMB 18810 (6). – *caudovittatus*, *Barbus* Boulenger, 1902. Syntypes: BMNH 1901.12.26.26 (1), MRAC 1168 (1). Synonyms: *Barbus euchilus* Boulenger, 1920. Holotype: BMNH 1919.7.24.7; *Barbus miochilus* Boulenger, 1920. Syntypes: BMNH 1919.7.24.8-9 (2), MRAC 6992 (1); *Varicorhinus stappersii* Boulenger, 1917. Syntypes: BMNH 1920.5.25.36-37 (2), MRAC 14197 (1), 14222 (2 instead of 1). – *clarkeae*, *Varicorhinus* Banister, 1984. Holotype: MRAC 164456. Paratype: MRAC 164457 (1). – *claudinae*, *Barbus* De Vos & Thys van den Audenaerde, 1990. Holotype: MRAC 86-01-P-501. Paratypes: MRAC 91755-756 (2), 92214 (1), 130310 (1),

85-44-P-141-144 (4), 85-44-P-269 (1), 85-44-P-281-289 (9), 86-01-P-502-508 (7), 86-09-P-444-451 (8), 87-11-P-1366-372 (7), 87-11-P-1373-384 (12). – *codringtonii*, *Barbus* Boulenger, 1908. Holotype: BMNH 1908.11.6.23. Synonyms: *Barbus altidorsalis* Boulenger, 1908. Holotype: BMNH 1908.11.6.26; *Barbus chilotes* Boulenger, 1908. Syntypes: BMNH 1908.11.6.24–25 (2). – *compiniei*, *Barynotus* Sauvage, 1879. Holotype: MNHN A-2845 (stuffed). Synonym: *Barbus labiatomimus* Pellegrin, 1914. Holotype: MNHN 1886-0395. – *crassibarbis*, *Barbus* Nagelkerke & Sibbing, 1997. Holotype: RMNH 32890 (*). – *dainellii*, *Barbus* Bini, 1940. Neotype: RMNH 32900 (*). – *dartevellei*, *Barbus* Poll, 1945. Holotype: MRAC 47781. – *dimidiatus*, *Varicorhinus* Tweddle & Skelton, 1998. Holotype: SAIAB 53080. Paratypes: SAIAB 53079 (15), SAIAB 53083 (21). – *ensifer*, *Varicorhinus* Boulenger, 1910. Syntypes: BMNH 1910.11.28.134–143 (10), NMW 48864 (10), ZMB 18213 (8). – *ensis*, *Barbus* Boulenger, 1910. Syntypes (partim): BMNH 1911.6.1.11–18 (8), NMW 54083 (4), ZMB 18217 (4). – *ethiopicus*, *Barbus* Zolezzi, 1939. Holotype: Not seen. Specimens: BMNH 1971.7.12.1–3 (3). – *fasolt*, *Barbus* Pappenheim, 1914. Holotype: ZMB 19061. – *fimbriatus*, *Varicorhinus sandersi* Holly, 1926. Syntypes (partim): NMW 7224–7226 (3). – *fritschii*, *Barbus* Günther, 1874. Syntypes: BMNH 1874.1.30.27–31 (5). Synonyms: *Capoeta atlantica* Boulenger, 1902. Syntypes: BMNH 1902.1.4.18–19 (2); *Barbus paytonii* Boulenger, 1911. Syntypes: BMNH 1903.10.29.17–20 (7 instead of 4); *Barbus riggenbachi* Günther, 1902. Syntypes: BMNH 1902.7.28.19 (1), 1902.7.28.20–21 (2); *Barbus rothschildi* Günther, 1901. Syntypes: BMNH 1901.4.26.6–7 (2); *Capoeta waldoi* Boulenger, 1902. Syntypes. BMNH 1902.1.4.16–17 (2). – *gananensis*, *Barbus* Vinciguerra, 1895. Holotype: MSNG 17525 (*). Paratypes: MSNG 17331 (4) (*). – *gestetneri*, *Barbus* Banister & Bailey, 1979. Holotype: BMNH 1976.10.12.98. Paratypes: BMNH 1976.10.12.86–97 (12). – *girardi*, *Barbus* Boulenger, 1910. Syntypes (partim): BMNH 1911.6.1.31–36 (6), NMW-54137 (1), ZMB 18215 (1). – *gorgorensis*, *Barbus intermedius* Bini, 1940. Neotype: RMNH 32910 (*). – *gorguari*, *Barbus* Rüppell, 1835. Holotype: SMF 2586 (stuffed) (*). – *gruveli*, *Barbus* Pellegrin, 1911. Holotype: MNHN 1911-0040 (*). – *gulielmi*, *Barbus* Boulenger, 1910. Syntypes (partim): BMNH 1911.6.1.29–30 (2). – *habereri*, *Barbus* Steindachner, 1912. Holotype: NMW 7274. – *harterti*, *Barbus* Günther, 1901. Syntypes BMNH 1901.7.26.4–5 (2). – *huloti*, *Barbus* Banister, 1976. Holotype: IRSNB 558. Paratypes: BMNH 1975.4.30.1 (1), IRSNB 563 (10). – *humphri*, *Barbus* Banister, 1976. Holotype: IRSNB 559. Paratypes: BMNH 1975.4.30.2 (1), IRSNB 564 (10). – *intermedius*, *Barbus* Rüppell, 1835. Holotype: SMF 6778 (stuffed) (*). Synonyms: *Capoeta bingeri* Pellegrin, 1905. Holotype: MNHN 1905-0252; *Barbus gudaricus* Boulenger, 1906. Syntypes: BMNH 1908.1.20.131–132 (6 instead of 2), 1908.1.20.133 (1); *Barbus leptosoma* Boulenger, 1902. Syntypes: BMNH 1902.12.13.300–302 (3); *Barbus macmillani* Boulenger, 1906. Syntypes: BMNH 1908.1.20.103–106 (4), 1937.4.20.68 (1) [a subsequently catalogued syntype (J Maclaine, pers. comm., 2015)]; *Barbus plagiostomus* Boulenger, 1902. Syntypes: BMNH 1902.12.13.271–272 (2), BMNH 1902.12.13.273 (1). – *iphthimostoma*, *Varicorhinus* Banister & Poll, 1973. Holotype: MRAC 179736. Paratypes: BMNH 1972.10.2.2 (1), IRSNB 644 (1), MRAC 179737 (1). – *iturii*, *Barbus* Holly, 1929. Holotype: NMW (lost). – *jaegeri*, *Varicorhinus* Holly, 1930. Holotype: NMW 13957. – *johnstonii*, *Barbus* Boulenger, 1907. Holotype: BMNH 1897.6.9.280. Synonyms: *Barbus globiceps* Worthington, 1933. Syntypes: BMNH 1932.11.15.275–282 (8); *Barbus njassae* Keilhack, 1908. Syntypes: ZMB 18163 (1); 18164 (1); *Varicorhinus nyasensis* Worthington, 1933. Syntypes: BMNH 1932.11.15.387–392 (6), 1932.11.15.393–395 (3). – *jubae*, *Varicorhinus* Banister, 1984. Holotype: BMNH 1976.7.1.13. Paratypes: BMNH 1976.7.1.14–15 (2). – *jubbi*, *Barbus* Poll, 1967. Holotype: Not seen. Paratypes (partim): MRAC 161065 (ex MD 2299) (1), 161066 (ex MD 6363) (1). – *kimberleyensis*, *Barbus* Gilchrist & Thompson, 1913. Holotype: SAIAB 134771 (ex SAM 9645). – *lagensis*, *Barynotus* Günther, 1868. Holotype: BMNH 1866.3.8.12. – *latirostris*, *Barbus intermedius* Keilhack, 1908. Lectotype: ZMB 18174. Paralectotype: ZMB 34766. *leleupanus*, *Varicorhinus* Matthes, 1959. Holotype: MRAC 92213. Paratypes: MRAC 92211 (1), 92212 (1), 130532–33 (2). – *longidorsalis*, *Varicorhinus* Pellegrin, 1935. Holotype: MNHN 1935-0065. – *longifilis*, *Barbus altianalis* Pellegrin, 1935. Lectotype: MNHN 1935-0150. Paralectotypes: 1935-0145 (1), 1935-0146 (1), 1935-0147 (1), 1935-0148 (1), 1935-0149 (1), 1935-0151 (1), 1935-0152 (1). – *longissimus*, *Barbus* Nagelkerke & Sibbing, 1997. Holotype: RMNH 32930 (*). – *lucius*, *Barbus* Boulenger, 1910. Syntypes: BMNH 1911.6.1.42–43 (2), NMW 54246 (1). – *lufupensis*, *Varicorhinus* Banister & Bailey, 1979. Holotype: BMNH 1975.9.5.1. Paratype: BMNH 1975.9.5.2 (1). – *macroceps*, *Barbus* Fowler, 1936. Holotype: ANSP 65759 (*). – *macrolepidotus*, *Varicorhinus* Pellegrin, 1928. Syntypes: MNHN 1928-0011 (1), MRAC 19945 (1), 138767 (1; ex MNHN 1928-0012), NMB 3983 (1), 3985 (1), 3988 (1), 3989 (1). – *macrolepis*, *Barbus* Pfeffer, 1889. Lectotype: ZMH H330 (ex 68 79). Paralectotypes: BMNH 1909.2.25.8 (ex ZMH)(1), ZMB 31672 (ex ZMH)(2), ZMH H331 (ex 68 79)(2), H332 (ex 380/7467)(4), H333 (ex 69 19)(1), H474 (ex 69 03)(1). – *macrophtalmus*, *Barbus gorguarii* Bini, 1940. Neotype: RMNH 32940 (*). – *malacanthus*, *Barbus* Pappenheim, 1911. Holotype: ZMB 18392. – *marequensis*, *Barbus* (*Cheilobarbus*) Smith, 1841. Holotype: BMNH 1845.7.3.95 (stuffed). Synonyms: *Barbus brucii* Boulenger, 1907. Holotype: BMNH 1907.3.15.34; *Varicorhinus nasutus* Gilchrist & Thompson, 1911. Holotype: SAIAB 134736 (ex SAM

8801); *Barbus oliphanti* Keilhack, 1910. Holotype: BMNH 1907.3.15.37; *Barbus sector* Boulenger, 1907. Holotype: BMNH 1907.3.15.35; *Barbus zambezensis* Peters, 1852. Syntypes: NMW 49730 (1); ZMB 3246 (2), 4744 (7). – *mariae*, *Varicorhinus* Holly, 1926. Syntypes: NMW-7221 (1), 7222–7223 (2). – *maroccana*, *Pterocapoëta* Günther, 1902. Syntypes: BMNH 1902.7.28.37–38 (2), 1902.7.28.39 (1). – *matris*, *Barbus* Holly, 1928. Holotype: NMW 8000. – *mawambi*, *Barbus* Pappenheim, 1914. Holotype: ZMB 19062. – *mawambiensis*, *Barbus hindii* Steindachner, 1911. Syntypes: NMW 54177 (2), 54286 (3), 54287 (2), 54288 (2). – *mbami*, *Barbus perplexicans* Holly, 1927. Holotype: NMW 7528. – *megastoma*, *Barbus* Nagelkerke & Sibbing, 1997. Holotype: RMNH 32950 (*). – *micronema*, *Barbus* Boulenger, 1904. Syntypes: BMNH 1904.2.29.37–38 (2). – *mirabilis*, *Barbus* Pappenheim, 1914. Holotype: ZMB 19059. – *mungoensis*, *Barbus* Trewavas, 1974. Holotype: BMNH 1973.5.14.163. Paratypes: BMNH 1973.5.14.164–182 (19). – *nanningsi*, *Labeobarbus* de Beaufort, 1933. Holotype: ZMA 113010. – *natalensis*, *Barbus* de Castelnau, 1861. Syntypes: lost. Synonyms: *Barbus bowkeri* Boulenger, 1902. Syntypes: BMNH 1862.8.28.3–7 (5), 1874.3.5.1–2 (2), 1894.7.10.4 (1) [Note: BMNH 1862.8.28.3–7 non-conspecific syntypes of *Enteromius gurneyi* (Günther, 1868)]; *Barbus lobochilus* Boulenger, 1911. Holotype: BMNH 1908.12.28.96; *Barbus mfongosi* Gilchrist & Thompson, 1913. Holotype: SAIAB 135057 (ex SAM 11392); *Barbus robinsoni* Gilchrist & Thompson, 1913. Holotype: SAIAB 135055 (ex SAM 11371); *Barbus zuluensis* Gilchrist & Thompson, 1913. Holotype: SAIAB 134939 (ex SAM 10745). – *nedgia*, *Labeobarbus* Rüppell, 1835. Holotype: SMF 2619 (stuffed) (*). – *nelspruitensis*, *Varicorhinus* Gilchrist & Thompson, 1911. Syntypes: SAIAB 134824 (ex SAM MB-F010518)(1), 135756 (ex SAM MB-F021698)(1). – *nthuwa*, *Labeobarbus* Tweddle & Skelton, 2008. Holotype: SAIAB 39341. Paratypes: SAIAB 39293 (1), 40787 (6), 51928 (2), 79494 (2). – *osseensis*, *Barbus* Nagelkerke & Sibbing, 2000. Holotype: RMNH 33721 (*). – *oxyrhynchus*, *Barbus* Pfeffer, 1889. Lectotype: ZMH H339 (ex 69 07). Paralectotypes: ZMH H340 (ex 69 07)(7). Synonyms: *Barbus babaulti* Pellegrin, 1926. Holotype: MNHN 1926-0285; *Barbus hindii* Boulenger, 1902. Syntypes (partim): BMNH 1902.5.26.25–28 (4); *Barbus* (*Labeobarbus*) *labiatus* Boulenger, 1902. Holotype: BMNH 1902.5.26.37; *Barbus perplexicans* Boulenger, 1902. Syntypes: 1902.5.26.35-35 (2); *Barbus tanensis* Günther, 1894. Syntypes (partim): BMNH 1893.12.2.24–29 (6), 1893.12.2.32–34 (3), BMNH 1893.12.2.37–39 (3). – *pagenstecheri*, *Barbus* Fischer, 1884. Lectotype: ZMH H341 (ex ZMH 3851). Paralectotypes: ZMH H342 (ex ZMH 3850)(1). – *parawaldroni*, *Barbus* Lévêque, Thys van den Audenaerde & Traore, 1987. Holotype: MRAC 73-10-P-2296. Paratypes: MRAC 73-10-P-2297 (1), 73-10-P-2298-2299 (2), 73-5-P-1936 (1). – *paucisquamata*, *Barbus altianalis* Pellegrin, 1935. Lectotype: MNHN 1935-0076. Paralectotypes: MNHN 1935-0077 (1), 1935-0078 (1), MRAC 42932 (1). Synonyms: *Barbus altianalis lobogenysoides* Pellegrin, 1935. Holotype: MNHN 1935-0154. – *pellegrini*, *Varicorhinus* Bertin & Estève, 1948. Holotype: MNHN 1932-0181. – *petitjeani*, *Barbus* Daget, 1962. Syntypes: MNHN 1959-0108 (3). – *platydorsus*, *Barbus* Nagelkerke & Sibbing, 1997. Holotype: RMNH 32970 (*). – *platyrhinus*, *Barbus* Boulenger, 1900. Holotype: BMNH 1906.9.6.12. – *platystomus*, *Varicorhinus* Pappenheim, 1914. Syntypes: ZMB 19051 (1), ZMB 34769 (1). – *pojeri*, *Barbus* Poll, 1944. Holotype: IRSNB 71. Paratype: IRSNB 599 (1). – *polylepis*, *Barbus* Boulenger, 1907. Holotype: BMNH 1907.3.15.33. – *progenys*, *Barbus* Boulenger, 1903. Holotype: BMNH 1902.11.12.127. – *pungweensis*, *Varicorhinus* Jubb, 1959. Holotype: SAIAB 120014 (ex AMG 850). Paratypes: SAIAB 120015 (ex AMG 851)(1), 120016 (ex AMG 852)(1), 120017 (ex AMG 853)(1), 120018 (ex AMG 854)(1). – *reinii*, *Barbus* Günther, 1874. Syntypes (partim): BMNH 1874.1.30.22–24 (3). – *rhinoceros*, *Barbus* Copley, 1938. Types?: BMNH 1936.12.22.35–39. Synonyms: *Barbus mariae* Holly, 1929. Lectotype: NMW 96552 (ex 6562)(designated by Banister, 1973). Paralectotypes: NMW 96553 (ex 6562)(1). – *rhinophorus*, *Barbus* Boulenger, 1910. Syntypes: BMNH 1911.6.1.37–38 (2). – *robertsi*, *Varicorhinus* Banister, 1984. Holotype: BMNH 1983.3.30.20. Paratypes: BMNH 1983.3.30.21-38 (18). – *rocadasi*, *Barbus* Boulenger, 1910. Syntypes (partim): BMNH 1911.6.1.19–20 (2), 1911.6.1.21–25 (6 instead of 5), 1911.6.1.27–28 (2), NMW 13347–13352 (6), ZMB 18214 (6). – *rosae*, *Barbus* Boulenger, 1910. Syntypes: BMNH 1911.6.1.39–41 (3), NMW 54500 (1), ZMB 18216 (1). – *roylii*, *Barbus* Boulenger, 1912. Syntypes (partim): BMNH 1912.4.1.338–340 (3), 1912.4.1.341–342 (2), MRAC 1533 (1), 1534 (1), 1535 (1), NMW 54501 (1), ZMB 18809 (1). – *ruandae*, *Varicorhinus* Pappenheim, 1914. Holotype: ZMB 19050. – *ruasae*, *Barbus* Pappenheim, 1914. Lectotype: ZMB 19053. Paralectotype: ZMB 22652 (1). – *ruwenzorii*, *Capoëta* (*Pterocapoëta*) Pellegrin, 1909. Syntypes: MNHN 1909-0583 (1), 1909-0584 (1), 1909-0585 (1). – *sacratus*, *Barbus* Daget, 1963. Syntypes: MNHN 1959-0119 (4), 1959-0139 (1). – *sandersi*, *Varicorhinus* Boulenger, 1912. Lectotype: BMNH 1912.4.1.333. Paralectotypes: BMNH 1912.4.1.334–336 (2 instead of 3), 1912.4.1.337 (1), MRAC 1525 (1), 1526 (1), 1527 (1), ZMB 18808 (1). – *seeberi*, *Barbus* Gilchrist & Thompson, 1913. Syntypes: BMNH 1936.8.4.6 (1), SAIAB 134867 (ex SAM 10672)(2). Specimens: SAIAB 54133 (1), 54688 (1), 58362 (1), 58418 (1), 65536 (6). – *semireticulatus*, *Varicorhinus* Pellegrin, 1924. Syntypes: MNHN 1924-0052 (2). – *somereni*, *Barbus* Boulenger, 1911. Holotype: BMNH 1911.7.26.1. – *stappersii*, *Barbus* Boulenger, 1915. Holotype: MRAC 14250. Synonyms: *Barbus curtus* Boulenger, 1915.

Holotype: MRAC 14172; *Barbus moeruensis* Pellegrin, 1922. Holotype: MRAC 14765; *Barbus oxycephalus* Boulenger, 1915. Holotype: MRAC 14233 (Note: not MRAC 14113 as in Lévêque & Daget, 1984; Eschmeyer, 2015). – *steindachneri*, *Varicorhinus* Boulenger, 1910. Syntypes (partim): BMNH 1910.11.28.147–155 (9), 1911.6.1.1–5 (5), NMW 48867 (3), ZMB 18212 (3). – *stenostoma*, *Varicorhinus* Boulenger, 1910. Holotype: BMNH 1910.11.28.145. – *surkis*, *Barbus* Rüppell, 1835. Neotype: RMNH 32980 (*). – *tornieri*, *Varicorhinus* Steindachner, 1906. Holotype: NMW 46053. – *trachypterus*, *Barbus* Boulenger, 1915. Holotype: MRAC 11830. Synonym: *Varicorhinus bredoi* Poll, 1948. Holotype: IRSNB 76. Paratypes: IRSNB 77 (2). – *tropidolepis*, *Barbus* Boulenger, 1900. Lectotype: BMNH 1906.9.6.19. Paralectotypes: BMNH 1906.9.6.20–21, MRAC 327 (1), 328 (1), 329 (1). Synonym: *Varicorhinus chapini* Nichols & La Monte, 1950. Holotype: AMNH 18785. – *truttiformis*, *Barbus* Nagelkerke & Sibbing, 1997. Holotype: RMNH 32990 (*). – *tsanensis*, *Barbus* Nagelkerke & Sibbing, 1997. Holotype: RMNH 33000 (*). – *upembensis*, *Varicorhinus* Banister & Bailey, 1979. Holotype: BMNH 1975.9.5.5. Paratypes: BMNH 1975.9.5.6–12 (7), 1975.9.5.13–17 (5). – *urotaenia*, *Barbus* Boulenger, 1913. Syntypes: BMNH 1912.12.6.5 (1), MRAC 1791 (1), 1792 (2). – *varicostoma*, *Varicorhinus* Boulenger, 1910. Holotype: BMNH 1910.11.28.146. – *versluysii*, *Barbus* Holly, 1929. Syntypes: NMW 13954–13956 (3). – *werneri*, *Varicorhinus* Holly, 1929. Syntypes: NMW 13948 (1). – *wittei*, *Varicorhinus* Banister & Poll, 1973. Holotype: MRAC 179738. Paratypes: BMNH 1972.10.2.3 (1), MRAC 179739–741 (3), 179742 (1), IRSNB 645 (1). – *wurtzi*, *Barbus* Pellegrin, 1908. Holotype: MNHN 1908-0097. Specimens cf. *wurtzi* [undescribed species (see text)]: MRAC 1986-13-P-114; MNHN 1959-0153 (2) (smallest of both: 127.5 mm SL), MNHN 1987-0689 (3), 1988-1955(3) (both largest specimens: 172.5 and 175.6 mm SL), 1991-0519. – *xyrocheilus*, *Varicorhinus* Tweddle & Skelton, 1998. Holotype: SAIAB 53082. Paratypes: SAIAB 34336 (11).

African nominal *Labeobarbus s.l.* species identified as hybrid phenotypes: list of examined specimens

alluaudi, *Barbus* Pellegrin, 1909. Syntypes: MNHN 1909-0586 (1), 1909-0587 (1). – *microbarbis*, *Barbus* David & Poll, 1937. Holotype: MRAC 41847. Paratypes: MRAC 41848–49 (2). – *microterolepis*, *Barbus* Boulenger, 1902. Holotype: BMNH 1902.12.13.220.

Acapoeta and *Sanagia*: list of examined specimens

tanganicae, *Capoeta* Boulenger, 1900. Lectotype: BMNH 1906.9.6.9. Paralectotype: BMNH 1906.9.6.10 (1). – *velifera*, *Sanagia* Holly, 1926. Syntypes: NMW 7261–7262 (2).

Non-*Labeobarbus s.l.* species: list of examined specimens

capensis, *Barbus* Smith, 1841. Not a *Labeobarbus* species (E. Vreven, E.R. Swartz & P.H. Skelton, unpubl. data). Holotype: BMNH 1845.7.3.99 (stuffed). – *capoetoides*, *Varicorhinus* Pellegrin, 1938. Not a *Labeobarbus* species (see present paper). Holotype: MNHN 1938-0030. – *litamba*, *Barbus* Keilhack, 1908. Not a *Labeobarbus* species (see present paper). Holotype (unique): ZMB 18162.

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