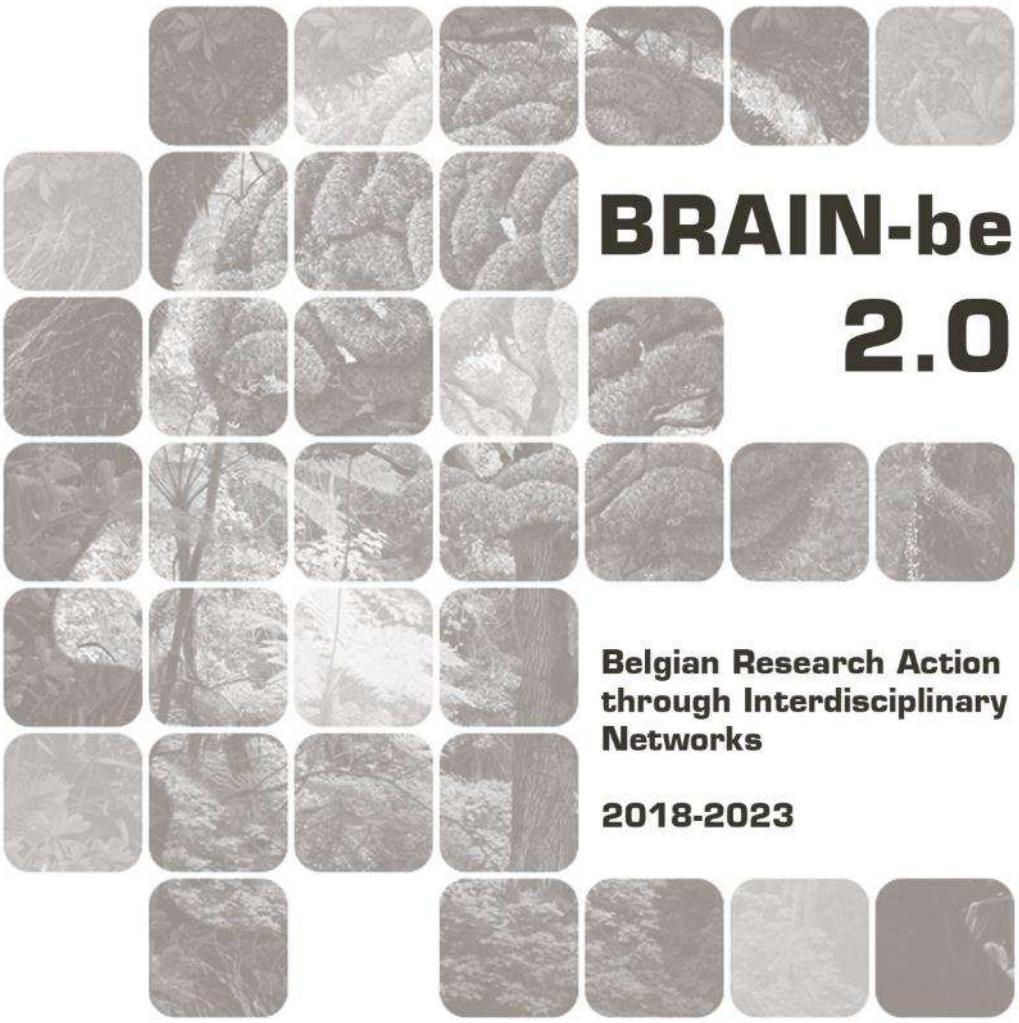

KEAFish

The biodiversity, biogeography and evolutionary history of the northern basins of the Great African Lakes: the enigmatic fish faunas of Lakes Kivu, Edward and Albert revisited.

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NETWORK PROJECT

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Contract - B2/202/P1/KeaFish

FINAL REPORT

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TABLE OF CONTENTS

ABSTRACT

Context: The ichthyofauna of the region of lakes Kivu, Edward and Albert (KEA) is poorly known. At the same time, it is one of the most enigmatic regions because of its complex ichthyo-geography as a result of its turbulent tectonic history and fluctuating climatic cycles.

Objectives: We wanted to evaluate to what extent geological and climatic events have shaped the ichthyo-diversity of the region. Via a multidisciplinary approach with morphometrics, DNA barcoding and genomics we studied the ichthyo-diversity and their evolutionary history.

Conclusions: A baseline barcoding study of the Lake Edward ichthyofauna and an account to the fishes from the Nyungwe National Park region were published. An annotated checklist and barcoding study for the Lake Albert system was prepared. Several Edward haplochromines have been (re)described and many examples of convergent evolution were found. Undescribed species diversity with a complex evolutionary history was found within *Enteromius*. A genomic approach to *C. gariepinus* and *O. niloticus* confirmed a geographic clustering pattern for the former but not for the latter. We did not find evidence for a general Out-of-Kivu hypothesis nor traces of the region acting as a refuge during megadroughts. Some new bio-geographic hypotheses have been proposed. The fish fauna was found to be threatened by overfishing and introductions.

Keywords: East Africa, fish diversity, barcoding, Lake Edward, biogeography.

1. INTRODUCTION

The region of the northern East African rift-valley lakes Kivu, Edward and Albert (KEA) is one of the most enigmatic regions because of its complex ichthyo-geography. It is situated at the intersection of three major ichthyo-geographic provinces (Nilo-Sudan, East Coast and Congo), featured a turbulent tectonic history and likely acted as a species reservoir during recent climatic changes, such as the major drought some 15.000 years ago. With a diversity of techniques ranging from classical taxonomic morphometry and molecular methods to biogeographical analyses and state-of-the-art genomics, we attempt to characterise the diversity and evolution of the understudied fish fauna of this region in order to evaluate the importance of the KEA lakes as origins and/or refuges in shaping the ichthyodiversity of the region.

2. STATE OF THE ART AND OBJECTIVES

Note: this state of the art concerns the situation at the start of the project and does not contain the new insights published by the project in the meantime.

Biogeographic framework: Ichthyo-geographic regions in Africa are large areas with similar fish species and genera, which differ from other such regions. The larger regions can then be subdivided in smaller regions (e.g. Thieme et al., 2005). Such areas form the basis for regional studies on fish communities and reflect, to a large extent, the geological history of the basins and their abiotic variables. On various scales, a well-defined ichthyo-geographic framework complements phylogenetic hypotheses and provides premises for evolutionary studies. On a small scale, ichthyo-geographic areas form the basis for conservation studies and sustainable management programmes. Specifically in Africa, they also provide the template to delineate areas of extraordinary species richness, endemism or threats (Thieme et al., 2005; Snoeks, et al., 2011; Kishe-Machumu et al., 2018).
One of the major hanging issues in the biogeography of African fishes, if not the most enigmatic, is the status of the basins of Lakes Kivu, Edward/George and Albert (KEA) as drivers for the fish diversity in the wider region. While hydrologically, Lake Kivu drains to the Congo and Lakes Edward and Albert to the Nile system, the two former lakes belong, from an ichthyo-geographical point of view, to the East Coast Province, while Lake Albert is part of the Nilo-Sudan Province (Snoeks et al. 1997). The situation of the Lake Edward system is special; it is connected to Lake Albert via its outlet, the Semliki, and hence part of the Nile Basin, but at the same time it is not fully separated from the Lake Victoria system (East Coast Province) by marshy areas on the Katonga and Ruizi Rivers (Decru et al., 2019). Pro and contra-arguments against the allocations of these basins to their respective bio-geographic provinces have been ventilated in the literature (e.g. Lévêque, 1997; Witte et al., 2009; Snoeks & Getahun, 2013; Decru et al., 2019). In an alternative attempt to define meaningful freshwater ecoregions in Africa, Lakes Kivu and Edward were placed in a separate Lake Victoria basin ecoregion, while Lake Albert was placed in the Upper Nile (Thieme et al., 2005; Abell et al., 2008).

The KEA lakes: cichlids and non-cichlids: Each of these three lakes harbours **an endemic assemblage of haplochromine cichlids** (Snoeks, 2000; Vranken et al., 2018, 2019, 2020), the evolutionary history of which is poorly known, certainly compared to those of the three major lakes: Victoria, Tanganyika and Malawi. Indeed, because of their explosive speciation and adaptive radiation, the cichlid faunas of the three major lakes are, for decades already, at the forefront of evolutionary research in vertebrates. The faunas of the smaller lakes are less studied, but because the systems are less complex and less species rich, may provide better opportunities to study certain evolutionary mechanisms (Vranken et al., 2019). Lake Kivu harbours some 15 haplochromine species (Snoeks, 1994) and Lake Edward an estimated 60 species (Vranken et al., 2018, 2019, 2020); currently only six valid species are known from the Lake Albert region though the estimated number may be up to twenty or more. In the three lakes, one to three native tilapias occur. In contrast to the haplochromine cichlids these are not endemic to the individual lakes.

Finally, and to complete the picture on cichlids, in the region two non-endemic taxa occur of the genera *Pseudocrenilabrus* and *Astatoreochromis*.

Even less attention is paid to **the non-cichlid fishes** of the KEA lake systems. However, because of the high endemism of the haplochromine cichlids (and therefore their distribution data are not informative), biogeographical considerations concerning the basins of these lakes are mainly based on the non-cichlids (Snoeks et al. 1997; Decru et al., 2019). As little progress has been made on the taxonomy, phylogeny and distribution patterns of these taxa during the last decennia, these considerations are essentially based on older taxonomic studies, for some groups even dating back to the book on the fishes of Uganda by Greenwood in 1966 (but see Maetens et al., 2020 for a recent contribution). In the Lake Kivu basin eight native non-cichlids occur (Snoeks et al., 2012); in the Lake Edward system 29 (Decru et al., 2019). For Lake Albert, between 37 and 45 species are recorded (Snoeks, 2000; Witte et al., 2009; Wandera & Balirwa, 2010). Several species and genera occur in two or all three lakes targeted, and in neighbouring basins, which makes them excellent target groups for our study (see below).

Numerous paleo-geological and paleo-climatic events have affected the African fish fauna and shaped their distribution. This is especially the case in the **African Rift valley** that has been influenced by major tectonic disruption, volcano eruptions and extreme drought. Indeed, an important and inconvenient truth in all studies on the evolutionary history of the fishes of this region is the report that **Lake Victoria dried up completely about 15.000 years BP** and that the region may have been without perennial river systems (Johnson et al., 1996; Stager & Johnson, 2008). From a biologist's point of view this is hard to reconcile with the presence of about 600 endemic haplochromine species in the lake (Verheyen et al., 2003; 2004) and the current distribution patterns and endemism of non-cichlids in the region. Even if the desiccation was not complete, higher and wetter areas in the region, probably including the deeper KEA lakes, will have acted as refuges for many species. Therefore, we hypothesise that these currently relatively species-poor regions of the KEA basins contained the genetic and taxic diversity that seeded **the extremely species-rich ichthyodiversity in the Lake Victoria basin**. Given the depth of these lakes, and given the high altitude of the basins and therefore cooler and wetter conditions, we can assume that they still contained all major freshwater habitats at a time when East Africa underwent profound droughts. Although the role of refuges in seeding biodiversity is well known for temperate zones, and although some studies looked at the role of refuges in explaining the current distribution of tropical terrestrial vertebrates (Hewitt, 2004), their role in explaining the biodiversity of tropical freshwater fishes has remained largely overlooked. This makes our study even more challenging and innovative.

Out-of-Kivu hypothesis: The position of Lake Kivu is special. While it is currently the most species-poor lake in the region, Lake Kivu is considered as the **centre of origin** of the about 700 species of haplochromine cichlids of the Lake Victoria Region Superflock (LVRS) (Verheyen et al., 2003; Elmer et al., 2009). From Lake Kivu, the ancestors dispersed into the various lakes and rivers in the KEA region. This scenario is partially in conflict with current ichthyo-geographic hypotheses and geological history scenarios. In addition, recent data pointing at a hybrid origin of the LVRS (Meier et al., 2017), suggested a more complicated scenario involving an ancient lineage from the Congo and one from the

Upper Nile (including *H. gracilior* from Lake Kivu and *H. pharyngalis* from Lake Edward). Therefore the evolutionary history of the haplochromines from the KEA lake basins is, most likely, even **more complicated than a simple out-of-Kivu hypothesis**. The out-of-Kivu scenario may also hold (partially) for *Clarias gariepinus*, as suggested by a cytb (mtDNA) analysis by Van Steenberge et al. (2020). This widespread catfish species is the only African freshwater fish with a near-continental distribution. Molecular clocks revealed that its spread throughout Africa could be linked to changes in climate and hydrology, but details on the KEA area are lacking. Studies on fish fossils have shown that Lakes Edward and Albert previously had much more similar fish faunas with Nilotic affinities. However, historic dry periods and volcanism have caused extinctions in large portions of the original faunas, probably also in the Lake Kivu fauna (Snoeks, 1994). Therefore, we use very **diverse fish groups** that are common to the basins of the three KEA lakes, to test the out-of-Kivu hypothesis: the highly adapted, specialised and recently speciated haplochromine cichlids; two wide-spread groups with relatively large species living in lakes and rivers (the tilapia cichlid *Oreochromis niloticus* and the two *Clarias* catfish, *C. gariepinus* and *C. liocephalus*), and a species-rich cyprinid genus with small-size representatives that mostly live in rivers (*Enteromius*).

Research hypothesis and objectives

Taken into account the (partial) out-of-Kivu origin for haplochromine cichlids and the suggestion of the existence of refuges in the region during the mega-drought causing the desiccation of the Lake Victoria region some 15.000 yrs BP, we hypothesise that both phenomena (Lake Kivu as a source, and the KEA area as a refuge) shaped to a large extent the ichthyo-diversity of the region. The overall objective of this project is to evaluate this hypothesis. Therefore we will first study the fish fauna, characterise its taxonomic and genetic diversity and revise some problematic key groups. This will provide the necessary data for a molecular phylogeography for some non-cichlid taxa of the area. However, we will need much more fine-grained data to fully test the hypothesis. Therefore we will use whole genome sequencing on some selected taxa (cichlids and non-cichlids).

3. METHODOLOGY

Fieldwork and specimens collected

The core of the study area encompasses the basins of the northern lakes of the western Rift Valley in Africa: Kivu, Edward and Albert, but in our research we sometimes had to extend this area to include the neighbouring basins and even to the whole of Africa. For more information on these basins, see above at the biogeographic framework.

To a large extent, specimens from the collections made during the HIPE (Brain) project were used for morphometric, molecular and genomic analyses.

Additional fish sampling was conducted by the **ISP-Bukavu team (DR Congo) within the Lake Kivu catchment** between October 2021 and September 2022, covering a total of eight campaigns. Three campaigns were carried out in the southern basin of Lake Kivu (Bukavu and Ishungu), four in the Ruzizi River, and one in the Lwiro influent. Sampling was extended to the Lwiro and the Ruzizi outlet to increase records of non-cichlid taxa in the catches. In Lake Kivu, fieldwork was conducted in both the littoral and pelagic zones. The Ruzizi River was explored from the lake outflow to the reservoir of the Ruzizi I dam, and further, downstream from the Ruzizi I dam (at the Ruzizi II bridge) to the reservoir of the Mumosho (Ruzizi II) dam.

Data collection methods were adapted to the size and characteristics of the waterbodies. In Lake Kivu and the Ruzizi River, multi-mesh gillnets (8, 10, 12, 20, 30, and 50 mm), each 30 m in length and 1.5 m in height, were used following the protocol of Kisekelwa et al. (2023). In contrast, cast nets were used in the Lwiro River basin, following Kisekelwa et al. (2020). Fishing activities in the Lwiro River basin and the littoral zone of Lake Kivu were conducted during daytime, whereas in the Ruzizi River and the pelagic zone of Lake Kivu, collections took place overnight. All specimens were identified to species level using taxonomic keys provided by Snoeks (1994) and Snoeks et al. (2012). A reference collection has been deposited at the Royal Museum for Central Africa.

Figure 1. Illustrations showing field activities in the Ruzizi reservoir (a), at the Ruzizi II bridge (b) and in the littoral zone of Lake Kivu (c &d) (Copyright T. Kisekelwa and W. Alimasi).

The **NaFIRRI team made a large and important collection, mainly of small barbs**, between June and September 2023 and sampled 46 localities within the basins of Lakes Edward, Albert and Victoria. This resulted in over 330 specimens of *Enteromius*. This proved to be a very valuable collection for the study on the biogeography of *Enteromius.*

Specimens were also collected during two summer schools in Rwanda, by respectively two and three Belgian team members. During the first one, fish were collected **in and near Nyungwe National Park,** in the Lake Kivu system and the Rusizi (Figure 2; Maetens et al., 2025); during the second one, collections were made **in the Akagera National Park** (Lake Victoria basin). During these expeditions, specimens were caught using gillnets, minnow traps, scoop net, backpack electrofisher or bought from local fishermen. All specimens were euthanised with an overdose of clove oil. For a selection of specimens, a fin clip was taken for molecular analyses and preserved in 100% ethanol. Specimens were fixed in 10% formalin. The relevant literature was checked to identify specimens on the field or in the laboratories of the Royal Museum for Central-Africa (RMCA) and Royal Belgian Institute for Natural Sciences (RBINS). Additionally, environmental samples (filtered water) were taken during the second survey from the same locations as where the nets were placed.

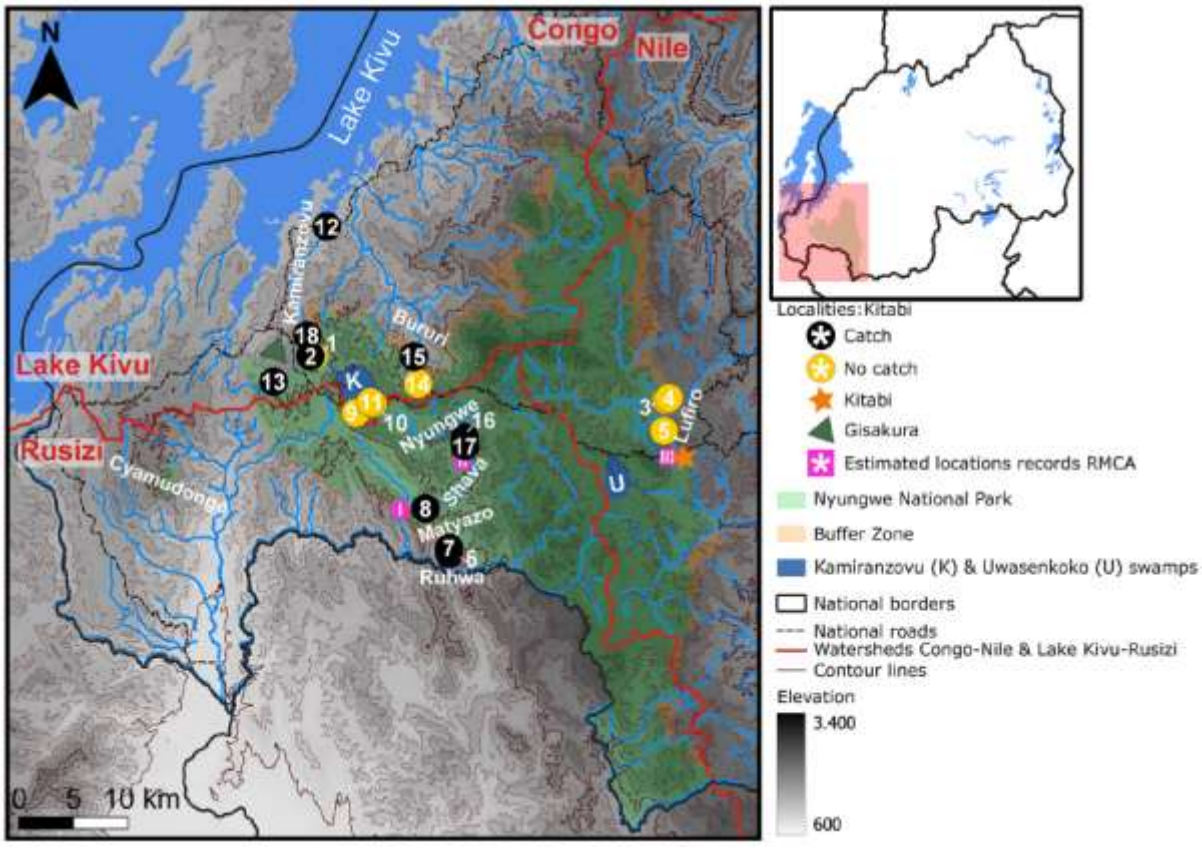

Figure 2: Hydrological map of the Nyungwe National Park (green) showing the sampling locations in Rwanda. Black circles denote sites where fishes were caught and yellow circles sites where, in spite of intensive sampling, no fish samples were retrieved. Roman numerals highlight the estimated localities of specimens of the historical collection of the RMCA (Maetens et al., 2025).

Samples from **the Mara wetlands** (Lake Victoria basin) were received via a collaboration with Dr. Ken Irvine and Dr. John Simaika, whose MSc student Leandro Antonio De La Cruz Alvares (Delft University, the Netherlands) did field work in the framework of the project 'Community-led fisheries management in the Mara Wetlands, Tanzania'.

WP1. Region-wide scan of non-haplochromines

DNA barcoding using the cytochrome c oxidase I (COI) gene, has proven to be an effective additional tool to morphology-based techniques for species identifications in many animal taxa, including African freshwater fishes. It has been successfully used in several regional studies of fish diversity (e.g. Decru et al., 2016; Sonet et al., 2019). Often, mismatches are discovered between morphology-based identifications and molecular results (Decru et al., 2016), resulting in the exposure of possible taxonomic problems. Barcoding is also a powerful tool to detect cryptic species (Van Ginneken et al., 2017).

For a successful application of barcoding as a identification tool, a **comprehensive database of COI** sequences needs to be available. In this project, we aimed at providing such a database. The team has been very well placed for this effort because it has a very large experience in fish taxonomy. This is necessary as the presence of correctly identified voucher specimens is a prerequisite for the database to be effective, especially for poorly known regions as the one studied during the project.
Different datasets were created for the lakes of the KEA-region. For the Lake Edward system, a dataset of 265 sequences (excluding *Enteromius* and *Haplochromis*) of the cytochrome oxidase I (COI) gene was available. For the Lake Albert basin, 260 barcodes (excluding *Enteromius* and *Haplochromis*) have been generated. For the Lake Kivu basin, we extracted and sequenced 70 samples (excluding *Enteromius* and *Haplochromis*) from the specimens collected during the field surveys in the course of the first two-week summer school in Rwanda. In addition, 30 barcodes (excluding *Enteromius* and *Haplochromis*) were generated form the specimens collected from the Lake Kivu basin by Alimasi Wilondja Ngalula. A total of 192 extractions were made (non-*Enteromius*) from the specimens collected during the second two-week summer school in the Akagera National Park. Of the Mara samples, 56 barcodes (non-*Enteromius*) were generated. Additionally, 150 barcodes from catfish species from Lakes Kivu and Tanganyika were also generated. These were collected during a UHasselt-RBINS PhD project.

For most species, COI sequences could be obtained using the universal primers of Ivanova et al. (2007), as detailed in Decru et al. (2022). For the genera *Labeobarbus, Enteromius, Brycinus* and (for some specimens of) *Alestes*, specialized primers were used from Sonet et al. (2019), which resulted in somewhat shorter sequences.
For the specific details on the methodology used to barcode the fish diversity of the Lake Edward system, we refer to Decru et al., 2022. Intra and interspecific distances were calculated to explore

barcoding gaps. In addition, identification success was assessed with three criteria: Best Match (BM), Best Close Match (BCM) and All Species Barcode (ASB) (Decru et al., 2022).

A total of 811 specimens of *Enteromius* from several sources were analysed genetically during the KEAFish project (Table I). In addition, collection material of the Royal Museum for Central-Africa (RMCA), Tervuren was examined and several tissues samples were donated by Dr. Ray Schmidt. The DNA barcoding study of *Enteromius* included three genetic markers: cytochrome oxidase I (COI, mtDNA), cytochrome b (Cytb, mtDNA) and recombination activating gene 1, exon 3 (RAG1, nDNA).

Table I. Overview of the specimens of *Enteromius* used for genetic analyses during the KEAFish project.

Location	**Project/Collection**	**Number of specimens**	**Collectors**	**Date**
Uganda (Lakes Albert, Edward and Victoria)	HIPE: RMCA_2016.036, RMCA_2017.006, RMCA_2018.008, RMCA_2019.002	453	Eva Decru, Jos Snoeks, Maarten Van Steenberge, Nathan Vranken	October 2016, March 2017, January 2018, March 2019
Uganda (Lakes Albert, Edward and Victoria)	KEAFish	142	Laban Musinguzi	August 2023, September 2023
Nyungwe National Park	KEAFish: RBINS_ IG34524	79	Heleen Maetens, Maarten Van Steenberge	August 2022
Akagera National Park	KEAFish: RBINS_ 34872	85	Heleen Maetens, Maarten Van Steenberge, Nathan Vranken	October 2023, November 2023
Lake Kivu	KEAFish	28	Alimasi Wilondja Ngalula	May 2019, June 2019
Mara wetlands	Community-led fisheries management in the Mara Wetlands, Tanzania	2	Leandro Antonio De La Cruz Alvares	September 2022,
Lake Victoria	RMCA_2019.016.P.0092-0093, RMCA_2019.016.P.0094-0139, RMCA_2019.016.P.0140-0141, RMCA_2019.016.P.0082-0086, RMCA_2019.016.P.0080-0081	15	Nagy B. et al.	May, 2019, June 2019

Kenya	FW/2342/1-5, FW/2325/1-9, FW/2278/1-3, FW/2289/1-6, FW/2273/1-101	7	Tissue samples donated by Ray Schmidt	2011

Extractions were made in the lab of the RMCA and the RBINS, using a 'NucleoSpin Tissue Kit' following the manufacturer's instructions (Macherey-Nagel, Germany).
For all specimens, the COI gene was isolated and amplified using the M13-tailed primer cocktail of Ivanova et al. (2007) and the protocol described by Decru et al. (2016). The amplification of the Cyt b gene for the specimens of *Enteromius* was done with the primers L14724 (5'- G A C T T G A A A A A C C A C C G T T G-3') and H15915 (5'- C T C C G A T C T C C G G A T T A C A A G A C-3') (Xiao et al., 2001), with the following temperature profile: 94 °C for 60 s (initial denaturation); 35 cycles of 94 °C for 30 s, 54 °C for 30 s, 72 °C for 90 s; 72 °C for 10 min (final extension). For the amplification of the nuclear marker RAG1, primers 2533 F (5'- C T G A G C T G C A G T C A G T A C C A T A A G A T G T-3') and 4090R (5'-CTGAGTCCTTGTGAGCTTCCATRAAYTT-3') were used, following the protocol from López et al. (2004). The amplified products were then visualised on a 1.2% agarose gel and purified using ExoSAP (Fermentas). Sequencing was executed bidirectionally by the company Macrogen. DNA sequences were assembled and visually checked in CodonCode Aligner (CodonCode Corporation) or Geneious 2024.0.5, aligned and further analysed with MEGA version 7.02 (Kumar et al., 2016) using Muscle Alignment (Edgar, 2004) or Geneious.

For all groups for which we obtained barcodes, we produced **Neighbour-Joining trees** incorporating all relevant published barcodes to evaluate barcode-based taxon diversity. First, the quality of the sequencing outputs were checked with Geneious Prime® (Biomatters Ltd. Auckland, New Zealand). A consensus sequence was generated for each specimen, and subsequently compared against the Identification System of BOLD, with Species Level Barcode Records option (www.boldsystems.org). COI consensus sequences were also used as queries to search for most similar sequences in GenBank (NCBI, National Centre for Biotechnology), using the Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).
Neighbour-Joining (NJ) trees (Tamura-Nei distance model, branch support assessed by 1,000 bootstrap replicates) were constructed to examine the clustering support of each identified species. To this end, generated sequences were first aligned with all publicly available COI sequences of species of its genus, or higher taxon level if required (see Figure legends), downloaded from BOLD and GenBank (www.ncbi.nlm.nih.gov/genbank), using MUSCLE in Geneious Prime®. Then duplicates (i.e., identical sequences) and sequences of less than 400 base pairs (bp) were discarded per species to limit the size of the database. Also, some misidentified sequences (i.e., blasting with high pairwise identity with sequences of species belonging to another genus) were also discarded. The final alignments were then trimmed to retain the 652 bp overlapping region, and sequences from sister taxa were included to root the NJ trees. Clustering of the generated sequences in relation to the other species in the dataset were examined. To this end, generated sequences were relabeled as follow: " '*Specimen code*'**_**'*morphological species identification*', GB: '*best species match on GenBank*'**,** '*sampling location*'".

The KEAfish team and our collaborators at IHE Delft, performed fieldwork at two wetlands in the Lake Victoria system: those of the Mara and Akagera river (Figure 3).

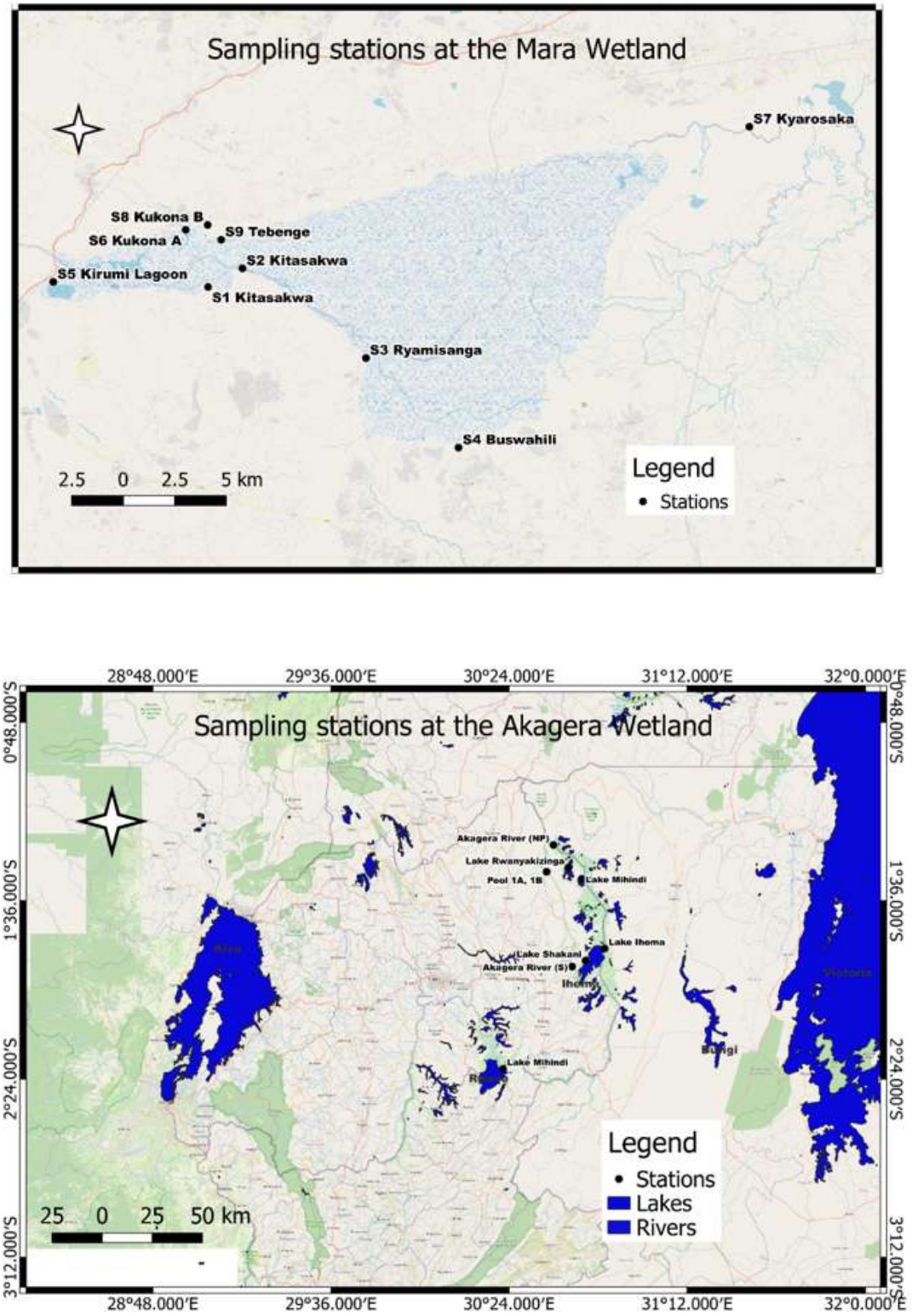

Figure 3: Distribution of sampling stations at the Mara and Akagera wetlands (image, Melvin Odiba, source: QGIS 3.30.3)

In order to assess the completeness of our sampling, traditional biodiversity monitoring methods (e.g., gill nets, morphological identification) were complemented with **environmental DNA (eDNA) metabarcoding approaches.** These offer a non-invasive, efficient alternative by detecting genetic material left by organisms in water, enabling comprehensive biodiversity assessments. eDNA metabarcoding was applied to 36 water samples collected from 22 sites in the Akagera National Park in Rwanda (9 sites) and the Mara Wetland in Tanzania (13 sites). Assessment of fish and vertebrate biodiversity was performed by amplifying part of the 12S mitochondrial gene and using two sets of primers: 12SV5 (targeting all vertebrates) and MiMammal (targeting mammals). We also barcoded over 200 fish specimens collected at the same locations using experimental gill nets for comparison.

One of the project's aspirations was to be able to get **genetic data from important museum collection specimens**. This would make it possible to cover species that are rare or for which it would not be possible, in the framework of the project, to acquire fresh tissue material. DNA extractions from such specimens, exposed to formalin to varying degrees and often preserved already for a long time in denatured alcohol is very challenging. We tested this on West-African *Enteromius* species in order to evaluate at the same time whether also in this region unrecognized diversity occurs as is found in the KEA region. We used an adapted protocol of Diedericks (2017) to obtain DNA extractions and then applied the protocol of Shokralla et al., (2015), to produce universal COI mini-barcodes (Figure 4).

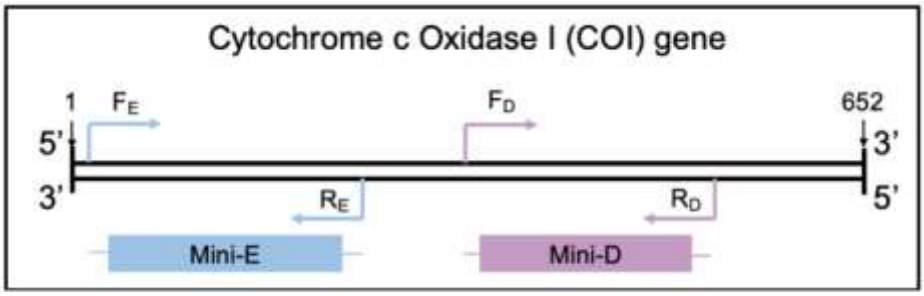

Figure 4: Schematic representation of the regions in the Cytochrome c Oxidase I (COI) gene (652bp) amplified by the mini barcodes: Mini-E and Mini-D. F = forward primer; R = reverse primer (from Bosmans, 2023, adapted from Shokralla et al., (2015)).

WP2. Diversity of non-haplochromines

As annotated checklists to the fishes of the Lakes Kivu and Edward basins were already published (De Vos et al., 2001; Decru et al., 2019), we concentrated our work on **the fish diversity of the Lake Albert system**. For this, we identified old and new collections and checked the relevant literature and databases. Voucher specimens were morphologically verified in order to link the specimen identifications with the results of the barcoding study (WP1).

We also **concentrated on the *Enteromius* species group with an ossified serrated dorsal spine** from the Lake Edward system which includes three species complexes: *E. apleurogramma*, *E. kerstenii* and

E. pellegrini (Maetens et al., 2024). The morphometric and genetic analyses included additional specimens from the neighbouring systems of Lakes Kivu, Albert and Victoria. For the genetic analyses, several maximum likelihood trees were made, a TCS haplotype network was created and a K2P (Kimura 2-P) distance matrix was calculated (Maetens et al., 2024). In addition, a molecular clock analysis was conducted using BEAST v1.8.4 (Drummond et al., 2012). All sequences of *Enteromius* (Table I) have been included in phylogeographical analyses with BioGeoBEARS (Dupin et al., 2016; Matzke, 2016). In addition, all relevant sequences of *Enteromius* from GenBank and BOLD were included. For the morphological part, both traditional morphometric (Figs. 5 & 6; Maetens et al., 2024) and geometric morphometric analyses were performed.

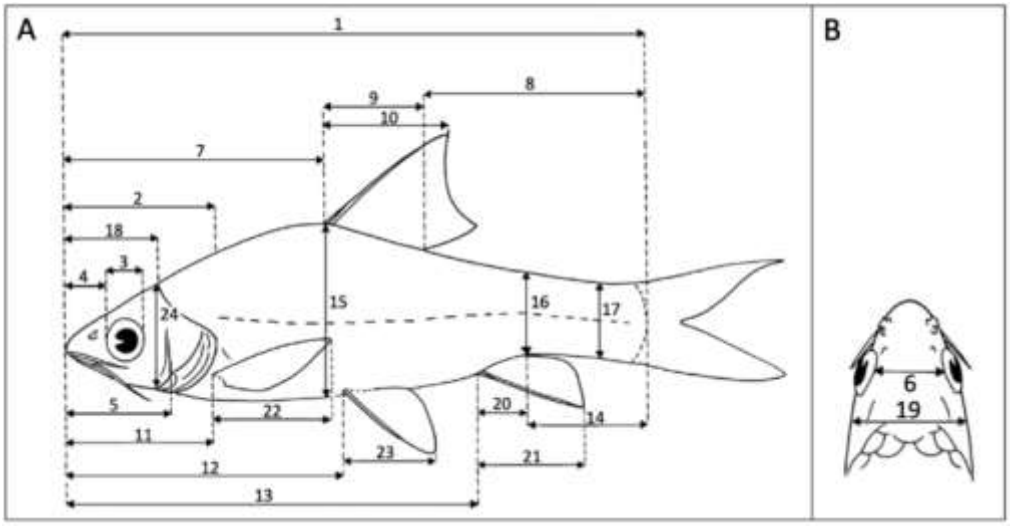

Figure 5: Schematic illustration of the measurements taken for the morphometric study on *Enteromius* (from Maetens et al., (2024), based on Bamba et al., (2011)).

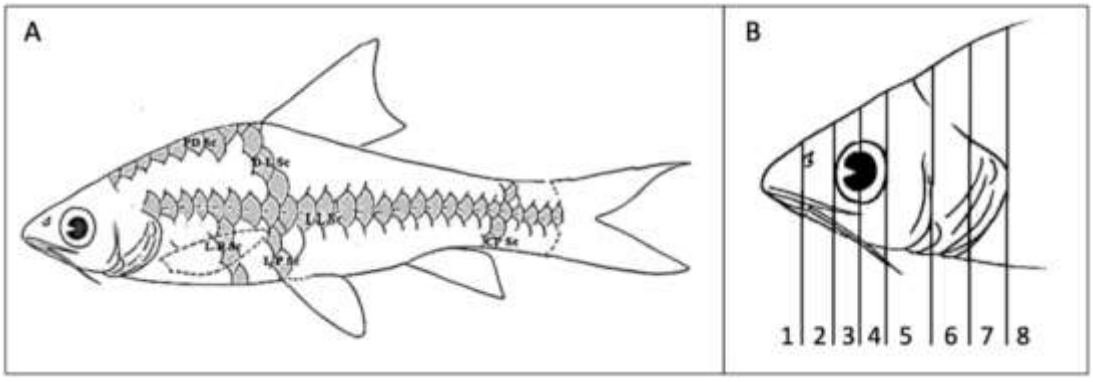

Figure 6: Schematic illustration of the meristics taken (A) and encoding scheme for barbel lengths (B) for the morphometric study on *Enteromius* (from Maetens et al., (2024), based on Bamba et al., (2011)).

We also did **an eco-morphological study** in order to examine the degree of trophic specialisation in populations of *Enteromius* with a serrated dorsal spine, using a 2D (head structures; Figure 7) and 3D (pharyngeal jaw and kinethmoid bone) geometric morphometric approach. For the 3D approach, µ-CT scans were made using a RX Solutions EasyTom Micro at the RBINS and segmentation was done

using 3D slicer. For the pharyngeal bone, a set of eight fixed homologous landmarks and 92 semilandmarks were selected (Figure 8). For the kinethmoid bone an automated approach was used that placed pseudo-landmarks on 3D surface meshes according to the method used by T. Liyandja in his unpublished PhD thesis (Liyandja, T., pers. comm.). R-Studio was used for further analysis and plotting.

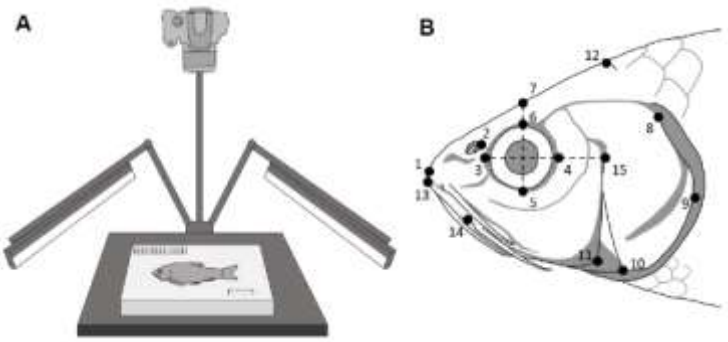

Figure 7: Illustration of the experimental set up (A) and of the 15 homologous landmarks taken (B) on the head for the 2D geometric morphometric study (Matté, 2023).

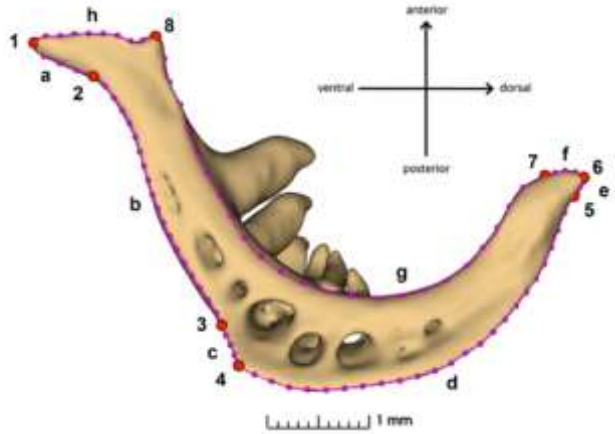

Figure 8: Visualization of the position of the eight fixed homologous landmarks (1-8) and 92 semilandmarks on a 3D reconstructed left pharyngeal arch in *Enteromius*.

We studied the morphometry of ***Clarias*** *liocephalus* and *C. gariepinus* within the KEA region. The latter species was also studied on a broader scale with a combination of traditional and geometric morphometrics, using new data and datasets used by Teugels (1986) and Rognon et al. (1998). Morphometric studies were also done on the ***Bagrus*** species of the area and the ***Amphilius*** *kivuensis* complex.

When it comes to examining the diversity of the fishes of the KEA region, there is an extra dimension that is important for the **economically important species**, which has to do with the possible occurrence of separate stocks within species. This is an important question for the management of fisheries and for conservation. We performed a microsatellite analyses on two commercially

important species from the connected but ecologically distinct Lakes George and Edward: *Bagrus docmak* and *Clarias gariepinus*. This work builds further on a study on two other fisheries target species, *Oreochromis niloticus* and *Oreochromis leucostictus*, which was previously performed by our team (Diedericks et al., 2021). For both species, six loci were used. Structure was used to determine the number of populations (K) and Fst-values were calculated via R.

WP3. Diversity of haplochromines

Standard morphometric and geometric morphometric were used to study the diversity of haplochromines. DNA-barcoding as a technique for species delineation and identification does not work in this group because of their recent explosive speciation.
The geometric morphometric approach allowed us to construct an eco-morphological framework of all currently recognised (described and undescribed) lacustrine species from Lakes Albert (12 species), Edward (52), and Kivu (15) together with 122 species from Lake Victoria. Via PCAs, we could examine the morphospace occupation of the species from each lake.

WP4. Genome-wide sequencing and analyses

Haplochromines: We implemented a whole-genome sequencing approach to investigate the evolutionary history and biogeography of the haplochromines from the KEA region, including representatives from Lake Victoria. We have sequenced 1–3 specimens of 80 target species from Lakes Kivu, Edward, Albert, and Victoria. The data were analysed via principal component analyses, maximum likelihood trees, and introgression metrics to evaluate the radiations of the Lake Victoria Region Superflock of haplochromines. AS most of these species were also included in our eco-morphological framework (see WP 3), we linked the genomic dataset to these results. Pairwise contrast-divergence plots were contrasted against Brownian motion simulated neutral trait evolution in order to evaluate the occurrence of convergence. In order to evaluate whether genomic regions can be identified that segregated across replicate radiations and sorted repeatedly into species with similar eco-morphologies, we implemented genome-wide association mapping of eco-morphological traits.

Enteromius: In a first step, we analysed and reconstructed mitochondrial genome sequences for twenty individuals from several *Enteromius* species, covering nine different mitochondrial lineages from the Edward Lake system. The approach relied on whole-genome sequencing effort, complemented by additional Oxford Nanopore sequencing experiments for a subset of individuals (n = 2, *E. alberti* and *E. kerstenii*), in collaboration with the teams of Olivier J. Hardy and Jean-François Flot (ULB).
We reconstructed the complete mitochondrial genome sequences, used Genomescope2 and FindGSE to estimate the genome sizes for the different samples, and reconstructed Neighbor-Net splits graphs.

In a second step, a total of 108 whole genomes (ca. 15-20X) for several *Enteromius* species were created to better explore species delineation, the relationships between species, and the genomic mechanisms that promote speciation. These analyses are ongoing and include ABBA-BABBA tests, an evaluation of structural variants and islands of differentiation, and analysing genome-wide Fst profiles.

Clarias: Genomic analyses were carried out on 103 specimens of *Clarias gariepinus s.l.* collected between 1996 and 2022. These samples originated from 20 African countries across five ichthyofaunal provinces. One sample from Israel and nine samples from Turkey were included as well. Two complementary methodological approaches were employed to investigate the population genomics and biogeography of African clariids. First, we analysed single nucleotide polymorphisms (SNPs) to explore population structure within *C. gariepinus*. Second, we generated an Ultra-Conserved Elements (UCE) dataset with broader taxonomic sampling, including 25 clariid taxa, to infer a time-calibrated phylogeny. Together, these approaches enabled us to address the research questions at both fine-scale and broad-scale resolutions. Most analyses were performed on the high-performance computing infrastructure of KU Leuven (VSC Genius cluster).
After trimming and filtering, the paired-end reads were aligned against the new high-quality *C. gariepinus* reference genome CGAR_prim_01v2 available on NCBI. Variant calling was performed, and the final dataset included 2.2 M SNPs for 103 specimens of *C. gariepinus* specimens. Population structure was inferred from the SNP dataset and the optimal number of ancestral populations was calculated to be K = 10. Each of the 10 populations was assigned a unique colour code and a name based on its location, which remained consistent across all plots. Principal component analysis (PCA), a phylogenetic tree and a network were then performed on the same SNP dataset.

To complement the SNP analyses and to perform accurate phylogenetic inferences with genomic data from multiple clariid species available on Genbank, two Ultra-Conserved Elements (UCEs) dataset were generated. The first dataset included all the *C. gariepinus* specimens and two *C. ngamensis* as the outgroup. The second, broader, phylogenetic inference was carried out on all taxa of Clariidae. A subset of 24 specimens of *C. gariepinus* was included with all the other species of *Clarias* sequenced for this project *C. ngamensis* (n = 3), *C. anguillaris* (n = 2), *C. camerunensis* (n = 1), *C. gabonensis* (n = 2), *C. liocephalus* (n = 3). To that list, we added all the clariid genomes assemblies available on NCBI and *Ictalurus punctatus* as an outgroup. Phylogenetic analyses were carried out to generate a tree and a phylogenetic network. Finaly, two fossil calibrated tree were generated using the two UCE dataset.

4. SCIENTIFIC RESULTS AND RECOMMENDATIONS

Scientific results

WP1. Region-wide scan of non-haplochromines

Quite early in the project we were able to produce a **barcoding paper on the fishes of the Lake Edward system** (Decru et al. 2022). This was not just another barcoding paper of a particular region In Africa, but its strength laid in the **nearly complete coverage** (91.2 %) of the non-*Haplochromis* fish diversity of the system, with 31 out of the 34 known species sequenced (table II). This is quite exceptional with this kind of studies. The endemic haplochromines were excluded, since barcoding for identification purposes cannot be used for this group (see position of haplochromines in Figure 10). This barcoding effort could profit from an earlier study that produced an annotated checklist from the Lake Edward system (Decru et al., 2019). In our study, we generated the first barcodes for many species, and found the Lake Edward populations of some widespread species to be genetically distinct. This means that the Lake Edward system went in a couple of years' time from a poorly known region in terms of its fish diversity to one of the better known areas in Central and East Africa (see also sampling map, Figure 9).

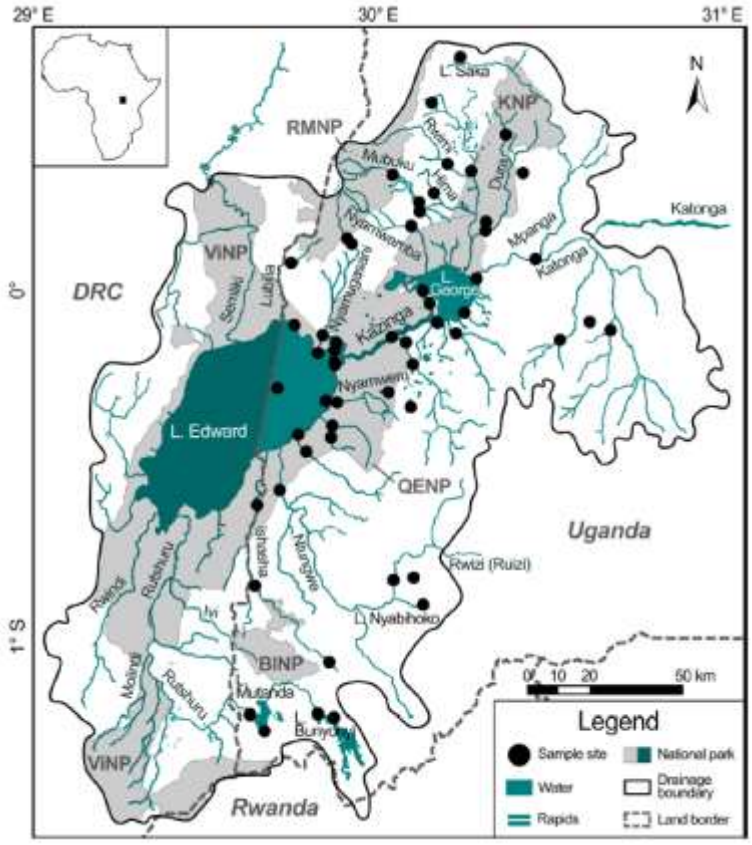

Figure 9: Map of the Lake Edward system indicating all localities with fish samples from which COI barcoding sequences were obtained (from Decru et al., 2022). Many additional samples, mostly of *Enteromius* species, were collected by the NaFIRRI team after the study had been published, so that the region has been even more densely sampled than illustrated here.

Table II: Maximum intraspecific diversity ('intra') (p-distance; values >2 in bold) and number of obtained sequences (n) for each species (excluding the genus *Haplochromis*) of the Lake Edward system. Ht denotes the total number of haplotypes. Species names ordered alphabetically (Decru et al., 2022).

Species	intra	n	ht
Amphilius cf. *kivuensis*	0.2	2	2
Amphilius jacksonii Boulenger, 1912	0.2	7	2
Astatoreochromis alluaudi Pellegrin, 1904	0.5	5	4
Bagrus docmak (Forsskal, 1775)	0	8	1
Clarias alluaudi Boulenger, 1906	NA	1	1
Clarias gariepinus (Burchell, 1822)	1.2	35	11
Clarias liocephalus Boulenger, 1898	0.8	6	5
Clarias werneri Boulenger, 1906	NA	1	1
Coptodon zillii (Gervais, 1848)	**2.3**	3	2
Ctenopoma muriei (Boulenger, 1906)	0.3	8	2
Enteromius alberti (Poll, 1939)	0.5	36	5
Enteromius apleurogramma (Boulenger, 1911)	**3.1**	36	13
Enteromius cf. *mimus*	**2.4**	101	43
Enteromius kerstenii (Peters, 1868)	**3.2**	93	32
Enteromius pellegrini (Poll, 1939)	**5.5**	103	31
Hypsopanchax modestus (Pappenheim, 1914)	0.3	11	4
Labeo forskalii Rüppel, 1835	0.8	12	4
Labeobarbus altianalis (Boulenger, 1900)	1.8	15	7
Labeobarbus ruwenzorii (Pellegrin, 1909)	1.3	8	7
Labeobarbus somereni (Boulenger, 1911)	0.2	8	2
Laciris pelagicus (Worthington, 1932)	0.3	7	4
Microctenopoma damasi (Poll & Damas, 1939)	0.3	2	2
Micropanchax vitschumbaensis (Ahl, 1924)	1.4	18	12
Mormyrus kannume Forsskal, 1775	0	8	1
Oreochromis leucostictus (Trewavas, 1933)	1.1	28	7
Oreochromis niloticus (Linnaeus, 1758)	0.8	26	5
Poecilia reticulata Peters, 1859	1.1	12	4
Pollimyrus nigricans (Boulenger, 1906)	0	4	1
Protopterus aethiopicus Heckel 1851	**2.5**	18	8
Pseudocrenilabrus multicolor (Schöller, 1903)	0.5	3	2
Zaireichthys rotundiceps (Hilgendorf, 1905)	0.6	9	3

This barcoding study was the first study to report the presence of **unexpected hidden diversity with *Enteromius*** that became one of the main study topics of the project (see large values of intraspecific divergence in Table II and the various lineages in Figure 10). At present, we are integrating the last *Enteromius* barcodes from the region. For more information on the barcoding results on *Enteromius*, see WP2: Diversity of non-haplochromines.

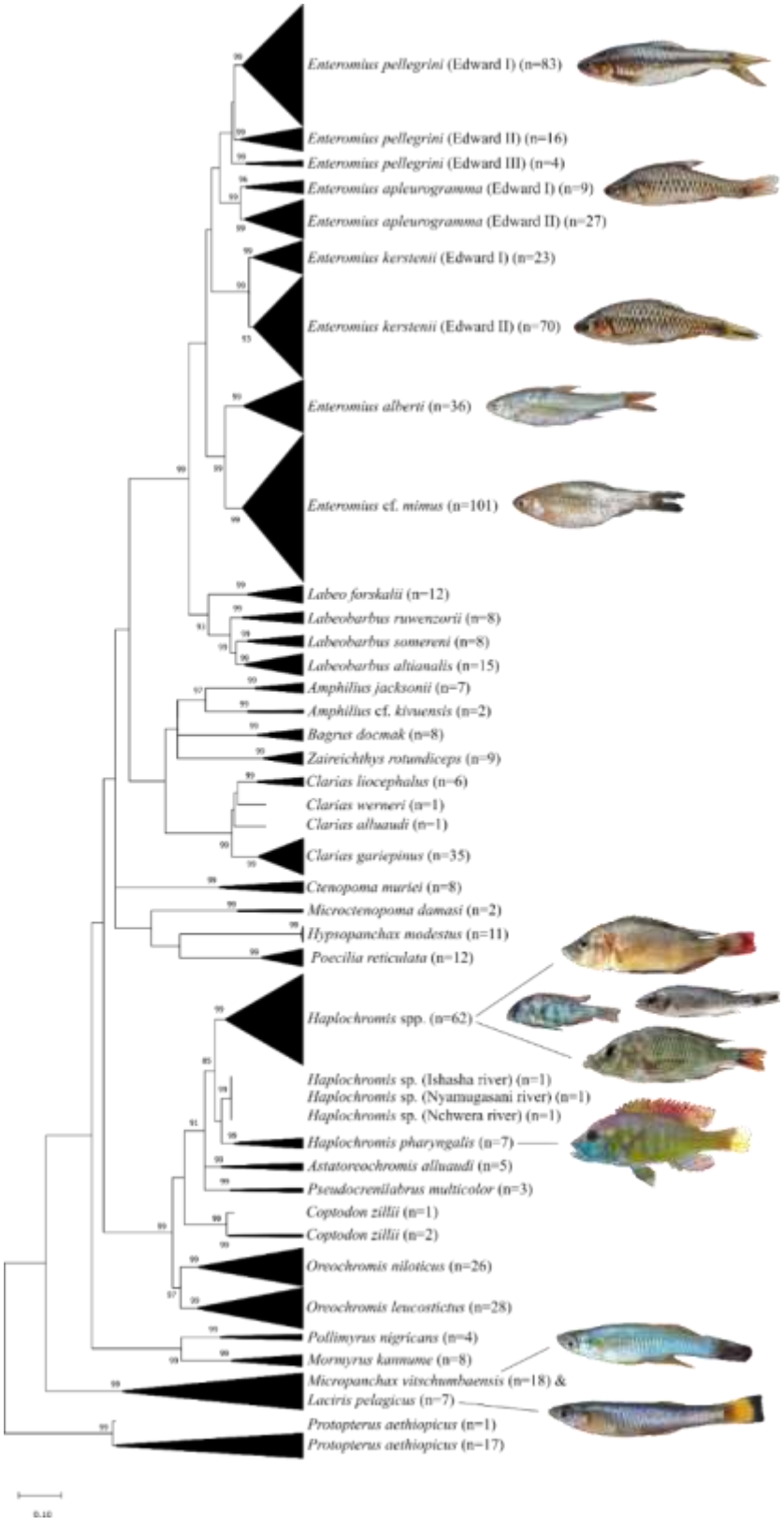

Figure 10. ML tree based on 696 COI barcode sequences from fish species of the Lake Edward system. Conspecific lineages with less than 2% genetic divergence are collapsed. Numbers in brackets indicate the number of specimens analysed per species, except for *Haplochromis* spp, which includes 32 valid species. Statistical node support values (100 bootstrap replications) >85 are visualized, branch lengths are measured in number of substitutions per site.

The main issue that remains unsolved after the publication of our results on the Lake Edward system is the fact that we found the killifishes *Micropanchax vitschumbaensis* and *Laciris pelagicus* to be indistinguishable based on COI (Figure 10). This was unexpected, as the two species are morphologically clearly different and differ profoundly in ecology. The genus *Laciris* has been erected only for the species *L. pelagicus*, previously place in *Micropanchax*. It is not excluded that the split between the two species occurred recently resulting in a pelagic lacustrine and a riverine form with no or little genetic differentiation. As a consequence, the validity of placing L*. pelagicus* in a separate genus is also questioned.

We also created a **barcoding database for the fishes of the Nyungwe forest** region and published the data as part of a fish diversity study of the area (Maetens et al., 2025). This study filled an important knowledge gap as no barcodes were available on the global databases for the fishes collected in Rwanda. This study also resulted in the discovery of undescribed diversity in the small barbs and in the amphiliid catfishes.

In analogy with the studies on the Lake Edward system and the Nyungwe area, **COI barcodes** were generated **for the Lake Albert system, and parts of the Lake Victoria (Akagera, Mara wetlands) and Kivu systems.** Those from the Lake Albert system will be used for a publication on the poorly known fish diversity of this basin that is being prepared.

All generated barcodes during the project were combined with all barcodes found on Genbank and Bold for the genera (for some even higher taxonomic groups) involved, including those previously published by the team. This resulted in a very large dataset. The analysis of the **Neighbour-Joining trees** resulted in **an enormous amount of new research questions**. Below we will comment on those that are important for the region. The order of presenting the results corresponds with alphabetical order of families in the annotated checklist of Lake Albert (see below, table III).

Alestidae: We combined the barcodes of the genera *Alestes*, *Brycinus* and *Brachyalestes* in one tree. Suprizingly, we found the specimens we identified as *Alestes dentex* with specimens identified as *Alestes baremoze* from Nigeria and our specimens of *A. baremoze* with specimens identified as *A. dentex* from Nigeria (Figure 11). We suspect an identification problem or a switch of names for the specimens of the Nigerian study.

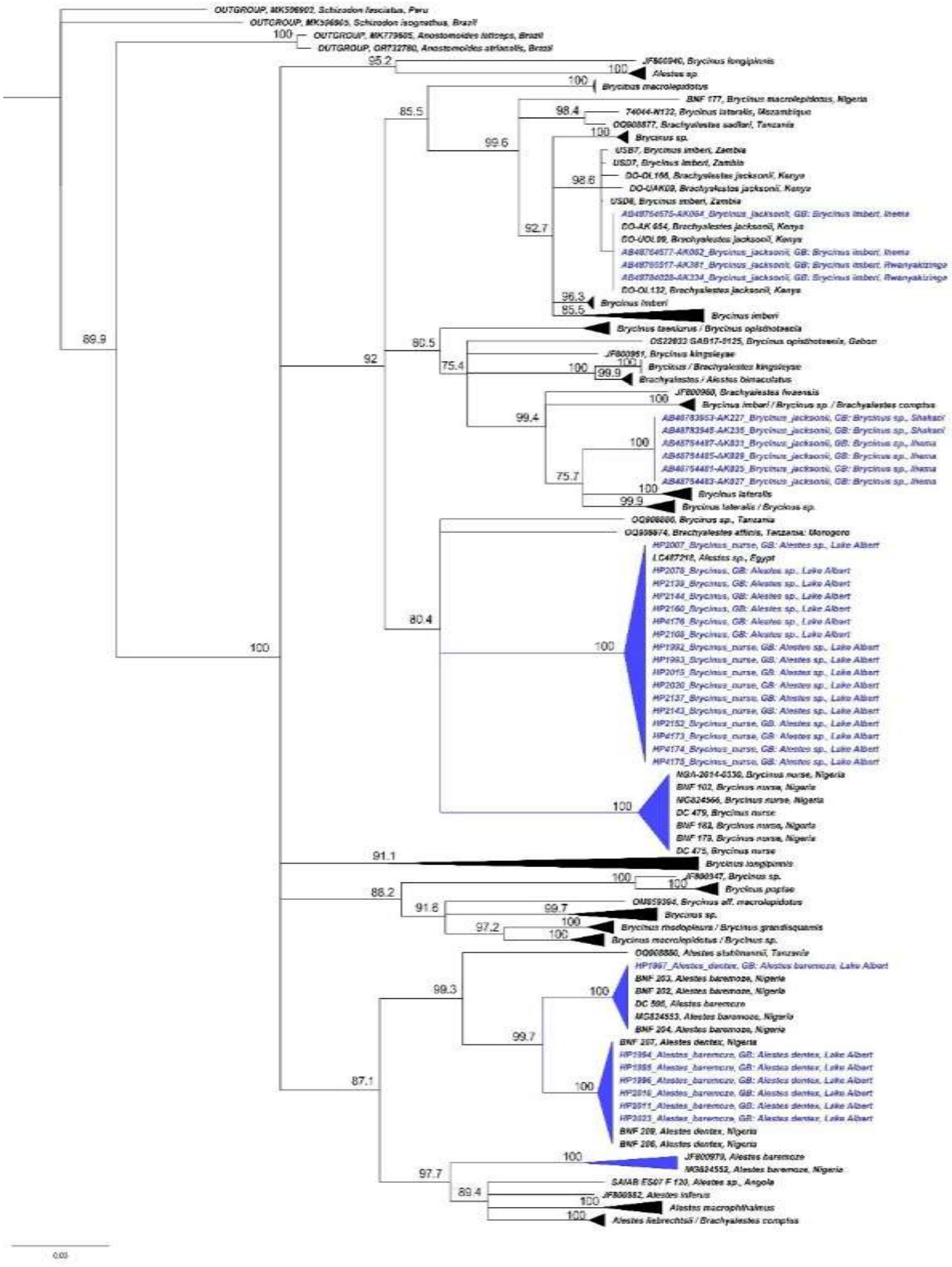

Figure 11 : Neighbour-Joining tree including species of the genera *Alestes*, *Brycinus* and *Brachyalestes* (family Alestidae), based on the cytochrome *c* oxidase subunit I (COI) gene (Tamura–Nei distance model; 652 bp fragment; 1,000 bootstrap replicates). Bootstrap values are shown at branch nodes, and the newly generated COI sequences are highlighted in blue. For clarity, clusters of non-target species were collapsed, and only bootstrap values ≥ 75 are shown.

The results for *Brachyalestes jacksonii* are surprising as well. There is a well-defined *B. jacksonii* clade that includes our specimens from the Middle Akagera in Rwanda (Victoria system) and other specimens from the Lake Victoria basin in Kenya, but also specimens identified as *Brachyalestes imberi* from the Lake Tanganyika basin in Zambia. The identification of the latter may be doubtful as there are two other clades containing specimens identified as *B. imberi*, one from southern Africa and one from the Congo basin. The polyspecific nature of what is currently regarded as *B. imberi* had already been reported (Decru et al., 2016). As the type locality is in the Zambezi system, the southern African specimens most probably represent the real *B. imberi*. The specimens of *B. imberi* from the Tanganyika system most probably either belong to *B. jacksonii* (but the species has never been reported from this basin) or belong to a closely related, possibly undescribed, species.
There is a second *B. jacksonii* clade from the Middle Akagera in Rwanda. These specimens probably represent *Brachyalestes sadleri*, the only other species reported from the system (De Vos et al., 2001). Finally, there is a clade representing *B. nurse* from the Lake Albert system and Egypt. However, this clade is well separated (large genetic divergence) from another *B. nurse* clade from West Africa. We suspect that *B. nurse*, as currently defined, is a polyspecific assemblage, similar to the situation in *B. imberi*, another widespread species (see above). As the type specimen comes from Cairo, Egypt, the Lake Albert population corresponds most probably to the real *B. nurse*, while the West Africa clade represents another species, the identity is unclear at the moment.

Our COI tree (Figure 12) confirmed the confused status of the taxonomy of *Hydrocynus* as already discussed extensively by Goodier et al. (2011). As expected, the identification of tigerfishes posed problems. Our specimens clustered with some specimens identified as *H. vittatus* from Nigeria and Egypt. However, when we compared our COI tree with the CytB tree of Goodier et al. (2011), we noticed that this cluster corresponds most probably with part of the *H. forskahlii* complex. This raises doubt as to the identification of the cluster containing our specimens, as *H. vittatus*. As the *H. vittatus* complex is the only one occurring in southern Africa (Goodier et al., 2011), it is clear that this complex corresponds with the large cluster of specimens from the Congo basin and southern Africa in our COI tree and not with the cluster including the Lake Albert specimens. The holotype of *H. forskahlii* was collected in the Nile River, Egypt. Therefore our specimens most probably belong to *H. forskahlii*.

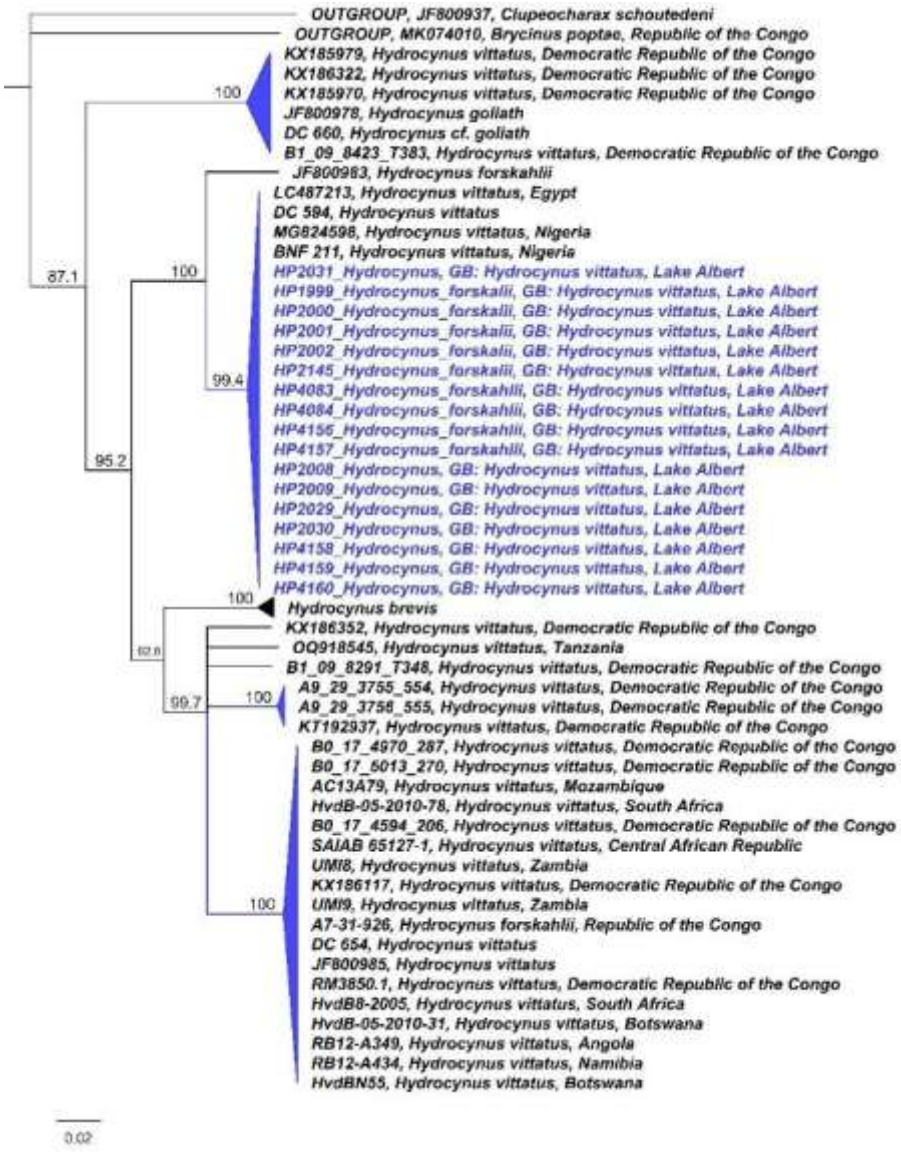

Figure 12 : Neighbour-Joining tree including species of the genus *Hydrocynus* (family Alestidae), based on the cytochrome *c* oxidase subunit I (COI) gene (Tamura–Nei distance model; 652 bp fragment; 1,000 bootstrap replicates). Bootstrap values are shown at branch nodes, and the newly generated COI sequences are highlighted in blue. For clarity, clusters of non-target species were collapsed, and only bootstrap values ≥ 75 are shown.

Anabantidae: Our specimens of *Ctenopoma muriei* from the Lake Albert basin and the Kafu system (Lake Victoria basin) form a separate cluster that is part of a monophyletic lineage (named here the *C. muriei* complex) with, surprisingly, a specimen from the Kilombero (Rufiji system) in Tanzania and our specimens from the Lake Edward system (Figure 13). Almost certainly, the Kilombero specimen represents a new species as its locality is far remote from the reported distribution area of *C. muriei*. In fact the area of the Rufiji system, is as far as we know, the only coastal river area in Eastern Africa where *Ctenopoma* occurs. One has to postulate an ancient connection between the Malagarasi and the Rufiji to explain its occurrence in the latter river system. What is of more interest to our study of the KEA region is the large genetic divergence between the Lake Edward specimens and those from

the Lakes Albert and Victoria systems. Such a large diverge raises the question whether they are conspecific. If they are different, then the Lake Edward populations may represent a new species, as the type locality of *C. muriei* is the White Nile in Sudan. As a side observation, we noticed major identification issues in the various clades from the Congo basin.

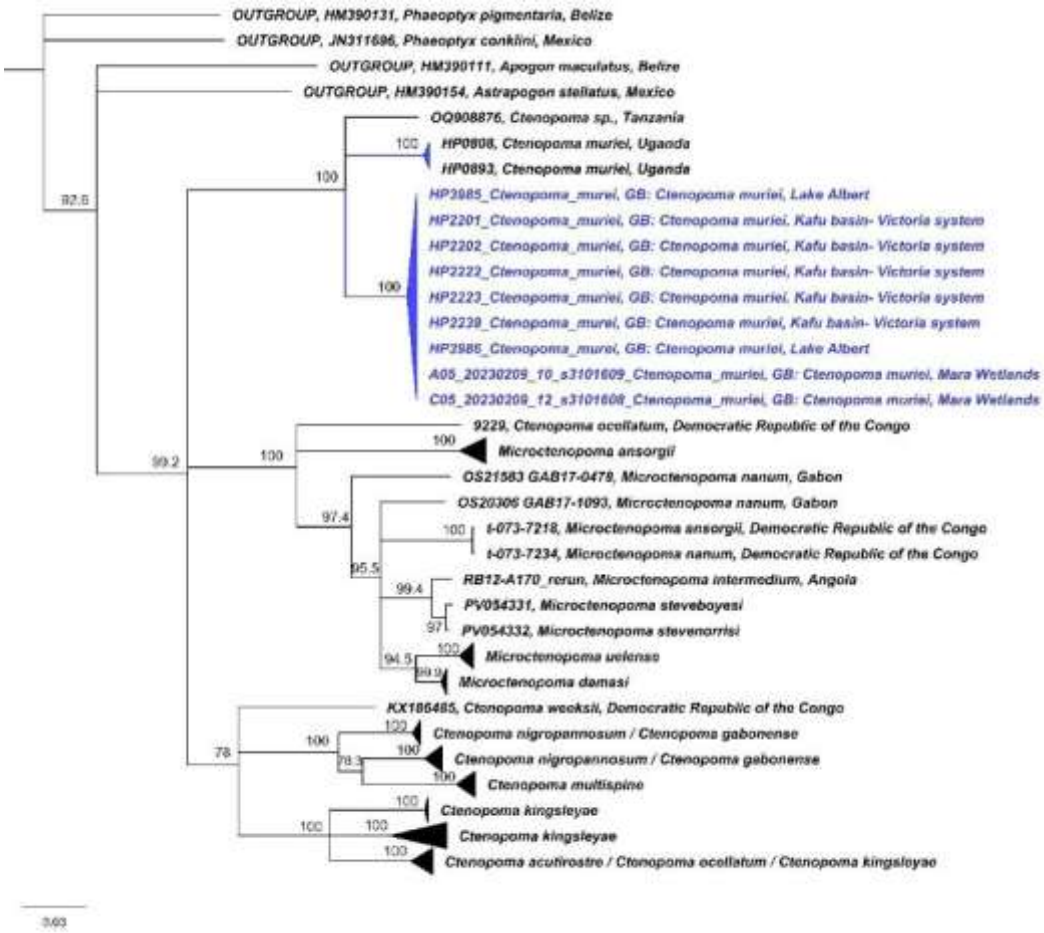

Figure 13 : Neighbour-Joining tree including species of the genera *Ctenopoma* and *Microctenopoma* (family Anabantidae), based on the cytochrome *c* oxidase subunit I (COI) gene (Tamura–Nei distance model; 652 bp fragment; 1,000 bootstrap replicates). Bootstrap values are shown at branch nodes, and the newly generated COI sequences are highlighted in blue. For clarity, clusters of non-target species were collapsed, and only bootstrap values ≥ 75 are shown.

Bagridae: We found a cluster of *B. docmak* from the systems of Lakes Tanganyika, Edward, Victoria and Albert, including also some specimens from the Nile system in Ethiopia and Egypt (Figure 14). This confirms its occurrence in a large part of its reported distribution area. The *B. docmak* population from Nigeria (representing the West Africa distribution) is genetically somewhat divergent. The sistergroup of the large *B. docmak* cluster is a large clade that harbours a *B. bajad* subclade from Lake Albert and the Nile system in Sudan and Egypt, that also includes a specimen identified as *B. filamentosus* from Nigeria. This is not surprising as *B. filamentosus* is a problematic species, classified as Data Deficient in the IUCN red list, living sympatrically in the Niger basin with *B. bajad*. No clear-cut differences have been found between the two species (Risch, 2003). The clade also includes another subclade with

specimens identified as *B. bajad* from West Africa (Nigeria and Ghana) but also a specimen from the Nile basin in Egypt. This subclade is well diverged from the other *B. bajad* subclade and at present, it is not known what species this represents. The clade harbours a third subclade with specimens from an affluent within the Juba system in Ethiopia identified as *B. urostigma*. This species, also classified as Data Deficient by IUCN, is only known from the Juba basin in Somalia.

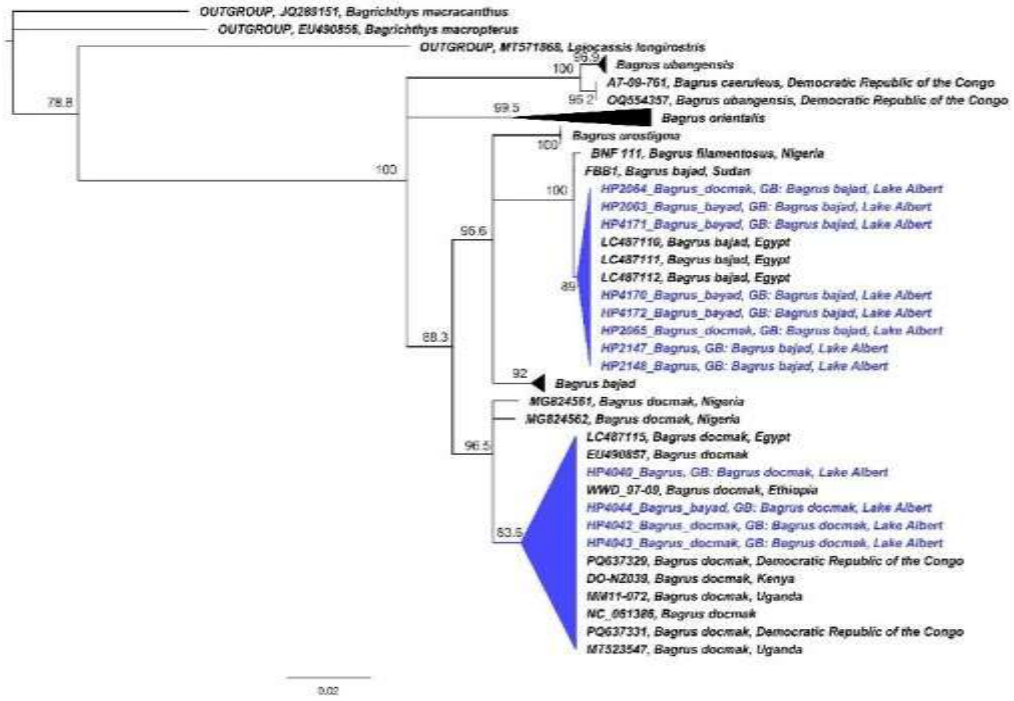

Figure 14 : Neighbour-Joining tree including species of the genus *Bagrus* (family Bagridae), based on the cytochrome *c* oxidase subunit I (COI) gene (Tamura–Nei distance model; 652 bp fragment; 1,000 bootstrap replicates). Bootstrap values are shown at branch nodes, and the newly generated COI sequences are highlighted in blue. For clarity, clusters of non-target species were collapsed, and only bootstrap values ≥ 75 are shown.

Cichlidae: Barcoding is not effective for haplochromine cichlids (see above), so their results are not include. We also did not include the tilapias in this effort. A separate study on *Oreochromis niloticus* was done by a PhD student of Hasselt University and the RBINS (see below under WP4, genome-wide sequencing).

Pseudocrenilabrus multicolor from the Lake Albert system clusters with the conspecifics from the Victoria system (Figure 15). There is a subclade made of all except one specimen from the Kafu basin and including a specimen from the Mpanga river in the Lake Edward system. The large Victoria/Edward/Albert cluster is clearly divergent from the populations from Egypt, confirming the morphological differences reported, which led to the split of the species in two subspecies (Seegers, 1990), *P. multicolor multicolor* for the Lower Nile populations and *P. multicolor victoriae* for the Victoria/Edward/Albert basin populations. It is likely that they need to be considered as separate species.

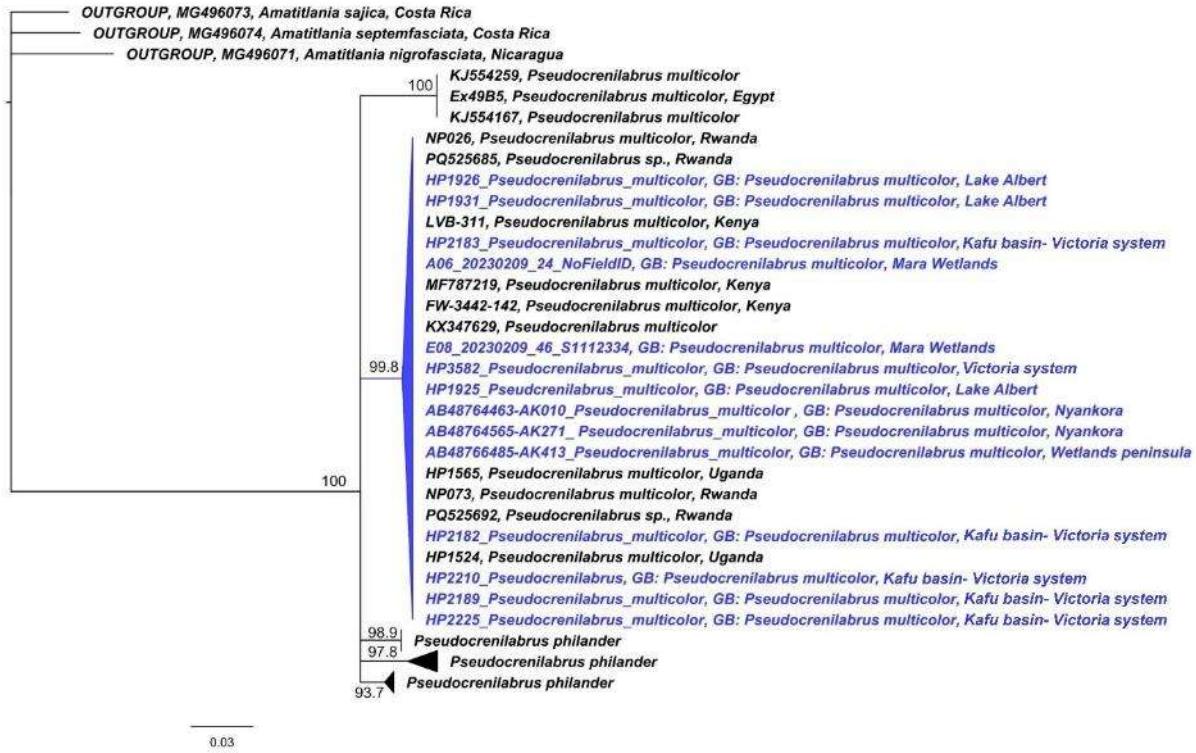

Figure 15 : Neighbour-Joining tree including species of the genus *Pseudocrenilabrus* (Cichlidae), based on the cytochrome *c* oxidase subunit I (COI) gene (Tamura–Nei distance model; 652 bp fragment; 1,000 bootstrap replicates). Bootstrap values are shown at branch nodes, and the newly generated COI sequences are highlighted in blue. For clarity, clusters of non-target species were collapsed, and only bootstrap values ≥ 75 are shown.

Clariidae: *Clarias gariepinus* is represented by one large cluster from the of the Congo basin, the Lakes Albert, Edward and Victoria systems, the Akagera, southern Africa, Egypt, Türkiye, West Africa, Lower Guinea, and some areas outside Africa, where it has been introduced (Figure 16).

The *Clarias liocephalus* clade includes specimens from the systems of lakes Kivu (including Rusizi), Edward, and Victoria, corresponding to the northern area of its distribution (Figure 17).

Not expected, but also not surprising, is the possible presence of a third *Clarias* species in Rwanda. In the Akagera system, two specimens that were difficult to identify are grouped with *C. alluaudi* barcodes from Genbank (Figure 17). Since the Lake Victoria basin is part of the natural distribution of this species, its presence in the Akagera is not unlikely. However, it constitutes the first report of this species in that system in Rwanda.

There is some confusion about the presence of the similarly looking *C. alluaudi* and *C. werneri* in the Lake Albert system. Both species are found in the systems of Lakes Edward and Victoria, but Teugels (1986) did not mention either of them from the Lake Albert system. The specimens we collected from the Albert system fall within the Genbank cluster of *C. alluaudi*, which also includes one of our specimens from in the Lake Edward system, and others from the Victoria system (Figure 17). The cluster of *C. werneri* includes our specimens from the Lakes Edward and Victoria systems and also a specimen from the Lake Tanganyika system.

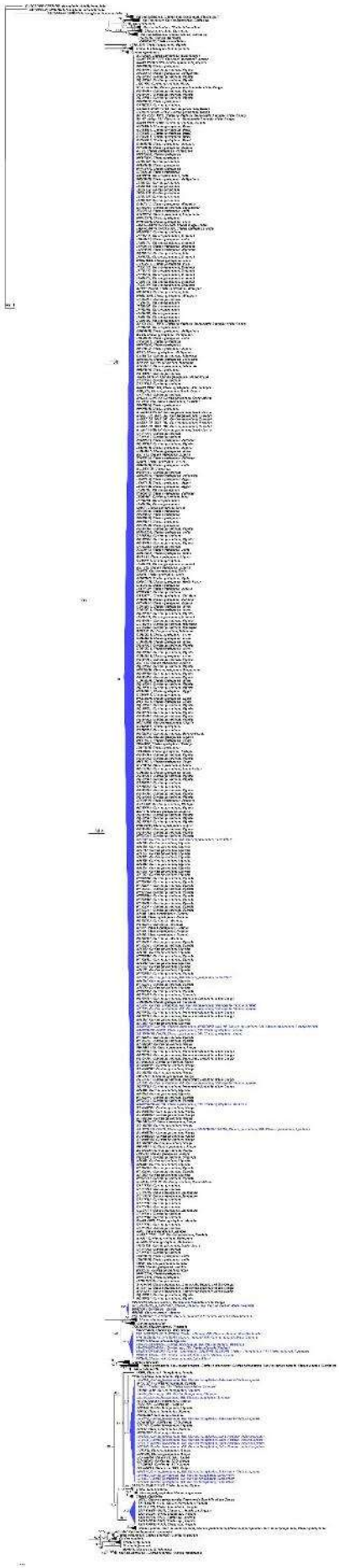

Figure 16 : Neighbour-Joining tree including species of the genus *Clarias* (Clariidae), based on the cytochrome *c* oxidase subunit I (COI) gene (Tamura–Nei distance model; 652 bp fragment; 1,000 bootstrap replicates). Bootstrap values are shown at branch nodes, and the newly generated COI sequences are highlighted in blue. For clarity, clusters of non-target species were collapsed, and only bootstrap values ≥ 50 are shown.

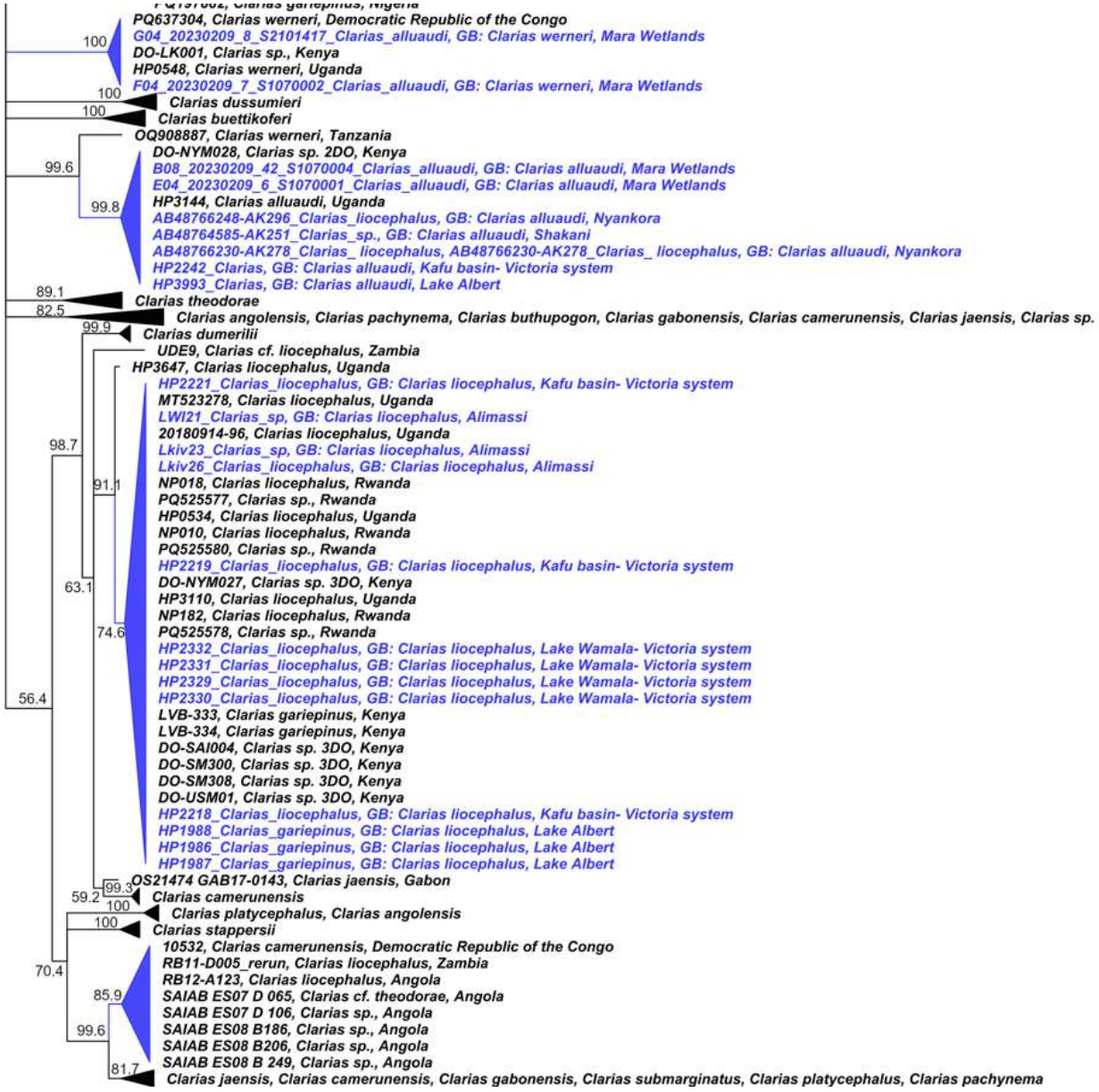

Figure 17 : Details for *C. liocephalus, C. allluaudi* and *C. werneri* of the Neighbour-Joining tree including species of the genus *Clarias* (Clariidae), based on the cytochrome *c* oxidase subunit I (COI) gene (Tamura–Nei distance model; 652 bp fragment; 1,000 bootstrap replicates). Bootstrap values are shown at branch nodes, and the newly generated COI sequences are highlighted in blue. For clarity, clusters of non-target species were collapsed, and only bootstrap values ≥ 50 are shown.

Cyprinidae: For the situation on *Enteromius*, see separate special discussion below.

The taxonomic history of *Labeo* in Africa is one of great confusion, despite several efforts to disentangle the prevailing issues (Reid, 1985; Tshibwabwa & Teugels, 1995; Liyandja et al., 2025). Therefore it came to no surprise to find the taxonomic situation of *Labeo* in the KEA region to be complex. The COI-tree for the genus (Figure 18) is scattered with large clusters with specimens with many different names, and it is hard to make some taxonomic sense of most of the African lineages. However we do see a major geographic logic as it seems the higher-level clusters correspond to relatively well-defined geographic areas.

When concentrating on the specimens of our study area, the specimens of *Labeo victorianus* from the Akagera basin cluster nicely with other specimens identified as *L. victorianus* from Kenya and Tanzania. However, this lineage forms only a subcluster of a larger cluster that also includes other specimens identified as *L. victorianus* from Kenya and Uganda. In that large cluster there are also many specimens with some eight other Genbank names. Two specimens from the Lake Albert system, one identified as *L. forskalii* and one as *L. sp.*, form a separate lineage within this large cluster. However, *Labeo victorianus* has not been reported from the Lake Albert system and it is unclear what the identity of these two specimens is. They most probably do not belong to *L. forskalli* as our specimens from the Lake Edward system identified as *L. forskalii* are situated in a genetically very divergent clade, including also specimens of *L. forskalii* from Ethiopia and specimens identified as *L. cylindricus* from Kenya. At this moment, it is difficult to speculate about the identity of the two Lake Albert specimens. Because of the relatively large genetic divergence (ca. 2%) with the specimens of *L. victorianus* from the Lake Victoria basin, it is possible the Albert specimens represent a different species.

Two other specimens from the Lake Albert system, which we identified as *L. horie*, are within a large cluster that appears to correspond to the *L. senegalensis* clade of Liyandja et al., (2025). As far as can be judged, their position corresponds remarkably well with the position of *L. horie* in the phylogenomic trees of Liyandja et al., (2025).

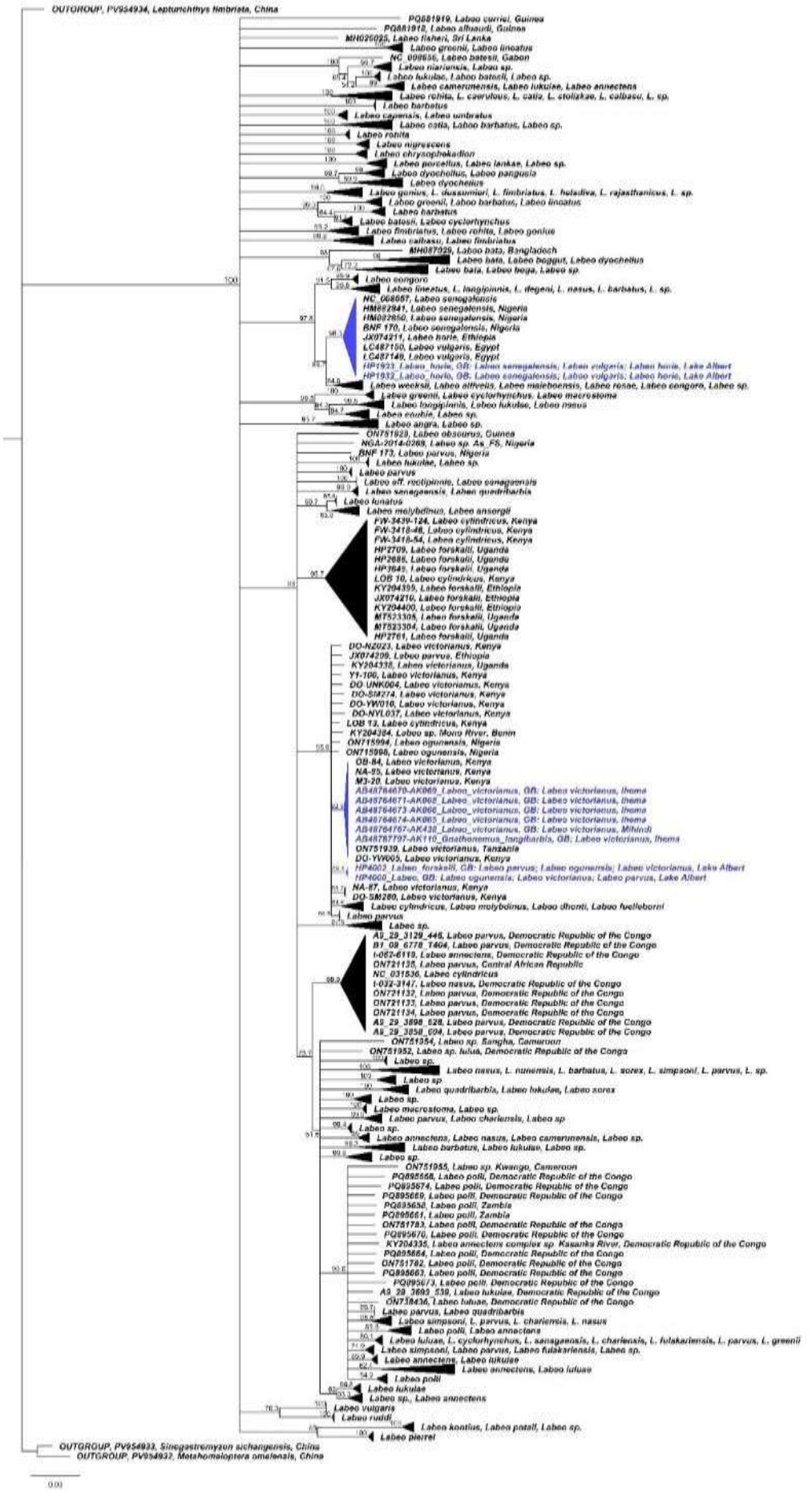
OUTGROUP, PV954934, Lepturichthys fimbriata, China
PQ881919, Labeo curriei, Guinea
PQ881918, Labeo alhaurdi, Guinea
MH026025, Labeo fisheri, Sri Lanka
Labeo greenii, Labeo lineatus
NC_009856, Labeo batesii, Gabon
Labeo niloticus, Labeo sp.
Labeo lukulae, Labeo batesii, Labeo sp.
Labeo camerunensis, Labeo lukulae, Labeo annectens
Labeo rohita, L. coeruleus, L. catla, L. stoliczkae, L. calbasu, L. sp.
Labeo barbatus
Labeo cameronensis, Labeo umbratus
Labeo catla, Labeo barbatus, Labeo sp.
Labeo rohita
Labeo nigrescens
Labeo chrysophekadion
Labeo porcellus, Labeo lankae, Labeo sp.
Labeo dyocheilus, Labeo pangusia
Labeo dyocheilus
Labeo gonius, L. dussumieri, L. fimbriatus, L. huladiva, L. rajasthanicus, L. sp.
Labeo greenii, Labeo barbatus, Labeo lineatus
Labeo barbatus
Labeo batesii, Labeo cyclorhynchus
Labeo fimbriatus, Labeo rohita, Labeo gonius
Labeo calbasu, Labeo fimbriatus
MH087029, Labeo bata, Bangladesh
Labeo bata, Labeo boggut, Labeo dyocheilus
Labeo bata, Labeo boga, Labeo sp.
Labeo congoro
Labeo lineatus, L. langipinnis, L. degeni, L. nasus, L. barbatus, L. sp.
NC_008857, Labeo senegalensis
HM822841, Labeo senegalensis, Nigeria
HM822850, Labeo senegalensis, Nigeria
BNF 170, Labeo senegalensis, Nigeria
JX074211, Labeo horie, Ethiopia
LC487150, Labeo vulgaris, Egypt
LC487149, Labeo vulgaris, Egypt
HP1933_Labeo_horie, GB: Labeo senegalensis; Labeo vulgaris; Labeo horie, Lake Albert
HP1932_Labeo_horie, GB: Labeo senegalensis; Labeo vulgaris; Labeo horie, Lake Albert
Labeo weeksii, Labeo altivelis, Labeo maieboensis, Labeo rosae, Labeo congoro, Labeo sp.
Labeo greenii, Labeo cyclorhynchus, Labeo macrostoma
Labeo langipinnis, Labeo lukulae, Labeo nasus
Labeo coubie, Labeo sp.
Labeo angra, Labeo sp.
ON751928, Labeo obscurus, Guinea
NGA-2014-0269, Labeo sp. As_FS, Nigeria
BNF 173, Labeo parvus, Nigeria
Labeo lukulae, Labeo sp.
Labeo parvus
Labeo aff. rectipinnis, Labeo senegaensis
Labeo senegaensis, Labeo quadribarbis
Labeo lunatus
Labeo molybdinus, Labeo ansorgii
FW-3439-124, Labeo cylindricus, Kenya
FW-3418-46, Labeo cylindricus, Kenya
FW-3418-54, Labeo cylindricus, Kenya
HP2709, Labeo forskalii, Uganda
HP2686, Labeo forskalii, Uganda
HP3645, Labeo forskalii, Uganda
LOB 10, Labeo cylindricus, Kenya
KY204395, Labeo forskalii, Ethiopia
JX074210, Labeo forskalii, Ethiopia
KY204400, Labeo forskalii, Ethiopia
MT523305, Labeo forskalii, Uganda
MT523304, Labeo forskalii, Uganda
HP2761, Labeo forskalii, Uganda
DO-NZ023, Labeo victorianus, Kenya
JX074209, Labeo parvus, Ethiopia
KY204338, Labeo victorianus, Uganda
Y1-106, Labeo victorianus, Kenya
DO_UNK004, Labeo victorianus, Kenya
DO-SM274, Labeo victorianus, Kenya
DO-YW010, Labeo victorianus, Kenya
DO-NYL037, Labeo victorianus, Kenya
LOB 13, Labeo cylindricus, Kenya
KY204384, Labeo sp. Moro River, Benin
ON715994, Labeo ogunensis, Nigeria
ON715998, Labeo ogunensis, Nigeria
OB-84, Labeo victorianus, Kenya
NA-95, Labeo victorianus, Kenya
M3-20, Labeo victorianus, Kenya
AB48764670-AK068_Labeo_victorianus, GB: Labeo victorianus, Ihema
AB48764671-AK068_Labeo_victorianus, GB: Labeo victorianus, Ihema
AB48764673-AK068_Labeo_victorianus, GB: Labeo victorianus, Ihema
AB48764674-AK065_Labeo_victorianus, GB: Labeo victorianus, Ihema
AB48764767-AK430_Labeo_victorianus, GB: Labeo victorianus, Mihindi
AB48787797-AK110_Gnathonemus_longibarbis, GB: Labeo victorianus, Ihema
ON751939, Labeo victorianus, Tanzania
DO-YW005, Labeo victorianus, Kenya
HP4002_Labeo_forskalii, GB: Labeo parvus; Labeo ogunensis; Labeo victorianus, Lake Albert
HP4000_Labeo, GB: Labeo ogunensis; Labeo victorianus; Labeo parvus, Lake Albert
NA-87, Labeo victorianus, Kenya
DO-SM280, Labeo victorianus, Kenya
Labeo cylindricus, Labeo molybdinus, Labeo dhonti, Labeo fuelleborni
Labeo parvus
Labeo sp.
AS_29_3129_445, Labeo parvus, Democratic Republic of the Congo
B1_09_6778_1404, Labeo parvus, Democratic Republic of the Congo
I-062-6119, Labeo annectens, Democratic Republic of the Congo
ON721135, Labeo parvus, Central African Republic
NC_031536, Labeo cylindricus
I-032-3147, Labeo nasus, Democratic Republic of the Congo
ON721132, Labeo parvus, Democratic Republic of the Congo
ON721133, Labeo parvus, Democratic Republic of the Congo
ON721134, Labeo parvus, Democratic Republic of the Congo
AS_29_3898_628, Labeo parvus, Democratic Republic of the Congo
AS_29_3858_604, Labeo parvus, Democratic Republic of the Congo
ON751954, Labeo sp. Sangha, Cameroon
ON751952, Labeo sp. lulua, Democratic Republic of the Congo
Labeo sp.
Labeo nasus, L. runenuis, L. barbatus, L. sorex, L. simpsoni, L. parvus, L. sp.
Labeo sp
Labeo quadribarbis, Labeo lukulae, Labeo sorex
Labeo sp.
Labeo macrostoma, Labeo sp.
Labeo parvus, Labeo charlenels, Labeo sp
Labeo sp.
Labeo annectens, Labeo nasus, Labeo camerunensis, Labeo sp.
Labeo barbatus, Labeo lukulae, Labeo sp.
Labeo sp.
ON751955, Labeo sp. Kwango, Cameroon
PQ895668, Labeo polli, Democratic Republic of the Congo
PQ895674, Labeo polli, Democratic Republic of the Congo
PQ895669, Labeo polli, Democratic Republic of the Congo
PQ835658, Labeo polli, Zambia
PQ895661, Labeo polli, Zambia
ON751783, Labeo polli, Democratic Republic of the Congo
PQ895670, Labeo polli, Democratic Republic of the Congo
KY204335, Labeo annectens complex sp. Kasanka River, Democratic Republic of the Congo
PQ895664, Labeo polli, Democratic Republic of the Congo
ON751782, Labeo polli, Democratic Republic of the Congo
PQ895663, Labeo polli, Democratic Republic of the Congo
PQ895673, Labeo polli, Democratic Republic of the Congo
AS_29_3893_539, Labeo lukulae, Democratic Republic of the Congo
ON758436, Labeo lukulae, Democratic Republic of the Congo
Labeo parvus, Labeo quadribarbis
Labeo simpsoni, L. parvus, L. charlenels, L. nasus
Labeo polli, Labeo annectans
Labeo lukulae, L. cyclorhynchus, L. senegaensis, L. charlenels, L. fulakariensis, L. parvus, L. greenii
Labeo simpsoni, Labeo parvus, Labeo fulakariensis, Labeo sp.
Labeo annectens, Labeo lukulae
Labeo annectens, Labeo lukulae
Labeo polli
Labeo lukulae
Labeo sp., Labeo annectens
Labeo vulgaris
Labeo ruddi
Labeo kontius, Labeo potaii, Labeo sp.
Labeo pierrei
OUTGROUP, PV954933, Sinogastromyzon sichangensis, China
OUTGROUP, PV954932, Metahomaloptera omeiensis, China
0.03

Figure 18 : Neighbour-Joining tree including species of the genus *Labeo* (Cyprinidae), based on the cytochrome *c* oxidase subunit I (COI) gene (Tamura–Nei distance model; 652 bp fragment; 1,000 bootstrap replicates). Bootstrap values are shown at branch nodes, and the newly generated COI sequences are highlighted in blue. For clarity, clusters of non-target species were collapsed, and only bootstrap values ≥ 75 are shown.

The taxonomy of *Labeobarbus* species from the KEA region and neighbouring areas is in a mess. While we obtained monophyletic clades of specimens from the area studied, it is difficult to allocate these clusters to species. There is a cluster containing specimens we identified as *L. somereni* from the Lake Edward and Kivu systems, together some identified on Genbank as *L. altianalis* from the Lake Victoria basin in Kenya. We assume this clade refers to *L. somereni* (lineage named *Labeobarbus altianalis, Labeobarbus somereni, Labeobarbus sp.* on Figure 19). However, *L. somereni* has not been reported from the Lake Kivu system yet. In view of the confused taxonomy of the large African barbs, it is not excluded that it also occurs in this system.

There is another huge clade including mainly specimens identified as *L. altianalis* from Uganda (including our specimens from the Lake Edward basin) and Kenya, together with others identified as *L. intermedius*, mostly from Ethiopia. This clade also includes some endemic species from Lake Tana Ethiopia, and a few other taxa. We consider this cluster to represent the *L. altianalis* complex. The cluster also includes specimens we identified as *L. bynni* from the Lake Albert basin. Specimens identified in Genbank as *L. bynni* from Egypt form a small separate lineage within this large cluster.

The specimens of *L. ruwenzorii* we found in the Lake Edward system form a another separate clade (*L. ruwenzorii* on Figure 19).

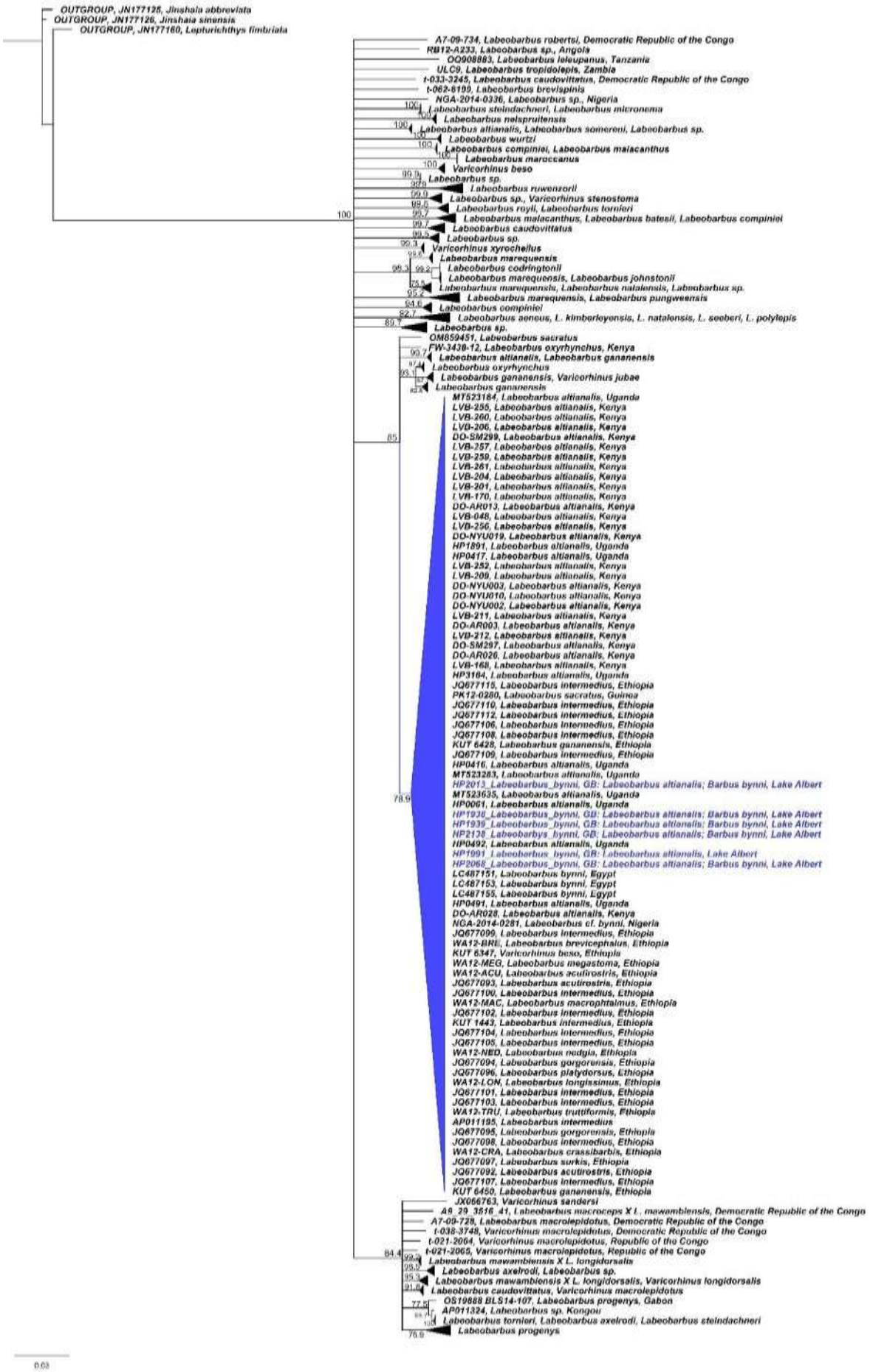
OUTGROUP, JN177125, Jinshaia abbreviata
OUTGROUP, JN177126, Jinshaia sinensis
OUTGROUP, JN177160, Lepturichthys fimbriata
A7-09-734, Labeobarbus robertsi, Democratic Republic of the Congo
RB12-A233, Labeobarbus sp., Angola
OQ908883, Labeobarbus leleupanus, Tanzania
ULC9, Labeobarbus tropidolepis, Zambia
t-033-3245, Labeobarbus caudovittatus, Democratic Republic of the Congo
t-062-8199, Labeobarbus brevispinis
NGA-2014-0336, Labeobarbus sp., Nigeria
Labeobarbus steindachneri, Labeobarbus micronema
Labeobarbus nelspruitensis
Labeobarbus altianalis, Labeobarbus somereni, Labeobarbus sp.
Labeobarbus wurtzi
Labeobarbus compiniei, Labeobarbus malacanthus
Labeobarbus maroccanus
Varicorhinus beso
Labeobarbus sp.
Labeobarbus ruwenzorii
Labeobarbus sp., Varicorhinus stenostoma
Labeobarbus royli, Labeobarbus torrieri
Labeobarbus malacanthus, Labeobarbus batesii, Labeobarbus compiniei
Labeobarbus caudovittatus
Labeobarbus sp.
Varicorhinus xyrocheilus
Labeobarbus marequensis
Labeobarbus codringtonii
Labeobarbus marequensis, Labeobarbus johnstonii
Labeobarbus marequensis, Labeobarbus natalensis, Labeobarbus sp.
Labeobarbus marequensis, Labeobarbus pungweensis
Labeobarbus compiniei
Labeobarbus aeneus, L. kimberleyensis, L. natalensis, L. seeberi, L. polylepis
Labeobarbus sp.
OM859451, Labeobarbus sacratus
FW-3438-12, Labeobarbus oxyrhynchus, Kenya
Labeobarbus altianalis, Labeobarbus gananensis
Labeobarbus oxyrhynchus
Labeobarbus gananensis, Varicorhinus jubae
Labeobarbus gananensis
MT523184, Labeobarbus altianalis, Uganda
LVB-255, Labeobarbus altianalis, Kenya
LVB-260, Labeobarbus altianalis, Kenya
LVB-206, Labeobarbus altianalis, Kenya
DO-SM299, Labeobarbus altianalis, Kenya
LVB-257, Labeobarbus altianalis, Kenya
LVB-259, Labeobarbus altianalis, Kenya
LVB-261, Labeobarbus altianalis, Kenya
LVB-204, Labeobarbus altianalis, Kenya
LVB-201, Labeobarbus altianalis, Kenya
LVB-170, Labeobarbus altianalis, Kenya
DO-AR013, Labeobarbus altianalis, Kenya
LVB-048, Labeobarbus altianalis, Kenya
LVB-256, Labeobarbus altianalis, Kenya
DO-NYU019, Labeobarbus altianalis, Kenya
HP1891, Labeobarbus altianalis, Uganda
HP0417, Labeobarbus altianalis, Uganda
LVB-252, Labeobarbus altianalis, Kenya
LVB-209, Labeobarbus altianalis, Kenya
DO-NYU003, Labeobarbus altianalis, Kenya
DO-NYU010, Labeobarbus altianalis, Kenya
DO-NYU002, Labeobarbus altianalis, Kenya
LVB-211, Labeobarbus altianalis, Kenya
DO-AR003, Labeobarbus altianalis, Kenya
LVB-212, Labeobarbus altianalis, Kenya
DO-SM297, Labeobarbus altianalis, Kenya
DO-AR026, Labeobarbus altianalis, Kenya
LVB-168, Labeobarbus altianalis, Kenya
HP3164, Labeobarbus altianalis, Uganda
JQ677115, Labeobarbus intermedius, Ethiopia
PK12-0280, Labeobarbus sacratus, Guinea
JQ677110, Labeobarbus intermedius, Ethiopia
JQ677112, Labeobarbus intermedius, Ethiopia
JQ677106, Labeobarbus intermedius, Ethiopia
JQ677108, Labeobarbus intermedius, Ethiopia
KUT 6428, Labeobarbus gananensis, Ethiopia
JQ677109, Labeobarbus intermedius, Ethiopia
HP0416, Labeobarbus altianalis, Uganda
MT523283, Labeobarbus altianalis, Uganda
HP2013_Labeobarbus_bynni, GB: Labeobarbus altianalis; Barbus bynni, Lake Albert
MT523635, Labeobarbus altianalis, Uganda
HP0061, Labeobarbus altianalis, Uganda
HP1936_Labeobarbus_bynni, GB: Labeobarbus altianalis; Barbus bynni, Lake Albert
HP1939_Labeobarbus_bynni, GB: Labeobarbus altianalis; Barbus bynni, Lake Albert
HP2138_Labeobarbys_bynni, GB: Labeobarbus altianalis; Barbus bynni, Lake Albert
HP0492, Labeobarbus altianalis, Uganda
HP1991_Labeobarbus_bynni, GB: Labeobarbus altianalis, Lake Albert
HP2068_Labeobarbus_bynni, GB: Labeobarbus altianalis; Barbus bynni, Lake Albert
LC487151, Labeobarbus bynni, Egypt
LC487153, Labeobarbus bynni, Egypt
LC487155, Labeobarbus bynni, Egypt
HP0491, Labeobarbus altianalis, Uganda
DO-AR028, Labeobarbus altianalis, Kenya
NGA-2014-0281, Labeobarbus cf. bynni, Nigeria
JQ677099, Labeobarbus intermedius, Ethiopia
WA12-BRE, Labeobarbus brevicephalus, Ethiopia
KUT 6347, Varicorhinus beso, Ethiopia
WA12-MEG, Labeobarbus megastoma, Ethiopia
WA12-ACU, Labeobarbus acutirostris, Ethiopia
JQ677093, Labeobarbus acutirostris, Ethiopia
JQ677100, Labeobarbus intermedius, Ethiopia
WA12-MAC, Labeobarbus macrophtalmus, Ethiopia
JQ677102, Labeobarbus intermedius, Ethiopia
KUT 1443, Labeobarbus intermedius, Ethiopia
JQ677104, Labeobarbus intermedius, Ethiopia
JQ677105, Labeobarbus intermedius, Ethiopia
WA12-NED, Labeobarbus nedgia, Ethiopia
JQ677094, Labeobarbus gorgorensis, Ethiopia
JQ677096, Labeobarbus platydorsus, Ethiopia
WA12-LON, Labeobarbus longissimus, Ethiopia
JQ677101, Labeobarbus intermedius, Ethiopia
JQ677103, Labeobarbus intermedius, Ethiopia
WA12-TRU, Labeobarbus truttiformis, Ethiopia
AP011195, Labeobarbus intermedius
JQ677095, Labeobarbus gorgorensis, Ethiopia
JQ677098, Labeobarbus intermedius, Ethiopia
WA12-CRA, Labeobarbus crassibarbis, Ethiopia
JQ677097, Labeobarbus surkis, Ethiopia
JQ677092, Labeobarbus acutirostris, Ethiopia
JQ677107, Labeobarbus intermedius, Ethiopia
KUT 6450, Labeobarbus gananensis, Ethiopia
JX066763, Varicorhinus sandersi
A6_29_3516_41, Labeobarbus macroceps X L. mawambiensis, Democratic Republic of the Congo
A7-09-728, Labeobarbus macrolepidotus, Democratic Republic of the Congo
t-038-3748, Varicorhinus macrolepidotus, Democratic Republic of the Congo
t-021-2064, Varicorhinus macrolepidotus, Republic of the Congo
t-021-2065, Varicorhinus macrolepidotus, Republic of the Congo
Labeobarbus mawambiensis X L. longidorsalis
Labeobarbus axelrodi, Labeobarbus sp.
Labeobarbus mawambiensis X L. longidorsalis, Varicorhinus longidorsalis
Labeobarbus caudovittatus, Varicorhinus macrolepidotus
OS19888 BL514-107, Labeobarbus progenys, Gabon
AP011324, Labeobarbus sp. Kongou
Labeobarbus torrieri, Labeobarbus axelrodi, Labeobarbus steindachneri
Labeobarbus progenys
0.03

Figure 19 : Neighbour-Joining tree including species of the genus *Labeobarbus* (Cyprinidae), based on the cytochrome *c* oxidase subunit I (COI) gene (Tamura–Nei distance model; 652 bp fragment; 1,000 bootstrap replicates). Bootstrap values are shown at branch nodes, and the newly generated COI sequences are highlighted in blue. For clarity, clusters of non-target species were collapsed, and only bootstrap values ≥ 75 are shown.

Danionidae: The neobolines constitute a group of relatively small sardine-like zooplanktivorous cypriniforms within the subfamily Chedrinae. Endemic species occur in several lakes of the rift valley including Lake Albert. At first, the position of the endemic *Engraulicypris bredoi* from this system came as a surprise, as it did not cluster with its congeners but formed a subcluster within a large cluster made up of specimens of *Rastrineobola argentea*, an endemic of the Lake Victoria/Kyoga system (Figure 20). For a long time, the genus *Engraulicypris* only included the endemic *E. sardella* from the Lake Malawi system (Howes, 1984). *Engraulicypris bredoi*, in turn, has longtime been included in the genus *Mesobola*, the sister genus to *Rastrineobola* (Howes, 1984). Later, the species was included in *Engraulicypris* via the synonymisation of *Mesobola* (Riddin et al., 2016). As such, *Engraulicypris* became a southern African clade with one representative in the Nile system, *E. bredoi*, a species that, however, was not included in the molecular analyses. With this biogeographic anomaly in mind, and the close phylogenetic position of *E. bredoi* with *R. argentea*, it is highly plausible that *E. bredoi* is actually a member of the genus *Rastrineobola*.

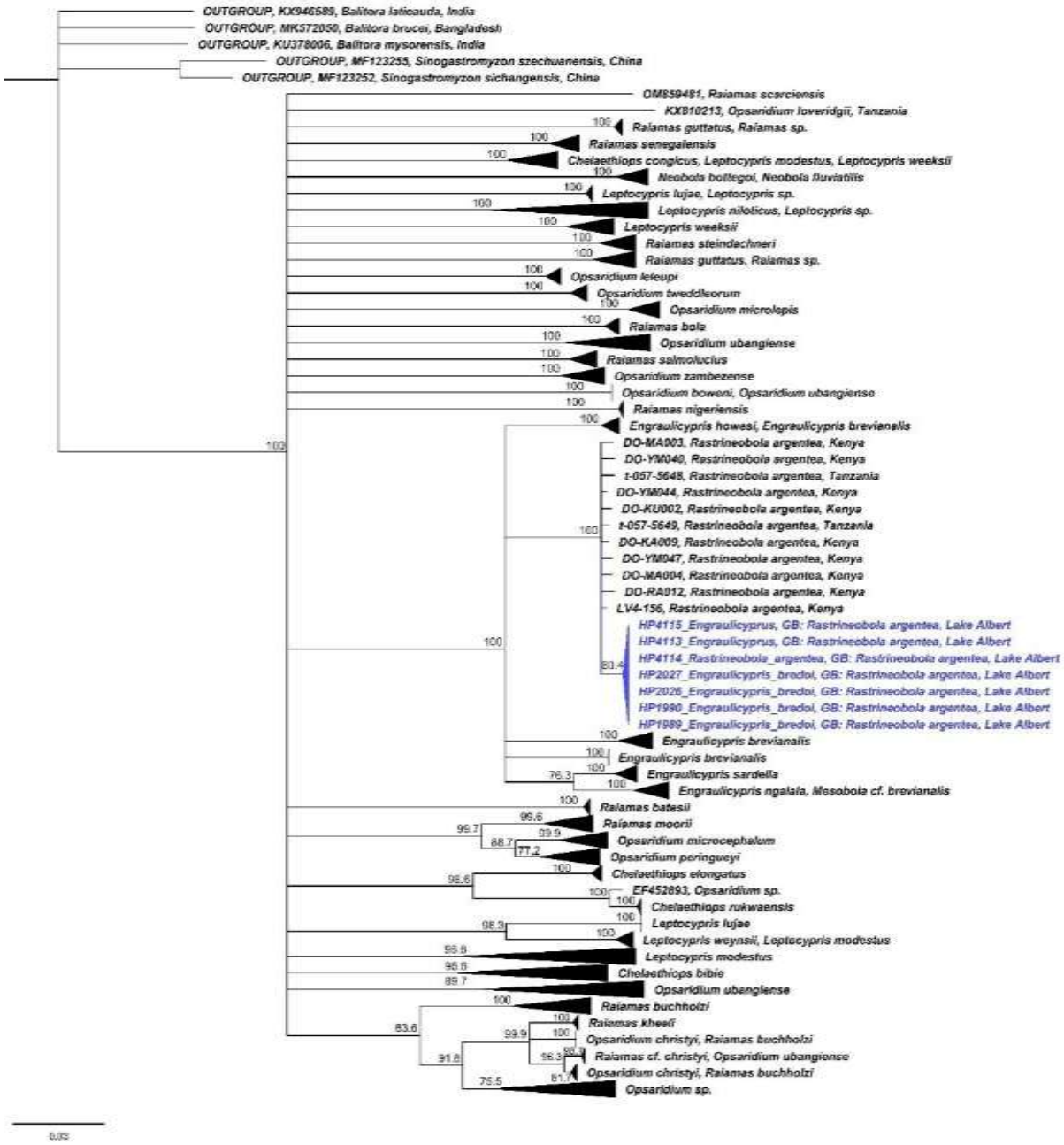

Figure 20 : Neighbour-Joining tree including species of the genera *Raiamas, Opsaridium, Engraulicypris, Rastrineonola, Neobola, Chelaethiops, Leptocypris* (Chedrinae, Danionidae), based on the cytochrome *c* oxidase subunit I (COI) gene (Tamura–Nei distance model; 652 bp fragment; 1,000 bootstrap replicates). Bootstrap values are shown at branch nodes, and the newly generated COI sequences are highlighted in blue. For clarity, clusters of non-target species were collapsed, and only bootstrap values ≥ 75 are shown.

Latidae: From the COI tree (Figure 21), it is clear that there are several clusters of *Lates niloticus*. All our samples (from Lakes Albert, Kyoga and Victoria) fall within a clade with Genbank specimens from Lake Victoria. The Egypt specimens in this cluster appear to originate from the commercial trade and most probably originate from Lake Victoria as well. A second *L. niloticus* clade includes specimens from the Nile and the Niger system, a third one from the Congo basin and a fourth one (*Lates aff. niloticus*) constitutes of one specimen from the Rokel in Sierra Leone. Koblmüller et al. (2021) already discussed the occurrences of multiple lineages within the large distribution area of what is currently considered as *L. niloticus*. Because of the genetic divergence of the Lake Albert cluster and the Nile/Niger cluster, it is not excluded that if further research finds indications that the differences are species specific, that the name *L. albertianus* needs to be resurrected for the Lake Albert populations.

There is no indication in our sample of a second species in the Lake Albert system, the endemic *L. macrophthalmus*. It is difficult to say whether this is due to the fact that there is only one in stead of two species in the lake or because we did not collect the species, possibly because of its deep benthic lifestyle (Worthington, 1929).

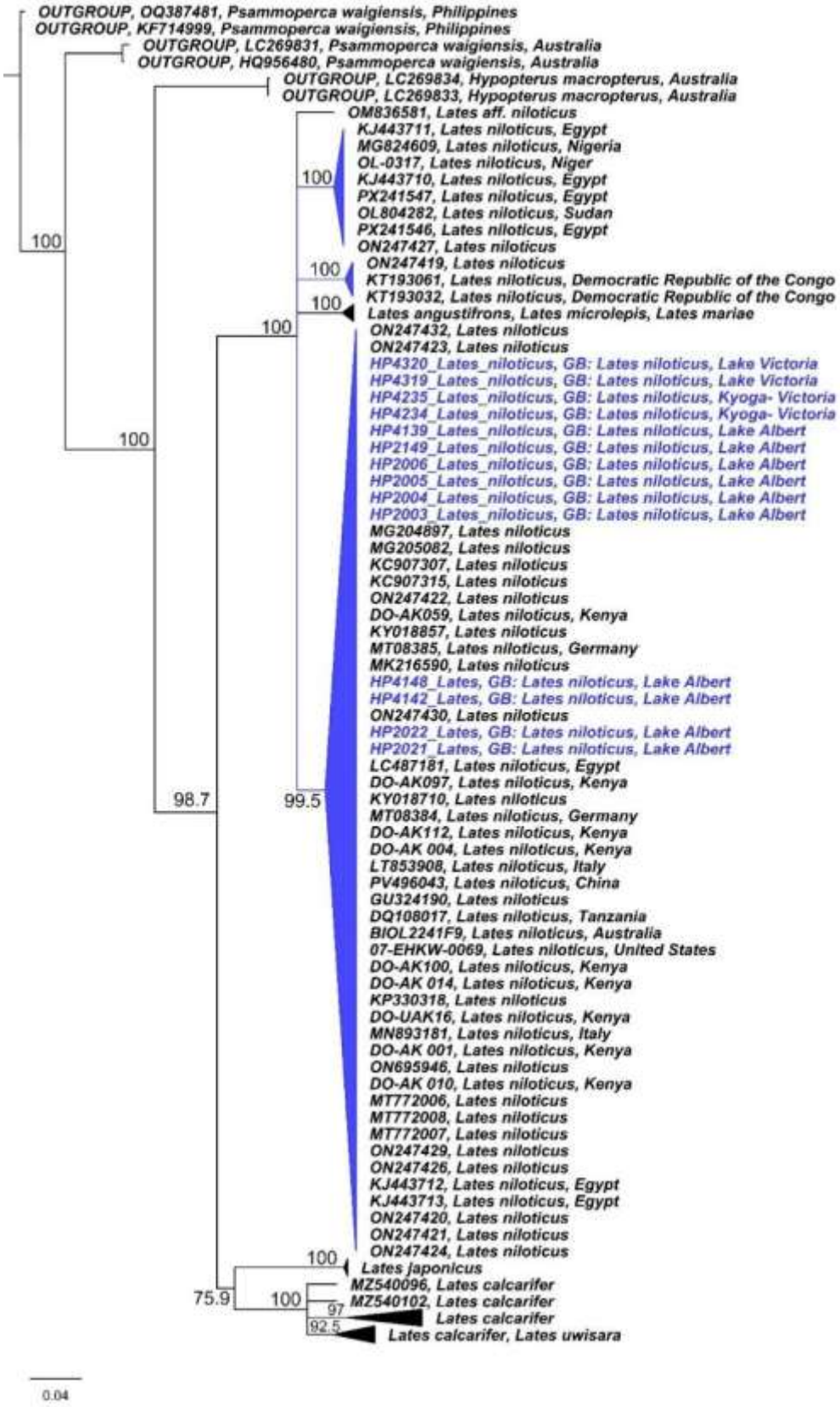

Figure 21 : Neighbour-Joining tree including species of the genus *Lates* (Latidae), based on the cytochrome *c* oxidase subunit I (COI) gene (Tamura–Nei distance model; 652 bp fragment; 1,000 bootstrap replicates). Bootstrap values are shown at branch nodes, and the newly generated COI sequences are highlighted in blue. For clarity, clusters of non-target species were collapsed, and only bootstrap values ≥ 75 are shown.

Mochokidae: Except for the *Synodontis schall/S. frontosus* group, the barcoding results of the representatives of the genus *Synodontis* are pretty straightforward (Figure 22).

The single specimen of *S. nigrita* from the Lake Albert system clusters with a conspecific specimen of the same system. This lineage is sister to another clade with other representatives of this Nilo-Sudanic species (Ethiopia, Sudan, Burkina Faso and Nigeria) and a specimen from the aquarium trade in South Africa.

All specimens we identified as S. afrofisheri and some as *S. sp.* originate from the Lake Victoria system and cluster together with others from the region.

A specimen we identified as *S. victoriae* from the Mara wetlands indeed clusters with Genbank conspecifics from other parts of the Victoria basin. There is, however, a separate clade of *S. victoriae* with specimens from the Malagarazi river, an affluent of Lake Tanganyika. This small clade is quite divergent from all other *S. victoriae* specimens. This lineage was named *S.cf. victoriae* by Englmaier et al. (2024) and considered a sister group of *S. victoriae* from the Lake Victoria system. Both clusters were considered as conspecific and the genetic divergence to be the result of a long geographic isolation between the two populations.

As said, the situation for *S. schall* and *S. frontosus* is not clear.

One specimen we identified as *S. schall* from Lake Albert is isolated from all others of the system in a lineage of specimens identified in Genbank as *S. sp.* from the Lake Victoria system in Kenya. It is unclear what the identity of this species is.

We found a large cluster that encompasses specimens from the Lake Albert system we identified as S. *schall* and a few as *S. frontosus*. This cluster includes also GenBank identifications of the same species, as well as specimens identified as *S. nigrita, S. serratus, S. caudovittatus,* and *S. khartoumensis*. This confusion is not surprising as Day et al. (2013) already indicated that *S. schall, S. frontosus* and *S. caudovittatus* are part of a monophyletic cluster of species. This large cluster of specimens from eastern Africa and the Nile system is a subclade of a larger clade that contains another subclade also encompassing specimens identified as *S. schall* and *S. caudovittatus* (amongst others), but that only includes specimens from West Africa. There is definitely a taxonomic problem here. The third and last subclade includes a specimen we collected in the Lake Albert basin in a cluster with *S. serratus* specimens from the Lake Albert system and the Nile system in Ethiopia and Egypt.

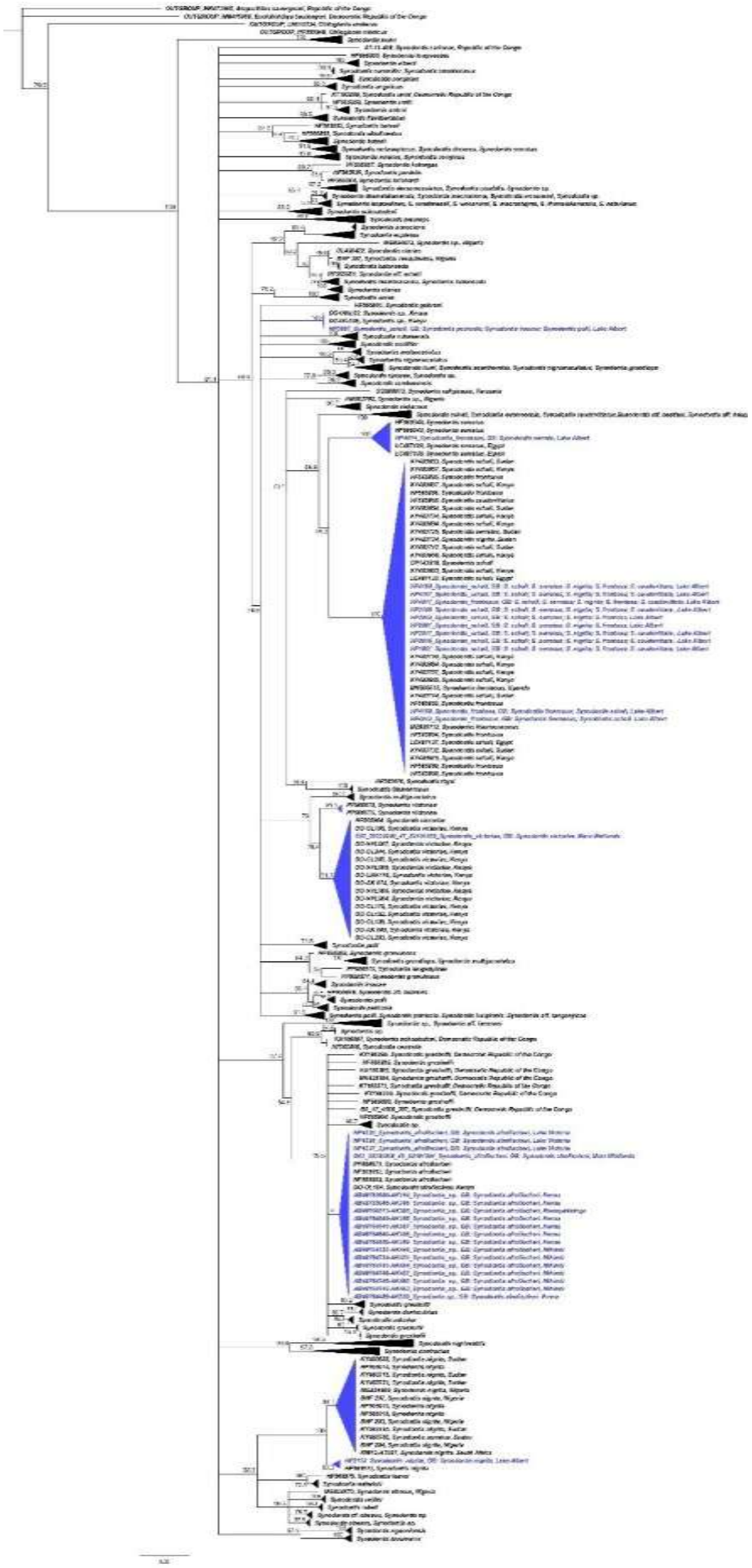

Figure 22 : Neighbour-Joining tree including species of the genus *Synodontis* (Mochokidae), based on the cytochrome *c* oxidase subunit I (COI) gene (Tamura–Nei distance model; 652 bp fragment; 1,000 bootstrap replicates). Bootstrap values are shown at branch nodes, and the newly generated COI sequences are highlighted in blue. For clarity, clusters of non-target species were collapsed, and only bootstrap values ≥ 50 are shown.

Mormyridae:

All our *Marcusenius* specimens cluster together and form one monophyletic lineage, sister to *M. cyprinoides* from Nigeria (Figure 23). This cluster represents *M. victoriae* from the Akagera, the Mara wetlands and the Kafu system, all within the Lake Victoria basin.

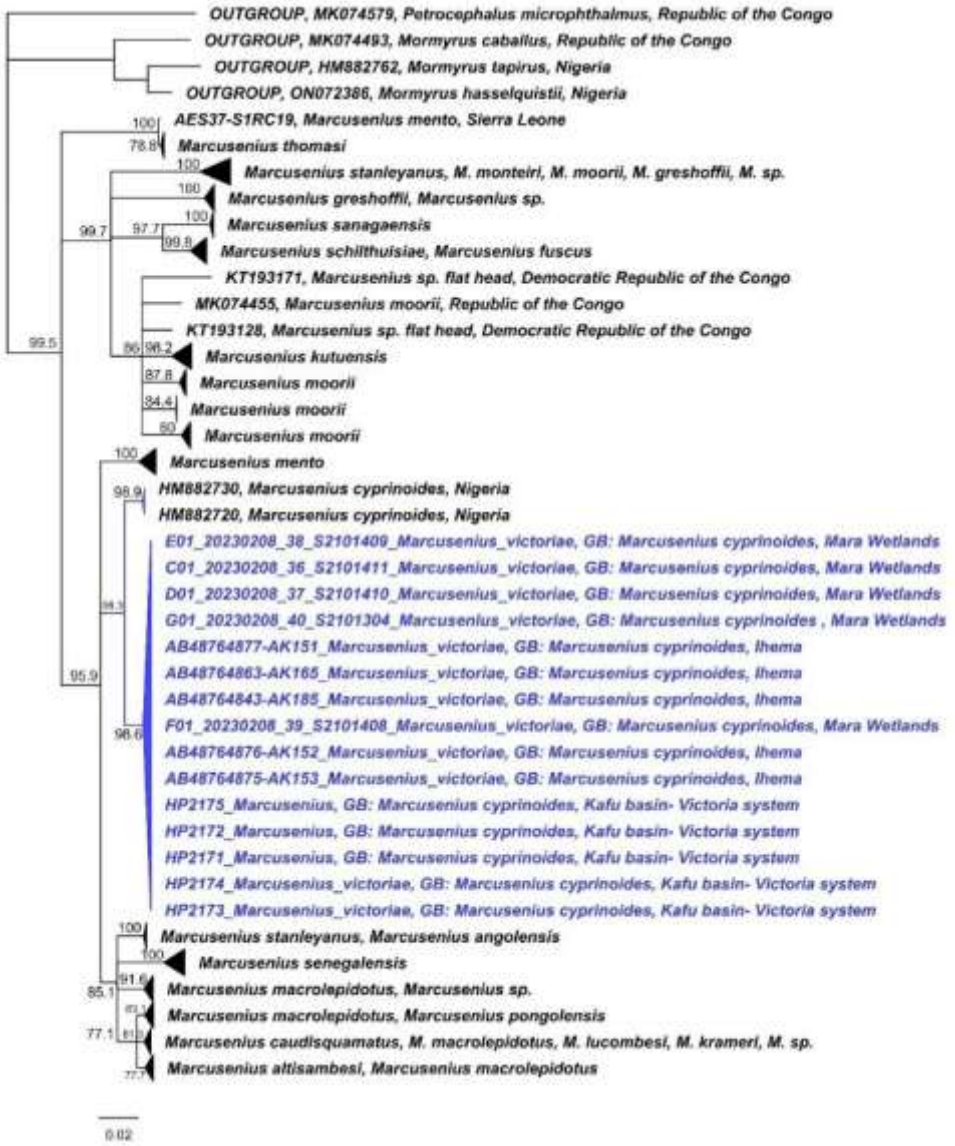

Figure 23 : Neighbour-Joining tree including species of the genus *Marcusenііus* (Mormyridae), based on the cytochrome *c* oxidase subunit I (COI) gene (Tamura–Nei distance model; 652 bp fragment; 1,000 bootstrap replicates). Bootstrap values are shown at branch nodes, and the newly generated COI sequences are highlighted in blue. For clarity, clusters of non-target species were collapsed, and only bootstrap values ≥ 75 are shown.

The *Mormyrus* tree (Figure 24) contains some well-defined species-specific lineages and a particular cluster that includes *M. tapirus* and some undescribed species from Cameroon (Sommer et al., 2024). There is a large clade containing all *M. kannume* specimens from the project, collected in the Lakes Edward and Albert systems and in the Middle Akagera in Rwanda (Lake Victoria basin). It also includes Genbank specimens from the Nile in Egypt. This all corresponds well to the currently known distribution area of the species. We observe a weak substructuring with our Victoria specimens clustering together and those of the Edward and Albert system also clustering together. However support is weak and both subclades contain a Genbank specimen from the Aswan dam in Egypt. A third subclade only contains specimens from the latter locality. At present, it is unclear whether this has something to do with the presence in the Nile basin, of the very similar *M. caschive*.

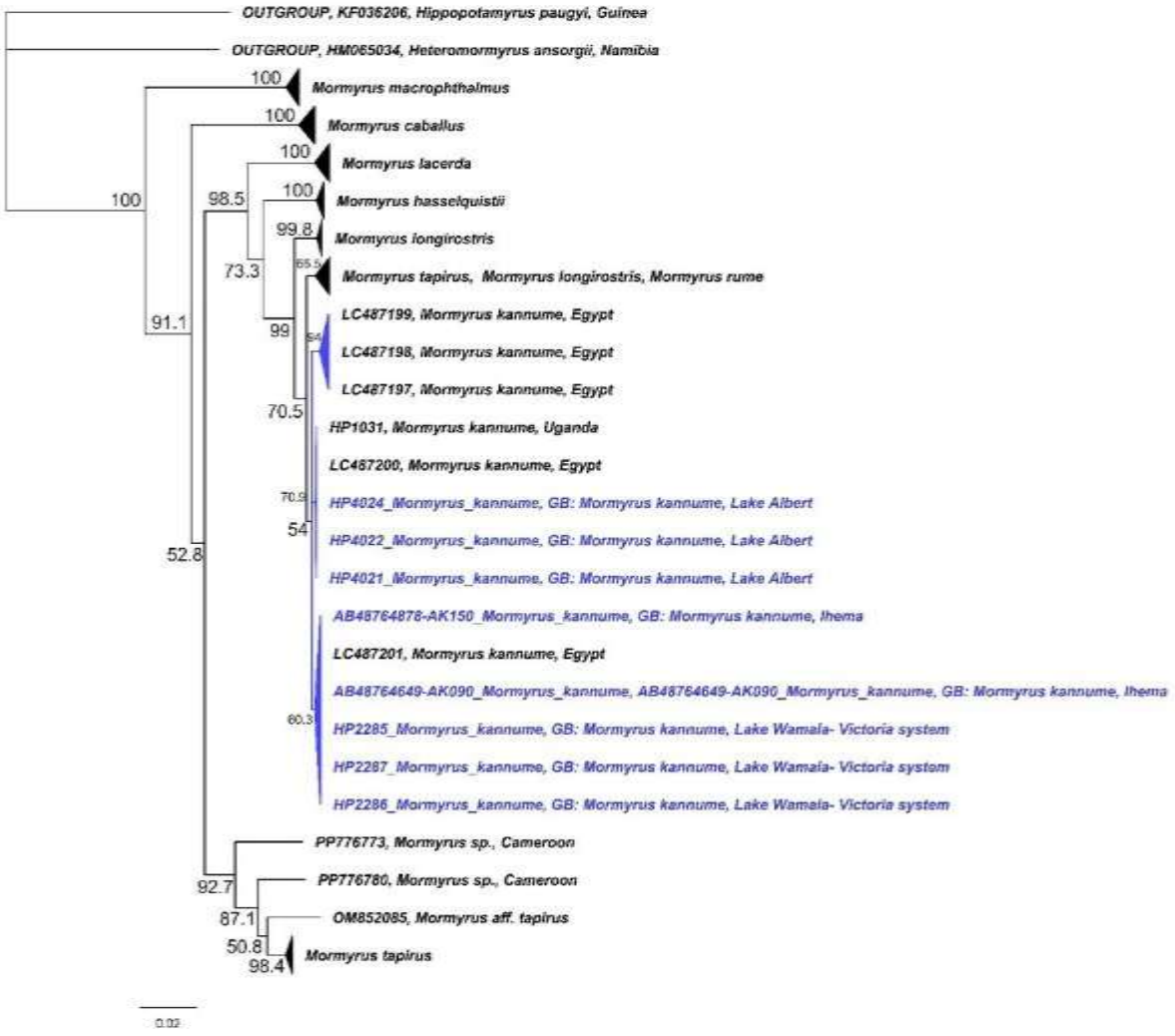

Figure 24 : Neighbour-Joining tree including species of the genus *Mormyrus* (Mormyridae), based on the cytochrome *c* oxidase subunit I (COI) gene (Tamura–Nei distance model; 652 bp fragment; 1,000 bootstrap replicates). Bootstrap values are shown at branch nodes, and the newly generated COI sequences are highlighted in blue. For clarity, clusters of non-target species were collapsed, and only bootstrap values ≥ 50 are shown.

Procatopodidae: We got barcodes of five species from this family from the area studied. Decru et al. (2022) reported three species from the Lake Edward system and already discussed the strange finding that the endemic *Laciris pelagicus* had the same COI-haplotype as *Micropanchax vitschumbaensis*. Both species form the sister clade to Genbank specimens identified as *Micropanchax kingii* from Sudan (Figure 25). This corresponds to the position of *M. cf. kingii* as a sister group of *M. vitschumbaensis* and *M. kassenjiensis* in the multimarker trees of Bragança & Costa (2019). The third species reported from the Lake Edward system was *Hypsopanchax modestus*. Its sistergroup relationship with *H. platysternus* has already been discussed by Bragança & Costa (2019) and bears testimony of the ancient hydrological connection between the Lake Edward region and the Aruwimi system.

All our samples from the Victoria basin are within a clade including *Lamprichthys tanganicanus, Lacustricola lualabaensis* and *L.acustricola pumilus*. They add two new lineages to a clade that consisted of the three above-mentioned species found by Bragança and Costa (2019). The samples from the Middle Akagera (Rwanda) formed a separate lineage, which we assumed to represent *Lacustricola centralis*, as it is the only known procatopodid from this area (De Vos et al., 2001). However, there is another *L. centralis* clade with a specimen from the Malagarasi, used in the study of Bragança & Costa (2019). In the latter study, this specimen is well established within a larger clade of southern African species. At present *L. centralis* is reported from Lake Rukwa, the Malagarazi and the Lake Victoria basin. It seems the Akagera specimens belong to another species, possibly *L. bukobanus*, reported from the Lake Victoria basin but not yet from the Middle Akagera system in Rwanda.

Our second Victoria system lineage includes samples from the Kafu river and Lake Kyoga (Lake Victoria system). These probably belong to the recently described species, *Lacustricola marginatus*, by Nagy & Watters (2022).

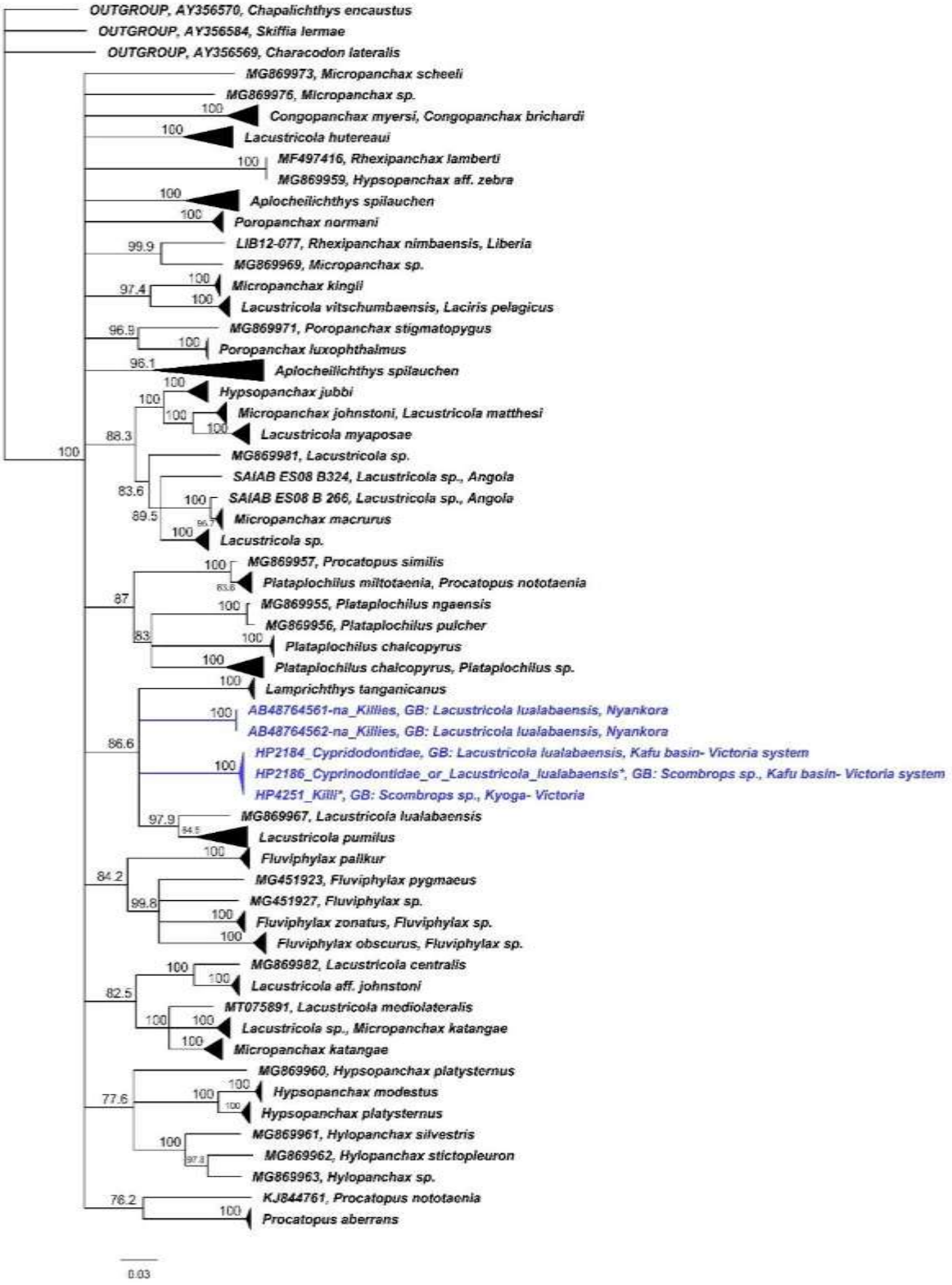

Figure 25 : Neighbour-Joining tree including species of the genera *Micropanchax, Procatopus, Hypsopanchax, Congopanchax, Lacustricola, Poropanchax, Rhexipanchax, Aplocheilichthys, Lamprichthys, Fluvihyloax, Hylopanchax* and *Plataplochilus* (Procatopodidae), based on the cytochrome *c* oxidase subunit I (COI) gene (Tamura–Nei distance model; 652 bp fragment; 1,000 bootstrap replicates). Bootstrap values are shown at branch nodes, and the newly generated COI sequences are highlighted in blue. For clarity, clusters of non-target species were collapsed, and only bootstrap values ≥ 75 are shown.

Polypteridae: No surprise with *Polypterus* (Figure 26). The Lake albert samples are situated within the clade of other *P. senegalus* specimens, most of which, as far as could be detected, originate from the aquarium trade.

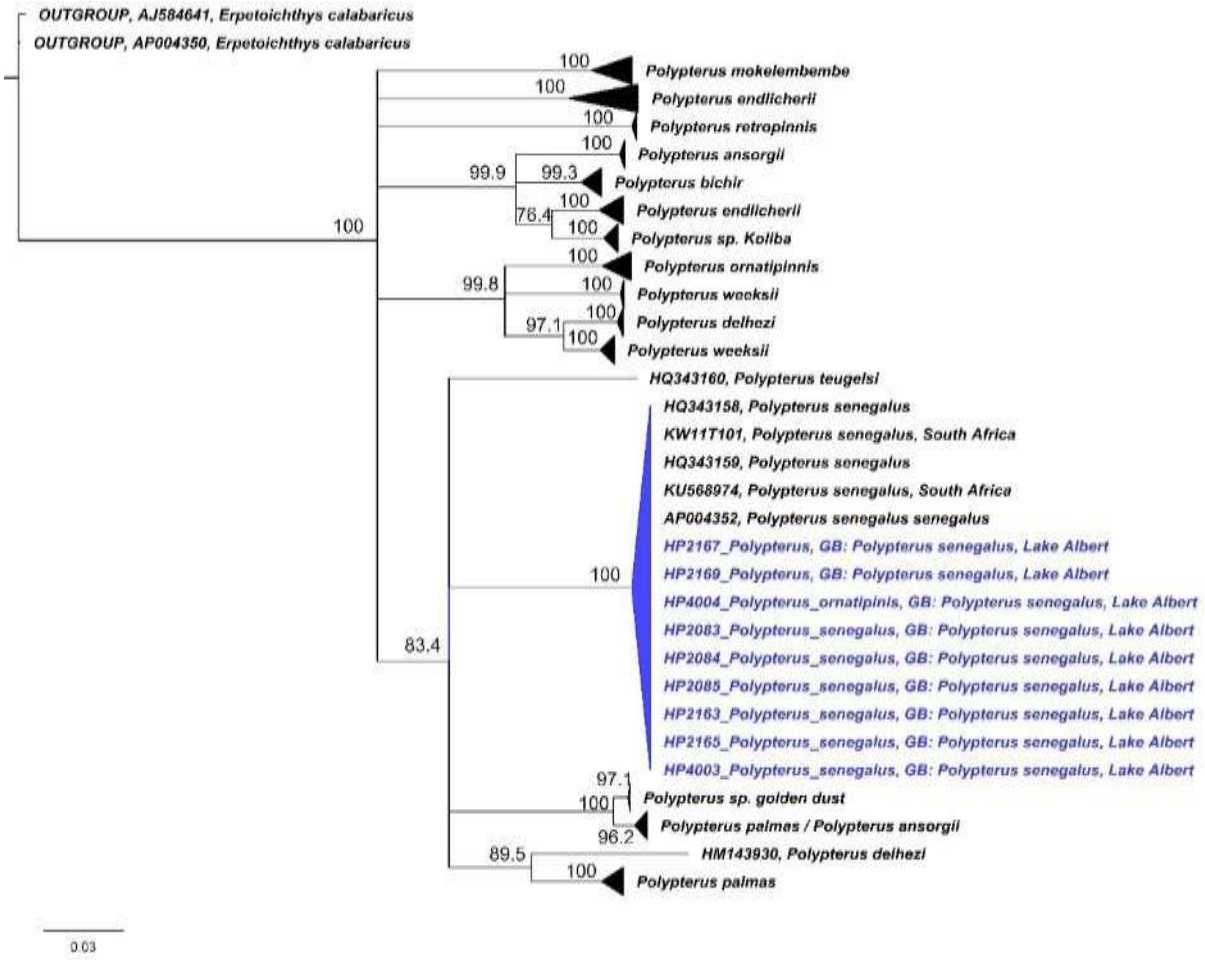

Figure 26: Neighbour-Joining tree including species of the genus *Polypterus* (Polypteridae), based on the cytochrome *c* oxidase subunit I (COI) gene (Tamura–Nei distance model; 652 bp fragment; 1,000 bootstrap replicates). Bootstrap values are shown at branch nodes, and the newly generated COI sequences are highlighted in blue. For clarity, clusters of non-target species were collapsed, and only bootstrap values ≥ 75 are shown.

The **eDNA metabarcoding survey** in the Mara and Akagera wetlands yielded a total of 19,196,347 paired-end DNA sequences using the two libraries. Subsequent analyses allowed us to detect 38 species, in 18 genera across 10 families. Both the 12SV5 and MiMammal primer sets successfully detected fish species, with 12SV5 exhibiting higher sensitivity across overall fish diversity. Species detection was performed by comparing a distance-based method with blasting against available datasets. As expected, this did not reveal sufficient resolution to distinguish between closely related species, mostly within the cichlids (Figure 27). Additional analyses are being performed to detect haplotype level diversity within these groups. These analyses form part of a PhD prepared at Université Mohammed V, Rabat (Melvin Odiba, Prof. Bouabid Babaoui), in collaboration with

colleagues of University of Graz (Dr. Tamara Schenekar), UHE Delft (Dr. Ken Irvine, Dr. J Simaika, Leandro de la Cruz) and the Tazanian Fisheries Research Institute (TAFIRI).

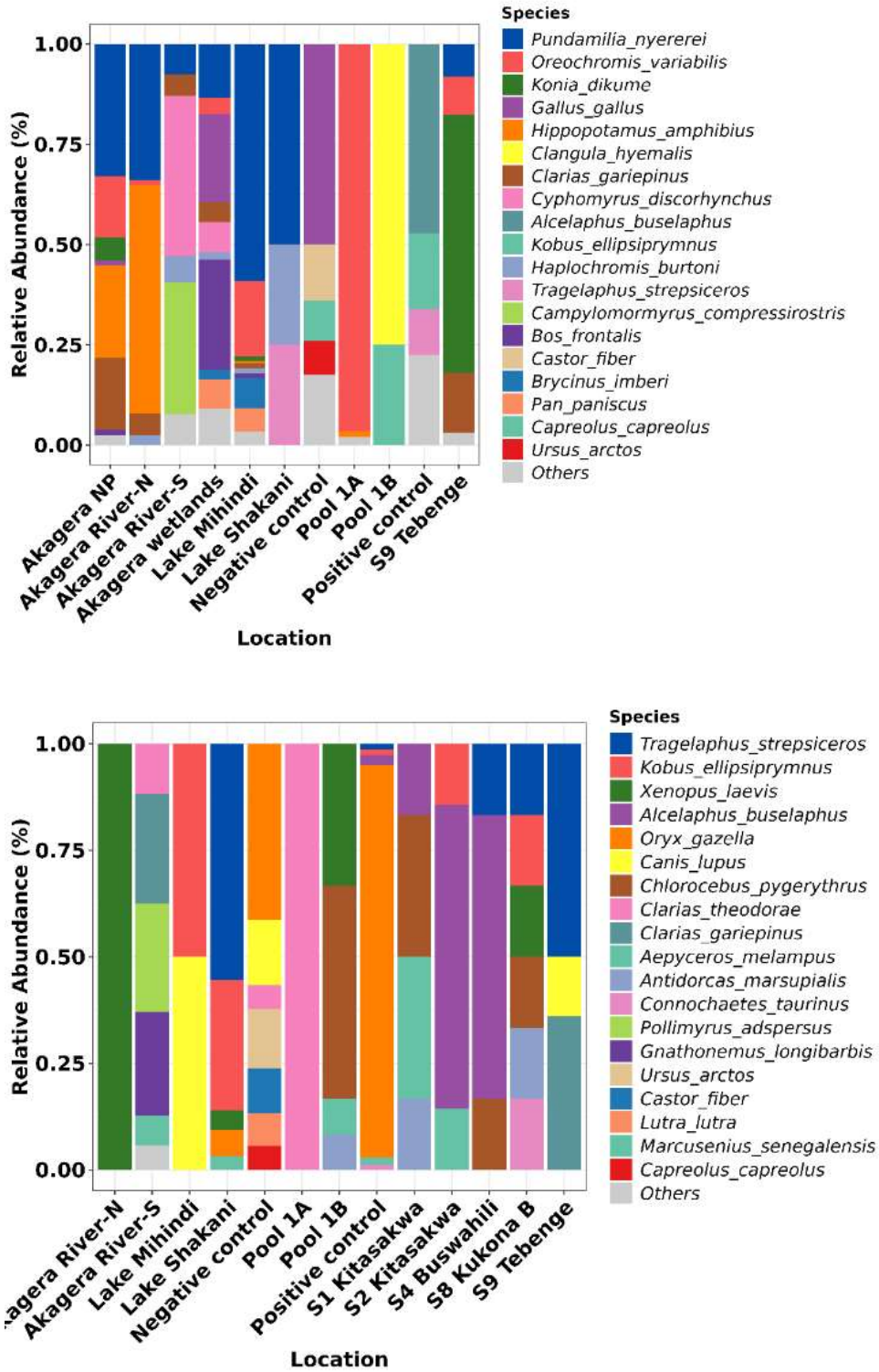

Figure 27: Preliminary results of relative read abundance at species-level detected through eDNA metabarcoding at each sampling location for the 12SV5 (upper figure) and MiMammal (lower figure) primers, negative controls, and positive controls. Species detection was done by blasting to available databases. (image Melvin Odiba).

Our attempt to mini-barcode preserved specimens (pilot study on *Enteromius* taxa), gave mixed results. DNA could successfully be extracted from most specimens. A collection effect was found, meaning that specimens from some collections resulted in higher concentrations DNA than others. This makes it difficult to predict which specimens will be best to use. However, a preservation-time effect was not found, meaning old specimens performed as well as recent ones.
The universal primers used failed to construct the sequences needed, as most sequences matched with *Homo sapiens*. This means that, in most cases, contaminant human DNA was amplified and sequenced. Our attempt to characterize the diversity of *Enteromius* via mini barcodes of preserved specimens was therefore not successful and was not further pursued in this project.

WP2. Diversity of non-haplochromines

At the start of the project the fish diversity of some parts of the KEA region was considered as relatively well known, while the ichthyofauna of others was very poorly documented.
One of the better known region was the Lake Kivu basin and by extension the whole of Rwanda based on the publications of Snoeks et al. (1997, 2012) and De Vos et al. (2001). However our study on the fish diversity of the **Nyungwe National Park** and its neighbouring aquatic systems (Maetens, 2024) demonstrated that the knowledge was incomplete for two main reasons. First of all, we found undiscovered species diversity in some groups such as the small barbs and amphiliid catfish. These catfishes are specialised inhabitants of fast running waters and seem to be endemic to much smaller distribution areas than previously reported. We found two taxa that we provisionally named *Amphilius cf. kivuensis* 'Kivu' and *Amphilius cf. kivuensis* 'Rusizi'.

Second, we discovered that much more species have been introduced in the area than reported. Introduced fish species are abundant in the region, especially in Rwanda. We discovered a new record of a translocated cichlid species (*Pseudocrenilabrus multicolor*) and a newly introduced species, the guppy (*Poecilia reticulatus*) in the Lake Kivu basin.
In the Nyungwe publication, we reported 14 **introduced species** from four fish families in Rwanda, representing 12% and 20% of the total fish diversity. This relatively high number masks the number of translocations within the country, and the number of introduced aquaculture strains of naturally occurring species, both of which can have effects on the native fauna (De Vos et al., 2001).
Introduced and translocated fish species have also been collected in the Akagera basin. In swamps outside of the park boundaries, the number of these fish species even surpassed that of native species. The results of this sampling trip will be published next year.

Our study revealed a large amount of **cryptic diversity within the genus *Enteromius*** in East Africa.
In the KEA region, the small diploid barbs all belong to the genus *Enteromius*. There are clearly two groups that are genetically and morphologically distinct. A group with a smooth flexible third unbranched dorsal fin ray (soft-rayed barbs) and a group in which this fin ray is hard and serrated

(sawfin barbs). While they are well-distinct groups in the region, on the pan-African level, they do not form monophyletic groups.

Within the Lake Edward system, two species of soft-rayed barbs were found prior to the project (Maetens et al. 2020), *Enteromius alberti* and *Enteromius cf. mimus*. The latter is very similar to *E. mimus* from the Ewaso Nyiro, (Kenya) flowing into the Indian Ocean. The former species was a synonym of *E. perince*, but was revalidated. At the same time, *E. cercops* from the Nzoia River (Kenya) in the Lake Victoria system was put in synonymy with *E. alberti*. Additional confirmation of this synonymy was found during this project when the COI haplotype of a specimen identified as *E. cercops* from the Lake Victoria system turned out to fall within the COI-lineage of *E. alberti* (Maetens unpubl.). During the project, three more lineages of the soft-fin rays barbs have been discovered within the region, all from the Lake Victoria / Awash system.

The highlight of the fish diversity component of the project was the unravelling of the cryptic diversity found in the sawfin barbs in the region. At the onset of the project we knew that the three species known from the Edward basin (Figure 28) actually included unrecognised diversity with seven distinct mtDNA lineages. At present, we have detected about 14 lineages within the KEA region and the Lake Victoria system.

Figure. 28: Photographs of representatives of the three species complexes of safwin barbs from the Lake Edward system: **a)** *E. apleurogramma* (HP3124), **b)** *E. kerstenii* (HP3123) and **c)** *E. pellegrini* (HP3127) (from Maetens et al., 2024).

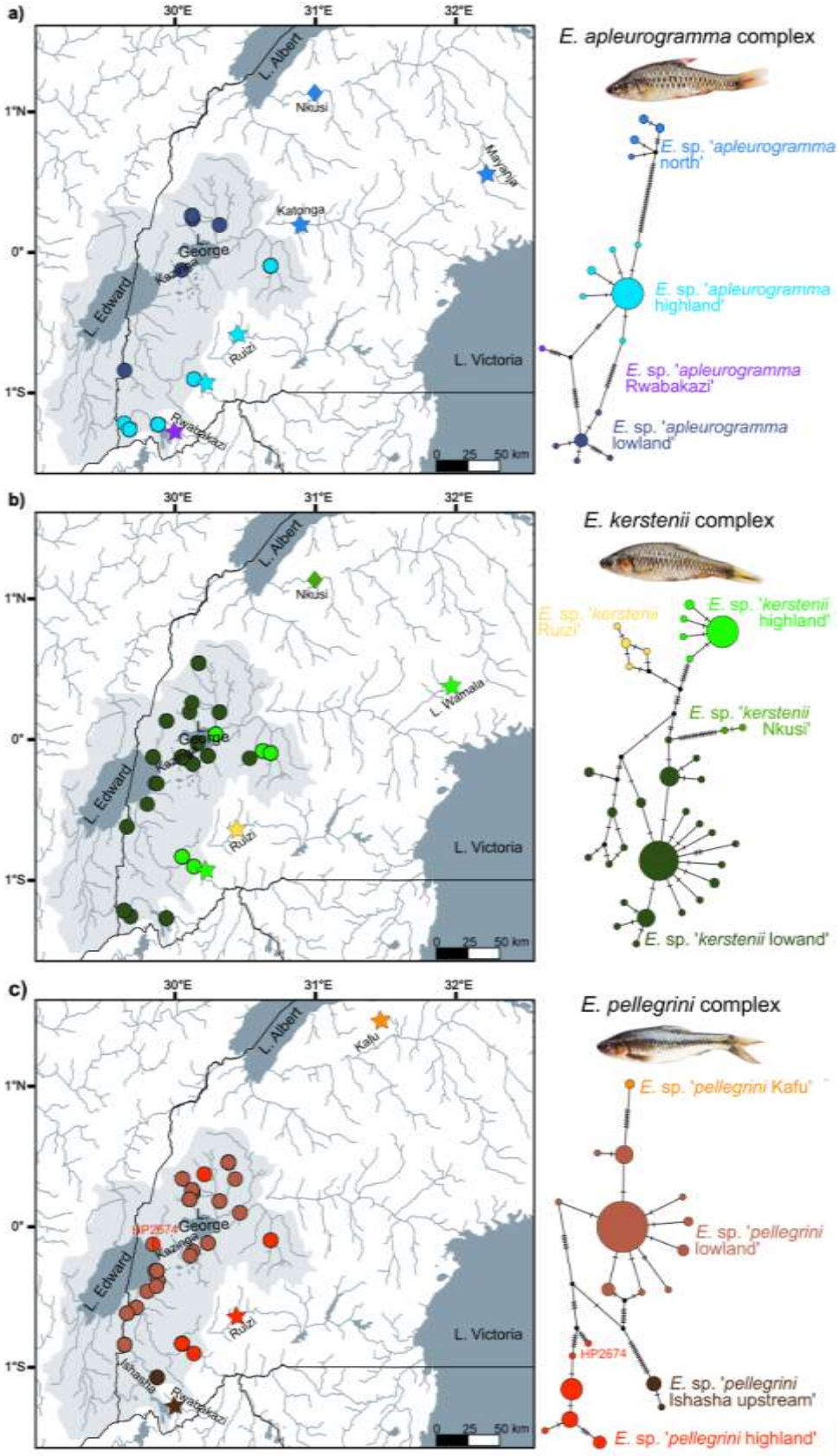

Figure 29. TCS haplotype network of the COI sequences and geographical distribution per species complex: **a)** *E. apleurogramma*, **b)** *E. kerstenii* and **c)** *E. pellegrini*. The Lake Edward system is highlighted in grey. Specimens from the drainages of Lakes Albert and Victoria are indicated with a diamond and a star respectively (From Maetens et al., 2024). Remarkably, each complex contains a lowland and a highland lineage.

Recently, we published an account to the sawfin barbs of the region (Maetens et al., 2024). Within each of the three complexes , we found four lineages that are regarded as putative species, illustrating the unrecognized diversity in the region (Figure 29). All putative species were genetically very divergent, while morphologically very similar within individual complexes. Putative species within a complex could not be distinguished by naked eye, only via multivariate morphometric analyses (Figure 30).

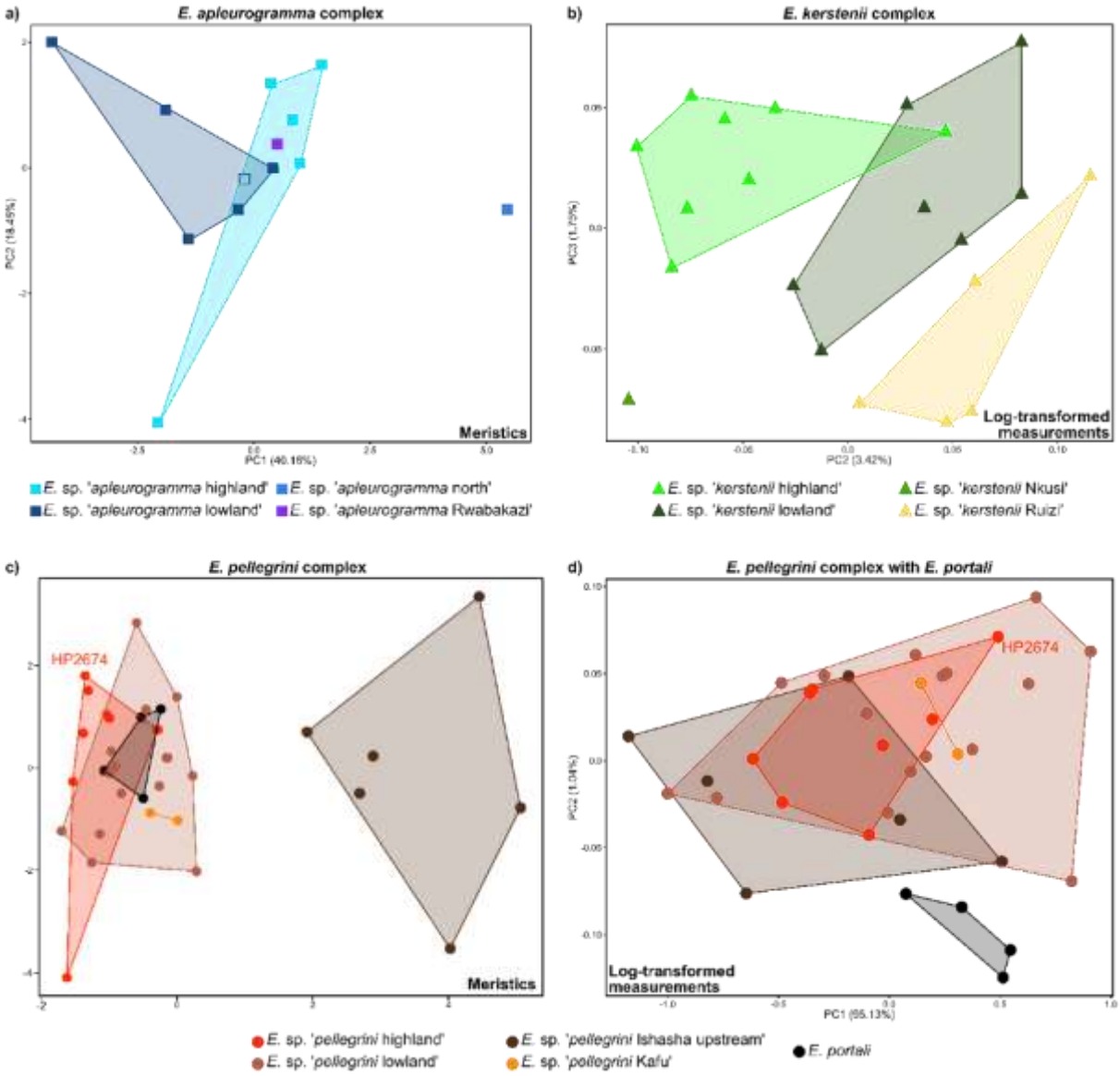

Figure 30. Scatterplots of PCAs on specimens from the Lakes Edward, Albert and Victoria systems with a**)** PC2 against PC1 of the PCA on 12 meristics of 14 specimens of the *E. apleurogramma* species complex, **b)** PC3 against PC2 of the PCA on 24 log-transformed measurements of 21 specimens of the *E. kerstenii* species complex, **c)** PC2 against PC1 of the PCA on 14 meristics of 31 specimens of the *E. pellegrini* species complex, and **d)** PC2 against PC1 of the PCA on 24 log-transformed measurements of 32 specimens of the *E. pellegrini* species complex and the four syntypes of *E. portali* (From Maetens et al., 2024).

Within each complex, we also found a remarkable consistent geographic pattern, with one species occurring at higher altitudes within the Lake Edward system and over the water divide in the Lake Victoria basin, and another closely related species occupying lower altitudes near Lakes Edward and George (Figure 29).

The resulting picture is that of a unique genus of fishes typified by a large unrecognised diversity displaying morphological stasis and high genetic divergence between species, with no indication of speciation processes involving sexual selection, eco-morphological adaptations or large-scale hybridization. The bio-geographic patterns suggest a predominant allopatric mode of speciation. Intriguingly, the revealed Pliocene-Pleistocene origin of nearly all species of sawfin barbs from the Lake Edward system and neighbouring regions largely predated the important geological events that reshaped the hydrology in the western rift. Therefore the evolutionary history of the genus in the region remains an enigma. At present, we are doing some further analyses involving whole-genome sequencing to unravel this puzzle.

The distribution areas found for the various species is clearly influenced by one of the most important hydrological features of the region: the abundance of marshy areas at watershed borders that may allow an inter-basin exchange of fish taxa during periods of high water (Figure 31).

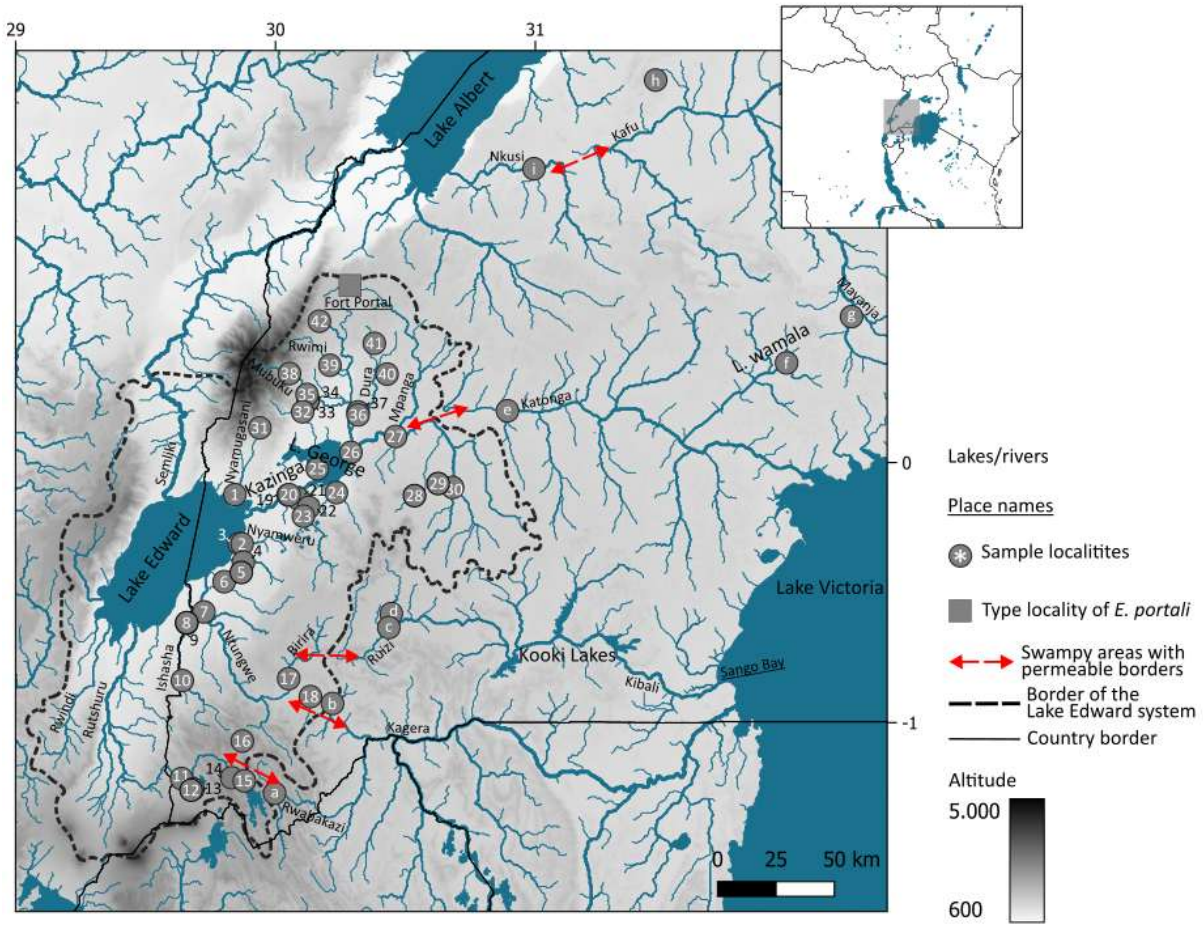

Figure 31: Map of the region sampled for the publication on the saw-fin barbs by Maetens et al. (2024). Areas of possible inter-basin exchange are indicated with red arrows.

In a recent MSc-thesis (Thuwis, 2025), even more areas were found to be inhabited by one of the new sawfin barb lineages discovered (Figure 32).

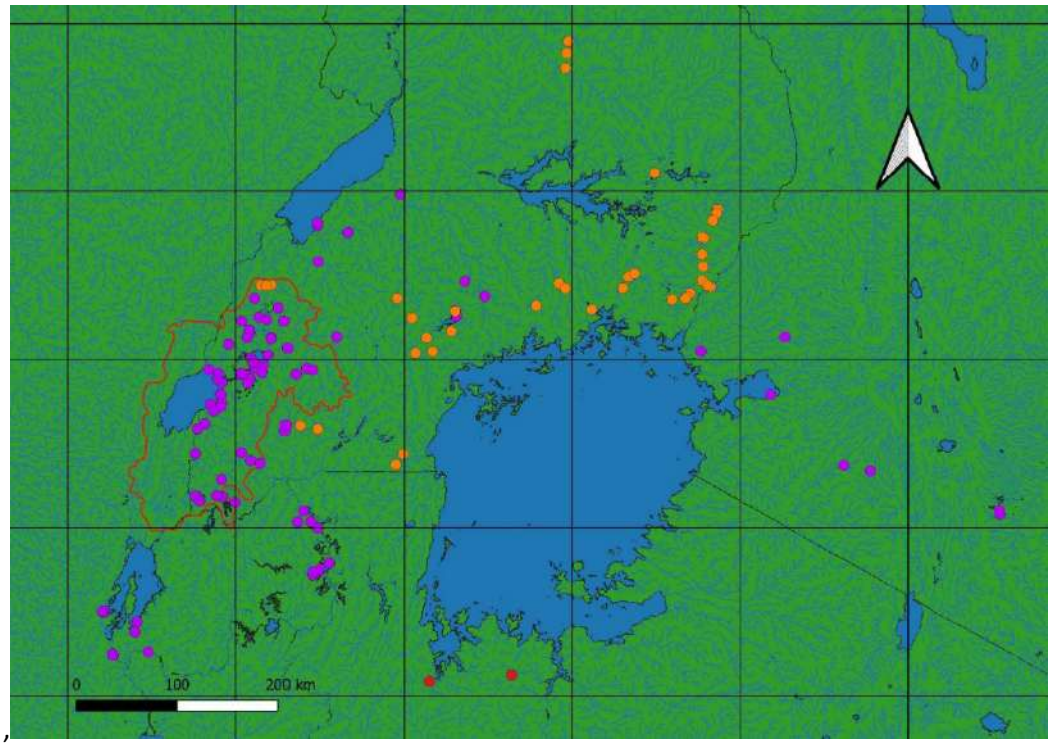

Figure 32: Sampling localities for the barcoding study on *Enteromius*. Purple dots refer to the localities analysed and reported in Maetens et al., (2024). The orange and red dots refer to localities sampled afterwards (Thuwis, 2025). This shows clearly that the region is well covered, with a much more detailed sampling than anticipated at the start of the project, as a result of collaborative effort of project partners and additional collaborators (see above for methods).

In addition, another new lineage was discovered within the *E. kerstenii* complex, at present limited to the Akagera system in Rwanda (Figure 33). New is also the discovery of three lineages within what we tentatively call the *E. paludinosus* complex, which together formed a sister-clade to a clade comprising the *E. apleurogramma* and *E. pellegrini* complexes. Three new lineages were also discovered outside the group of sawfin barbs. These lineages form part of the cluster of smooth-fin barbs already including *E. alberti* and *E. cf. mimus*.

When we complemented our COI dataset of sawfin barbs with those from GenBank, we discovered that many of the sequences came from specimens with a doubtful identification. This complicates the reconstruction of phylogenetic relationships and biogeographic patterns of the sawfin barbs inthe region and in Africa as a whole (Figure 33).

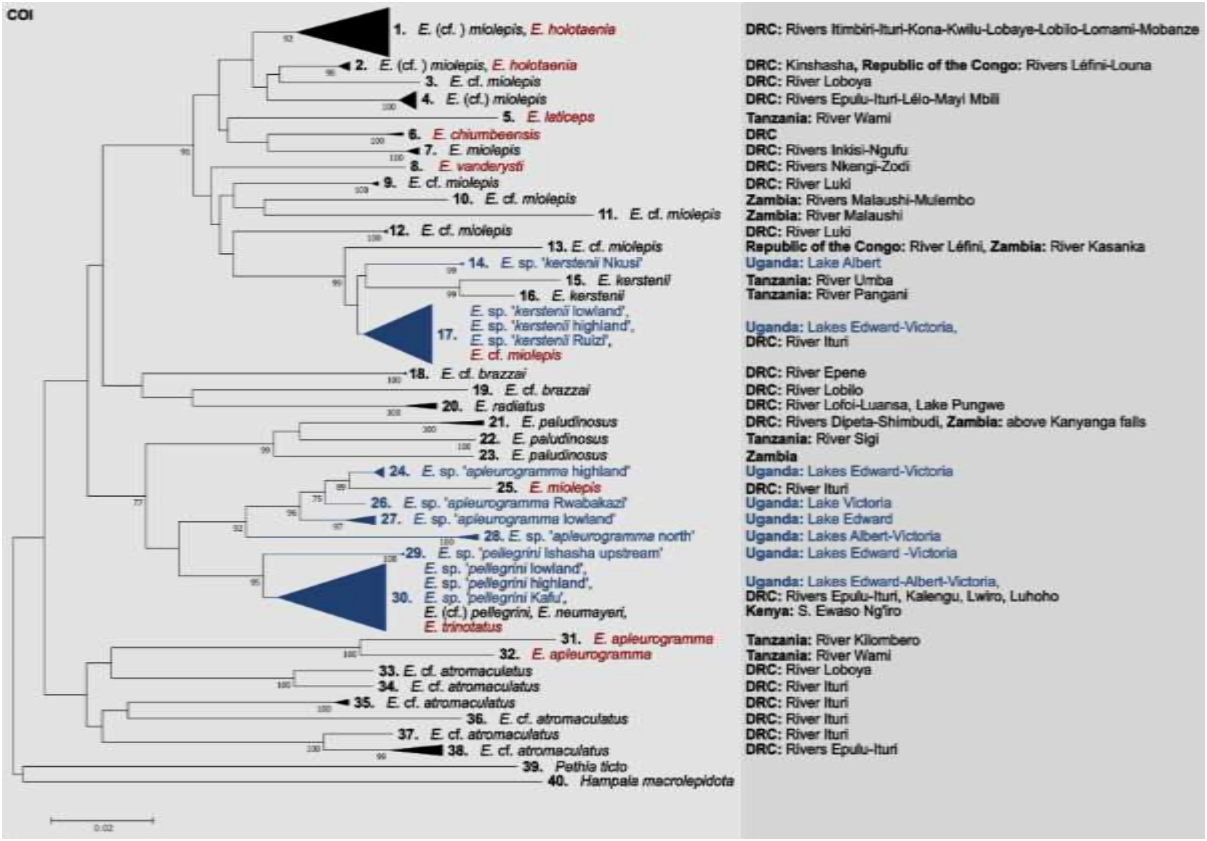

Figure 33. NJ tree of the mitochondrial COI gene of the 'sawfin barbs' from the Lake Edward system and the neighbouring basins of the Nile, Congo and other regions in the East Coast ichthyofaunal provinces. Lineages with less than 2% genetic divergence were collapsed and given a number. Bootstrap values (>75%) are given under the branches. Specimens from the study of Maetens et al. (2024) are in blue, specimens with a doubtful GenBank identification are in red.

To complete the picture, we are awaiting the results of the barcoding of some complementary specimens from the NaFIRRI collection. This all will allow us to more correctly infer distribution patterns and refine the biogeographical analysis. As a result, this detailed and intensive sampling effort also multiplicated the amount of work to be done, which is still ongoing.

From the above it is clear that one of the major results concerning the diversity in the KEA region is the large amount of undescribed diversity of the small barbs. While the existence of cryptic diversity in *Enteromius* was already known as one of the outcomes of the previous HIPE project, the discovery rate of new lineages was beyond expectations. During our project it has become clear that in a consistent way, the large genetic divergence that is found between lineages within the various complexes, is accompanied with morphological stasis. Most of the lineages can morphologically only be distinguished based on detailed multivariate analyses. In order to get some ideas as to how this diversity originated and persisted in the region, we foresaw a genomic component to the project (see below WP4). But because of the enigmatic nature of the diversity of small barbs, we also decided to have a look at eventual trophic adaptations. The idea behind this was that if sexual selection was not important as a driver for speciation (no significant differences in colour pattern between putative

species and no sexual colour dimorphism), then may be ecological speciation has been important. If so, then we should find clear adaptive differentiation in the morphological structures important for food gathering.

Therefore, we added to the project a component to look for indications of trophic adaptations. As such, we did **a geometric morphometric study** on the head structures (2D), the pharyngeal jaw (3D) and the kinethmoid bone (3D). The latter bone is a special structure unique in carp-like fish and was selected based on its importance in a study on another group of cyprinid fishes of the genus *Labeo* in the Congo basin (lyandja, pers. comm.) While some differences could be found between the three complexes of sawfin barbs, none of the three studies allowed us to detect meaningful differences between lineages within a complex. We therefore conclude that the **large genetic divergence not only is accompanied by morphological stasis, but also by a lack of trophic differentiation**.

Our research on *Enteromius* has implications outside the KEA region. After having found such cryptic diversity in the Congo basin (Van Ginneken et al., 2017) and in the KEA region (Maetens et al., 2024), we liked to know whether this phenomenon can be considered as a hallmark of the genus or whether it was rather linked to region-specific geological events. In an MSc study we attempted to verify if such extra-ordinary diversity patterns were also present in West African river systems. Based on the results of a largely unpublished PhD thesis (Bamba, 2012), we selected the *E. ablabes / E. guildi* group as a case study. The existence of two undescribed species already found by Bamba (2012) was confirmed. In addition, a third new species was found, meaning that undescribed diversity also occurs in West Africa. Some geographic structuring was found in the two described species, but on the basis of our results, it was impossible to decide whether these results point at additional cryptic diversity or not. These observations are off course very tentative, as no samples were available for DNA barcoding. The mini barcoding approach on preserved specimens did not result in a meaningful outcome (see above WP1).

We are in a process of writing up a manuscript on the **fish diversity of the Lake Albert system**. Table III gives an overview of this ichthyofauna. The presence of 77 species has been confirmed, while we have indications for another three that may be present in the system. Some 29 species mentioned in the literature or in public databases are not found in the system, while there are five more species that, most probably, are also not present. For five other species their present is possible.

Table III: Annotated list of the fish species present in the Lake Albert system based on the relevant literature, public databases (FishBase, GBIF, IUCN, NaFIRRI freshwater biodiversity portal) and collections at the RMCA. + = presence confirmed, +? = presence likely, ? = presence possible, -? = presence unlikely, - = presence not confirmed. Families ordered alphabetically; undescribed species of *Haplochromis* included as one entry.

	Presence	**Comments**
Alestidae		
Alestes baremoze	**+**	
Alestes dentex	**+**	
Brycinus macrolepidotus	**+**	
Brachyalestes jacksonii	**-?**	Species very similar to B. sadleri. So maybe confused with this species? Mentioned by Nakiyende 2025 but not by Akoth et al., 2022. No preserved specimens available.
Brachyalestes nurse	**+**	
Brachyalestes sadleri	**-?**	Species very similar to B. jacksonii. Not reported from the Lake Albert basin, but reported from the Aswa river (Upper Nile basin). Possible misidentification?
Hydrocynus forskahlii	**+**	
Hydrocynus goliath	**-**	
Hydrocynus vittatus	**+**	
Micralestes acutidens	**-**	Specimens in collection from Kasenyi, re-identified as M. elongatus
Micralestes elongatus	**+**	
Amphiliidae		
Amphilius cf. jacksonii	**+**	Rivers in southern part of basin
Amphilius sp. 'Albert Nile'	**+**	There is a GBIF record from the intake of Lake Okurachere (near the Albert Nile). This area is situated far north of the distribution area of A. jacksonii. Therefore it is here referred to as A.sp. 'Albert Nile'
Anabantidae		
Ctenopoma muriei	**+**	
Microctenopoma damasi	**+**	Found in Semliki
Auchenoglanididae		
Auchenoglanis occidentalis	**+**	Should probably be called A. acuticeps, currently still a synonym
Bagridae		
Bagrus bajad	**+**	
Bagrus docmak	**+**	
Cichlidae		
Coptodon congica	**-**	A specimen from an affluent of the Semliki was identified as C. tholloni congica, but this is probably an error for C. zillii
Coptodon zillii	**+**	
Haplochromis albertianus	**+**	
Haplochromis ampullarostratus	**-**	Lake Victoria species
Haplochromis avium	**+**	

Haplochromis bullatus	+	
Haplochromis commutabilis	-	Lake Victoria species
Haplochromis exspectatus	-	Lake Victoria species
Haplochromis lanceolatus	-	Possible synonym
Haplochromis loati	+	
Haplochromis mahagiensis	+	
Haplochromis nigrescens	-	Lake Victoria species
Haplochromis nigricans	-	Lake Victoria species
Haplochromis perrieri	-	Lake Victoria species
Haplochromis petronius	-	Lake Edward species
Haplochromis prognathus	-	Lake Victoria species
Haplochromis simotes	-	Lake Victoria species
Haplochromis victorianus	-	Lake Victoria species
Haplochromis wingatii	?	
Haplochromis spp.	+	Seven undescribed species
Oreochromis esculentus	-	There are three specimens at Peabody Museum, Yale University, misidentifications
Oreochromis leucostictus	+	
Oreochromis niloticus	+	
Oreochromis urolepis	-	There are three specimens at Peabody Museum, Yale University, misidentifications
Oreochromis cf. variabilis	+	Based on photographs of Yves Fermon, Congolese side
Pseudocrenilabrus multicolor	+	
Sarotherodon galilaeus	+	
Citharinidae		
Citharinus citharus	+	
Citharinus latus	+	
Clariidae		
Clarias alluaudi	+	Specimens have been confused with C. werneri in the past. Our barcoding study confirms the presence of this species
Clarias gariepinus	+	
Clarias hilli	+?	Seegers (2008) mentions specimen from Lake Albert system; no other collections known
Clarias liocephalus	+?	Not reported from the lake, but five specimens identified by G. Teugels from Hohwa river near Kaseeta
Clarias werneri	+	No specimens available from the basin, but present in the Victoria-Kyoga basin and in Upper Nile. Therefore presence in Lake Albert basin is logical.
Heterobranchus longifilis	+	
Cyprinidae		
Enteromius apleurogramma	+	Complex of genetically distinct lineages
Enteromius kerstenii	+	Complex of genetically distinct lineages
Enteromius neglectus	-	Species from the Nile delta
Enteromius neumayeri	+	
Enteromius paludinosus	?	Two observations in an affluent of the Semliki are only indications of presence in Lake Albert basin
Enteromius pellegrini	+	Complex of genetically distinct lineages

Enteromius perince	**+**	
Enteromius stigmatopygus	**+**	
Enteromius trinotatus	**?**	Two specimens from Ndrige river, near mouth in Lake Albert at Congolese side are only records. Maybe misidentification of a species otherwise limited to the upper reaches of the Aruwimi and Ituri basins
Labeo annectens	**-**	Species known from the Lower Guinea Province and the Congo basin; one old record at Harvard from an affluent of the Semliki is most probably a misidentification
Labeo coubie	**+**	
Labeo cylindricus	**-**	Two small specimens from an affluent of the Semliki have probably been misidentified as this species, which occurs in southern Africa, coastal rivers in East Africa and the upper and middle parts of the Congo basin. Strangely enough also reported from Nilotic basins and rift lakes in Ethiopia
Labeo forskalii	**+**	
Labeo horie	**+**	
Labeo niloticus	**-**	Not mentioned from the area but there are six old specimens from two localities in two musea identified as this species, which otherwise is only known from the Nile system. Probably misidentifications.
Labeobarbus altianalis	**-?**	Reported from the basin, but taxonomy is in a mess and confusion with L. bynni is possible.
Labeobarbus bynni	**+**	
Labeobarbus huloti	**+**	Only known from type locality
Labeobarbus pellegrini	**-**	Only known from type locality, probably the Lowa river (Congo basin); specimen from Talya is probably L. ruwenzorii.
Labeobarbus ruwenzorii	**+**	Found during project, meaning the species is present on the two basins at the foot of the Rwenzori mountains (systems of Lakes Edward and Albert)
Danionidae		
Engraulicypris bredoi	**+**	
Leptocypris niloticus	**+**	
Raiamas sp.	**+**	Raiamas was found by Y. Fermon on the Congolese side. Also mentioned by Akoth et al. (2022)
Distichodontidae		
Distichodus nefasch	**+**	
Distichodus rostratus	**+**	
Nannocharax niloticus	**+**	
Nannocharax fasciatus	**-**	Species from West Africa and Lower Guinea, probably misidentification for N. niloticus
Latidae		
Lates niloticus	**+**	
Lates macrophthalmus	**+**	

Malapteruridae		
Malapterurus electricus	**+**	
Mastacembelidae		
Mastacembelus frenatus	**+**	Species has a very large and strange distribution. Possibly, the Nilotic/Albert populations belong to separate species
Mochokidae		
Synodontis afrofischeri	**?**	Species reported from the Lake Rukwa and Malagarazi basins and those of Lakes Victoria/Kyoga. Only one observation was known from downstream of Murchisons falls; possible misID.
Synodontis frontosus	**+**	
Synodontis khartoumensis	**+**	
Synodontis macrops	**-**	Only known from Aswa river (Upper Nile)
Synodontis nigrita	**+**	
Synodontis schall	**+**	
Synodontis serratus	**+**	
Synodontis victoriae	**?**	Species reported from the Malagarazi basins and Lakes Victoria/Kyoga basins. Only one observation downstream of Murchisons falls; possible misID.
Mormyridae		
Cyphomyrus grahami	**+?**	Possible misidentification of C. petherici? Also put as uncertain by Akoth et al. 2022.
Cyphomyrus petherici	**+**	
Gnathonemus longibarbis	**-?**	Probably not present, but old record in Peabody Museum, Yale University
Hyperopisus bebe	**+**	
Marcusenius cyprinoides	**+**	
Marcusenius victoriae	**-?**	Possible misidentification of M. cyprinoides. Not mentioned from Albert system by Akoth et al., 2022, but records from the system present in the Nafirri database.
Mormyrops anguilloides	**+**	
Mormyrus caschive	**+**	
Mormyrus kannume	**+**	One specimen in the RMCA collection confirmed identification;
Mormyrus macrocephalus	**-**	
Mormyrus niloticus	**+**	
Petrocephalus catostoma	**-**	Specimens are P. degeni according to revision of Kamer et al. (2012)
Petrocephalus degeni	**+**	
Pollimyrus nigricans	**+**	
Nothobranchiidae		
Nothobranchius albertinensis	**+**	
Nothobranchius elucens	**-**	Present in Achwa system, but not in Lake Albert basin
Nothobranchius robustus	**+**	
Nothobranchius ugandensis	**-**	Present in Achwa system, but not in Lake Albert basin

Procatopodidae		
Lacustricola bukobanus	+	
Lacustricola pumilus	-	Only present in Lake Tanganyika system
Micropanchax kassenjiensis	+	
Micropanchax loati	-	Misidentification for M. kassenjiensis
Micropanchax vitschumbaensis	+	According to Wildekamp (1995) present in the Mbuga Creek in Lower Semliki
Polypteridae		
Polypterus senegalus	+	
Protopteridae		
Protopterus aethiopicus	+	
Schilbeidae		
Schilbe intermedius	+	
Schilbe mystus	+	

We looked at intraspecific diversity in *Clarias gariepinus* and *Bagrus docmak* by using microsatellites analyses, to found out if these highly **commercial fish species** contain **different stocks** in the Lake Edward system. We did not find a clear difference between the populations from the two lakes, Edward and George, though the environment is quite different. For *C. gariepinus*, we found a small differentiation between specimens caught in rivers and those caught in the lakes (Eyckens, 2025).

Our morphometric study of ***C. gariepinus*** revealed that this species exhibits a large and underreported morphological variation over its large distribution area (Maebe, 2022). The populations from Lake Victoria and Swaziland were morphologically different from those inhabiting the Nilo-Sudan ichthyofaunal province (Egypt, Mali, Chad and Senegal). This corresponds to the findings of Van Steenberge et al. (2020) who found that *C. gariepinus* populations from Lake Victoria and Swaziland were genetically distinct from the Nilo-Sudan populations. Important morphological variation was also discovered within the Congo basin. A group with shorter barbels and a shorter vomerine tooth plate was found in the upper part of the Congo basin, i.c. upstream from the Kamalondo Depression and in the Bangweulu-Mweru area and was associated with specimens form the Middle Zambezi. Again, this observation is in line with the genetic results of Van Steenberge et al. (2020) which indicated that the different *C. gariepinus* populations from the southern part of the Congo basin (i.e. Bangweulu-Mweru and Upper Lualaba ecoregions) are genetically much similar to each other than to the populations within the central part of the basin. This also supports the hypothesis of river connections between the southernmost affluents of the Congo and the Zambezi system as has been demonstrated for the Upper Luapula and the Upper Zambezi (Van Steenberge et al., 2014).
As we were particularly interested to see how diverse the populations from the various systems within the KEA region are, we analysed two new data sets, one with traditional measurements and meristics and a second one with homologous landmarks. Small differences were found between the three KEA-lake systems. The Kivu population has shorter barbels than those of Edward and Albert. The populations of the latter differed in the size of the vomerine tooth plate. No differences were found between riverine and lacustrine specimens, nor between the sexes.

***Clarias liocephalus**:* We found some geographic variation in this species, mainly in the length of the barbels. The longest barbels were found in specimens from the Lake Kivu and the Malagarazi systems, while the shortest ones in specimens from the Lakes Edward and Albert basins, and the northern part of the Lake Victoria system.

Bagrus: Traditionally, one species, *B. docmak* has been reported from the Lake Edward system and two, *B. docmak* and *B. bajad*, from the Lake Albert system. The two species are not easy to distinguish, at least not in the KEA region, and the discriminating characters described in the literature are sometimes ambivalent or contradictory. We have found indications, based on morphology, that *B. bajad* may also be present in the Lake Edward system.

WP3. Diversity of haplochromines

In Lake Kivu, 15 endemic haplochromine cichlid species occur. The Lake Edward system is estimated to be inhabited by about 80 *Haplochromis* species. So far, we (re)described 26 species, 19 of which are new to science. This brings the total number of valid species of *Haplochromis* from the Lake Edward system to 46 (Vranken 2024). We recognise an additional 10 undescribed lacustrine species in the system for which a revision is ongoing.

Based on a geometric morphometric study and detailed morphological and anatomical comparison, a total of **12 species from Lake Albert** are recognised, seven of which are undescribed new species in addition to five already described ones (Table III), together representing a large variation in eco-morphologies. The species assemblage of *Haplochromis* from the Lake Albert system is generally regarded as a species-poor assemblage with less ecological diversity as in other lakes. Nevertheless, two undescribed species were discovered with the morphology of epilithic algae scrapers: *H.* sp. 'black scraper' and *H.* sp. 'red scraper'. These species have very broad oral jaws set with many rows of blunt teeth that are used to scrape algae from hard surfaces.

We constructed an eco-morphological framework of all currently recognised (described and undescribed) lacustrine species from Lakes Albert (12 species), Edward (52), and Kivu (15) together with 122 species from Lake Victoria using a geometric morphometric approach. From the morphospace occupation of the species from each lake (Figure 34), we could infer many instances of **strong eco-morphological similarity** between species from the different lakes.

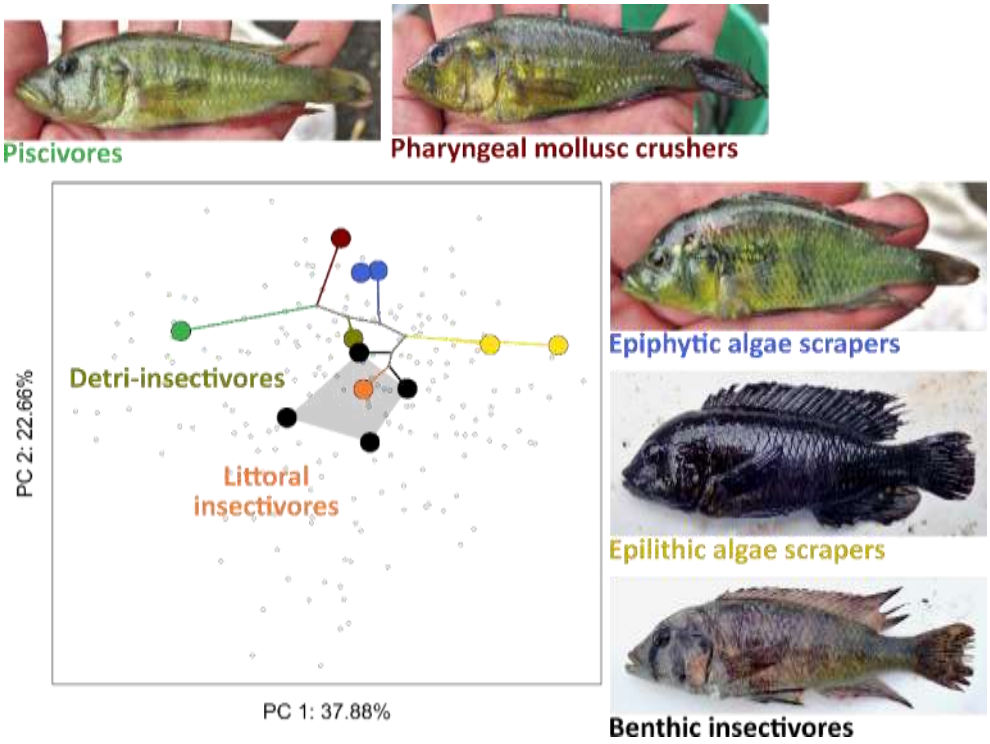

Figure 34. Phylomorphospace projection of the species tree of all species of *Haplochromis* from Lake Albert (n=12) with photographs of representative species shown. The morphospace occupation is indicated in the morphospace of 204 species from all four lakes of the Lake Victoria Region Superflock. Species, branches and example photographs are coloured by eco-morphological group; undetermined branches and ancestral nodes are coloured in grey; species for which no genomic data are available are included but no branches are shown. All species that do not belong to Lake Albert are shown in small grey circles. Smaller values on PC 1 correspond to larger head and oral jaw lengths, smaller values on PC 2 to more elongate bodies (Vranken, 2024).

WP4. Genome-wide sequencing and analyses

Haplochromines: Various principal component analyses, maximum likelihood trees, and introgression metrics confirmed the existence of three replicate radiations within the Lake Victoria Region Superflock of haplochromines (LVRS), with limited signals of recent introgression between them. The species of the basins of Lakes Albert and Victoria form two independent monophyletic radiations, while most species of the basins of Lakes Edward and Kivu form a single radiation. A fourth small lineage contains only specimens from Lake Edward (Figure 35). *Haplochromis squamipinnis* from the Lake Edward system is another exception forming the sisterclade to the Lake Victoria radiation.

We found some endemic haplochromine species from Lakes Kivu and Edward back in a more basal clade. Two species of *Astatotilapia, A. gracilior* from Lake Kivu and *A. pharyngalis* from Lake Edward, are known to belong to the pharyngalis clade (= Upper Nile lineage), ancestral to the LVRS (Figure 35). In addition, we discovered four other species from the Lake Edward system to also belong to this species flock, a finding that might improve our understanding of the evolutionary origin and biogeography of the LVRS. All seven species are confirmed to show uniform introgression signals into all members of the LVRS. In addition, also *Astatotilapia flaviijosephi,* a species from the Jordan river

system that belongs to a lineage basal to the pharyngalis flock and the LVRS, shows a uniform introgression signal with all members of the LVRS.

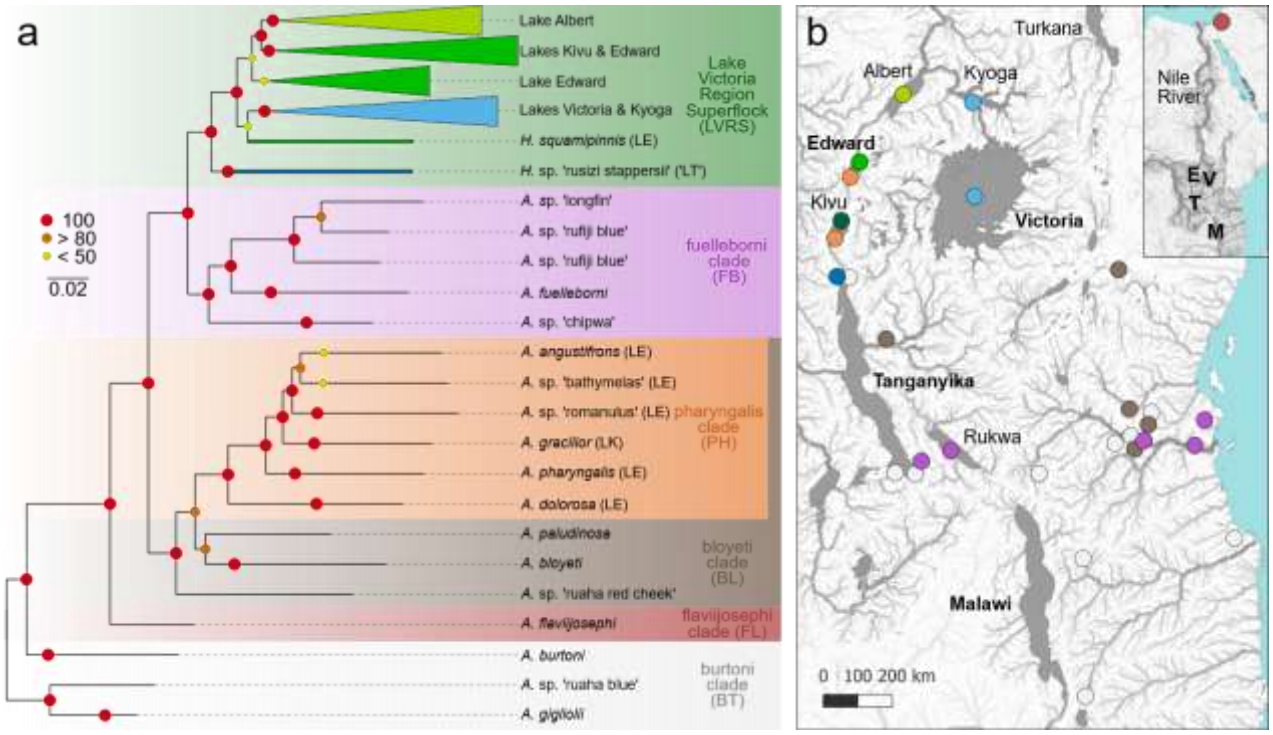

Figure 35. Evolutionary relationships between species of the Lake Victoria Region Superflock and closely related clades. (a) Summary of an IQ-TREE and (b) concise geographical distribution of all specimens, all coloured by clade and, for the LVRS, coloured by lake (Vranken 2024).

We contrasted our genomic dataset against the eco-morphological framework of the LVRS (see WP3). Phylomorphospace projections suggest that all flocks diverged rapidly, which coincides with an efficient expansion of morphospace. Pairwise contrast-divergence plots were contrasted against Brownian motion simulated neutral trait evolution, which shows **strong signals of convergent evolution between flocks and rapid divergence within flocks**, in addition to a weaker signal of convergence within flocks (Figure 36). Numerous examples of strikingly similar species belonging to different radiations were identified.

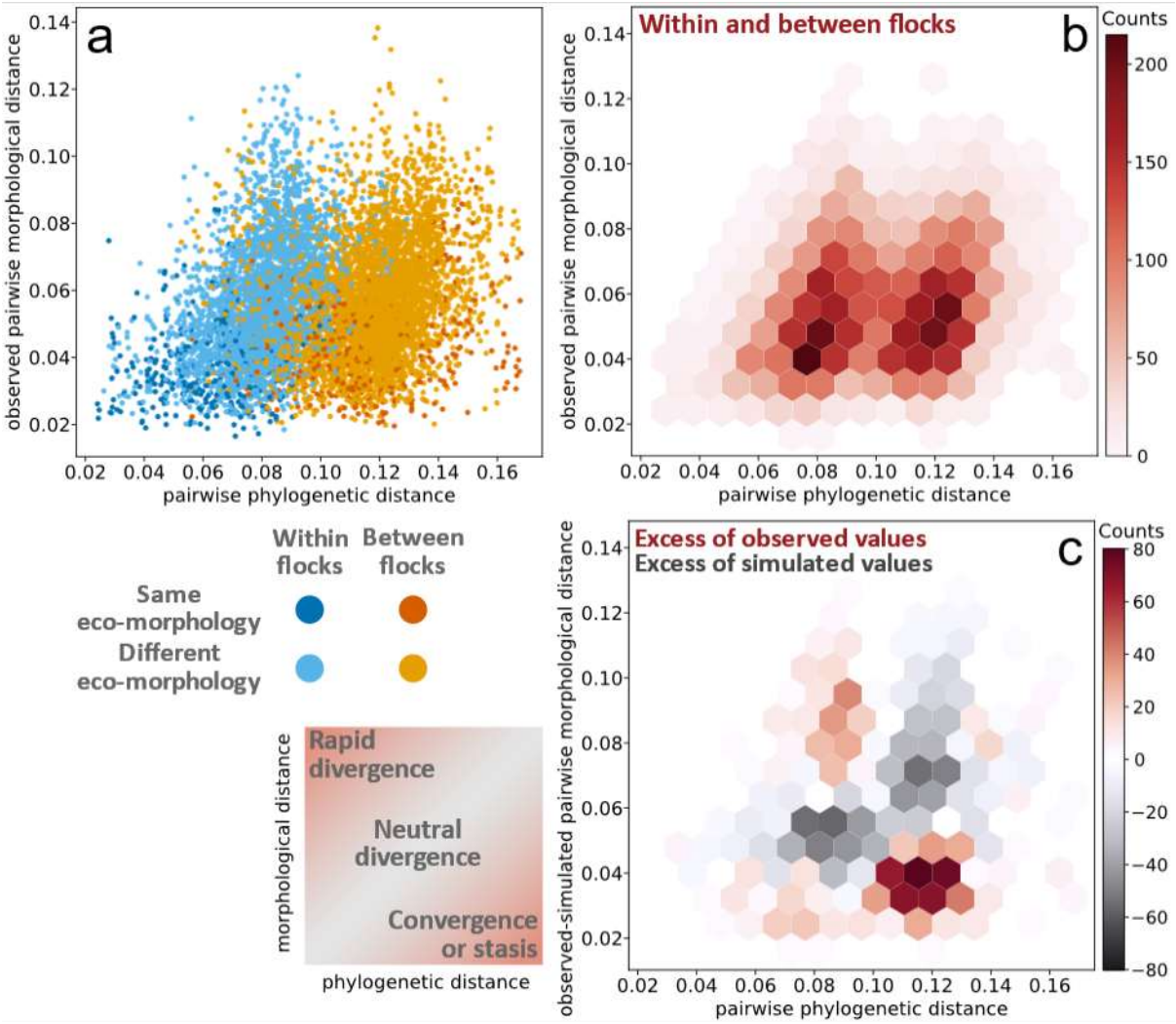

Figure 36. Pairwise distance-contrast plots for 128 species from the LVRS. Pairwise morphological distances (procrustes distances between the mean shapes of species) are contrasted against pairwise phylogenetic distances (interspecific distances in the species tree). (a) The scatter plot and (b) density distributions for all observed pairwise morphological distances within and between species flocks of the LVRS and (c) the density distribution contrasted against the mean density distribution of 100 iterations of Brownian motion simulated neutral trait evolution (Vranken, 2024).

The evolutionary mechanisms underlying convergent evolution remain understudied. We investigated whether genomic regions can be identified that segregated across replicate radiations and sorted repeatedly into species with similar eco-morphologies. In a previous study, little support for this hypothesis had been found for some piscivorous species from different lakes, which all shared the same indel (McGee et al., 2020). However, it remains to be tested how this shared indel is linked to a piscivorous eco-morphology and to what degree similar shared genomic regions are present in LVRS species with other eco-morphologies.

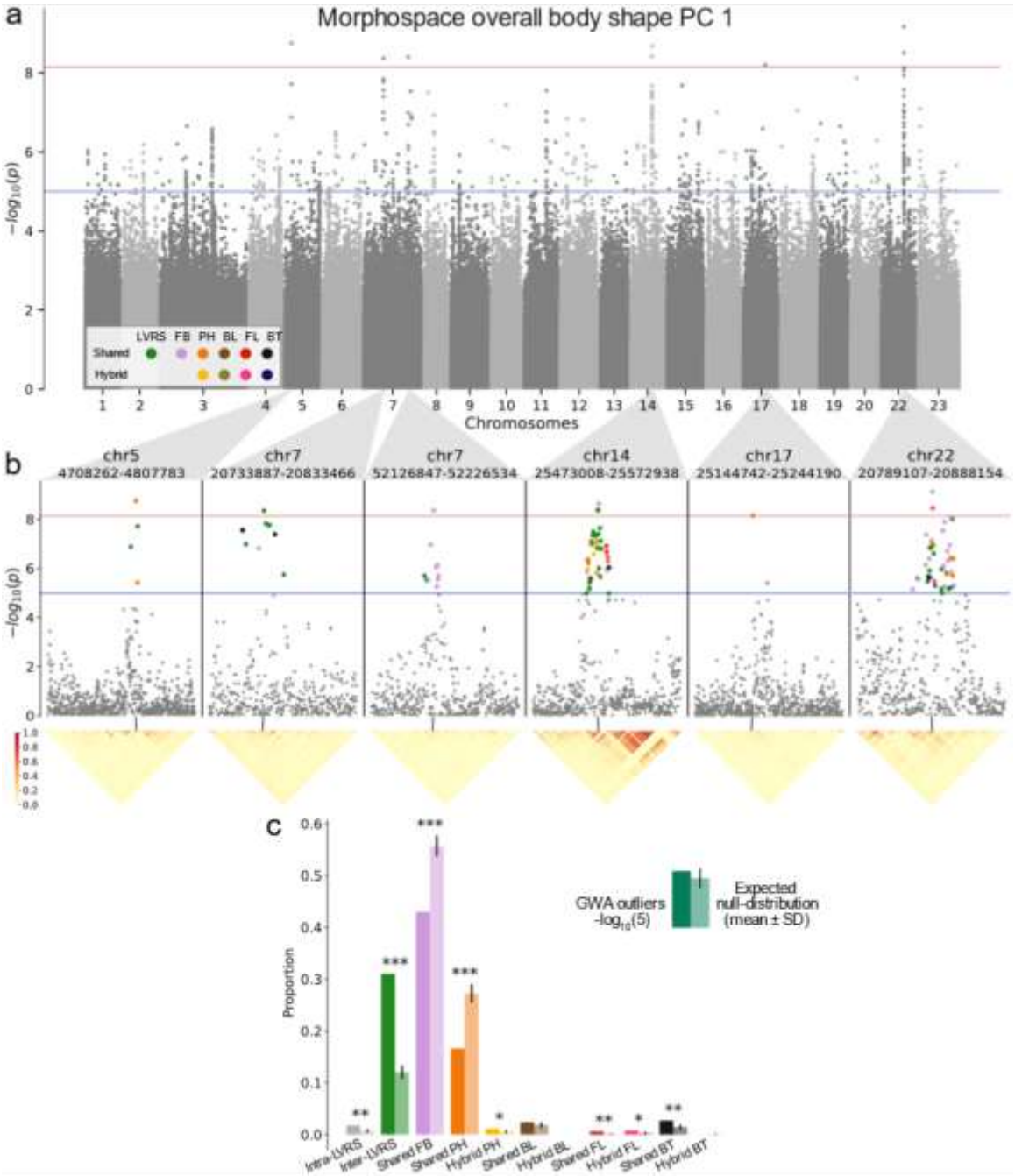

Figure 37. Genome-wide association (GWA) mapping of PC 1 of the morphospace of body shape, corresponding to variation in trophic morphology (head and oral jaw lengths). (a) Manhattan plot in which all positive outlier SNPs ($P < 1x10^{-5}$; blue line) are coloured by SNP category (except for intra-LVRS). (b) Zoom-ins of all six genomic regions (100 kb each) that contain significant SNPs (FWER < 0.05 after Bonferroni correction; red line) with plots of linkage disequilibrium (LD) of the same regions; for each LD plot, the most significant SNP is indicated by a black line. (c) The proportions of all positive outlier SNPs are shown per SNP category and compared to a null-distribution represented by 1000 random samples of genome-wide SNPs with the same allele frequency distribution as the positive outlier SNPs. For each category, significance was determined based on the rank of the true proportion against those of the null-distribution; * < 0.05, ** < 0.01, *** < 0.001. LVRS, Lake Victoria Region Superflock; FB, fuelleborni-clade; PH, pharyngalis-flock; BL, bloyeti-clade; FL, flaviijosephi-clade; BT, burtoni-clade (Vranken, 2024).

For this, we implemented genome-wide association mapping of eco-morphological traits. **Candidate adaptive genetic variation** associated with eco-morphological traits was found to be **highly polygenic** for all investigated traits. These genetic variants were found to be enriched in presumed *de novo* mutations unique to the LVRS (Figure 37; intra-LVRS), hybrid-derived variation (hybrid PH & FL), and ancient standing variation shared across haplochromine cichlids over relatively large phylogenetic distances (shared PH, FL & BT). We identified candidate genes that appear to be involved in body shape, trophic morphology, metabolism, and behaviour, suggesting a strong genetic correlation between morphological variation, ecology, and behaviour in haplochromine cichlids.

An additional 85 riverine specimens of haplochromines from the Lake Victoria Region were whole-genome sequenced. Phylogenomic inference, genomic PCA analyses, and introgression statistics suggest that most riverine specimens of the Lake Edward and Lake Victoria systems constitute **sister lineages of the lacustrine radiation within the same system.** Surprisingly, specimens from the Nkusi (Lake Albert system), Ntungwe, and Oruyubu Rivers (Lake Edward system) resolved as belonging to lineages that are sister to the Lake Victoria radiation. Specimens from the Akagera River (Lake Victoria system) and Lakes Nyabihoko, Bunyonji, and Mutanda (Lake Edward) resolved as belonging to the Lake Victoria radiation. This suggests that Lake Nyabihoko was relatively recently connected to the Akagera River system, allowing the lake to be colonised by lineages from the Lake Victoria radiation. Lakes Bunyonji and Mutanda, located on high altitude between Lakes Edward and Kivu, have no such likely recent connection to the Lake Victoria system. However, between 1928 and 1953, various species of *Oreochromis* and *Haplochromis* from Lake Victoria have been introduced into Lake Bunyonji (Green, 2009). These haplochromines seem to have established stable populations as all specimens from this lake and the connected Lake Mutanda resolve as belonging to the Lake Victoria radiation.

Enteromius. The whole genome approach for *Enteromius* concentrated first on the **mitochondrial genome**. Mitochondrial genomes in vertebrates are characterised by their conserved gene content and compact size. However, several cases in various taxa support **a more diverse architecture** for this organellar genome, including gene duplications and the presence of repeat motifs. The extent of such phenomena across taxa, and their evolutionary or functional significance, remains largely elusive. In our data, we detected signatures of at least two independent events involving the duplication of the control region, tRNA and ribosomal gene(s), differing in their specific modalities (Figure 38). Given the relatively high prevalence of these cases within the limited number of *Enteromius* species investigated, our results suggest that the occurrence of such phenomena may be widespread in this species-rich genus. It conclusively highlights the potential of the genus to serve as a model for better understanding the significance of such events. Although tandem duplications of substantial mitochondrial genome regions have already been documented in birds, reptiles and fishes, our findings provide further evidence that such events are probably more frequent than generally recognised. These results are currently being prepared for publication.

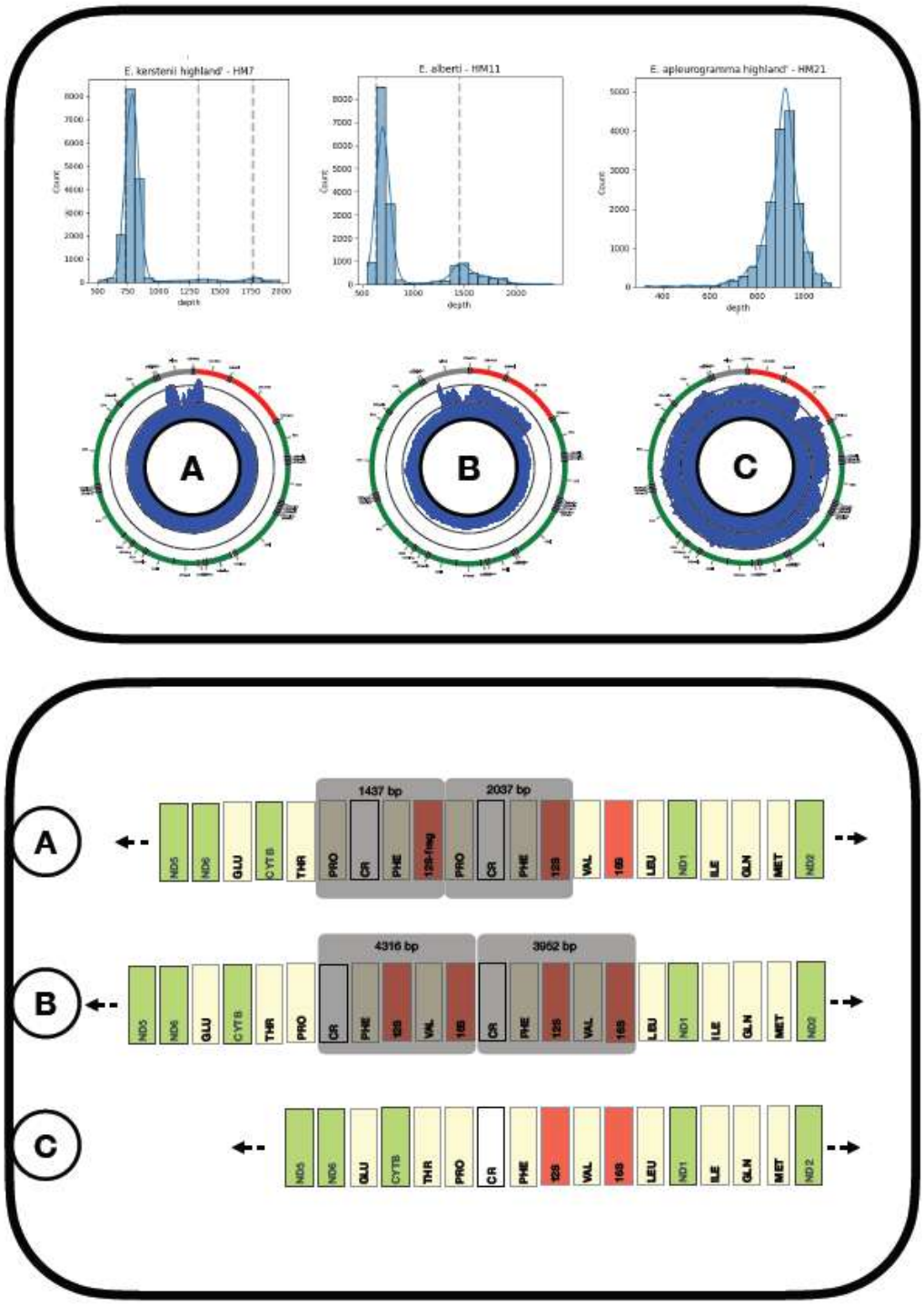

Figure 38: Top panel: histograms representing the distribution of sequencing depth (Illumina) for the mitochondrial genomes of three *Enteromius* species. From left to right: *E. kerstenii* "highland", *E. alberti*, and *E. apleurogramma* "highland". The graphical representations for corresponding assemblies are shown below the histograms. Both *E. kerstenii* and *E. alberti* clearly display multimodal distributions (grey dashed lines), in contrast with *E. apleurogramma*. Sequencing depth also appears to be approximately twice as high around the control regions and ribosomal RNA regions, which is consistent with the presence of duplicated regions, and confirmed by Oxford Nanopore reads. Bottom panel: schematic representation of a portion of the gene order for the three species (obtained by Illumina for *E. apleurogramma*, and by Oxford Nanopore for *E. kerstenii* and E. alberti). Duplicated regions are highlighted in grey (Boom et al., in prep.).

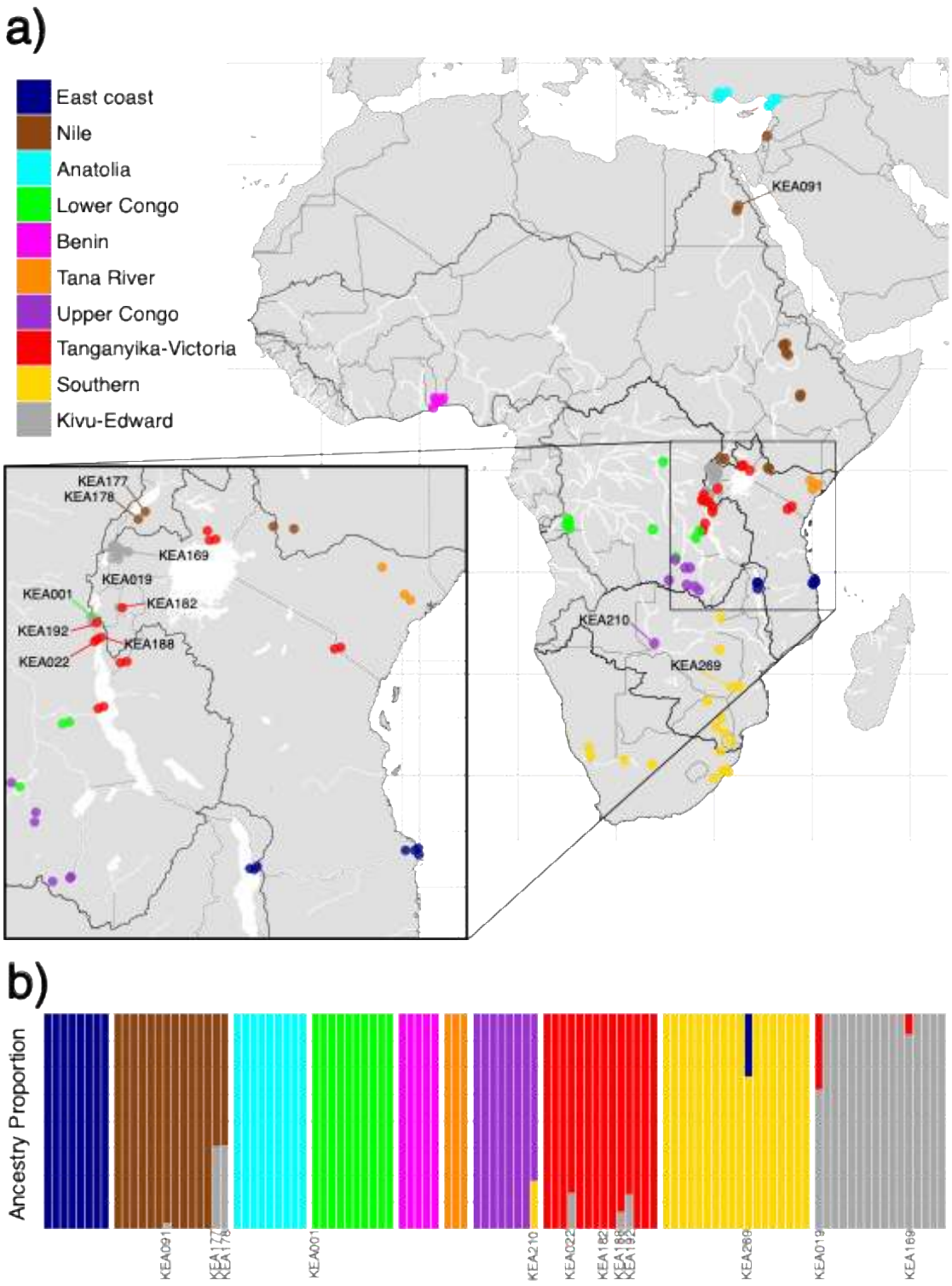

Figure 39: a) Localities of specimens studied. Each colour corresponds to a group determined with Admixture on SNP data. b) Admixture plot showing the ancestry components of 103 *C. gariepinus* specimens. A model with ten ancestral components (K = 10) was the most parsimonious to explain the variation and similarities of the genome-wide genotype data (Gaspar et al., in prep.).

Clarias gariepinus. For *C. gariepinus*, the population structure (Figure 39) reveals strong genetic clustering, with most individuals predominantly associated with a single ancestry component. Each specimen was assigned to one population, the individuals exhibiting multiple ancestry were assigned to the population with dominant proportion. Notably, the **clustering patterns align well with the geographic origin** of the specimens. Evidence of gene flow is observed among the three populations pertinent for the project: Nile, Tanganyika-Victoria and Kivu-Edward, which is consistent with their proximity.

Principal Component Analysis (PCA) of the SNP dataset (Figure 40) shows that PC1 separates the specimens into two main groups**,** with the Southern/Congolese populations (Southern, East coast, Upper and Lower Congo) clustering on the left, and all others on the right. One notable case is the specimen KEA019 (population Kivu-Edward), which is situated a bit outside the Kivu-Edward cluster towards the Tanganyika-Victoria population, an observation also supported by the admixture plot.

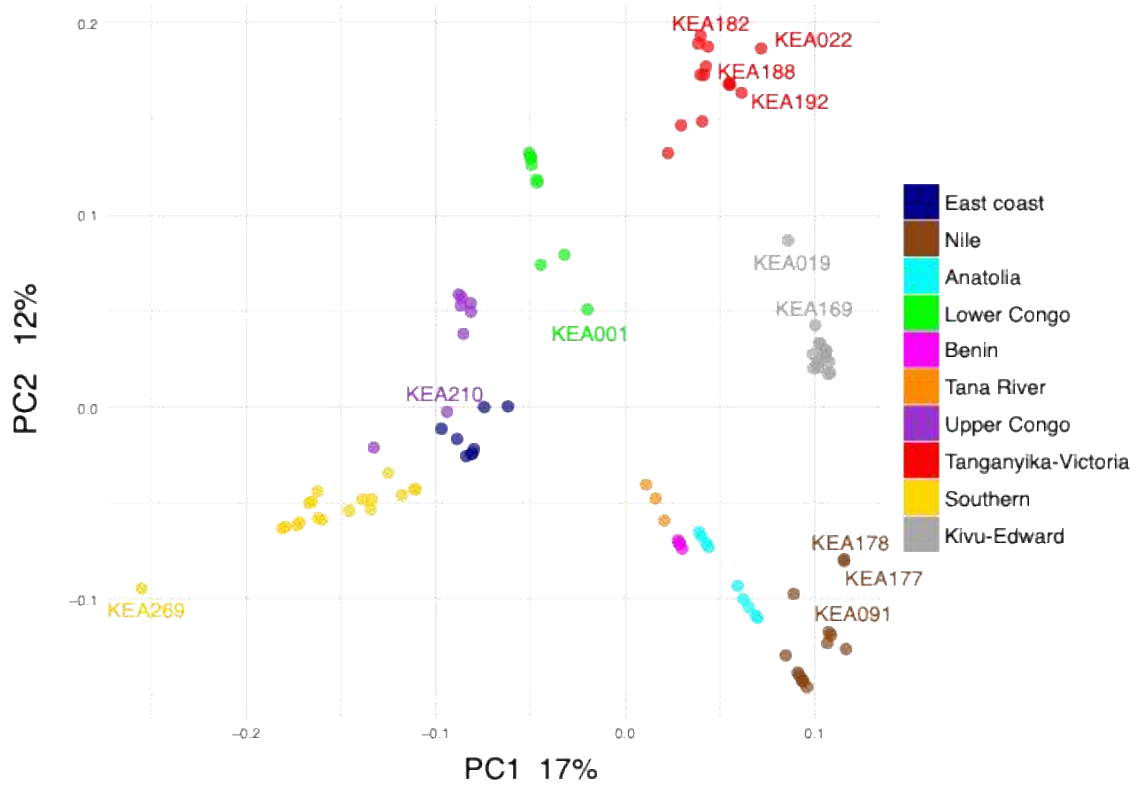

Figure 40. Principal Component Analysis on SNP dataset of 105 *C. gariepinus* specimens. PC1 explains 17% of the variation and PC2 12% (Gaspar et al., in prep.).

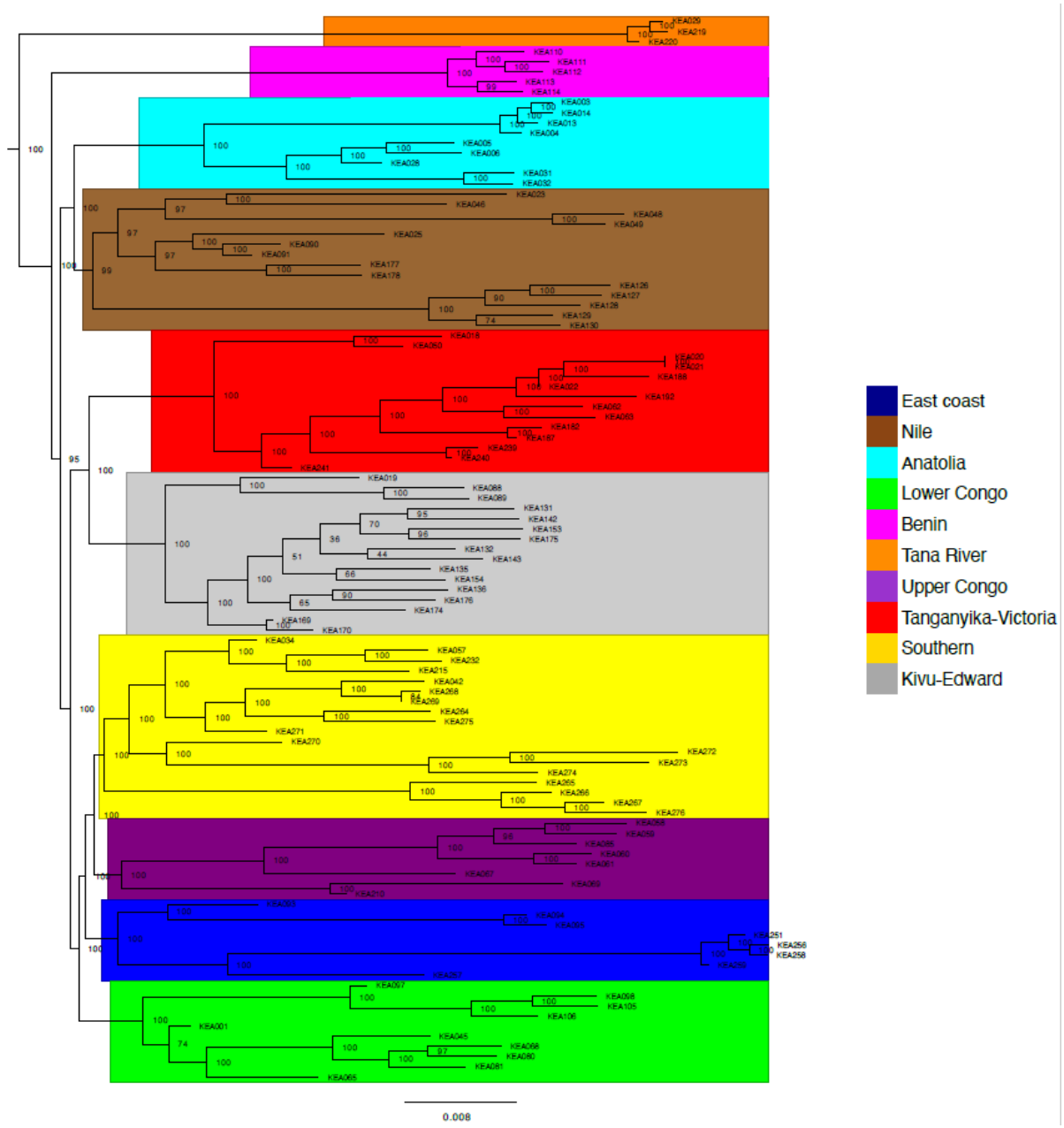

Figure 41: Maximum Likelihood Phylogenetic tree inferred from SNP data of 105 *C. gariepinus* specimens (Gaspar et al., in prep.).

A maximum likelihood phylogeny inferred from the SNP dataset (Figure 41) reveals a topology consistent with population clustering. The most basal lineage is the Tana River population (Kenya), followed by the Benin population. The remaining populations form two main groups: a northern clade (Nile an Anatolia populations) and a larger clade comprising central and southern populations. Within this larger clade, a distinct branch leads to the Southern/Congolese populations (Southern, Lower and Upper Congo and East coast). The phylogenetic network derived from the same SNP matrix (Figure 42) reveals conflicting signals not captured in the tree. It shows long branches leading to early diverging clades in populations Benin and Tana River, suggesting early isolation. A bottleneck separates the southern clades and the rest of the population although the Lower Congo population

might be considered intermediate. The reticulation observed among populations Nile, Tanganyika-Victoria and Kivu-Edward suggests ongoing or historical gene flow, likely due to geographic proximity.

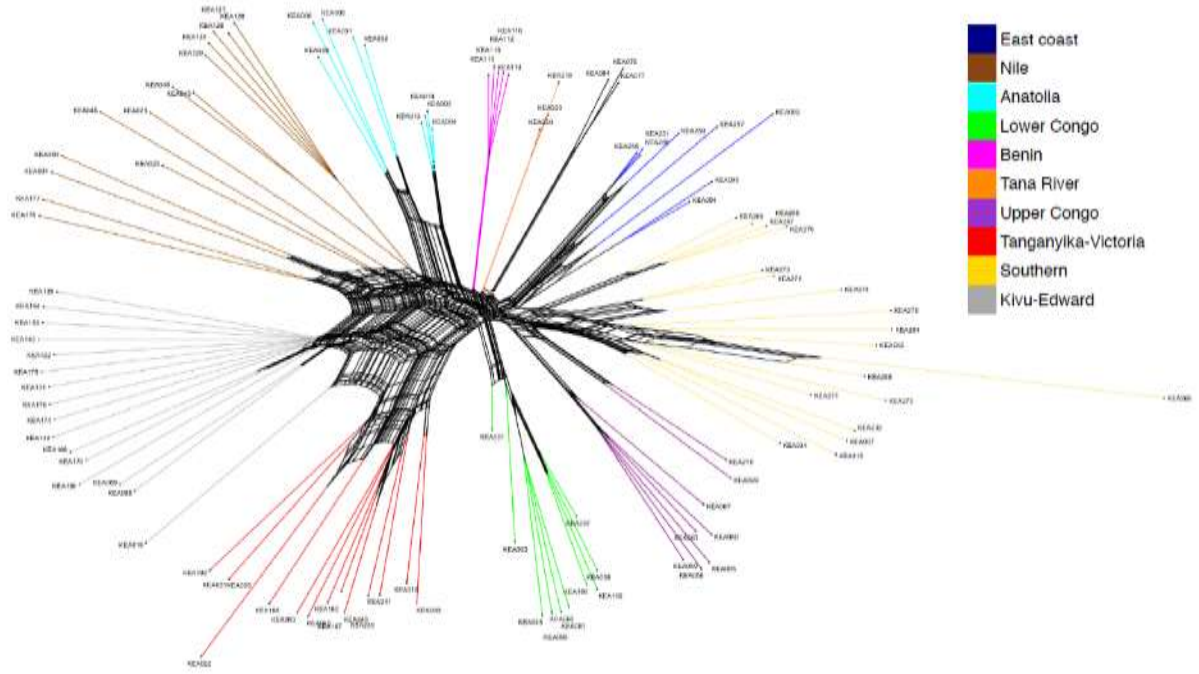

Figure 42. Phylogenetic network of 105 C. gariepinus specimens generated from a genome scale SNP dataset generated on Splitstree (Gaspar et al., in prep.).

The phylogeny inferred from the UCE dataset (Figure 43) broadly reflects the SNP tree topology. For instance, the Southern/Congolese clade is again recovered. The main difference resides with the fact that the clade formed by the Tanganyika-Victoria and Kivu-Edward populations in the SNP tree is separated in the UCE tree with the former being closer to the southern clade and the latter being closer to the northern group. The KEA019 specimen (Kivu-Edward population) clusters with the Tanganyika-Victoria population, supporting its intermediate genetic status. Unlike the SNP phylogeny, the Benin population is positioned closer to the northern clade. The Nile population, which includes a broad sampling range, is divided into five distinct monophyletic groups. The UCE-based phylogenetic network (Figure 44) shows fewer reticulations than the SNP network, consistent with the slower evolutionary rate of UCE loci. The separation between southern and non-southern clades is even more pronounced in this network.

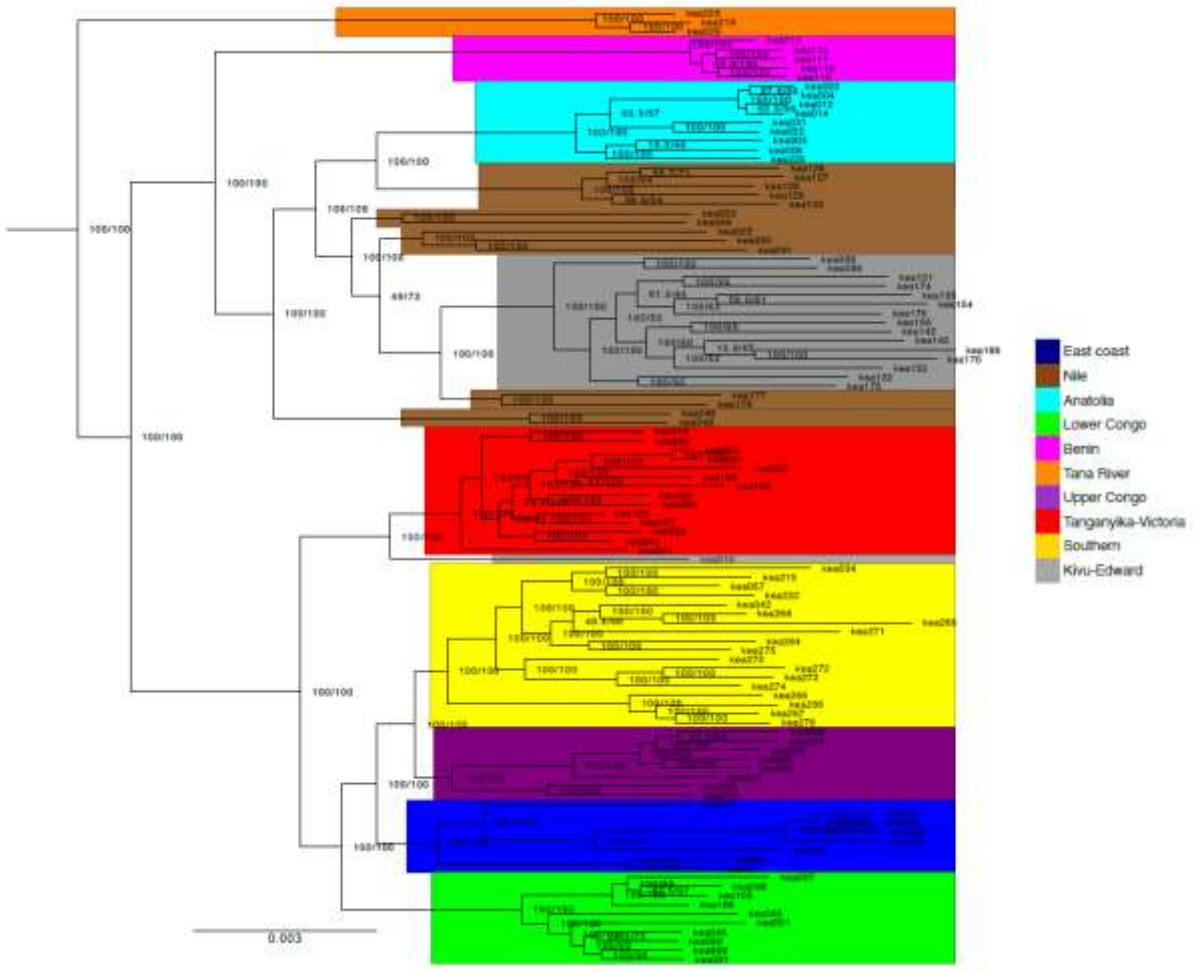

Figure 43: UCE Maximum Likelihood phylogenetic tree including 105 *C. gariepinus*. *C. ngamensis* was used as an outgroup (not shown on the tree) (Gaspar et al., in prep.).

A UCE matrix including 25 species of Clariidae was used to estimate divergence times. The tree (Figure 45) places the origin of *C. gariepinus* to approximately 4.3 million years ago (3.32-5.54). The divergence between the African *Clarias* lineage and its closest-related Oriental species (*C. dussumieri*) is estimated at 34.8 MYA (29.24-40.30).

A second, more focused calibrated tree (Figure 46) based solely on *C. gariepinus* reveals that the basal lineage from the Tana River diverged 4.3 MYA**.** The subsequent split between the northern and southern clades occurred around 3.6 MYA**.**

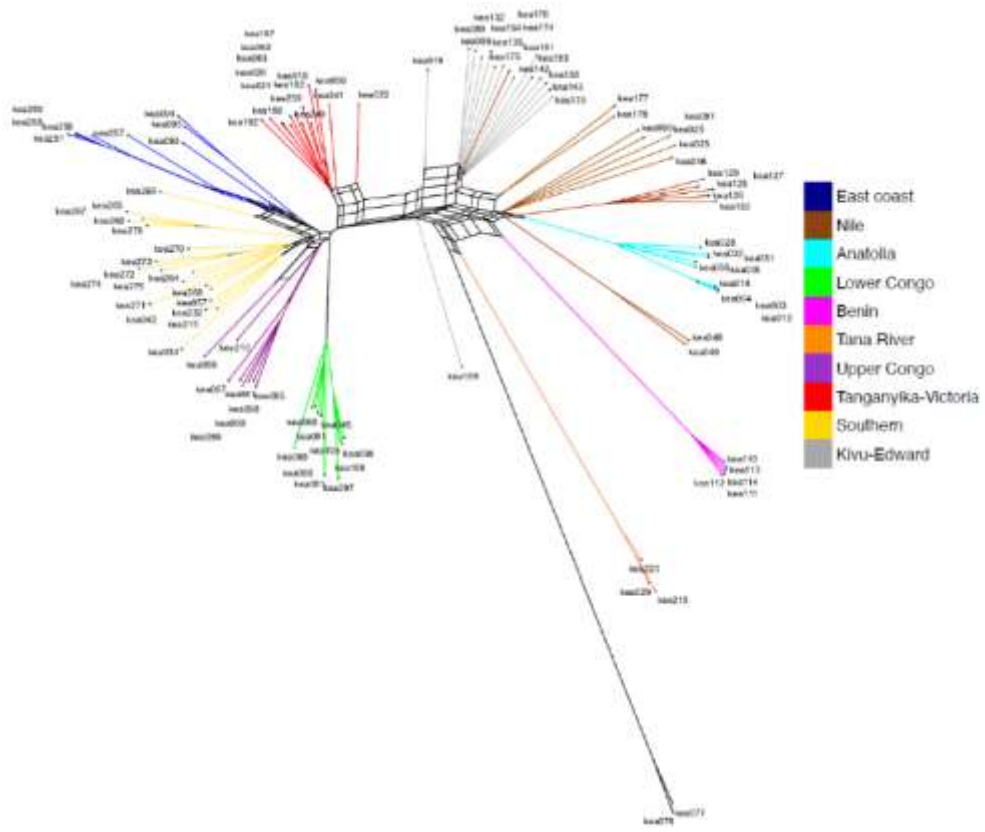

Figure 44: UCE Phylogenetic network of 105 C. gariepinus generated on Splitstree (Gaspar et al., in prep.).

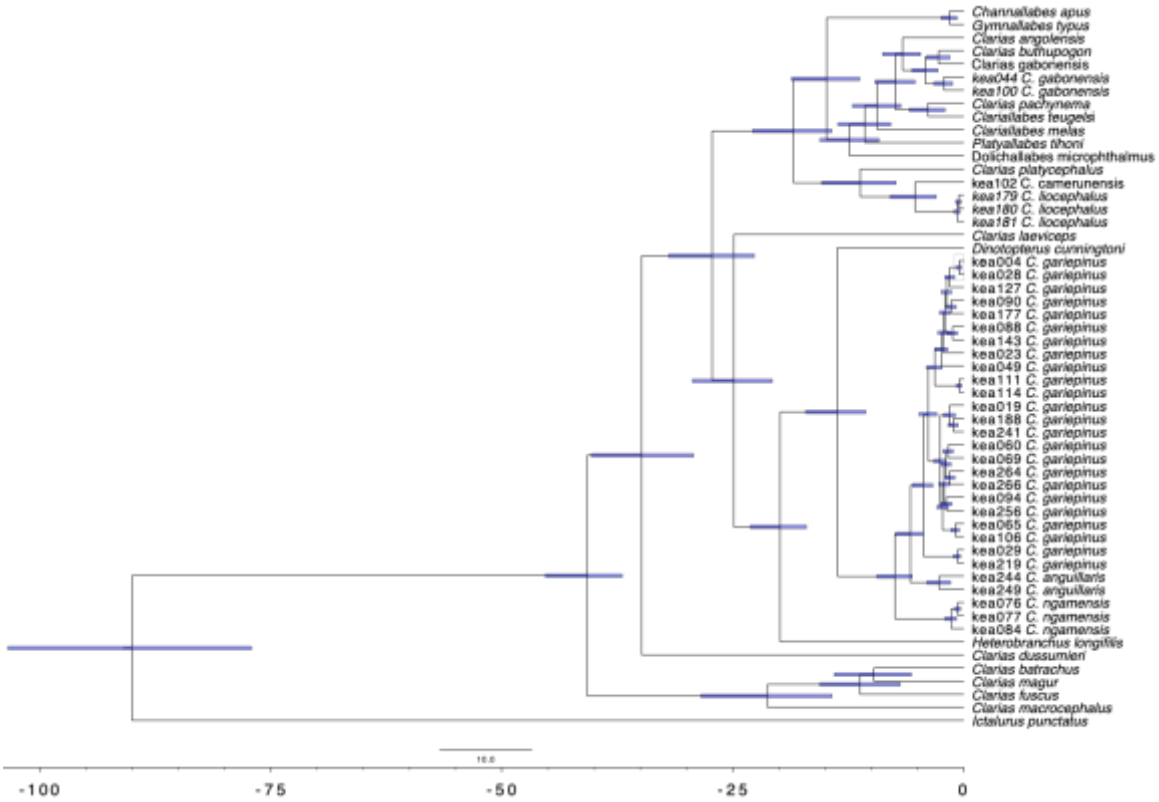

Figure 45: Time calibrated tree on Beast2 including 25 species of Clariidae (Gaspar et al., in prep.).

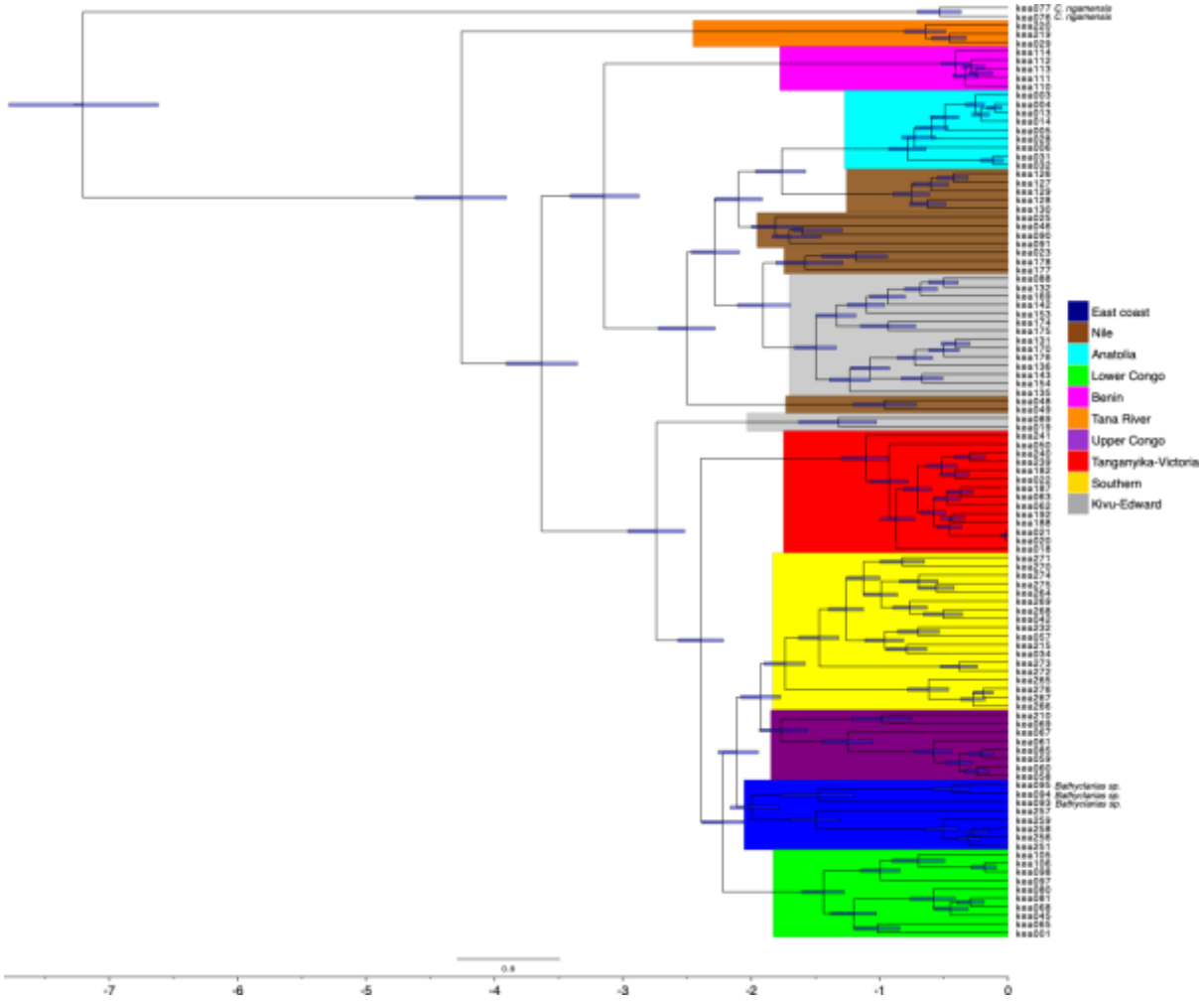

Figure 46: Time-calibrated tree on BEAST2, 105 *C. gariepinus* specimens (Gaspar et al., in prep.).

The phylogenetic analysis of Clariidae (Figure 45) includes 25 species distributed across Asia and Africa. Clariidae originated in Southeast Asia (Lavoué et al. 2024), with a subsequent divergence of the African lineage approximately 35 million years ago (MYA). Within the Oriental clariids, *Clarias dussumieri* is recovered as the sister lineage to the Afrotropical Clariidae, rendering the Asian clariids paraphyletic. Among the African taxa, our tree identifies *C. anguillaris* as the closest relative of *C. gariepinus* s.l., followed by *C. ngamensis*. These three species, together with *Dinotopterus cunningtoni*, form a monophyletic clade which is sister to *Heterobranchus longifilis*. In agreement with Jansen et al., (2006), our robust phylogenomic tree, based on 1,687 UCE loci (3.5 Mb in total), resolves the placement of *D. cunningtoni* within clariids. This finding contrasts with some earlier studies based on shorter molecular markers (Pouyaud et al. 2009; Lavoue et al. 2024), which had suggested a closer affinity of *D. cunningtoni* with *Heterobranchus*.

A closer examination of *Clarias gariepinus* (Figs. 44, 46) indicates that the species **originated approximately 4.3 MYA (3.90–4.61 MYA)**. This corresponds to the earliest split within the species, separating the Tana River population (southeastern Kenya) from all other populations. The timing of

this divergence coincides with the formation of the Gregory Rift, which extends from Ethiopia through Kenya to Tanzania, and is recognized as a major vicariance barrier for terrestrial animals (Lehmann et al. 1999; Lohay et al. 2023). South of the Turkana region, the Gregory Rift developed progressively from north to south between 7 and 4 MYA (Baker 1986; Macgregor 2015), and its emergence likely isolated the basal Tana River lineage from the other *C. gariepinus* populations.

Our data therefore suggest that ***C. gariepinus* originated in eastern Africa**, within or near the African Great Lakes region. This inference is close to the hypothesis of Van Steenberge et al. (2020) who proposed an origin in East Coast ichthyofaunal province with the Lake Kivu lineage as a basal lineage. Our results place the basal lineage further east, between the coastal zone and the Lake Victoria basin, implying that early populations occurred on both sides of the developing Gregory Rift.

The complex hydrological history of the Great Lakes region appears to have facilitated lineage mixing. In our analyses, the Kivu–Edward population shows shared ancestry with both the Nile and the Tanganyika–Victoria populations in the admixture results (Figure 39). This is further supported by the SNP phylogenetic network (Figure 42), which exhibits the most reticulations between these three populations.. This pattern can be explained by the paleogeographic history of the northern Albertine Rift: between approximately 8 and 2.5 MYA, Lakes Edward, George, Albert, and likely Kivu were part of a single paleolake, Lake Obweruka (Dumont 2009). Specimens collected from Lakes Kivu and Edward cluster within the same clade, a pattern also observed in freshwater gastropods from the region (Dusabe et al. 2025) and in haplochromine cichlids (Vranken, 2024). The high intraspecific diversity in that area has been observed in other taxa, such as the Nile crocodile, which shows high haplotype diversity in the Great Lakes region, suggesting it harbours some of the oldest crocodile lineages in Africa (Asch et al. 2019).

The phylogenetic position of the Benin population remains uncertain: SNP-based analyses suggest it represents the second most basal split within *C. gariepinus*, whereas the UCE dataset places it within the broader northern clade. All remaining populations are divided into two major groups: a northern clade including Anatolia, the Nile basin and most individuals from the Kivu–Edward region, and a southern/Congolese clade encompassing Tanganyika–Victoria, Lower and Upper Congo, the East Coast, and southern African populations. The divergence between these two major lineages occurred approximately 3.6 MYA (3.35–3.9 MYA), coinciding with disrupted hydrology connections between the East Coast and Congo ichthyofaunal provinces due to rift shoulder uplift in Albertine area (Macgregor 2015).

Following this vicariant event, populations east of the Rift either remained within the Great Lakes region or dispersed northward into the Nile basin. The two specimens from Lake Awasa (Ethiopia) represent a first split around 2.1 MYA (1.91–2.28 MYA), indicating an early north-eastward dispersal. A later divergence separated the Kivu–Edward populations from a lineage that followed the Nile drainage northward. This northern lineage comprises two lineages that independently reached the Middle East: one including specimens from Kenya (Turkana basin), Egypt, and Israel, and another one including specimens from Lake Tana (Ethiopia) and the Anatolian population. Specimens collected in Lake Tana (KEA126, 127, 128, 129, 130) display a strong genetic differentiation in all analyses.

A similar pattern has been reported for **Nile tilapia** *Oreochromis niloticus* (Geraerts et al. 2022) (see Figure 7) and is generally attributed to the isolation of the Lake Tana fish community from the lower Nile basin, caused by the Blue Nile or Tis Abay Falls. It is worth noting that, apart from Lake Tana, Nile tilapia do **not show a strong correlation between phylogeny and geographic location** (Figure 47), especially when compared to *C. gariepinus*. This weaker pattern may be due to the high level of introgression in tilapias, which can blur phylogeographic signals.

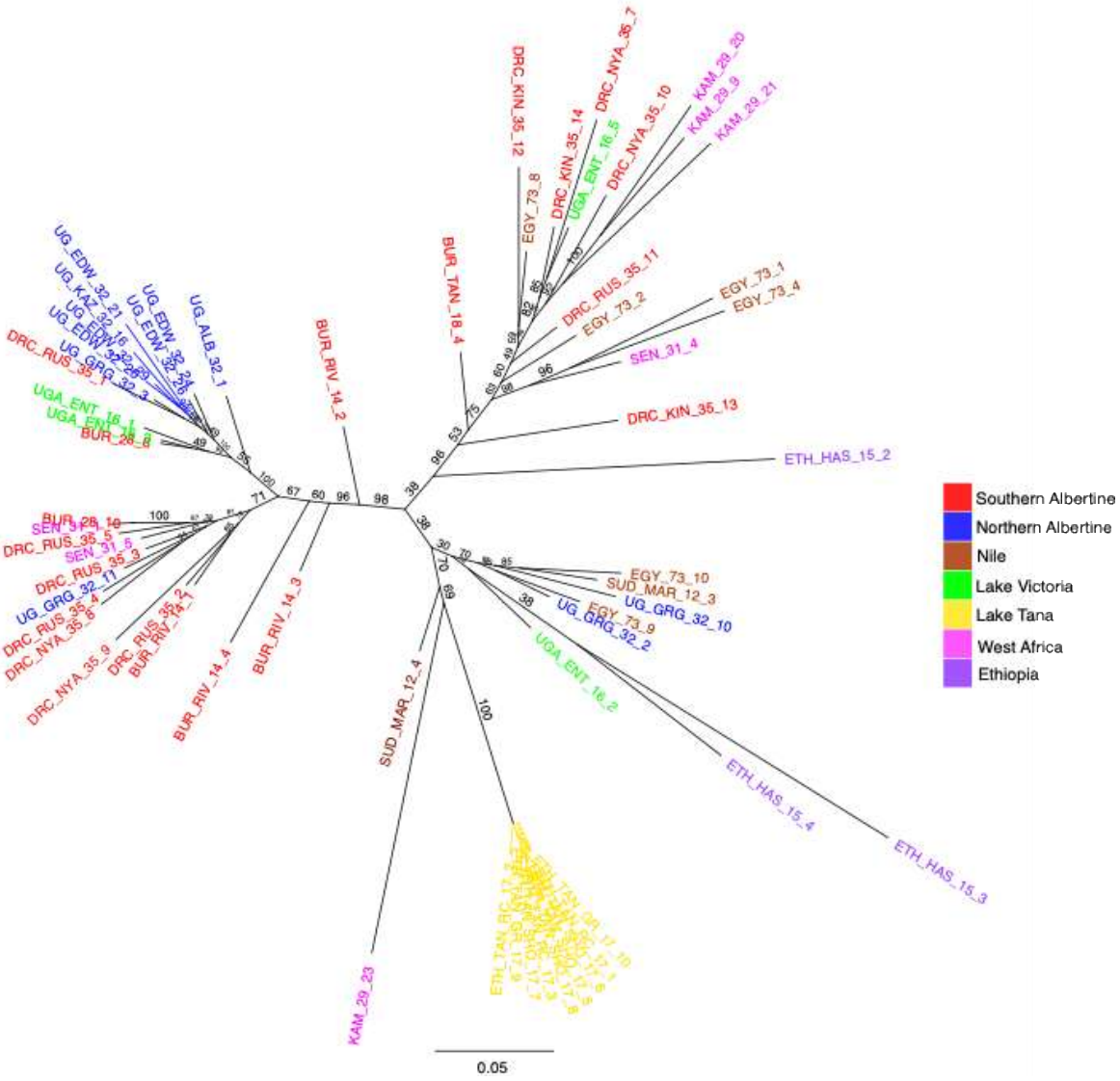

Figure 47: Maximum Likelihood Phylogeny of the Nile tilapia, *Oreochromis niloticus*, based on 27,611 SNP (reconstructed by Julien Gaspar based on data of Geraerts et al. (2022)).

The Tanganyika–Victoria population in *C. gariepinus* remains enigmatic, as these two lakes are not currently connected. Its phylogenetic placement varies depending on the dataset used: in the SNP-based tree it clusters with the northern clade, whereas in the UCE-based analysis it affiliates with the southern/Congolese clade. Once again, populations from the Great Lakes region display shared ancestry and evidence of recent gene flow with the adjacent Kivu–Edward population. We propose

that this group represents a remnant of an ancestral population belonging to the southern/Congolese lineage that subsequently colonized Lake Victoria in a secondary dispersal event. This dispersal pattern aligns with the "out of Tanganyika" hypothesis, which proposes that cichlids from Lake Tanganyika seeded radiations in Lake Victoria, as supported by documented phylogenetic proximity between haplochromines from both lakes (Meyer 1993; Meyer et al. 2015; Sturmbauer and Meyer 1993). Within the southern/Congolese clade, the earliest lineage to diverge (excluding the Tanganyika–Victoria group) is the Lower Congo population, which split around 2.2 MYA (2.05–2.38 MYA). This was followed by the divergence of the East coast population, including specimens from Lake Malawi and the Mozambique coastal region, approximately 2.1 MYA (1.94–2.28 MYA). The three *Bathyclarias* specimens (KEA 093, 094, 095) in Lake Malawi diverged around 1.5 MYA (1.19–1.75 MYA). This timing coincides with a major glacial drought that caused extremely low lake levels in Malawi (Lyons et al. 2015; Ivory et al. 2016; Dusabe et al. 2025), followed by a humid phase that led to lake overfilling between ~950 and 760 ka (Lyons et al. 2015; Ivory et al. 2016). These alternating arid and humid phases likely played an important role in driving *Bathyclarias* diversification through habitat fragmentation and subsequent re-expansion. One final split between the Southern population, and the Upper Congo population happened 1.9 MYA (1.76 – 2.08). The Upper Congo population occupies the Kalahari Plateau, which is a vast, elevated area that distinguishes the topography of southern and central Africa (Cotterill & Goodier 2008).

Amphilius: A manuscript with the description of a new species of the *Amphilius jacksoni* complex has been drafted with Avantino Kasangaki from Uganda. This species is present in the Rutshuru River (Lake Edward basin) within the Bwindi Impenetrable National Park in Uganda.
We have also done a first morphometric analysis of specimens of the *Amphilius kivuensis* group from the Lake Edward region in Uganda and the drainages of Kivu (Rwanda and DRC) and Akagera in Rwanda. We found indications for the presence of at least five species in stead of one, clearly illustrating that what is currently considered as *A. kivuensis* represents actually a species complex. Remarkably there were two species found within the Bwindi river system (Lake Edward basin) in two affluents at some 12 km in a bird's-eye view.

WP5. Synthesis, integration and formation of ichthyogeographic scenarios

It is clear that **no uniform scenario** can be developed for all fish groups in the region. Especially the unexpected and intriguing results on *Enteromius* have forced us to reconsider ichthyogeographic scenarios.
If we start with the two scenarios available at the start of the project, the ones on the haplochromine cichlids and *Clarias gariepinus*, our results clearly show that the **situation is more complex.**
The scenario with two ancient lineages (Congo and Upper Nile) at the hybrid origin of the haplochromines of the LVRS (Meier et al., 2017) is more complicated then previously reported. We found the species diversity and geographic origin of these ancient lineages to be much larger and adapted the names to respectively the fuelleborni clade and the pharyngealis clade (Figure 37).

Specifically for our region, we found four supplementary species from the Lake Edward basin to belong to the pharyngealis clade. This may indicate that the centre of distribution of the Upper Nile lineage sensu Meier et al. (2017) is actually in Lake Edward. In addition, introgression signals with the LVRS were also found in a very remote species from the Jordan River, *Astatotilapia flaviijosephi*. In the meantime, the scenario of the hybrid origin of the LVRS has also been fine-tuned by Meier et al., (2023) reporting on multiple fusion and fission cycles to explain the rapid and extensive radiations of the LVRS.

New is that also for *C. gariepinus* we observed gene flow, notably among the three lineages pertinent for the project: Nile (including Lake Albert), Tanganyika-Victoria and Kivu-Edward. Sister-group relationships between the lineages of these adjacent regions vary according to the methods used. More importantly, the most basal clade within *C. gariepinus* can be found in the Tana, a coastal river in the East Coast Ichthyofaunal Province. This lineage diverged about 4.3 million years ago during the formation of the Gregory Rift, which probably split the ancient lineage in populations at both the eastern and the western side in eastern Africa.

The clear split between a Kivu-Edward group and a Lake Albert group as well in the haplochromine cichlids as in *C. gariepinus*, together with the genetic divergence between populations of *Ctenopoma muriei* from Lakes Albert and Edward leads us to the conclusion that the presence of the **ancient Lake Obweruka** encompassing the areas of the present Lakes Edward and Albert is of **much less importance** to explain current distribution patterns then expected. This lake existed from the Late Miocene to the Pliocene (7.5 – 2.5 Mya, Van Damme & Pickford 2003). During its subsequent history, very long periods of effective separation must have occurred between the two lakes to explain the observed differences in species diversity. Lake Kivu, the area of which traditionally is not pictured as part of Lake Obweruka, is in terms of its fish diversity much nearer to Lake Edward than Lake Albert is.

The impoverished ichthyofauna of Lakes Kivu and Edward, which both lack major taxonomic groups that are present in Lake Albert, represents additional indications for the **close ichthyo-geographic relationship between Lakes Kivu and Edward**. On the family level, these are Alestidae, Auchenoglanidae, Citharinidae, Distichodontidae, Latidae, Mastacembelidae, Latidae, Malapteruridae, Mastacembelidae, Mochokidae, Nothobranchidae, Polypteridae and Schilbeidae. Also the family Danionidae could be used as an example as *Raiamas moorii* is considered to be a relatively recent invader into Lake Kivu from the Lake Tanganyika system (Snoeks et al., 1997).

These observations seem to confirm two important ichthyogeographic considerations. While the current Lake Kivu is of very recent origin and with a southwards outlet towards Lake Tanganyika, a proto-Lake Kivu and northwards running rivers existed in the area from about 5 Mya until the eruptions of the volcanoes blocked the connection with the Lake Edward region in the Late Pleistocene, (Pouclet, et al., 2016), some 25000 -11000 years ago (Snoeks et al., 1997). It is clear that the relative recency of this connection has shaped the current diversity of the KEA region and in particular the similarities between the ichthyofaunas of Lakes Kivu and Edward. The second issue is the splitting of the Obweruka lake due to the development of the Rwenzori volcanoes around 2.5 Mya, into a northern (Lake Albert area) and a southern (Lake Edward area) lake (Van Dame & Van Bocxlaer,

2009). At that time, both proto-lakes were still connected to the Congo system. However, it must have been this separation that is at the origin of the large difference in ichthyofaunas of both lakes.
During the project we obtained a large amount of new data on *Enteromius*. This genus is becoming a key genus for the study of the evolution of riverine fishes in Africa. Studies are continuing with the PhD of Heleen Maetens and at this stage, the biogeographic analysis is its last phase. Our results seem to indicate that the **soft-rayed species from the Lake Edward are more related to those from the East Coast than to those from the Nile system** (*E. prince* and *E. stigmatopygus*), confirming the hypothesis that the Lake Edward ichthyofauna is part of the East Coast Ichthyofaunal Province rather that the Nilo Sudan Province (Greenwood, 1983; Snoeks et al., 1997).
As is shown by the distribution areas of the various sawfin species, the abundance of marshy areas at watershed borders that may allow **an inter-basin exchange** of fish taxa during periods of high water (Figure 31) is a neglected but important element to understand the biogeography of the region.
One other important issue turned up, namely the **link between the KEA region and the Congo basin**. The COI-clades of three complexes of sawfin barbs, discussed above (apleurogramma, kerstenii & pellegrini), contain either individual specimens or small monophyletic lineages with specimens from the Ituri/Epulu rivers within the Aruwimi basin. Similarly, the *Enteromius* species of the KEA region with a smooth flexible dorsal spine (*E. cf. mimus* and *E. alberti*) are part of a larger clade with lineages from the same Aruwimi area. These findings appear to be at odds with the findings of Decru et al. (2017), who could not confirm the hypothesis that an ancient connection between the north-eastern part of the Congo basin and the region of the Albertine Rift is still reflected in the present ichthyofauna of the upper part of the Aruwimi. Our findings hence illustrate the importance of a intensive sampling effort and thorough multidisciplinary study the of all available data. As such, the KEA area went from one of the least known areas in terms of its fish diversity in East and Central Africa to one of the better known ones.

WPX. Coordination, project management and reporting
No further comments

WPY. Data management
New collections and finclips are curated either at the RMCA or the RBINS.
All data on extractions and lab protocols have been digitized in the standardized virtual lab book of the RBINS-RMCA.
DNA barcodes of the Lake Edward species have been submitted on GenBank and BOLD. Additional DNA barcodes (Victoria, Kivu, Albert) will be submitted on GenBank and BOLD after publication. . The latter platform includes additional metadata connected to the specimens (e.g. photographs, voucher ID, collection dates and localities) and is considered the golden standard for barcoding.
WGS data are stored on the VSC (Flanders supercomputer network).

WPZ. Valorisation, dissemination, exploitation

The results of this work package are discussed below under section "5. Dissemination and valorisation".

Added value, training and collaborations of the project

The project and its numerous outputs confirmed role of Belgium and especially the tandem RMCA & RBINS, in leading fish diversity and evolutionary studies in central and eastern Africa.

The project has engaged in a lot of collaborations with other projects and research initiatives. Team members Nathan Vranken, Heleen Maetens and Laban Musinguzi obtained PhD grants (FWO and FishBase) at KU Leuven on subjects of direct importance to the project.

Collections made during the Hipe and KEAFish projects were not only studied by KEAFish collaborators but also used for various studies on the parasites of fishes (collaboration RBINS and U Hasselt).

Three KEAFish collaborators (Maarten Van Steenberge, Heleen Maetens and Nathan Vranken) have been invited to conduct fieldwork in Rwanda as instructors during two multidisciplinary summer schools.

We collaborated with the FishBase-for-Africa programme at the RMCA, which funded the local MSc programme of Toussaint Josaphat Omombo Mulamba at the 'Université Officielle de Bukavu' (DRC) on the fisheries at the Congolese part of Lake Edward.

We also strengthened the existing collaboration between the KEAFish and the BOPCO teams (RMCA/RBINS) on barcoding analyses.

Alimasi Wilondja and Kamya Ashiraf from our African partner institutes, respectively the 'Université Officielle de Bukavu' (DRC) and NaFIRRI (Uganda) successfully completed the specialized training on "Identification and Taxonomy of East African Fishes", held from 02/10 – 25/11/2022 at the Royal Museum for Central Africa (Belgium). This programme enhanced theirs expertise in species-level identification and provided an introduction to DNA barcoding.

Richard Ddungu from NaFIRRI did a study visit to the museum to be trained in the taxonomy of haplochromines. This visit was financially supported by NaFIRRI, illustrating the good collaborative relationships between both institutes.

Societal relevance and the importance for decision making.

KEAfish Team members (Maarten Van Steenberge and Heleen Maetens) prepared an advice for the Belgian Delegation at the UNESCO summit in Saudi Arabia (10-20 september 2023) to evaluate the Rwandan bid to inscribe Nyungwe Forest National Park as a world heritage site.

The KEAfish project allowed for the activities of Maarten Van Steenberge in the ACARE (African Center of Aquatic Research and Education) network as an advisor in the Lakes Edward and Albert advisory group. This network serves as a meeting place for African experts on the Great Lakes and formulates advice for the littoral countries' decision makers in the management of their natural resources. Several collaborators and students involved in the KEAFish project are part of this network, either as members of advisory groups or as members of the annual African Women in Science programme.

The KEAfish project gave a boost to ichthyological research at RBINS, which contributed to its involvement in the Fishbase/Sealifebase consortium. The RBINS is currently an advisor in the consortium and is on the road to full membership in 2026. Maarten Van Steenberge is also a co-applicant (team member) of a successful FWO-Funded ICA project (promotor Prof. Maarten Vanhove, UHasselt) that will integrate parasitological data into Fishbase. Insights in the biogeography of fishes (and their parasites), learned in the KEAfish project, will be included in the implementation of this project. This application both strengthens the link between RBINS and UHasselt as well as the link between these institutions and the FishBase/SeaLifeBase consortium.

Due to its focus on a commercially important part of the African ichthyofauna, the KEAfish project also strengthened the connection between the ichthyological research teams at RBINS and RMCA and the DGD-funded CEBioS project. The samples collected during the expeditions financed by the HIPE and KEAfish projects also contributed to scientific capacity building activities that align with the priorities of DGD.

Three African students and collaborators: Elysée Nzigire Rutakaza, Melvin Odiba, and Alphonse Nzarora benefitted from the GTI (Global Taxonomy Initiative)-funded project “Valorising intra-African networks of young researchers to study ecosystem functioning of wetlands in the Lake Victoria drainage.” (promotors Maarten Van Steenberge and Christine Cocquyt) to undertake an internship at the Royal Belgian Institute for Natural Sciences and the Botanic Garden Meise in the spring of 2023.

The samples collected during the expeditions financed by the HIPE and KEAfish projects also contributed to scientific capacity building activities that align with the priorities of DGD. Two PhD projects, financed via the BILA-BOF ‘Bilateral Scientific Cooperation- Bijzondere Onderzoeks Financiering’ (UHasselt) were allocated to Congolese researchers and depend heavily on the samples in Belspo-funded expeditions. A large part of the training and supervision was done by KEAfish partners and staff members:

Dr. Archimède Mulega, UHasselt- Université Mohammed V, Rabat. « African catfishes and their monogenean gill parasites: barcoding, diversity and biogeography” , UHasselt, 365p defended juli 2025. Maarten Van Steenberge, Co-promotor. In collaboration with team members Arthur Boom and Julien Gaspar.

Ms. Elysée Nzigire Rutakaza UHasselt- Université Mohammed V, Rabat. » The algal flora of the Albertine Rift Lakes. Using stomach content analyses to compare of the dietary overlap of herbivorous fishes in native and introduced conditions, with a focus on diatom taxonomy. Planned defence 2027. Maarten Van Steenberge, promotor.

While the project concentrated on the fish diversity of the region, it did contribute to **more applied research questions**. The project did not have the goal to further analyse fisheries issues in the region. However, overfishing is omnipresent in the region and has important socio-economic implications. In addition, it has a large impact on the fish diversity studied in the project. Some other team members were involved with lead author, Laban Musinguzi (NaFIRRI, Uganda) in an assessment of some stocks of economically important species from Lake Edward (Musinguzi et al., 2021), which estimated poor stock status with the stocks defined as either collapsed, recruitment impaired or overfished. In a follow-up study with new data and including other water bodies in the region, this poor status was

confirmed in 22 of the 24 stocks studied from Lakes Edward, George, Albert, Kyoga and Victoria (Musinguzi, 2024).

In addition, the available information on the ecosystem functioning of the Lake Edward system has been reviewed. Data and information gaps exist on the abundance of biotic communities, fish life history, quantitative trophic ecology and fisheries management reference points (Musinguzi et al., 2024).

Jos Snoeks is much involved in Red List assessments and reviews of the African freshwater fish fauna for IUCN. During the project he has been asked by IUCN to review some old assessments and assess newly described species from eastern Africa. Information from the project was hence fed into the IUCN Red List database and the project benefitted from information provided by other assessors.

References:

Abell, R., Thieme, M. L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., Coad, B., Mandrak, N., Balderas, S. C., Bussing, W., Stiassny, M. L. J., Skelton, P., Allen, G. R., Unmack, P., Naseka, A., Ng, R., Sindorf, N., Robertson, J., Armijo, E., Higgins, J. V., Heibel, T. J., Wikramanayake, E., Olson, D., López, H. L., Reis, R. E., Lundberg, J. G., Sabaj Pérez, M. H., Petry, P. (2008). Freshwater Ecoregions of the World: A New Map of Biogeographic Units for Freshwater Biodiversity Conservation. BioScience, 58 (5): 403-414.

Asch, B. van, Versfeld, W. F., Hull, K. L. Leslie, A. J., Matheus, T. I., Beytell, P. C., du Preez, P., Slabbert, R. & Rhode, C. (2019). Phylogeography, Genetic Diversity, and Population Structure of Nile Crocodile Populations at the Fringes of the Southern African Distribution. PLoS One 14 (12): e0226505. https://doi.org/10.1371/journal.pone.0226505.

Baker, B. H. (1986). Tectonics and Volcanism of the Southern Kenya Rift Valley and Its Influence on Rift Sedimentation. Geological Society, London, Special Publications 25 (1): 45–57. https://doi.org/10.1144/GSL.SP.1986.025.01.05.

Bamba, M. (2012). The '*Barbus*' species (Cypriniformes, Cyprinidae) from Côte d'Ivoire: A systematic revision of some West African species complexes. PhD Tesis, KU Leuven, Belgium.

Bamba, M., Vreven, E. J. & Snoeks J. (2011). Description of *Barbus teugelsi* sp. nov. (Cypriniformes: Cyprinidae) from the Little Scarcies basin in Guinea, Africa. Zootaxa 2998: 48–65.

Bosmans, D. (2023). Cryptic species diversity in *Enteromius* (Cyprinidae) from West Africa. A morphometric and molecular approach. MSc thesis, KU Leuven, 65 pp.

Bragança, P. H. N. & Costa, W. J. E. M. (2019). Multigene fossil-calibrated analysis of the African lampeyes (Cyprinodontoidei: Procatopodidae) reveals an early Oligocene origin and Neogene diversification driven by palaeogeographic and palaeoclimatic events. Organisms Diversity & Evolution 19: 303-320.

Cotterill, F. P. D. & Goodier, S. A. (2008). DNA clocks for dating landforms. Quest, 4 (2), 19-21.

Day, J. J., Peart, C. R., Brown, K. J., Friel, J. P., Bills, R. & Moritz, T. (2013). Continental diversification of an African catfish radiation (Mochokidae: *Synodontis*), Systematic Biology, 62 (3): 351–365.

De Vos, L., Snoeks, J. & Thys van den Audenaerde, D. (2001). An annotated checklist of the fishes of Rwanda (East Central Africa) with historical data on introductions of commercially important species. Journal of East African Natural History. 90: 41-68.

Decru, E., Moelants, T., De Gelas, K., Vreven, E., Verheyen, E. & Snoeks, J. (2016). Taxonomic challenges in freshwater fishes: a mismatch between morphology and DNA barcoding in fish of the north-eastern part of the Congo basin. Molecular Ecology Resources, 16 (1): 342-352.

Decru, E., Vranken, N., Bragança, P. H. N., Snoeks, J. & Van Steenberge, M. (2019). Where ichthyofaunal provinces meet: the fish fauna of the Lake Edward system. Journal of Fish Biology 96 (5): 1186-1201.

Decru, E., Vranken, N., Maetens, H., De Vry, A. M., Kayenbergh, A., Snoeks, J. & Van Steenberge, M. (2022). DNA barcoding the Lake Edward basin: high taxonomic coverage of a tropical freshwater ichthyofauna. Hydrobiologia, 849: 1743-7962. doi: 10.1007/s10750-022-04812-0.

Decru, E., Vreven, E., De Gelas, K., Verheyen, E., & Snoeks, J. (2017). Species richness in the African pike genus *Hepsetus:* a perfect match between genetics and morphology. Journal of Fish Biology, 91 (2): 617-627.

Diedericks, G. (2017). Determining the mechanisms driving the invasion success of the smallmouth bass (*Micropterus dolomieu* , Lacepède 1802). PhD thesis, Stellenbosch University, 146 pp.

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, *29*, 1969–1973. https://doi.org/10.1093/molbev/mss075

Dumont, H. J. (2009). A Description of the Nile Basin and a Synopsis of Its History, Ecology, Biogeography, Hydrology, and Natural Resources. In: Dumont, H. J. (Eds.), The Nile: Origin, Environments, Limnology and Human Uses. Monographiae Biologicae: 1-21. Springer, Dortrecht, the Netherlands. https://doi.org/10.1007/978-1-4020-9726-3_1.

Dupin, J., Matzke, N. J., Sarkinen, T., Knapp, S., Olmstead, R., Bohs, L. & Smith, S. (2016). Bayesian estimation of the global biogeographic history of the Solanaceae. Journal of Biogeography, 44(4): 887-899. http://dx.doi.org/10.1111/jbi.12898

Dusabe, M. C., Clewing, C., Kagoro-Rugunda, G., Rwibutso, M., Ndeo, O. W., Tumusiime, J., & Albrecht, C. (2025). Historical Drainage Evolution and Water Level Fluctuations in the African Great Lakes Shaped Phylogeny and Biogeography of Freshwater Gastropods. Journal of Biogeography, 52 (12): 1-19. e70074. https://doi.org/10.1111/jbi.70074.

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acid Research*, *32*(5): 1792–1797. https://doi.org/10.1093/nar/gkh340

Elmer, K. R., Reggio, C., Wirth, T., Verheyen, E., Salzburger, W., Meyer, A. (2009). Pleistocene desiccation in East Africa bottlenecked but did not extirpate the adaptive radiation of Lake Victoria haplochromine cichlid fishes. Proceedings of the National Academy of Sciences, 106 (32): 13404-13409.

Englmaier, G.K., Blažek, R., Zimmermann, H., Bartáková, V., Polačik, M., Žák, J., Mulokozi, D. P., Katongo, C., Büscher, H. H., Mabo, L., Koblmüller, S., Palandačić, A. & Reichard, M. (2024). Revised taxonomy of *Synodontis* catfishes (Siluriformes: Mochokidae) from the Lake Tanganyika basin reveals lower species diversity than expected. Zoological Journal of the Linnean Society, 202 (3): 38 pp.

Eyckens, B. 2025. Bepalen van de stockstructuur voor *Clarias gariepinus* en *Bagrus docmak* in het Edward-Georgesysteem. MSc thesis, KU Leuven, 50pp.

Geneious Prime 2024.0.5.(https://www.geneious.com)

Geraerts, M., Vangestel, C., Artois, T., Fernandes, J., Jorissen, M., Choca Manda, A., Danadu Mizani, C., Smeets, K., Snoeks, J., Sonet, G., Tingbao, Y., Van Steenberge, M., Vreven, E., Lunkayilakio Wamuini, S., Vanhove, M. & Huyse, T. (2022). Population genomics of introduced Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) in the Democratic Republic of the Congo: Repeated introductions since colonial times with multiple sources. Molecular ecology, 31 (12): 3304-3322. doi: 10.1111/mec.16479.

Goodier, S. A. M., Cotterill, F. P. D., O'Ryan, C., Skelton, P. H. & de Wit, M. J. (2011). Cryptic Diversity of African Tigerfish (Genus *Hydrocynus*) Reveals Palaeogeographic Signatures of Linked Neogene Geotectonic Events. Plos One, 6 (12): 1–15.

Green, J. 2009. Nilotic Lakes of the Western Rift. In : H.J. Dumont (ed.). The Nile: origin, environments, limnology and human use: 263-286. Monographiae Biologicae, Springer, Dordrecht, the Netherlands.

Greenwood, P.H. (1966). The fishes of Uganda. The Uganda Society, Kampala, 131 pp.

Greenwood, P. H. (1983). The zoogeography of African freshwater fishes: bioaccountancy or biogeography? Evolution, time and space : the emergence of the biosphere. In: R.W. Sims, J.H. Price & P.E.S. Whalley (Eds): 179-199. Systematics Association Sp. Vol. 23. Academic Press, London & New York.

Hewitt, G. M. (2004). The structure of biodiversity – insights from molecular phylogeography. Frontiers in Zoology: 1: 4.

Howes, G.J. **(**1984). A review of the anatomy, taxonomy, phylogeny and biogeography of the African neoboline cyprinid fishes. Bulletin of the British Museum of Natural History (Zoology), 47(3): 151–185.

Ivanova, N. V., Zemlak, T. S., Hanner, R. H., & Hebert, P. D. (2007). Universal primer cocktails for fish DNA barcoding. Molecular Ecology Notes, 7: 544–548. https://doi.org/10.1111/j.1471-8286.2007.01748.x

Ivory, S. J., Blome, M. W. King, J. W., McGlue, M. M., Cole, J. E. & Cohen, A. S. (2016). Environmental Change Explains Cichlid Adaptive Radiation at Lake Malawi over the Past 1.2 Million Years. Proceedings of the National Academy of Sciences 113 (42): 11895–900. https://doi.org/10.1073/pnas.1611028113.

Jansen, G., Devaere, S., Weekers, P. H. H., & Adriaens, D. (2006). Phylogenetic Relationships and Divergence Time Estimate of African Anguilliform Catfish (Siluriformes: Clariidae) Inferred from Ribosomal Gene and Spacer Sequences." Molecular Phylogenetics and Evolution 38 (1): 65–78. https://doi.org/10.1016/j.ympev.2005.09.011.

Johnson, T. C., Scholz, C. A., Talbot, M. R., Kelts, K., Ricketts, R. D., Ngobi, G., Beuning, K., Ssemmanda, I. & McGill, J. W. (1996). Late Pleistocene desiccation of Lake Victoria and rapid evolution of cichlid fishes. Science, 273 (5278): 1091-1093.

Kisekelwa, T., Alimasi, W., Mazambi, L., Mwaijengo, G. N., Hyangya, L., Muzungu, H., Joyeuse, M., Lubala, A., Kubota, M., Wüest, A., Muvundja, A., & Masilya, M. (2023). Fish Diversity in Relation to Littoral Habitats in Three Basins of Lake Kivu (East Africa). Diversity, 15 (9): 1014. https:// doi.org/10.3390/d15091014.

Kisekelwa, T., Snoeks, J., & Vreven, E. (2020). An annotated checklist of the fish fauna of the river systems draining the Kahuzi-Biega National Park (Upper Congo: Eastern DR Congo). Journal of Fish Biology, 96(3): 700–721. https://doi.org/10.1111/jfb.14264

Kishe-Machumu, M. A., Natugonza, V., Nyingi, D. W., Snoeks, J., Carr, J. A., Seehausen, O. & Sayer, C. A. (2018). Chapter 4: The status and distribution of freshwater fishes in the Lake Victoria Basin. In: Sayer, C. A., Máiz-Tomé, L. & Darwall, W. R. T. (Eds). Freshwater biodiversity in the Lake Victoria Basin: Guidance for species conservation, site protection, climate resilience and sustainable livelihoods: 41-64.

Koblmüller, S., Schöggl, C. A., Lorber, C. J., Van Steenberge, M., Kmentová, N., Vanhove, M. P., & Zangl, L. (2021). African lates perches (Teleostei, Latidae, *Lates*): Paraphyly of Nile perch and recent colonization of Lake Tanganyika. Molecular Phylogenetics and Evolution, 160, 107141.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Lavoué, S, Jamaluddin, J. A. F., Muhammad-Rasul, A. H., Ilham-Norhakim, M. L. & Zain, K. M. (2024). Mitochondrial evidence on the phylogenetic position of the Southeast Asian catfish genus *Encheloclarias* Myers, 1937 (Actinopterygii: Siluriformes: Clariidae): Evolutionary and conservation implications. Acta Ichthyologica et Piscatoria 54: 235-241. https://doi.org/10.3897/aiep.54.122366

Lehmann, T., Hawley, W. A., Grebert, H., Danga, M., Atieli, F. & Collins, F. H. (1999). The Rift Valley Complex as a Barrier to Gene Flow for Anopheles Gambiae in Kenya. Journal of Heredity 90 (6): 613–621. https://doi.org/10.1093/jhered/90.6.613.

Lévêque, C. (1997). Biodiversity dynamics and conservation. The freshwater fish of tropical Africa. Cambridge University Press, 1-451.

Liyandja, T. L. D., Smith, B. T., Alter, S. E., Sidlauskas, B. L., & Stiassny, M. L. J. (2025). Phylogenomics of African Labeo(Cypriniformes: Cyprinidae) emphasizing central African species. Molecular Phylogenetics and Evolution. 213: 1-17.

Lohay, G. G., Lee, D. E., Wu-Cavener, L., Pearce, D.L., Hou, X. & Bond, M.L. (2023). Genetic Evidence of Population Subdivision among Masai Giraffes Separated by the Gregory Rift Valley in Tanzania. Ecology and Evolution 13 (6): e10160. https://doi.org/10.1002/ece3.10160.

López, J. A., Chen, W. J., & Ortí, G. (2004). Esociform Phylogeny. Copeia 2004 (3): 449–464. https://doi.org/10.1643/CG-03-087R1.

Lyons, R. P., Scholz, C. A., Cohen, A. S., King, J. W., Brown, E. T., Ivory, S. J., Johnson, T. C., Deino, A. L., Reinthal, P. N., McGlue, M. M., & Blome, M. W. (2015). Continuous 1.3-Million-Year Record of East African Hydroclimate, and Implications for Patterns of Evolution and Biodiversity." Proceedings of the National Academy of Sciences 112 (51): 15568–73. https://doi.org/10.1073/pnas.1512864112.

Maebe, T. 2022. Morphometric variation in *Clarias gariepinus*. A traditional and geometric morphometric approach. MSc thesis, KU Leuven, 69 pp.

Macgregor, D. (2015). History of the Development of the East African Rift System: A Series of Interpreted Maps through Time. Journal of African Earth Sciences 101: 232–52. https://doi.org/10.1016/j.jafrearsci.2014.09.016.

Maetens, H. (2019). The *Enteromius* species of the Lake Edward system: A genetic and morphometric approach. KU Leuven, unpublished MSc thesis, 1-64.

Maetens, H., Decru, E., Boom, A. F., Vranken, N., Van Steenberge, M., & Snoeks, J. (2024). Diving into diversity: The complex evolutionary history and species richness of the 'sawfin barbs' from Lake Edward and Adjacent systems. Evolutionary Biology, 51: 366–383. https://doi.org/10.1007/s11692-024-09640-9

Maetens, H., Van Steenberge, M., Snoeks, J. & Decru, E. (2020). Revalidation of *Enteromius alberti* and presence of *Enteromius cf. mimus* (Cypriniformes: Cyprinidae) in the Lake Edward system, East Africa. European Journal of Taxonomy, 700: 1-28.

Maetens, H., Mukungilwa, P., Kasangaki, A., Boom, A.F., Nshimiyumuremyi, T., Munyandamutsa, P.S., Clausnitzer, V., Vranken, N., Snoeks, J. & Van Steenberge, M. (2025). An ichthyological borderland: the fishfauna of Nyungwe National Park and surroundings (Rwanda, East Africa). Journal of fish biology, 107 (6): 1898-1909. doi: 10.1111/jfb.70185.

Matté, A. (2023). Eco-morphological divergence in the species of *Enteromius* Cope, 1867 (Cyprinidae) from the Lake Edward basin (East Africa). A geometric morphometric approach. MSc thesis, KU Leuven, 64 pp.

Matzke, N. J. (2016). Stochastic mapping under biogeographical models. PhyloWiki BioGeoBEARS website, 2016. http://phylo.wikidot.com/biogeobears#stochastic_mapping.

McGee, M. D., Borstein, S. R., Meier, J. I., Marques, D. A., Mwaiko, S., Taabu, A., Kishe-Machumu, M. A., O'Meara, B., Bruggmann, R., Excoffier, L., & Seehausen, O. (2020). The ecological and genomic basis of explosive adaptive radiation. Nature, 586: 75–79. https://doi.org/10.1038/s41586- 020-2652-7.

Meier, J. I., Marques, D. A., Mwaiko, S., Wagner, C. E., Excoffier, L. & Seehausen, O. (2017). Ancient hybridization fuels rapid cichlid fish adaptive radiations. Nature Communications, 8: 1-11.

Meier, J. I., McGee, M. D., Marques, D. A., Mwaiko, S., Kishe, M., Wandera, S., Neumann, D., Mrosso, H., Chapman, L. J., Chapman, C. A., Kaufman, L., Taabu-Munyaho, A., Wagner, C. E., Bruggmann, R., Excoffier, L. & Seehausen, O. (2023). Cycles of fusion and fission enabled rapid parallel adaptive radiations in African cichlids. Science, 381 (6665): 1-13. doi: 10.1126/science.ade2833.

Meyer, A. (1993). Phylogenetic Relationships and Evolutionary Processes in East African Cichlid Fishes. Trends in Ecology & Evolution 8 (8): 279–84. https://doi.org/10.1016/0169-5347(93)90255-n.

Meyer, B. S., Indermaur, A., Ehrensperger, X., et al. (2015). Back to Tanganyika: A Case of Recent Trans-Species-Flock Dispersal in East African Haplochromine Cichlid Fishes. Royal Society Open Science 2 (3): 140498. https://doi.org/10.1098/rsos.140498.

Musinguzi, L. (2024). Towards Ecosystem-Based Fisheries Management in the Lake Edward System: A Reappraisal of the Fisheries and Aquatic Ecosystem Status. PhD thesis, KU Leuven: 316 pp.

Musinguzi, L., Bassa, S., Natugonza, V., Van Steenberge, M., Okello, W., Snoeks, J., & Froese, R. (2021). Assessment of exploited fish species in the Lake Edward System, East Africa. Journal of Applied Ichthyology, 37(2): 216-226.

Musinguzi, L., Vranken, N., Natugonza, V., Okello, W., van Steenberge, M. & Snoeks, J. (2024). State of knowledge of aquatic ecosystem and fisheries of the Lake Edward System, East Africa. Aquaculture, fish and fisheries, 4(1): 1-25. doi:10.1002/aff2.140.

Nagy, B. & Watters, B.R. (2022). *Lacustricola margaritatus*, a new species of lampeye from the Lake Victoria and Lake Kyoga basins in eastern Africa (Cyprinodontiformes: Procatopodidae). Zootaxa 5128 (1): 44-60.

Pouclet, A., Bellon, H. & Bram, K. (2016). The Cenozoic volcanism in the Kivu rift: Assessment of the tectonic setting, geochemistry, and geochronology of the volcanic activity in the South-Kivu and Virunga regions. Journal of African Earth Sciences 121: 219-246.

Pouyaud, L., Sudarto, & Paradis, E. (2009). The Phylogenetic Structure of Habitat Shift and Morphological Convergence in Asian *Clarias* (Teleostei, Siluriformes: Clariidae). Journal of Zoological Systematics and Evolutionary Research 47 (4): 344–56. https://doi.org/10.1111/j.1439-0469.2008.00507.x.

Ren, Q., & Mayden, R. L. (2016). Molecular phylogeny and biogeography of African diploid barbs, 'Barbus', and allies in Africa and Asia (Teleostei: Cypriniformes). Zoologica Scripta, 45 (6): 642–649.

Riddin, M. A., Bills, I. R. & Villet, M. H. (2016) Phylogeographic, morphometric and taxonomic reevaluation of the river sardine, *Mesobola brevianalis* (Boulenger, 1908) (Teleostei, Cyprinidae, Chedrini). ZooKeys 641: 121–150. https://doi.org/10.3897/zookeys.641.10434

Risch, L. M. (2003). Bagridae. In: C. Lévêque, D. Paugy and G.G. Teugels (eds.) Faune des poissons d'eaux douce et saumâtres de l'Afrique de l'Ouest, Tome 2: 54-59. Coll. Faune et Flore tropicales 40. Musée Royal de l'Afrique Centrale, Tervuren, Belgique, Museum National d'Histoire Naturalle, Paris, France and Institut de Recherche pour le Développement, Paris, France.

Rognon, X., Teugels, G. G., Guyomard, R., Galbusera, P., Andriamanga, M., Volckaert, F., & Agnèse, L. F. (1998). Morphometric and allozyme variation in the African catfishes *Clarias gariepinus and C. anguillaris*. Journal of Fish Biology, 53: 192‑207.

Seegers, L. (1990). Bemerkungen zur gattung *Pseudocrenilabrus*. Teil 2: *Pseudocrenilabrus multicolor victoriae nov. subsp.*. D.A.T.Z. 43(2): 99-103.

Seegers, L. (2008). The catfishes of Africa. A handbook for identification and maintenance. Aqualog & Tetra Verlag Pfeill: 680 pp.

Seehausen, O., Koetsier, E., Schneider, M. V., Chapman, L. J., Chapman, C. A., Knight, M. E., Turner, G. F., van Alphen, J. J., Bills, R. (2003). Nuclear markers reveal unexpected genetic variation and a Congolese-Nilotic origin of the Lake Victoria cichlid species flock. Proceedings of the Royal Society B: Biological Sciences, 270: 129–137.

Shokralla, S., Hellberg, R. S., Handy, S. M., King, I. & Hajibabaei, M. (2015). A DNA Mini-Barcoding System for Authentication of Processed Fish Products. Scientific Reports, 5: 1‑11.

Snoeks, J. (1994). The haplochromine fishes (Teleostei, Cichlidae) of Lake Kivu, East Africa: a taxonomic revision with notes on their ecology. Annales Musee Royal de L'Afrique Centrale - Sciences Zoologiques, 270: 221 pp.

Snoeks, J. (2000). How well known is the ichthyodiversity of the ancient African lakes? In: Rossiter, A. & Kawanabe, H. (Eds) Ancient lakes: biodiversity, ecology and evolution. Advances in ecological research, 31: 17-38.

Snoeks, J., De Vos, L., Thys Van Den Audenaerde, D. F. E. (1997). The ichthyogeography of Lake Kivu. South African Journal of Science, 93: 579–584.

Snoeks, J. & Getahun, A. (2013). African Fresh and Brackish Water Fish Biodiversity and their Distribution: more unknowns than knowns. In: Snoeks J. & Getahun A. (Eds.) Zoological Documentation Online Series, Proceedings of the 4th International Conference on African Fish and Fisheries, Addis Ababa, Ethiopia 22-26 September 2008: 67-76. RMCA, Tervuren, Belgium.

Snoeks, J., Harrison, I. J. & Stiassny, M. L. J. (2011). The status and distribution of freshwater fishes. In: Darwall, W. R. T., Smith, K. G., Allen, D. J., Holland, R. A., Harrison, I. J. & Brooks, E. G. E. (Eds), The diversity of life in African freshwaters: under water, under threat. an analysis of the status and distribution of freshwater species throughout mainland Africa: 42-73. IUCN, Gland, Switzerland and Cambridge, UK.

Snoeks, J., Kaningini, B., Masilya, P., Nyina-Wamwiza, L. (2012). Fishes in Lake Kivu: Diversity and Fisheries. In: Descy, J-P., Darchambeau, F., Schmid, M. (Eds). Lake Kivu: limnology and biogeochemistry of a tropical great lake: 127-152. Aquatic Ecology Series. Springer, Dordrecht.

Sommer, G. M., Njom, S. D., Indermaur, A., Nyom, A. R. B., Jandová, K., Kukla, J., Petrtyl, M., Horka, P. & Musilova, Z. (2024). Trophic ecology of the African riverine elephant fishes (Mormyridae). Ecology and Evolution, 14 (8): 17 pp.

Sonet, G., Snoeks, J., Nagy, Z., Vreven, E., Boden, G., Breman, F., Decru, E., Hanssens, M., Ibala Zamba, A., Jordaens, K., Mamonekene, V., Musschoot, T., Van Houdt, J., Van Steenberge, M., Lunkayilakio Wamuini, S., Verheyen, E. (2019). DNA barcoding fishes from the Congo and the Lower Guinean provinces: assembling a reference library for poorly inventoried fauna. Molecular Ecology Resources, 19 (3): 728–743.

Stager, J. C. & Johnson, T. C. (2008). The late Pleistocene desiccation of Lake Victoria and the origin of its endemic biota. Hydrobiologia, 596 (1): 5-16.

Sturmbauer, C. & Meyer, A. (1993). Mitochondrial Phylogeny of the Endemic Mouthbrooding Lineages of Cichlid Fishes from Lake Tanganyika in Eastern Africa. Molecular Biology and Evolution 10 (4): 751–68. https://doi.org/10.1093/oxfordjournals.molbev.a040042.

Teugels, G. G. (1986). A systematic revision of the African species of the genus *Clarias* (Pisces; Clariidae). Annales-Musee Royal de l'Afrique Centrale. Sciences Zoologiques, 10: 372 pp.

Thieme, M. L., Abell, R., Burgess, N., Lehner, B., Dinerstein, E., Olson, D., Teugels, G., Kamdem-Toham, A., Stiassny, M. L. J. S. & Skelton, P. (2005). Freshwater ecoregions of Africa and Madagascar: A conservation assessment. Island Press: 452 pp.

Tshibwabwa, S. M., Teugels, G. G. (1995). Contribution to the systematic revision of the African cyprinid fish genus *Labeo*: species from the lower Zaire River system. Journal of Natural History, 29: 1543–1579.

Van Damme, D. & Pickford, M. (2003). The late Cenozoic Thiaridae (Mollusca, Gastropoda, Cerithioidea) of the Albertine Rift Valley (Uganda-Congo) and their bearing on the origin and evolution of the Tanganyikan thalassoid malacofauna. Hydrobiologia 498: 1–83.

Van Damme, D., & Van Bocxlaer, B. (2009). Freshwater molluscs of the Nile Basin, past and present. In H. J. Dumont (Ed.), The Nile: 585–629. Springer, Dordrecht, the Netherlands.

Van Ginneken, M., Decru, E., Verheyen, E. & Snoeks, J. (2017). Morphometry and DNA barcoding reveal cryptic diversity in the genus *Enteromius* (Cypriniformes: Cyprinidae) from the Congo basin, Africa. European Journal of Taxonomy, 310: 1-32.

Van Steenberge, M., Vanhove, M. P. M., Chocha Manda, A., Larmuseau, M. H. D., Swart, B. L., Khang'Mate, F., Arndt, A., Hellemans, B., Van Houdt, J., Micha, J-C., Koblmüller, S., Roodt-Wilding, R. & Volckaert, F. A. M. (2020). Unravelling the evolution of Africa's drainage basins through a widespread freshwater fish, the African sharptooth catfish *Clarias gariepinus*. Journal of Biogeography, 2020 (47): 1739–1754.

Van Steenberge, M., Vreven, E. J. & Snoeks J. (2014). The fishes of the upper Luapula area (Congo Basin): a fauna of mixed origin. *Ichthyological Exploration of Freshwaters* 24: 329–345.

Verheyen, E., Salzburger, W., Snoeks, J. & Meyer, A. (2003). The origin of the superflock of cichlid fishes from Lake Victoria, East Africa. Science, 300 (5617): 325-329.

Verheyen, E., Salzburger, W., Snoeks, J. & Meyer, A. (2004). Response to comment on "Origin of the superflock of cichlid fishes from Lake Victoria, East Africa". Science, 304: 963.

Vranken, N. (2024). Patterns of phenotypic similarity in the haplochromine cichlids of the Lake Victoria Region Superflock. An eco-morphological and genomic approach. Unpublished PhD thesis, KU Leuven, 426 pp.

Vranken, N., Van Steenberge, M., Kayenbergh, A. & Snoeks, J. (2019). The lobed-lipped species of *Haplochromis* (Teleostei, Cichlidae) from Lake Edward, two instead of one. Journal of Great Lakes Research, 46 (5): 1079-1089.

Vranken, N., Van Steenberge, M. & Snoeks, J. (2018). Grasping ecological opportunities: not one but five paedophagous species of *Haplochromis* (Teleostei: Cichlidae) in the Lake Edward system. Hydrobiologia, 832 (1): 105-134.

Vranken, N., Van Steenberge, M. & Snoeks, J. (2020). Similar ecology, different morphology: the oral mollusc shellers from Lake Edward. Journal of Fish Biology, 96 (5): 1202-1217.

Wandera, S. B. & Balirwa, J. S. (2010). Fish species diversity and relative abundance in Lake Albert—Uganda. Aquatic Ecosystem Health and Management, 13(3): 284–293.

Witte, F., van Oijen, M. J. P., Sibbing, F. A. (2009). Fish Fauna of the Nile. In: Dumont, H. J. (Eds.), The Nile: Origin, Environments, Limnology and Human Uses. Monographiae Biologicae, 647–675. Springer, Dortrecht, the Netherlands.

Worthington, E. B. (1929). New species of fish from the Albert Nyanza and Lake Kioga. Proceedings of the Zoological Society of London: 429-440.

Xiao, W., Zhang, Y., & Liu, H. (2001). Molecular Systematics of Xenocyprinae (Teleostei: Cyprinidae): Taxonomy, Biogeography, and Coevolution of a Special Group restricted in East Asia. Molecular Phylogenetics and Evolution, 18(2): 163–173.

5. DISSEMINATION AND VALORISATION

Team members have taken part in **two summer schools in Rwanda**, where they have sampled for the project. On the basis of these sampling efforts, species lists of the Nyungwe and Akagera National Parks have been compiled. These have been shared, together with policy recommendations, with the organisers of the summer school and with the relevant Rwandan authorities (National Park managers and Centre of Excellence in Biodiversity and Natural Resource management of the University of Rwanda). The KEAfish team was involved in the review of the bid of the Rwandan government in the inscription of Nyungwe forest national park on the UNESCO world heritage list.
The KEAFish project was presented at the Akagera National Park headquarters.
In total, the project has now been presented at **eight international conferences**, including 12 lectures at the largest conference on African fishes, held every five year, PAFFA (Pan African Fish and Fisheries Association) organised in September 2023 in Brazzaville, Republic of the Congo.
Two PhD theses were finished by project members: Laban Musinguzi (NaFIRRI, Uganda) defended his thesis, "Towards Ecosystem-Based Fisheries Management in the Lake Edward System: A Reappraisal of the Fisheries and Aquatic Ecosystem Status" on 17/04/2024 at the KU Leuven. Nathan Vranken defended his thesis, "Patterns of phenotypic similarity in the haplochromine cichlids of the Lake Victoria Region Superflock: an eco-morphological and genomic approach" on 19/04/2024 also at the KU Leuven. A third PhD will be presented mid 2026 by Heleen Maetens, "Swimming in the shadow of cichlids: the enigmatic species richness and evolutionary history of the African small barbs".
The project's goals and results have been discussed by the coordinator in yearly seminars for FishBase trainees, and for MSc students of the KU Leuven and of the University of Namur.

The aquatic environment of the Lake Edward system is under continuing pressure, leading to a severe decline in fish catches (Musinguzi et al. 2021). The baseline data collected by the project and the information gathered during the PhD of Laban Musinguzi on life history parameters of the economically important fishes of the Lake Edward system and on the ecosystem functioning of the system are valuable tools for future management actions to build a more sustainable fisheries and to protect the fish fauna of the system.
At present, team members and additional scientists from NaFIRRI (Uganda) are finishing a publication on an **Ecopath/Ecosim model for the Lake Edward system**, as a vital step in promoting ecosystem-based fisheries management in the region.

<u>Presentations at scientific meetings</u>

Antolini P., Vranken N., Mondal N., Van Steenberge M., Svardal H., Boom A., Gante H.F. & Snoeks J. (2022). Evolution of genome size and body size in a fast adaptive radiation. *Cichlid Science 2022*, 06-09/09/2022, University of Cambridge, UK.

Boom A.F., Bosmans D., Maetens H. & Snoeks J. (2023). Exploring universal mini barcodes for fish: trials, pitfalls, and future prospects on formalin fixed, fluid-preserved specimens of *Enteromius*

species from western Africa. *Seventh International Conference of the Pan African Fish and Fisheries Association (PAFFA),* 18-23/09/2023, University of Marien Ngouabi, Brazzaville (Republic of the Congo).

Boom A.F., Maetens H., Decru E., Gante H., Van Steenberge M. & Snoeks, J. (2023). Whole mitochondrial sequence of *Enteromius* species from Lake Edward: duplications and methodological considerations. *Seventh International Conference of the Pan African Fish and Fisheries Association (PAFFA),* 18-23/09/2023, University of Marien Ngouabi, Brazzaville (Republic of the Congo).

Decru E., Van Steenberge M., Maetens H., Vranken N., Geerts M., Boom A., Musinguzi L., Gante H. & Snoeks J. (2023). Exploring the East African Rift Lakes: investigating the ichthyofauna and biogeography in lakes Kivu, Edward and Albert. *Seventh International Conference of the Pan African Fish and Fisheries Association (PAFFA),* 18-23/09/2023, University of Marien Ngouabi, Brazzaville (Republic of the Congo).

Gaspar, J., Mushagalusa Mulega, A., Snoeks, J. & Van Steenberge, M. (2025). Reconstructing freshwater biogeography using catfish genomes. *23rd FishBase and SeaLifeBase Symposium*, 2-3/09/2023, Royal Belgian Institute of Natural Sciences, Brussels (Belgium).

Geerts M., Boom A., Van Steenberge M., Gante H. & Snoeks J. (2023). The biogeography of the widespread African sharptooth catfish using WGS. *Zoology 2023,* 31/05/2023, NATURALIS Biodiversity Center, Leiden, The Netherlands.

Geerts M., Boom A., Van Steenberge M., Gante H. & Snoeks J. (2023). The biogeography of the widespread African sharptooth catfish using WGS. *Phylogenomics and Population Genomics course 2023*, 14/07/2023, University of Barcelona, Spain.

Geraerts M., Jorissen M., Huyse T., Barson M., . . . Decru E., . . . Snoeks J., Van Steenberge M., . . . & Vanhove M. (2023). Museum collections, barcoding and mitogenomics to detect co-introduction, transmission, and magnifying glass potential of invasive Nile tilapia parasites in African freshwater systems. In *Seventh International Conference of the Pan African Fish and Fisheries Association (PAFFA)*. Brazzaville, Republic of the Congo.

Kasanganki A. & Snoeks J. (2023). Description of a new species of catfish (family Amphiliidae) from the Lake Edward Basin, Uganda. *Seventh International Conference of the Pan African Fish and Fisheries Association (PAFFA),* 18-23/09/2023, University of Marien Ngouabi, Brazzaville (Republic of the Congo).

Maetens H., Boom A., Decru E., Van Steenberge M. & Snoeks J. (2023). *Enteromius*, a new model organism to study speciation in riverine fishes. *21st FishBase/SeaLifeBase Symposium*, 04/09/2023, Royal Museum for Central Africa, Tervuren, Belgium.

Maetens H., Decru E., Van Steenberge M., Boom A. & Snoeks J. (2023). Exploring the success of *Enteromius*: insights from the Lake Edward system. *Seventh International Conference of the Pan African Fish and Fisheries Association (PAFFA),* 18-23/09/2023, University of Marien Ngouabi, Brazzaville (Republic of the Congo).

Maetens H., Decru E., Van Steenberge M. & Snoeks J. (2025). Diving into diversity: the species richness of the sawfin barbs from the Lake Edward system. *Research Day 2025 Biology Department, KU Leuven*, 10/01/2025, KU Leuven, Leuven (Belgium).

Maetens H., Decru E., Van Steenberge M., Boom A. & Snoeks J. (2025). Se plonger dans la diversité : la richesse en espèces des barbeaux à nageoires sciées du système du lac Edouard. *9e Rencontres de l'Ichtyologie en France*, 24-28/03/2025, FIAP Jean Monnet, Paris (France).

Maetens H., Decru E., Van Steenberge M., Boom A. & Snoeks J. (2025). Fish without borders: unravelling hidden diversity in sawfin barbs from the Lake Edward system. *23rd FishBase and SeaLifeBase Symposium*, 2-3/09/2023, Royal Belgian Institute of Natural Sciences, Brussels (Belgium).

Mushagalusa Mulega A., Vanhove M., Van Steenberge M. Morphological and phylogenetic analyses of species of *Bagrobdella* (Monogenea: Dactylogyridae) parasitising African auchenoglanidid catfishes combined with barcoding of *Auchenoglanis occidentalis* provide insight into host taxonomy. *23rd FishBase/SeaLifeBase Symposium*, 02-03/09/2025, Royal Belgian Institute of Natural Sciences, Brussels, Belgium.

Musschoot T., Boden G. & Snoeks J. (2023). AfriBasins: a framework to analyse African freshwater fish distributions. *Seventh International Conference of the Pan African Fish and Fisheries Association (PAFFA),* 18-23/09/2023, University of Marien Ngouabi, Brazzaville (Republic of the Congo).

Musinguzi L., Kamya A., Nsega M., Natugonza V., Okello W., Van Steenberge M. & Snoeks, J. (2023). A reappraisal of the growth parameters and status of exploited fish stocks in lakes Edward and George, East Africa *Seventh International Conference of the Pan African Fish and Fisheries Association (PAFFA),* 18-23/09/2023, University of Marien Ngouabi, Brazzaville (Republic of the Congo).

Musinguzi L., Natugonza V. (2025). The integration of FishBase into decision support tools for the conservation of fishes of Uganda. *23rd FishBase/SeaLifeBase Symposium*, 02-03/09/2025, Royal Belgian Institute of Natural Sciences, Brussels, Belgium.

Nzigire Rutakaza E., Cocquyt C., Bazaire H., Van Steenberge M. (2025). Resource partitioning and dietary shifts in *Oreochromis niloticus* and *Oreochromis leucostictus* in Lake Edward and Lake George

(East Africa). *23rd FishBase/SeaLifeBase Symposium*, 02-03/09/2025, Royal Belgian Institute of Natural Sciences, Brussels, Belgium.

Rutakaza Nzigire E., Van Steenberge M., Bazaire H., Cocquyt C. (2025). Observations *d'Afrocymbella* Krammer (Cymbellales, Bacillariophyta) dans le lac Edward, le lac George et le canal de Kazinga, en Afrique de l'Est. *43rd conférence of the Association des Diatomistes de Langue Française (ADLaF),* 09-11-09-2025, Rouan,France.

Oben L., Van Steenberge M., Vanhove MPM., Gobbin T. Darwin's nightmare revisited: a dual decline of cichlids and their parasites. *23rd FishBase/SeaLifeBase Symposium*, 02-03/09/2025, Royal Belgian Institute of Natural Sciences, Brussels, Belgium.

Snoeks J. (2022). Fish diversity studies in Central and Eastern Africa: from morphology to genomics. *20th FishBase–SeaLifeBase Symposium*. 5-6/09/2022, Worldfish, Penang, Malaysia.

Snoeks J. (2023). African freshwater fish diversity and human utilisation: does anybody know what is happening?. In *Seventh International Conference of the Pan African Fish and Fisheries Association (PAFFA)*. Brazzaville, Republic of the Congo.

Snoeks J. (2024). African fish diversity and inland fisheries: the quiet crisis. *Fishing in Changing Ecosystems, 22nd Annual FishBase Symposium*, 2-3/09/2024, University of Thessaloniki, Greece.
Snoeks J. (2025). Études sur la diversité des poissons en Afrique centrale et orientale : de la morphologie à la génomique. *9e Rencontres de l'Ichtyologie en France*, 24-28/03/2025, FIAP Jean Monnet, Paris (France).

Thys K., Vanhove M., Kmentova N & Van Steenberge M. (2023). Rethinking the Nile Perch Species Complex: Diversification and Phenotypic Plasticity in the most widespread fisheries keystone species in African Freshwaters. *Seventh International Conference of the Pan African Fish and Fisheries Association (PAFFA),* 18-23/09/2023, University of Marien Ngouabi, Brazzaville (Republic of the Congo).

Van Steenberge M. (2025). Darwin's hofvijvers herbekeken: ichthyologisch onderzoek om veranderingen in Oost- Afrikaanse Grote meren te ontraferelen. 27 05 2025. Uitgenodigde presentatie bij zitting van de Konkinklijke Academie voor Overzeese Wetenschappen.

Van Steenberge M., Geerts M., Kelly T., Maetens H., Maebe T., Mulega Mushagalusa M., . . . & Snoeks J. (2023). Contrasting biogeographic patterns of two wide-spread Congolese fish species. *Seventh International Conference of the Pan African Fish and Fisheries Association (PAFFA),* 18-23/09/2023, University of Marien Ngouabi, Brazzaville (Republic of the Congo).

Van Steenberge M., Gobbin T., Vranken N., Decru E., Rutakaza E., Thys K., Maetens H., Cocquyt C., Kmentová N., Vanhove M., Musinguz L., & Snoeks J. (2024). The changing ecosystem of East Africa's Mare Nostrum: Using ichthyology collections to identify the changes in the Lake Victoria region. *Fishing in Changing Ecosystems, 22nd Annual FishBase Symposium*, 2-3/09/2024, University of Thessaloniki, Greece.

Vranken N., Van Steenberge M., Svardal, H. & Snoeks J. (2022). The genomic basis of eco-morphological similarity in the cichlid fishes of the Lake Victoria Region Superflock. *Cichlid Science 2022*. Cambridge, UK, 6–8 September 2022.

Vranken N., Van Steenberge M., Svardal, H. & Snoeks J. (2022). The genomic basis of eco-morphological similarity in the cichlid fishes of the Lake Victoria Region Superflock. ESEB 2022. Prague, Czech Republic, 14–19 August 2022.

Vranken N., Van Steenberge M., Svardal, H. & Snoeks J. (2023). Diversity and striking similarity in the cichlid fishes of the Lake Victoria Region Superflock. Fishbase Symposium 2023. Tervuren, Belgium, 4 September 2023.

Vranken N., Van Steenberge M., Svardal, H. & Snoeks, J. (2023). The origin of eco-morphological similarity in rapidly radiating cichlids. *Seventh International Conference of the Pan African Fish and Fisheries Association (PAFFA),* 18-23/09/2023, University of Marien Ngouabi, Brazzaville (Republic of the Congo).

6. PUBLICATIONS

Project peer-reviewed publications & PhD theses

Vranken, N., Van Steenberge, M., Heylen, A., Decru, E. & Snoeks, J. (2022). From a pair to a dozen: the piscivorous species of *Haplochromis* (Cichlidae) from the Lake Edward system. *EUROPEAN JOURNAL OF TAXONOMY*, 815: 1-94. doi:10.5852/ejt.2022.815.1749.

Decru, E., Vranken, N., Maetens, H., De Vry, A.M., Kayenbergh, A., Snoeks, J. & Van Steenberge, M. (2022). DNA barcoding the Lake Edward basin: high taxonomic coverage of a tropical freshwater ichthyofauna. *HYDROBIOLOGIA, 849: 1743-7962*. doi: 10.1007/s10750-022-04812-0.

Vranken, N., Van Steenberge, M., Mbalassa, M. & Snoeks, J. (2023). Just below the surface, the pelagic haplochromine cichlids from the Lake Edward system. *HYDROBIOLOGIA*, 850 (14): 3173-3195. doi:10.1007/s10750-023-05246-y.

Musinguzi, L. (2024). *Towards Ecosystem-Based Fisheries Management in the Lake Edward System: A Reappraisal of the Fisheries and Aquatic Ecosystem Status*. PhD thesis, KU Leuven: 316 pp.

Vranken, N. (2024). *Patterns of phenotypic similarity in the haplochromine cichlids of the Lake Victoria Region Superflock: an eco-morphological and genomic approach*. PhD thesis, KU Leuven: 426 pp.

Musinguzi, L., Vranken, N., Natugonza, V., Okello, W., van Steenberge, M. & Snoeks, J. (2024). State of knowledge of aquatic ecosystem and fisheries of the Lake Edward System, East Africa. *AQUACULTURE, FISH AND FISHERIES*, 4(1): 1-25. doi:10.1002/aff2.140.

Maetens, H., Decru, E., Boom, A.F., Vranken, N., Van Steenberge, M. & Snoeks, J. (2024). Diving into Diversity: The Complex Evolutionary History and Species Richness of the 'Sawfin barbs' from Lake Edward and Adjacent Systems. *EVOLUTIONARY BIOLOGY*, 51: 366-383. doi:10.1007/s11692-024-09640-9.

Musinguzi, L., Kamya, A., Nsega, M., Natugonza, V., Okello, W., Snoeks, J. & Van Steenberge, M. (2025). Estimates of life-history and growth parameters of exploited fish species in lakes Edward and George: Implications on exploitation status, population dynamics, management, and conservation of native species. *JOURNAL OF FISH BIOLOGY*, 106(2): 201-215. doi:10.1111/jfb.15929.

Maetens, H., Mukungilwa, P., Kasangaki, A., Boom, A.F., Nshimiyumuremyi, T., Munyandamutsa, P.S., Clausnitzer, V., Vranken, N., Snoeks, J. & Van Steenberge, M. (2025). An ichthyological borderland: the fishfauna of Nyungwe National Park and surroundings (Rwanda, East Africa). *JOURNAL OF FISH BIOLOGY:* 1-11. doi: 10.1111/jfb.70185.

Other peer-reviewed publications linked to the project by team members

Diedericks, G., Maetens, H., Van Steenberge, M. & Snoeks, J. (2021). Testing for hybridization between Nile tilapia (*Oreochromis niloticus*) and blue spotted tilapia (*Oreochromis leucostictus*) in the Lake Edward system. JOURNAL OF GREAT LAKES RESEARCH 47 (5): 1446-1452. doi:10.1016/j.jglr.2021.06.005.

Musinguzi, L., Bassa, S., Natugonza, V., Van Steenberge, M., Okello, W., Snoeks, J., & Froese, R. (2021). Assessment of exploited fish species in the Lake Edward System, East Africa. *Journal of Applied Ichthyology*, *37*(2), 216-226.

Musinguzi, L., Kamya, A., Nsega, M., & Okello, W. (2022). Common water hyacinth (Pontederia crassipes) invades Lakes Edward, George, and Kazinga Channel: A call for immediate action. *Journal of Great Lakes Research*, *48*(6), 1723-1727.

Thys, K.J.M., Vanhove, M.P.M., Custers, J.W.J., Vranken, N., Van Steenberge, M. & Kmentová, N. (2022). Co-introduction of *Dolicirroplectanum lacustre*, a monogenean gill parasite of the invasive Nile perch *Lates niloticus*: intraspecific diversification and mitonuclear discordance in native versus introduced areas. *International Journal for Parasitology* 52: 775–786. Doi: 10.1016/j.ijpara.2022.09.001.

Geraerts, M., Vangestel, C., Artois, T., . . . Snoeks, J., . . . Van Steenberge, M., . . . Vanhove, M. & Huyse, T. (2022). Population genomics of introduced Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) in the Democratic Republic of the Congo: Repeated introductions since colonial times with multiple sources. *MOLECULAR ECOLOGY*, 31 (12): 3304-3322. doi: 10.1111/mec.16479.

Musschoot, T., Boden, G. & Snoeks J. (2023). AfriBasins: a new framework in FishBase for the analysis of African fresh and brackish water fish distributions, with a discussion on the Congo basin fauna. *CYBIUM,* 47(3): 287-308. doi: 10.26028/cybium/2023-024.

Kisekelwa, T., Alimasi, W., Mazambi, L., Mwaijengo, G. N., Hyangya, L., Muzungu, H., Joyeuse, M., Lubala, A., Kubota, M., Wüest, A., Muvundja, A., & Masilya, M. (2023). Fish diversity in relation to littoral habitats in three basins of Lake Kivu (East Africa). *Diversity*, *15* (9), 1014, 23 pp.. doi: 10.3390/d15091014.

Nakiyende, H., Basooma, A., Ikwaput Nyeko, J., . . . Okello, W., . . . Van Steenberge, M., . . . & Migeni, Z.A. (2023). Limitations for informed decision making and better management of the transboundary Lake Albert fisheries resources. *JOURNAL OF GREAT LAKES RESEARCH*, 49 (6): 1-15. doi:10.1016/j.jglr.2023.02.006.

Plisnier P., Kayanda R., MacIntyre S., Obiero K., Okello W., Vodacek A., Cocquyt C., Abegaz H., Achieng A., Akonkwa B., Albrecht C., Balagizi C., Barasa J., Bashonga R. A., Bashonga Bishobibiri A.,

Bootsma H., Borges A. V., Gavula G., Dadi T., De Keyzer E. L., Doran P. J., Gabagambi N., Gatare R., Gemmell A., Getahun A., Haambiya L. H., Higgins S. N., Hyangya B. L., Irvine K., Isumbisho M., Jonasse C., Katongo C., Katsev S., Keyombe J., Kimirei I., Kisekelwa T., Kishe M., Koding S. O., Kolding J., Kraemer B. M., Limbu P., Lomodei E., Mahongo S. B., Malala J., Mbabazi S., Masilya P. M., McCandless M., Medard M., Migeni Ajode Z., Mrosso H. D., Mudakikwa E. R., Mulimbwa N., Mushagalusa D., Muvundja F. A., Nankabirwa A., Nahimana D., Ngatunga B. P., Ngochera M., Nicholson S., Nshombo M., Ntakimazi G., Nyamweya C., Ikwaput Nyeko J., Olago D., Olbamo T., O'Reilly C. M., Pasche N., Phiri H., Raasakka N., Salyani A., Sibomana C., Silsbe G. M., Smith S., Sterner R. W., Thiery W., Tuyisenge J., Van der Knaap M., Van Steenberge M., van Zwieten P. A., Verheyen E., Wakjira M., Walakira J., Ndeo Wembo O. & Lawrence T. (2023). Need for harmonized long-term multi-lake monitoring of African Great Lakes. *Journal of Great Lakes Research*, 49:101988.

Sayer, C.A., Fernando, E., Jimenez, R.R., Macfarlane, N.B.W., Rapacciuolo, G., Bohm, M., . . . Snoeks, J., . . . & Darwall, W.R.T. (2025). One-quarter of freshwater fauna threatened with extinction. *NATURE*, 638: 138-145. doi:10.1038/s41586-024-08375-z

Mushagalusa Mulega, A.M., Boom, A.F., Rahmouni, I., Bazairi, H., Benhoussa, A., Vanhove, M. P. M. & Van Steenberge, M. (2025). Catfishes from the north-western part of Lake Tanganyika: contribution to a reference library of DNA Barcodes. *Diversity*, 17(1): 8.

Other publications

Stalmans, V. 2022. The detritivorous and phytoplanktivorous *Haplochromis* species of the Lake Edward system: a morphometric revision. MSc thesis, KU Leuven, 47 pp.

Maebe, T. 2022. Morphometric variation in *Clarias gariepinus*. A traditional and geometric morphometric approach. MSc thesis, KU Leuven, 69 pp.

Matté, A. 2023. Eco-morphological divergence in the species of *Enteromius* Cope, 1867 (Cyprinidae) from the Lake Edward basin (East Africa). A geometric morphometric approach. MSc thesis, KU Leuven, 64 pp.

Antolini, P. 2023. Computational estimation of genome size and its evolution in relation to body size in the fast adaptive radiation of African cichlids. MSc thesis, Università degli Studi di Padova, 56 pp.

Bosmans, D. 2023. Cryptic species diversity in *Enteromius* (Cyprinidae) from West Africa. A morphometric and molecular approach. MSc thesis, KU Leuven, 65 pp.

Eyckens, B. 2025. Bepalen van de stockstructuur voor *Clarias gariepinus* en *Bagrus docmak* in het Edward-Georgesysteem. MSc thesis, KU Leuven, 50pp.

Thuwis, S. 2025. Unravelling the cryptic diversity of *Enteromius* (Cyprinidae) in Eastern Africa. A geometric morphometric and genetic approach. MSc thesis, KU Leuven, 58 pp.

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