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TRACING FISH INTRODUCTIONS AND LATERAL PARASITE TRANSFER TO INDIGENOUS AQUATIC FAUNA

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Microscopic photo of the gill parasite *Cichlidogyrus dokoui* isolated from the tilapia *Coptodon rendalii* from the RMCA collection (Photo: Michiel Jorissen).

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SUMMARY

Context

The Nile Tilapia, *Oreochromis niloticus*, is the most commonly farmed tilapia species in the world. Native to Africa, this highly invasive species became established worldwide. With a yearly production of 20 million tonnes this species forms an essential part of food security. It is however a highly invasive species, being a strong competitor and fast grower. Its introduction resulted in the decline of endemics in e.g. Lakes Victoria and it by hybridizing with autochtonous cichlid species it threatens the genetic integrity of native fish resources. Fish introductions can also lead to the introduction of new parasite species that can threaten local fish species. This co-introduction is often overlooked despite the fact that it can strongly impact on the local fauna. Some parasite species can easily switch hosts and by doing so causing new epidemics. Introduction of a Monogenean *Gyrodactylus* parasite with live fish led to the decimation of the European salmon population in Norway, both in nature and in aquaculture. Other parasites are more host-specific and will not switch hosts after introduction. They can be used a biological tag of their host. Parasitic organisms usually have a much higher mutation rate than their hosts because of their shorter generation time. By genetically characterizing the parasite population more information can be obtained on the migration history of the host it was isolated from. This is the so-called 'magnifying glass principle'. Nile tilapia has also been introduced in the DR Congo. It started in the colonial times and was followed by natural and human-assisted dispersal throughout the Congo Basin. These fish introductions are not well documented and the consequences for the local fauna are unknown.

Objectives

Here we want to study the stocking history of the Nile Tilapia in the DRC by means of their monogenean parasites. More specifically we want to 1) test whether the introduction of nile tilapia in the Congo basin has led to the introduction of new parasite species and 2) whether parasite genetics can help to decipher the complex stocking history.

Conclusions

This study took an innovative approach by using an underexploited source of information (parasites) to tackle a worldwide problem (study impact and stocking history of invasive fish species). Using museum collections to reconstruct historical parasite faunas was the first important novel benchmark of the project. In this way, we refuted most potential parasite transmission scenarios, as most parasites shared by Nile tilapia and native tilapias appeared to already occur in the respective part of the Congo Basin prior to Nile tilapia introduction. Nevertheless, co-introduction of the parasite *Cichlidogyrus tilapiae* into the Lower Congo Basin, and transmission to the local cichlid *Coptodon tholloni* was observed. Secondly, a geographically well-spread dataset of Nile tilapia parasite barcodes is being developed based on the material collected within this project. As a third innovative benchmark, high-resolution markers for monogenean parasites of cichlids are developed. Given the recent advantages in next-generation sequencing techniques since the start of this project, we opted for a mitogenomic rather than a microsatellite approach. The project furthermore serves as a basis for future studies on invasive species. The results were published in international peer review journals and presented at national and international conferences.

Keywords

cichlids – Congo Basin – parasites – invasion biology – Monogenea – Nile tilapia

SAMENVATTING

Context

De Nijltilipia (*Oreochromis niloticus* L.) is een van de meest gekweekte vissoorten wereldwijd. Hij is endemisch in bepaalde delen van Afrika maar is ondertussen in meer dan 100 landen succesvol geintroduceerd. Met een jaarlijkse productie van 20 miljoen ton vormt hij een belangrijke component van de wereldwijde voedselzekerheid. De Nijltilapia is echter een sterk invasieve soort die in competitie treedt met lokale vissoorten voor voedsel en habitat. Doordat hij kan hybridiseren met lokale soorten wordt de genetische integriteit van deze soorten bedreigd. Daarenboven kunnen visintroducties ook leiden tot introducties van nieuwe parasieten die de lokale fauna kunnen bedreigen. Deze co-introductie van parasitaire organismen wordt veelal over het hoofd gezien, ook al kunnen de gevolgen voor de lokale fauna groot zijn. Sommige van deze parasieten kunnen namelijk van gastheer wisselen en daardoor lokale soorten infecteren en nieuwe epidemieën veroorzaken. Zo heeft de introductie van de Monogenea-platworm *Gyrodactylus salaris* in Noorwegen geleid tot de decimering van de natuurlijke en gekweekte Europese zalmpopulaties. Andere parasieten soorten zijn zeer gastheer-specifiek, en zullen na introductie niet van gastheer wisselen. Daardoor kunnen ze als een extra biologische merker voor hun gastheer dienen. Parasitaire organismen evolueren namelijk veel sneller dan hun gastheer door hun kortere generatietijd. Daardoor zullen ze in eenzelfde tijdspanne meer genetische mutaties opstapelen dan hun gastheer. Door deze parasieten genetisch te karakteriseren kunnen we meer informatie over de migratiegeschiedenis van de gastheer verkrijgen. Dit wordt het principe van het ‘vergrootglas-effect’ genoemd. Ook in de Democratische Republiek Congo werd de nijltilapia tijdens de koloniale periode op grote schaal ingevoerd voor aquacultuur-doeleinden. Deze introducties zijn echter slecht gedocumenteerd en de gevolgen ervan op de lokale visfauna onbekend.

Doelstellingen

Doel van dit project is om de impact van nijltilapia introducties op de lokale tilapiasoorten te bestuderen. Meer bepaald willen we achterhalen of 1) de introductie van nijltilapia in het Congo-bekken geleid heeft tot de introductie van nieuwe parasietensoorten en 2) of genetische analyses van de parasietengemeenschap inzicht verschaffen in de geschiedenis van nijltilapia introducties.

Besluiten

Het gebruik van museumcollecties om historische parasietenfauna’s te reconstrueren was de eerste belangrijke mijlpaal van dit project. Op deze manier konden we de meeste waarschijnlijke transmissiescenario’s verwerpen, aangezien de parasietsoorten die gedeeld werden tussen Nijltilapia en inheemse cichliden, in de meeste gevallen reeds aanwezig bleken in het respectieve deel van het Congobekken. Wel werd co-introductie van de parasiet *Cichlidogyrus tilapiae*, gevolgd door transmissie naar de inheemse cichlide *Coptodon tholloni* waargenomen in het Beneden-Congobekken. Ten tweede stelden we een geografisch uitgebreide barcoding-databank van Nijltilapiaparasieten samen op basis van specimens verzameld binnen dit project. Als derde innovatieve mijlpaal ontwikkelen we hoge resolutie-genetische merkers voor de Monogenea-parasieten van cichliden. Gezien de recente vorderingen in “next-generation sequencing”-technieken opteerden we voor een mitogenomische aanpak, in plaats van de voorziene aanpak op basis van microsatelliet DNA merkers. Deze studie zal een model zijn voor toekomstige studies omtrent introducties en invasieve soorten. De resultaten zijn reeds voor een deel gepubliceerd in internationale peer-reviewed tijdschriften en werden eveneens meegedeeld op nationale en internationale congressen en bijeenkomsten.

Trefwoorden

cichliden – Congobekken – invasiebiologie – Monogenea – Nijltilapia – parasieten

RESUME

Contexte

Le Tilapia du Nil (*Oreochromis niloticus*) figure parmi les espèces de poissons les plus produites en pisciculture. Il est natif de certaines régions d'Afrique, mais a été introduit à grande échelle dans plus de 100 pays. Avec une production annuelle de 20 millions de tonnes, il constitue une part importante de la sécurité alimentaire mondiale. Cependant, il s'agit d'une espèce fortement invasive qui entre en compétition avec les populations de poissons locales, pour la nourriture et l'habitat. Comme le Tilapia du Nil peut s'hybrider avec des espèces locales, l'intégrité génétique de ces espèces est menacée. De plus, les introductions de poissons peuvent aussi introduire de nouveaux parasites qui peuvent menacer la faune locale. Cette co-introduction d'organismes parasitaires est souvent ignorée, bien que les conséquences pour la faune locale puissent être importantes. En effet, certains de ces parasites peuvent changer d'hôte et, dès lors, infecter des espèces locales, voire causer de nouvelles épidémies. Ainsi, l'introduction du ver plat monogène *Gyrodactylus salaris* en Norvège a mené à la décimation des populations de saumon naturelles et captives. D'autres espèces de parasites sont très spécifiques à leur hôte et semblent ne pas changer d'hôte après être introduites. Par conséquent, ils peuvent servir comme marqueur biologique additionnel pour leur hôte. En effet, les organismes parasitaires évoluent beaucoup plus rapidement que leur hôte, en raison de leur cycle de vie plus court. Ainsi, ils accumuleront, pour une même période de temps, davantage de mutations génétiques que leur hôte. En caractérisant ces parasites de façon génétique, nous pouvons donc acquérir plus d'informations sur l'histoire de migration de l'hôte. Ce principe est connu comme « l'effet-loupe ». Le Tilapia du Nil (*Oreochromis niloticus*) a également été introduit à grande échelle en République démocratique du Congo pour l'aquaculture. Néanmoins, ces introductions sont peu documentées, et leurs conséquences sur l'ichtyofaune locale sont inconnues.

Objectifs

Le but de ce projet est d'améliorer les connaissances sur l'histoire des introductions et leur impact sur les espèces locales de tilapia. Notre objectif est de découvrir si l'introduction du Tilapia du Nil dans le bassin du Congo a mené à l'introduction de nouvelles espèces parasitaires en République démocratique du Congo.

Conclusions

Dans cette étude nous avons utilisé une approche novatrice en exploitant une source négligée d'information (des parasites) afin d'adresser une problématique globale (l'analyse de l'impact et de l'histoire du peuplement d'espèces invasives de poisson). Utiliser des collections de musée afin de reconstruire des faunes parasitaires à travers l'histoire a été le premier accomplissement novateur du projet. De cette façon, nous avons pu réfuter la plupart des scénarios potentiels de transmission de parasites, étant donné que la majorité des parasites partagés par le tilapia du Nil et les tilapias locaux étaient déjà présents dans les différentes parties du Bassin du Congo avant l'introduction du tilapia du Nil. Cependant, la co-introduction du parasite *Cichlidogyrus tilapiae* dans la Bas Congo, et sa transmission au cichlide local *Coptodon tholloni* a été observée. Deuxièmement, une base de donnée comprenant les codes-barres des parasites du tilapia du Nil sur une large aire géographique est en cours de développement sur base du matériel collecté à travers ce projet. Comme troisième accomplissement novateur, nous développons des marqueurs haute-résolution ciblant les parasites monogènes des cichlides. Au vu des avantages offerts par les technologies de séquençage de nouvelle génération (Next Generation Sequencing), nous avons décidé d'opter pour une approche basée sur la mitogénomique plutôt que sur des microsatellites. Le projet sert par ailleurs de référence à d'autres études traitant d'espèces invasives. Les résultats ont été publiés dans des journaux internationaux révisés par des pairs, et présentés au cours de conférences nationales et internationales.

Mots-clés
bassin du Congo – biologie de l'invasion – cichlidés – monogènes – tilapia du Nil – parasites

1. INTRODUCTION

Aquaculture and species introductions

Aquaculture is the fastest growing animal-food producing sector worldwide (Naylor *et al.* 2001), with fish representing a major source of food and livelihood in the Global South (Casal 2006). Despite the crucial role of aquaculture in food production, there are important repercussions. A side effect of aquaculture and restocking is the introduction of alien species. Apart from intentional stocking for capture fisheries, a variety of mechanisms can lead to unintentional introductions, e.g. escapes from floating cages, aquaculture ponds and flooding of paddies for integrated rice-fish culture (Canonico *et al.* 2005). While many alien species cannot establish, certain species may persist and become invasive species that pose major environmental and economic problems (Pimentel *et al*. 2000). The introduction of invasive species is regarded the second leading cause of species extinction and endangerment worldwide, following habitat destruction, and is listed first among the threats to freshwater fishes (Cowx 2002; Snoeks *et al.* 2011). They often have negative impacts on the native fauna as they compete for food and habitat, but they can also introduce alien parasites, with sometimes devastating consequences. Despite their potential impact, studies on introduced parasite species are scarce.

Due to increased globalization, the major threat of anthropogenic introductions to African freshwater biodiversity is expected to rise (Dudgeon *et al.* 2011), as for freshwater ecosystems in general (Strayer 2010). Concomitant pathogen introduction is therefore an ever-more important aspect and needs to be carefully monitored (Hecht & Endemann 1998). Nevertheless, studies measuring the impact of invasive species are hampered by taxonomic difficulties because of the cryptic status of many introduced species (Carlton 1996).

Tilapias

After the sharptooth catfish *Clarias gariepinus*, tilapias are the second most important aquaculture fish in sub-Saharan Africa (FAO 2012). Tilapias are affordable, relatively undemanding in terms of food source and environment (oxygen level, salinity…), fast-growing and have a high reproductive rate. Therefore they are nicknamed “aquatic chickens”. “Tilapia” traditionally refers to a non-monophyletic group within the cichlid fish family, the most well-known genera being *Tilapia*, *Oreochromis* and *Sarotherodon* (Thys van den Audenaerde 1964; Trewavas 1983; Schwarzer *et al.* 2009). Although representatives of the three main genera are used, the most important species to aquaculture are *Oreochromis niloticus*, *O. mossambicus*, *O. aureus* and certain of their hybrids, accounting for 99.5 % of worldwide tilapia production (FAO 2012). While tilapias originate from Africa and the Middle East, they have been anthropogenically introduced worldwide since 1923 (Trewavas 1983), for biological control of aquatic weeds and insects, as baitfish, ornamental fish and food fish. They are the most widely distributed exotic fishes worldwide, as they have settled in about every ecosystem where they are raised or have been introduced (Courtenay 1997; Costa-Pierce 2003; Canonico *et al.* 2005).

The issue of Nile tilapia in the Democratic Republic of Congo (DRC)

With more than 60% of its population lacking access to adequate food resources (WFP 2013) and the country hosting a major share of undernourished people worldwide (FAO 2012), inland fisheries and aquaculture hold an important potential for food security in the DRC. The most important aquaculture species in the DRC is the Nile tilapia *Oreochromis niloticus*. It is also a highly invasive fish species, being a strong competitor and fast grower. Also, *Oreochromis niloticus* can hybridize with autochtonous cichlid species (Trewavas 1983; Nyingi & Agnèse 2007) and, in experimental conditions, even with tilapias belonging to other genera (Bezault *et al.* 2012), threatening genetic integrity of native fish stocks (van der Waal & Bills 2000; Canonico *et al.* 2005).

The species’ original distribution includes the Nile Basin and West Africa, roughly from Senegal to Egypt and south to Lake Tanganyika (Thys van den Audenaerde 1964; Trewavas 1983). Although it is native to certain parts of the DRC (basins of Lakes Albert, Edward, Kivu and Tanganyika), it has also

been widely introduced elsewhere in the country. Other tilapias introduced in many areas in the Congo Basin and beyond are *O. macrochir* and *Coptodon rendalli*, which are native to the ex-Katanga province but constitute another important source for tilapia aquaculture throughout the DRC. Moreover, many introductions remained undocumented (Thys van den Audenaerde 1988; Welcomme 1988). These many and poorly inventoried introductions, starting from colonial times and still ongoing, render monitoring tilapia introductions in the DRC an important task. The historical Congo freshwater fish collections of the Royal Museum for Central Africa (RMCA) are a rich source of information to fill the existing data gap.
In view of the well-documented negative effects that *O. niloticus* introduction can have on native fish species, it is paramount to identify and trace stocking events. Molecular tools can help to discriminate fish populations and strains, although the phylogeographic signal can be weak in case of recent colonization or large population sizes (Hauser & Carvalho 2008). In case of Nile tilapia, microsatellite analyses demonstrated only limited genetic differentiation on a local scale within hydrographic basins (Bezault *et al.* 2011). Therefore, additional sources are needed to increase the resolution. One source of information is provided by the parasites they carry. Every species on earth is parasitized by a unique set of parasite species, but their potential in biodiversity research or stock assignment remains underexplored.

Parasites as tools
The arguments behind a parasitological approach are:
(1) Parasites are very common; each host species is associated with at least one parasite species, and most parasites are host-specific to a certain extent; hence it easily follows that parasitism globally is the most common lifestyle in terms of species numbers(Price 1980; Windsor 1998). Therefore, parasites are important players in the ecosystem (Marcogliese 2004).
(2) Parasites live in intimate association with their host, an association that is often highly specific (Nieberding & Olivieri 2007).
(3) Because of the dependence on intermediate host species and/or the trophic transmission of certain parasite species, presence or absence of parasites can inform about diet and migration of their host: parasites may act as biological tags (MacKenzie 2002).
(4) Because of their usually short generation time and higher mutation rate compared to the host, parasites accumulate mutations much faster, providing a ‘magnifying glass’ to study their host’s evolutionary history (Nieberding & Olivieri 2007).

The choice of parasites is however very important. They are preferably host specific, have limited dispersal capability and have a fast generation time (Nieberding & Olivieri 2007). A group of choice are therefore parasites with a direct life cycle (i.e. no intermediate hosts), such as monogenean flatworms. We therefore focus on gyrodactylid and ancyrocephalid monogeneans.

Parasites as pests: introduction of non-native pathogens
In addition to the scientific advances of the direct (one host) lifecycle of monogeneans in using them as parasitological tools for tracing their introduced hosts’ origins, this lifecycle also makes them potentially harmful in the context of host introduction. Indeed, parasites lacking intermediate hosts stand a higher chance of being anthropogenically introduced with their hosts, in comparison to parasites whose more complex lifecycle requires multiple hosts (Bauer 1991). Despite ample proof of devastating effects, the introduction of diseases with non-native species is poorly studied in freshwater invasion ecology (Strayer 2010), particularly in Africa (Hecht & Endemann 1998).

In this project, we explored the potential of a parasitological approach to elucidate the origins of introduced Nile tilapia, and the risk of disease introduction in DRC tilapia stocks. As such, parasites will be approached both as a tool in fisheries biology and for the assessment of risks to fish stock conservation. Questions addressed were:

1) Can the parasite fauna of introduced Nile tilapia strains provide information about population identity and sources of introduction?
2) Was the introduction of Nile tilapia accompanied by the introduction of alien parasite species, and did these parasites manage to infect the autochthonous cichlid fauna ("parasite spillover")? Conversely, did local parasites manage to infect the introduced tilapia stocks? When native parasites infect introduced hosts, these may act as additional reservoirs for infection of native hosts, increasing disease impact on autochthonous fauna ("parasite spillback": Kelly *et al.* 2009). Bi-directional interactions between cultured and native fish species have been reported (Raynard *et al.* 2007) and cultured tilapias have been observed to host non-tilapia parasites acquired locally (Kaneko *et al.* 1988).

This pilot project focused on the Congo Basin in the DRC, serving as a proof-of-principle of the utility of a parasitological approach to fundamental and applied questions in ichthyology.

2. METHODOLOGY AND RESULTS

Work package (WP) 1: parasite community composition and detecting parasite introduction

Field expeditions to the three respective parts of the Congo Basin (Lower, Middle & Upper) occurred from 2014 to 2016 in collaboration with local partners (respectively, the Institut Supérieur Pédagogique de Mbanza-Ngungu with the team of Prof. Dr. Soleil Wamuini Lunkayilakio; the Centre de Surveillance de la Biodiversité at the Université de Kisangani with the team of Prof. Dr. Célestin Danadu Mizani; the Université de Lubumbashi with the team of Prof. Dr. Ir. Auguste Chocha Manda). This fieldwork was co-financed by the University Development Cooperation of the Flemish Interuniversity Council (VLIR-UOS) (South Initiative *Renforcement des capacités locales pour une meilleure évaluation biologique des impacts miniers au Katanga (RDC) sur les poissons et leurs milieux aquatiques*, ZRDC2014MP084), by travel grant K220314 N from the Research Foundation – Flanders (FWO-Vlaanderen), by OCA type II project S1_RDC_TILAPIA (*Un atelier ichtyo(parasito)logique au Bas-Congo en appui à un réseau centrafricain en ichtyoparasitologie et pour le renforcement de la collaboration scientifique au sein de la R.D.Congo*) and the Mbisa Congo project, which are both projects under the framework agreement of the RMCA with the Belgian Development Cooperation.

At fish markets fresh fish were bought from the target species *Oreochromis niloticus*. Additionally, local fish farms or ponds with Nile tilapia culture were sampled. Furthermore, in areas where Nile tilapia is established (escapees from fish farms), fishes were caught with gill nets, with special attention to regions where these escapees co-occur with native tilapias or other cichlids. Indeed, local cichlids (e.g. *Coptodon rendalli*, *Tilapia sparrmanii*, *Hemichromis elongatus*, *Hemichromis stellifer*, *Oreochromis mweruensis*, *Coptodon tholloni*,…) were sampled and contributed to tracing a possible spillover effect of parasites from *O. niloticus* to native species.

We opted to first study the native cichlid hosts from our recent field expeditions and subsequently publish a paper on each region, serving as a baseline for the hitherto unstudied monogenean fauna of these regions' cichlids. Such a baseline is necessary to evaluate whether alien parasite species have been introduced together with Nile tilapia. In the meantime the parasite fauna of the introduced *O. niloticus* in the three areas has been studied as well and will be bundled in an additional paper so we can study differences between regions and possibly find evidence for the enemy-release hypothesis, which proposes that the loss of part of their parasite species after introduction contributes to the success of invasive species. By studying both the native and introduced hosts on recent field expeditions we can identify which host species are infected by parasite species that occur on *O. niloticus* as well. On this basis, we sampled historical batches of those native hosts from the museum collections from the same (or nearby) localities, collected before Nile tilapia was present in the area. This allows us to verify if these parasites have effectively been co-introduced into the region and subsequently have spilled over or not. From the museum collections, the native cichlid species *C. rendalli* (18 specimens), *T. sparrmanii* (23), *O. mweruensis* (12) (Upper Congo), *Coptodon tholloni* (12) and *Hemichromis stellifer* (9) (Lower Congo) have been sampled.

A manuscript regarding the parasite fauna of native cichlids in the Upper Congo region, more specifically the Mweru-Luapula sub region has been published in *Journal of Helminthology* (Jorissen et al. 2018) (Fig. 1). In this manuscript we have assessed the present-day parasite fauna of 12 species of native cichlids (e.g. *Coptodon rendalli*, *Tilapia sparrmanii*, *Pseudocrenilabrus philander*, *Serranochromis macrocephalus*,…). For eight of these, this represents the first information on their gill parasites: *Oreochromis mweruensis*, *Orthochromis katumbii*, *Sargochromis mellandi*, *Serranochromis thumbergi*, *Serranochromis stappersi*, *Serranochromis angusticeps* and *Tylochromis mylodon*. Additionally, we described one species new to science, *Cichlidogyrus consobrini* (See Fig. 2) on *Sargochromis mellandi* and *Orthochromis katumbii* and characterized a putative endemic morphotype of *Cichlidogyrus halli* on *Oreochromis mweruensis*. This morphotype differed from the specimens of *C. halli* found on *O. niloticus* in the morphology of its dorsal anchors. The specimens of the typical *C. halli* occurred on both *O. niloticus* and *O. mweruensis*, but the new morphotype only occurred when *O. niloticus* was not present in the area.

At the species level, *C. halli*, *Cichlidogyrus tilapiae*, *Cichlidogyrus cirratus* and *Cichlidogyrus sclerosus* were shared between native *O. mweruensis* and introduced *O. niloticus* in our Upper Congo Basin samples. However, all four species also occurred on historical specimens of *O. mweruensis* collected prior to the introduction of *O. niloticus* in the region, refuting the possibility that these species are non-native to the region and stem from co-introduction with *O. niloticus*.

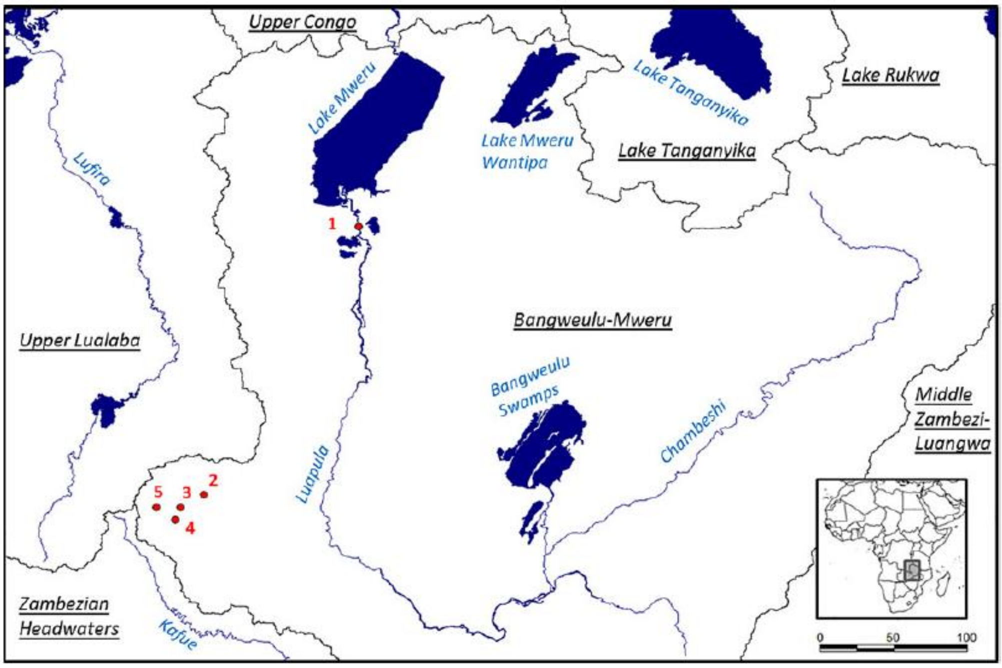

Fig. 1. Map of Bangweulu-Mweru and neighbouring ecoregions in underlined font. Rivers and water bodies in blue font. Sampling localities in red. The inset shows the location of Bangweulu-Mweru on the African continent. Sampling localities: 1, Luapula River off Kashobwe; 2, Futuka

Farm; 3, Bumaki Farm; 4, Lubumbashi Zoo; and 5, Kipopo. Scale in kilometres. From Jorissen et al. (2018).

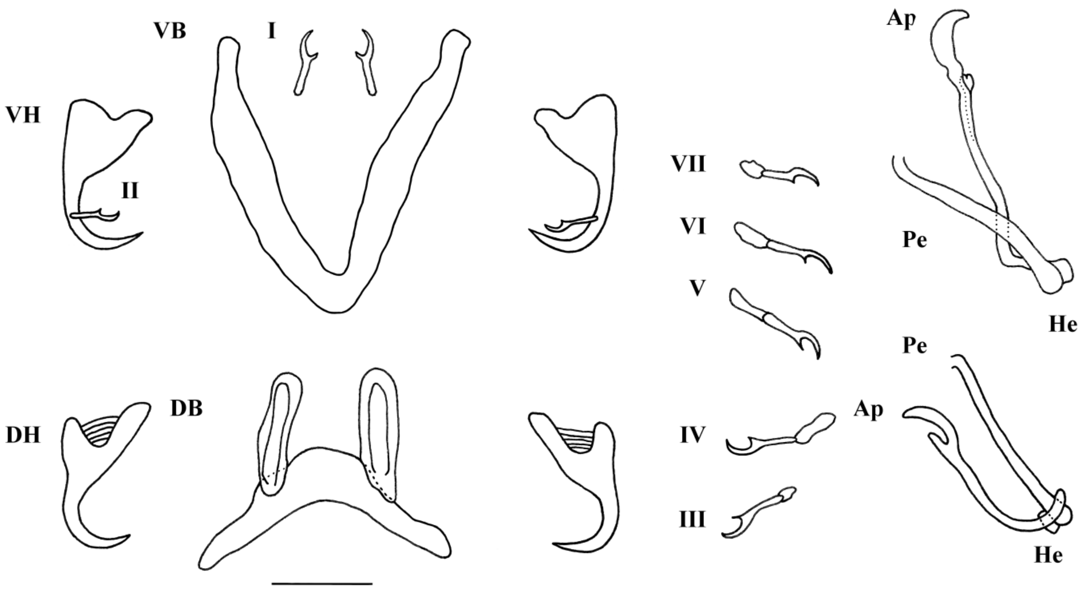

Fig. 2. Haptoral and genital sclerotized elements of Cichlidogyrus consobrini*. MCO (top right and bottom) depicted from two different specimens from two different angles. Abbreviations: I-VII (hooklets pair I-VII, pair III-VII only one of both drawn), Ap (accessory piece), DB (dorsal transverse bar), DH (dorsal hook), He (heel), Pe (penis), VB (ventral transverse bar) and VH (ventral hooks). Scale bar: 20 µm. From Jorissen et al. (2018).*

The samples from the Lower Congo expedition have been completely analyzed (Fig. 3). 382 parasites were collected from 66 host specimens. A manuscript concerning the parasite fauna of the native hosts (*Coptodon tholloni*, *Hemichromis stellifer*, *H. elongatus* and *Tylochromis praecox*) and the introduced *Coptodon rendalli* has been prepared and has been resubmitted to *Parasite*. In this manuscript, six new species of parasites were described. Additionally, possible parasite host-switches were discussed. We discovered that *C. rendalli* was infected by *Cichlidogyrus flexicolpos*, *C. cubitus* and *C. berradae*, which possibly translocated from *C. tholloni* to *C. rendalli* through a spillback effect. Furthermore, one individual of *C. tholloni* was infected by *Cichlidogyrus tilapiae*, which was also found on *O. niloticus* from the same locality. *Cichlidogyrus tilapiae* is known to infect *O. niloticus* in other African regions. It is therefore likely that the infection of *C. tilapiae* on *C. congica* is a result of parasite spillover.

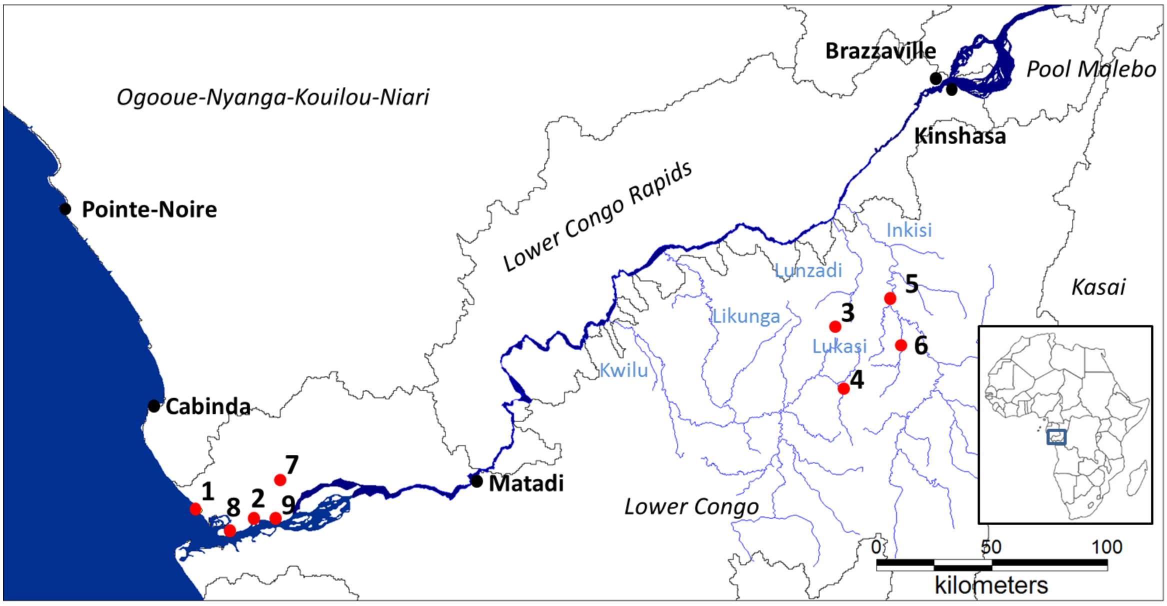

Fig. 3. Overview of sampling localities (red dots) in the Lower Congo Basin, with the ecoregions in italics. Localities 3 and 5 are located within the cities Mbanza-Ngungu and Kisantu respectively. The inset shows the Lower Congo region on the African continent. Sampling localities: 1, Tondé Estuary near Muanda; 2, Muila Kaku, mangroves near Lower Congo River; 3, Ndimba Leta ponds, Mbanza-Ngungu, in between the Lunzadi and Lukasi rivers of which the latter flows into the Kwilu and Likunga; 4, Pond near Kila Kindinga and Lukasi river; 5, Inkisi River, off le jardin botanique de Kisantu; 6, Mvuazi River, Inkisi; 7 Mbola River near Tshianya village; 8 Congo River near Nganda Flash station, 9 Muila Nzenze, Kibamba village, mangroves near Congo River. Rivers in blue. Scale in kilometres.

From the Middle Congo Basin, due to the relatively low prevalence of cichlid parasites, material from several expeditions was combined, including the 2010 Boyekoli Ebale Congo expedition, a dedicated sampling in June 2014, and specimens retrieved during other RMCA expeditions. This led to the discovery of seven new parasite species. Descriptions and analyses, including sequence data from six regions (28S, 18S and 5.8S rDNA genes, ITS-1 and ITS-2, and the “barcoding gene” mitochondrial cytochrome *c* oxidase subunit one) are ongoing.

In addition to our work on the taxonomy and faunistics of cichlid-infecting monogeneans of the Congo Basin, our team also contributed two papers on (the methodology for) scanning electron microscopy as a diagnostic tool for this fauna (Fannes *et al.* 2015, 2017).

Nile Tilapia in the Congo Basin

So far over 100 specimens of Nile tilapia underwent parasitological examination, but more will follow. All specimens from the Lower Congo expedition have been processed. From Upper Congo around 70% of the samples are processed.

WP2: tracing introduced Nile tilapia by means of fish genetics

By 2017, 120 DNA samples of Nile tilapia from a range of countries were collected (including China, Israel, Jordan, Zimbabwe, Cameroon, Madagascar, Ethiopia, Uganda, Sudan, Benin) through our international network. These samples are an addition to the collected finclips from the three major TILAPIA expeditions (Upper, Middle and Lower Congo respectively) and the material available from the Royal Museum for Central Africa. In total we now have genetic material from 5 out of 7 subspecies of *O. niloticus* and material from 37 populations from 13 countries. This constitutes native wild *O. niloticus* and farmed, escaped or introduced specimens. With regards to our initial proposal we have upgraded and updated our analysis to a larger geographical scale and more recent techniques. We have chosen to analyze the finclips of *O. niloticus* with RAD-sequencing. This is a novel, cost-efficient method for population genomics. It has already been successfully applied to study demographics and population structure of fisheries target species with relatively shallow population structure. RADseq and other genome reduction methods (i.e. they enzymatically digest the whole genomic DNA to sample consistent portions of it to compare individuals) are gaining popularity among population geneticists. It will allow us to calculate the genetic variability of *O. niloticus* within the DRC. Additionally, this will provide information about hybridization between local tilapias and *O. niloticus*. Lastly, because of our broad sampling it will be possible, at least partially, to reconstruct the introduction routes of *O. niloticus* into the DRC or to identify the native population that is genetically the closest to the introduced populations. Analyses are ongoing within the PhD of Mare Geraerts (see below).

WP3: tracing introduced Nile tilapia by means of parasite genetics

Whilst collecting parasites for morphological study from the gills of fresh fish, several specimens were cut in two. For representatives of Dactylogyridae we used the posterior body part (haptor) for parasite genetics. The anterior half of the animal would then still be prepared on a slide and identified based on the morphology of the MCO and, when sclerotized, the vagina. For representatives of Gyrodactylidae the anterior body part was used for parasite genetics.

Around 300 genetic samples of parasites have been taken of which 286 have been extracted. We opted to study these parasites with 4 markers that have been used effectively in literature in the past: the mitochondrial COI, the nuclear ribosomal 18S and 28S and ITS-1 regions. In these animals the 18S and 28S genes are the least variable, followed by ITS-1 and COI. So, for studies on a population level or between closely related species the latter two will be the most informative. The two other genes function more as verification of our morphological identification, and to phylogenetically situate the newly described or newly sequenced species of cichlid monogeneans. Analyses are ongoing within the PhD of Michiel Jorissen (see below).

Moreover, to reliably compare parasite genetic structure to the genomic host data collected under WP2, molecular markers for monogeneans, with a higher resolution than those currently available, are needed. With the advance of next-generation sequencing methodologies for non-model organisms, we opted to develop these through a mitogenomic approach, rather than developing microsatellites as foreseen in the original project proposal. In collaboration with the Natural History Museum (London, UK) (Dr. D.T.J. Littlewood and Dr. A.G. Briscoe) and with EU SYNTHESYS funding (GB-TAF-4940) the mitochondrial genomes of four African monogeneans, three of which infecting cichlids (*Cichlidogyrus halli*, *Cichlidogyrus mbirizei* and *Gyrodactylus nyanzae*), were assembled starting from Illumina reads. Their characterization (two entire and two partial mitogenomes), annotation and phylogenetic position were published in *BMC Genomics* (Vanhove *et al.* 2018). This will serve as the basis for a mitogenomic approach to the phylogeography and population genetics of the monogenean parasites of African introduced and native tilapias, within the ongoing PhD of Mare Geraerts (see below).

3. DISSEMINATION AND VALORISATION

Publications and scientific conferences:

In total, the results of the TILAPIA project have contributed to 10 international conference presentations and two posters in the DRC, Spain, Austria, Germany, Belgium, Czech Republic, Italy, Malawi and the Netherlands (see under "5. Publications").

Among these, a presentation was given in August 2017 on the 8th International Symposium for Monogenea in Brno, Czech Republic by Michiel Jorissen. This won the award for best student's presentation, which included a publishing voucher for the peer-reviewed open access journal *Parasite*.

In order to draw the attention of the scientific world to the parasitological aspects of tilapia introductions, we devoted a section to it in an invited review in the leading parasitological journal *Trends in Parasitology* (Vanhove *et al.* 2016).

In addition, the results of this project have been presented in six invited lectures, either under the form of research seminars or conference contributions, in Germany, Czech Republic, Ireland and Finland.

Science communication to the wider public:

In fall 2015 Michiel Jorissen and Tine Huyse set-up an exposition for a broad public in the frame of the Science Day at the University of Leuven. The public was introduced to the Nile tilapia, where it occurs, where it was introduced and how their parasites look like and the possible consequences of parasite introductions. They could have a look at a new species of parasite, *Cichlidogyrus consobrini* Jorissen, Pariselle and Vanhove, 2017 and explore its morphology under a light microscope.

Training of students:

Further dissemination and exposure for this project was created by the involvement of several BSc and MSc students in this research: Fidel Muterezi Bukinga (MSc, Université de Lubumbashi with prof. Chocha Manda and dr. Antoine Pariselle, 2016-present: Diversity and parasite ecology of the monogeneans of native *Oreochromis niloticus* and introduced *O. macrochir* in the Nyangara wetland), Michiel Jorissen (MSc 2014-2015, KU Leuven with prof. Filip Volckaert: Testing for parasite host-switching between invasive Nile tilapia (*Oreochromis niloticus*) and native tilapias in the Upper Congo Basin), Wouter Van Sever (BSc, KU Leuven with prof. Jos Snoeks, 2014-2015: Introduced Nile tilapia (*Oreochromis niloticus*) in the Congo Basin: historical reconstruction and the use of parasites (Monogenea) as biogeographical markers) and Thomas Kusters (BSc, Hasselt University with prof. Tom Artois, 2016-2017: Monogenea of Lower Congo).

Organized trainings in the Global South:

As a form of dissemination in the Global South, the results and expertise gathered during the TILAPIA project were incorporated into several capacity building workshops, either combined with sampling campaigns with Congolese partner institutes (VLIR-UOS South Initiative at KU Leuven and Université de Lubumbashi, supervised by prof. Jos Snoeks, Auguste Chocha Manda and Filip Volckaert: *Renforcement des capacités locales pour une meilleure évaluation biologique des impacts miniers au Katanga (RDC) sur les poissons et leurs milieux aquatiques*; OCA type II project at RMCA at ISP Mbanza-Ngungu: *Un atelier ichtyo(parasito)logique au Bas-Congo en appui à un réseau centrafricain en ichtyoparasitologie et pour le renforcement de la collaboration scientifique au sein de la R.D.Congo*) or in a summer school of KU Leuven and Université Mohammed V in Rabat, Morocco, supervised by prof. Ouafae Berrada-Rkhami and Filip Volckaert (VLIR-UOS Short Training Initiative *Building an African network for sustainable management of aquatic biological* *resources supported by genetics and parasitology*). This exposed dozens of young and established African scholars (biologists, agronomists, veterinarians…) to the issues surrounding the parasitology of tilapias. Consequently, our research was expanded to other aspects of tilapia parasitology, such as the use of fish parasites as indicators for pollution, as in the MSc thesis of Gyrhaiss Kapepula Kasembele: "Identification of gill monogeneans as bio-indicators of aquatic mining pollution in Upper Katanga, Lufira River" (Université de Lubumbashi, with Prof. Dr. Ir. Auguste Chocha Manda, Prof. Dr. Jos Snoeks). Recently, this student's poster was awarded the first prize for a senior student poster presentation at the 2018 meeting of the Parasitological Society of Southern Africa (PARSA).

4. PERSPECTIVES

The research under the TILAPIA project has fed into several other research projects that have since started. At Hasselt University, specimens collected within the project were used for the BSc thesis of Charlotte Goeyers (Explorative study of native Monogenea in African lakes), in which serial sections of representatives of *Cichlidogyrus* and *Scutogyrus* were studied in order to update the diagnoses of these genera. On a longer term, and also at Hasselt University, the TILAPIA project led to two PhD projects under the supervision of Prof. Tom Artois: « Tracing parasite transfer between invasive Nile tilapia and native cichlids in Central Africa after anthropogenic introductions » (Michiel Jorissen, BOF reserve fellowship until 2019) and « Reconstructing Nile tilapia stocking in Africa: parasites, next-generation-sequencing and museum collections » (Mare Geraerts, BOF doctoral fund until 2021). These PhDs are follow-up projects ensuring that the research piloted under the TILAPIA pioneer project will be continued and expanded for years to come. Moreover, our experience in the parasitology of tilapias has meanwhile also been applied in other regions (e.g. Madagascar, in collaboration with prof. Andrea Šimková of Masaryk University (Czech Republic) and dr. Jeanne Rasamy (Université d'Antananarivo) ; paper resubmitted).

5. PUBLICATIONS

Peer-reviewed journal publications

Vanhove M.P.M., Briscoe A.G., Jorissen M.W.P., Littlewood D.T.J. & Huyse T. (2018) The first next-generation sequencing approach to the mitochondrial phylogeny of African monogenean parasites (Platyhelminthes: Gyrodactylidae and Dactylogyridae). *BMC Genomics* **19**: 520 *(I.F. 2017: 3.730)*

Jorissen M.W.P., Pariselle A., Huyse T., Vreven E.J., Snoeks J., Volckaert F.A.M., Chocha Manda A., Kapepula Kasembele G., Artois T. & Vanhove M.P.M. (2018) Diversity, endemicity and host-specificity of monogenean gill parasites (Platyhelminthes) of cichlids in the Bangweulu-Mweru ecoregion. *Journal of Helminthology* **92**(4): 417-437 *(I.F. 2017: 1.344; 3 citations)*

Fannes W., Vanhove M.P.M. & Huyse T. (2017) Redescription of *Cichlidogyrus tiberianus* Paperna, 1960 and *C. dossoui* Douëllou, 1993 (Monogenea: Ancyrocephalidae), with special reference to the male copulatory organ. *Systematic Parasitology* **94**(1): 133-144 *(I.F. 2017: 1.273; 2 citations)*

Vanhove M.P.M., Hablützel P.I., Pariselle A., Šimková A., Huyse T. & Raeymaekers J.A.M. (2016) Cichlids: a host of opportunities for evolutionary parasitology. *Trends in Parasitology* **32**(10): 820-832 *(I.F. 2016: 6.333; 23 citations)*

Fannes W., Vanhove M.P.M., Huyse T. & Paladini G. (2015) A scanning electron microscope technique for studying the sclerites of *Cichlidogyrus*. *Parasitology Research* **114**(5): 2031-2034 *(I.F. 2015: 2.027; 12 citations)*

Invited lectures

Vanhove M.P.M. From parasitology to policy: stories of hidden biodiversity, overlooked introductions and capacity building. Department of Environmental and Biological Sciences, University of Eastern Finland, March 8th, 2018, Joensuu, Finland

Vanhove M.P.M. From parasitology to policy: stories of hidden biodiversity, overlooked introductions and capacity building. Dublin University Zoological Society, February 8th, 2018, Dublin, Ireland

Vanhove M.P.M. Cichlid parasites as models in evolutionary biology: stories of hidden biodiversity, overlooked introductions and capacity building. Parasitology seminar, Department of Botany and Zoology, Masaryk University, November 30th, 2017, Brno, Czech Republic

Vanhove M.P.M. Cichlid parasites as models in evolutionary biology: stories of hidden biodiversity, overlooked introductions and capacity building. Plenary lecture, *Cichlid Science 2017*, September 4th – 7th, Prague, Czech Republic

Vanhove M.P.M., Briscoe A., Fannes W., Jorissen M.W.P., Littlewood D.T.J. & Huyse T. Exploring the use of mitogenomics for phylogenetic reconstruction of African monogeneans (Gyrodactylidea and Dactylogyridea). Invited lecture, *8th International Symposium on Monogenea*, August 6th – 10th, 2017, Brno, Czech Republic

Vanhove M.P.M. From parasitology to policy: examples of research and capacity development regarding African (aquatic) biodiversity. Colloquium on evolution and biodiversity, Zoological Research Museum Alexander Koenig, January 19th, 2017, Bonn, Germany

International conference lectures

Vanhove M.P.M., Artois T., Briscoe A.G., Chocha Manda A., Decru E., Gelnar M., Geraerts M., Huyse T., Jorissen M.W.P., Kapepula Kasembele G., Kmentová N., Koblmüller S., Littlewood D.T.J., Muterezi Bukinga F., Pariselle A., Rahmouni C., Šimková A., Snoeks J., Sturmbauer C., Van Steenberge M., Volckaert F.A.M., Vreven E.J., Wamuini Lunkayilakio S. (2018) Monogenean parasites of cichlids in the Congo Basin: species inventories, next-generation sequencing and invasion biology. *6th International Conference of the Pan-African Fish and Fisheries Association (PAFFA 6)*, September 24th-28th, Mangochi, Malawi

Vanhove M.P.M., Briscoe A.G., Jorissen M.W.P., Littlewood D.T.J. & Huyse T. (2018) Exploring mitogenomics for the phylogenetic reconstruction of African monogeneans (Gyrodactylidae and Dactylogyridae). *XIVth International Symposium on Flatworm Biology (ISFB 2018)*, August 27th-31st, Alghero, Italy

Jorissen M.W.P., Pariselle A., Huyse T., Vreven E.J., Snoeks J., Decru E., Volckaert F.A.M., Kusters T., Wamuini Lunkayilakio S., Muterezi Bukinga F., Chocha Manda A., Kapepula Kasembele G., Artois T. & Vanhove M.P.M. (2018) Tracing parasite host switching in the Congo Basin: A tale of monogeneans and their cichlid hosts. *XIVth International Symposium on Flatworm Biology (ISFB 2018)*, August 27th-31st, Alghero, Italy

Jorissen M.W.P., Pariselle A., Huyse T., Vreven E.J., Snoeks J., Volckaert F.A.M., Chocha Manda A., Kapepula Kasembele G., Kusters T., Wamuini Lunkayilakio S., Artois T. & Vanhove M.P.M. (2017) The TILAPIA project: Parasite host switching in the Congo basin? *8th International Symposium on Monogenea*, August 6th – 10th, 2017, Brno, Czech Republic

Jorissen M., Pariselle A., Huyse T., Vreven E., Snoeks J., Volckaert F., Chocha Manda A., Kapepula Kasembele G., Artois T. & Vanhove M. (2017) Dusty baseline: the merit of museum collections in biological invasion studies. *European Conference of Tropical Ecology: (re)connecting tropical biodiversity in space and time*, February 6th-10th, Brussels, Belgium

Jorissen M.W.P., Pariselle A., Huyse T., Vreven E.J., Snoeks J., Volckaert F.A.M., Chocha Manda A., Kapepula Kasembele G., Artois T. & Vanhove M.P.M. (2016) A hitchhiker's guide to tilapia: How parasites take a ride on introduced Nile tilapia in the DR Congo. *Zoology 2016*, December 16th-17th, Antwerp, Belgium

Vanhove M.P.M. (2016) Collection valorisation and stakeholder involvement for the sustainable management of african aquatic ecosystems as best practices in capacity building. *Group on Earth*

Observations Biodiversity Observation Network (GEO BON) Open science conference & all hands meeting. *Biodiversity and ecosystem services monitoring for the 2020 targets and beyond*, July 4th-8th, Leipzig, Germany

Vanhove M.P.M., Snoeks J., Bamps J., Gelnar M., Grégoir A.F., Hablützel P.I., Jorissen M., Kmentová N., Koblmüller S., Mendlová M., Muterezi Bukinga F., Pariselle A., Přikrylová I., Raeymaekers J.A.M., Rahmouni C., Řehulkova E., Šimková A., Sturmbauer C., Van Sever W., Van Steenberge M., Volckaert F.A.M., Vreven E.J. & Huyse T. (2015) Central African cichlid parasites: exploring their biodiversity, phylogeny, ecology and introductions. *Cichlid Science 2015*, September 6th-9th, Graz, Austria

Vanhove M.P.M., Šimková A., Pariselle A., Van Steenberge M., Řehulková E., Muterezi Bukinga F., Přikrylová I., Mendlová M., Gelnar M., Koblmüller S., Sturmbauer C., Volckaert F.A.M., Snoeks J. & Huyse T. (2015) Monogenean parasites of cichlid fishes: from adaptive radiation to a tool in introduced species research. *9th International Symposium on Fish Parasites*, August 31st – September 4th, Valencia, Spain

Vanhove M.P.M., Pariselle A., Van den Broeck F., Vreven E.J., Volckaert F.A.M., Snoeks J. & Huyse T. (2014) The monogenean fauna of cichlids from the Congo River. *1re Conference internationale sur la biodiversite du bassin du Congo*, June 6th-10th, Kisangani, Democratic Republic of the Congo

International conference posters

Geraerts M., Vanhove M.P.M., Jorissen M., Pariselle A., Muterezi B.F., Chocha M.A., Huyse T. & Artois T. (2018) Monogeneans belonging to *Cichlidogyrus* Paperna 1960 on two cichlid fish species from the Lomami River Basin (DR Congo), with description of five new species. *XIVth International Symposium on Flatworm Biology (ISFB 2018)*, August 27th-31st, Alghero, Italy

Jorissen M.W.P., Pariselle A., Kusters T., Goeyers C., Wamuini Lunkayilakio S., Decru E., Huyse T., Vanhove M.P.M. & Artois T. (2017) Lower Congo, lower diversity? *Zoology 2017*, November 23rd-24th, Wageningen, The Netherlands

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ANNEXES