MASARYK
UNIVERSITY
FACULTY OF SCIENCE

The parasite fauna of economically important pelagic fishes in Lake Tanganyika

Ph.D. Thesis

NIKOL KMENTOVÁ

Supervisor: Maarten Vanhove, Ph.D.

Department of Botany and Zoology

Brno 2019

Bibliographic Entry

Author	Mgr. Nikol Kmentová Faculty of Science, Masaryk University Department of Botany and Zoology
Title of Thesis:	The parasite fauna of economically important pelagic fishes in Lake Tanganyika
Degree programme:	Ecological and Evolutionary Biology
Specialization:	Parasitology
Supervisor:	Maarten Vanhove, Ph.D.
Academic Year:	2019/2020
Number of Pages:	350 + 72
Keywords:	*Kapentagyrus, Dolicirrolectanum*, Cryptogonimidae, Clupeidae, Latidae, Bathybatini

Bibliografický záznam

Autor:	Mgr. Nikol Kmentová Přírodovědecká fakulta, Masarykova univerzita Ústav botaniky a zoologie
Název práce:	Parazité ekonomicky významných ryb pelagické zóny jezera Tanganika
Studijní program:	Ekologická a evoluční biologie
Specializace:	Parazitologie
Vedoucí práce:	Maarten Vanhove, Ph.D.
Akademický rok:	2019/2020
Počet stran:	350 + 72
Klíčová slova:	*Kapentagyrus, Dolicirrolectanum*, Cryptogonimidae, Clupeidae, Latidae, Bathybatini

ABSTRACT

Biodiversity is a well-known term characterising the variety and variability of life on Earth. It consists of many different levels with species richness as the most frequently used measure. Despite its generally lower species richness compared to littoral zones, the global importance of the pelagic realm in marine and freshwater ecosystems lies in the high level of productivity supporting fisheries worldwide. In terms of endemicity, Lake Tanganyika is one of the most exceptional freshwater study areas in the world. While dozens of studies focus on this lake's cichlids as model organisms, our knowledge about the economically important fish species is still poor. Despite their important role in speciation processes, parasite taxa have been vastly ignored in the African Great Lakes including Lake Tanganyika for many years. In the framework of this PhD thesis, the fauna of parasitic flatworms (Platyhelminthes, Neodermata) infecting economically important pelagic fish species in Lake Tanganyika (bathybatine cichlids, clupeids and lates perches) was characterised to analyse parasites' host-specificity, population structure and historical origin.

Overall, a low parasite host-specificity in the lake's pelagic zone was documented in all examined fish taxa. While clupeids and bathybatine cichlids are infected by representatives of the dactylogyrid monogenean genera *Kapentagyrus* and *Cichlidogyrus*, respectively, three of the four latid species are parasitized by the same diplectanid monogenean species of *Dolicirroplectanum*. The origin of the primarily marine diplectanids in African freshwaters is proposed to be connected with their hosts' marine ancestry. Interestingly, the digenean diversity recovered from three species of lates perches clearly surpassed the single monogenean species retrieved from these host species. Parasite populations were analysed by means of combined morphological and molecular approaches. Their genetic population structure does not show a clear north-south gradient. The observed geographically dependent phenotypic plasticity in monogenean species is therefore assumed to be induced by environmental differences during ontogenetic development. Nevertheless, incipient speciation related to host species identity is assumed for *Kapentagyrus tanganicanus*. Host size related infection dynamics of two species of *Kapentagyrus* were proposed to explain their contrasting success of co-introduction to non-native areas. Recent demographic expansion in monogenean species infecting clupeid and cichlid hosts was detected and can be linked with paleogeographic events and climate change, respectively. Finally, this thesis showed the importance of a combined approach in order to correctly discern between phenotypic plasticity and interspecific boundaries and highlighted the potential of parasites in better understanding the pelagic ecosystem.

ABSTRAKT

Biodiverzita je globálně používaný termín charakterizující rozmanitost a variabilitu života na Zemi. Doposud bylo popsáno mnoho různých úrovní a definic biodiverzity, avšak nejvíce rozšířeným ukazatelem biologické rozmanitosti je počet druhů. I přes nižší úroveň biodiverzity oproti litorálu, globální význam pelagické zóny mořských i sladkovodních ekosystémů tkví především v její vysoké produktivitě, která je hnací silou celosvětového rybářského průmyslu. Jezero Tanganika je se svou vysokou úrovní endemismu jedním z nejvíce studovaných sladkovodních ekosystémů na Zemi. Zatímco nespočet prací byl věnován cichlidám, jakožto známému modelu pro studium evolučních mechanismů, znalosti o ekonomicky významnějších skupinách ryb jsou stále nedostačující. Též parazitické organismy ve Velkých afrických jezerech, včetně jezera Tanganiky, i přes jejich důležitou úlohu v diverzifikačních procesech svých hostitelů, jsou stále přehlíženy. Předložená dizertační práce se zabývá parazitickými ploštěnci (Platyhelminthes, Neodermata) infikující ekonomicky významné druhy ryb v jezeře Tanganika z čeledí Clupeidae (sleďovití) a Latidae (latesovití), a cichlidy ze skupiny Bathybatini, za účelem studia jejich hostitelské specificity, populační struktury a historického původu.

Snížená hostitelská specifita parazitů oproti litorální zóně byla prokázána u všech tří vybraných skupin hostitelů. Zatímco studované druhy cichlid a sleďovitých ryb jsou parazitovány rody *Kapentagyrus* a *Cichlidogyrus* (Monogenea, Dactylogyridae), tři ze čtyř druhů latesovitých ryb jsou hostitelé druhu rodu *Dolicirroplectanum*, patřící do skupiny Diplectanidae. Původ této primárně mořské skupiny parazitů v jezeře Tanganika je s největší pravděpodobností spojen s mořským původem hostitelů. Druhová diverzita motolic infikující latesovité ryby (šest druhů čeledi Cryptogonimidae) převyšuje jediný druh výše zmíněné skupiny žábrohlístů. Genetická populační struktura vybraných parazitických druhů se zdá být zcela nezávislá na gradientu zeměpisné šířky nebo hostitelském druhu. Zjištěná vnitrodruhová fenotypová plasticita je tedy je pravděpodobně ovlivněna podmínkami prostředí během ontogeneze. Nicméně, počáteční speciace poháněná vzájemnou izolací hostitelských druhů byla zjištěna druhu *K. tanganicanus*. Míra parazitace podmíněná velikostí hostitele byla navržena jako důvod absence jednoho z druhů *Kapentagyrus* v nepůvodních oblastech výskytu hostitele. Zaznamenaná expanze vybraných parazitických druhů v demografickém měřítku je pravděpodobně spjata s paleogeografickou historií oblasti jezera, včetně klimatických změn. Předložená dizertační práce zdůrazňuje důležitost využití multidisciplinárního přístupu pro studium mezidruhových rozdílů a populační struktury, a možnost využití parazitů k lepšímu porozumění pelagického ekosystému.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Maarten Vanhove, a supervisor full of patience who kept me motivated for all these years and whose selfless time and care were sometimes all that kept me going. My deepest appreciation goes to Milan Gelnar who introduced me to the fascinating world full of ingenious parasitological strategies and ignited the profound love for parasites in my heart. A very special gratitude goes out to Stephan Koblmüller from the University of Graz for help with population genetic analyses, greatly tolerant to all my questions. I was more than lucky having such experienced ichthyologists as Maarten Van Steenberge from the Royal Museum for Central Africa in Tervuren, Pascal Masilya Mulungula and Théophile Mulimbwa N'sibula from the Centre de Recherche en Hydrobiologie in Uvira, mentoring me through the biological aspects of different fish groups. I am very grateful to Tom Artois, my mentor at the University of Hasselt, who gave me the opportunity to be part of the zoology lab and thought me to look at flatworms and taxonomy in general from a very different perspective. I have greatly benefited from the introduction to digenean taxonomy and leading through morphological descriptions given by Simona Georgieva from the University of Valencia and Rodney A. Bray from the Natural History in London. I appreciate the feedback on statistical analyses offered by Joost A. Raeymaekers from the University of Oslo and Pascal Hablützel from the Flanders Marine Institute. With a special mention to all my colleagues in Brno, it was great sharing laboratory with all of you during last four years full of fruitful discussions with Jiří Vorel, Michal Benovics, Tomáš Pakosta, Jana Ilgová, Magdaléna Kováčiková, Šárka Mašová, Eliška Jirounková, Radka Pecková, Chahrazed Rahmouni, Lucie Škorpíková and Nikol Reslová. I would like also to acknowledge my colleagues at the University of Hasselt, namely Michiel Jorissen, Marlies Monnens, Armando Cruz Laufer, Mare Geraerts. as well as at the Katholieke Universiteit Leuven, namely my mentor Filip Volckaert, Els De Keyzer, Henrik Christiansen and Federico Calboli. My research would have been impossible without the financial support of Czech Grant Agency, projects P505/12/G112 and GA19-13573S, and University of Hasselt. Last but not least, my deepest thanks go to my partner for his patience and mental support and my parents who encouraged me during all the years of studying.

Contents

1 INTRODUCTION 3

2 AIMS 5

3 LITERATURE OVERVIEW 6

3.1 The African Great Lakes as a study system 6

3.1.1 Biodiversity hotspot 6

3.1.2 Diversification in the East African Great Lakes 6

3.1.3 Paleohydrological history of the African Great Lakes 9

3.2 The overlooked importance of the pelagic realm 9

3.2.1 Pelagic zone of African Great Lakes 10

3.3 Lake Tanganyika 12

3.3.1 Economically important fish species 13

3.3.2 The clupeid species of the Lake Tanganyika 14

3.3.3 Lates perches in Lake Tanganyika 17

3.3.4 Bathybatine cichlids 19

3.3.5 Fisheries practice and management 19

3.4 The parasitic part of the story in African Great Lakes 22

3.4.1 Parasitic flatworms (Platyhelminthes, Neodermata) 22

3.4.2 Parasites' host-specificity and co-evolutionary scenarios 24

3.5 State of the art of parasitic flatworms in Lake Tanganyika 26

3.6 Parasitic flatworms infecting fisheries targets in the pelagic zone of Lake Tanganyika 28

3.6.1 Diversification and population structure of parasites in pelagic zones 29

3.6.2 Parasites' population structure and their potential as tags of host' biogeography 30

4 MATERIAL & METHODS 34

4.1 Data collection 34

4.1.1 Sampling design 34

4.1.2 Staining procedure 34

4.1.3 Morphometrics and geomorphometrics 37

4.1.4 DNA extraction and genetic characterisation 38

4.2 Data analysis 40

4.2.1 Morphometrics 40

4.2.2 Geomorphometrics 41

4.2.3 Population dynamics 41
4.2.4 Phylogenetic reconstruction 42
4.2.5 Population genetic structure and demographic history 43
5 SUMMARY OF RESULTS 44
5.1 Clupeids 44
5.2 Latidae 51
5.3 Bathybatine cichlids 53
6 DISCUSSION 57
7 CONCLUSIONS AND FUTURE PERSPECTIVES 71
8 REFERENCES 75
9 LIST OF PUBLICATIONS 108
10 APPENDIX 351

1 INTRODUCTION

The remarkable species diversity of the African Great Lakes has been attracting scientists for many decades mainly in the field of evolutionary biology. So far, research in these hotspots of biodiversity has revealed key principles and mechanisms of speciation, adaptive radiation or historical biogeography. While ichthyological as well as malacological research has been initiated as far back as the second part of the 19th century, information about parasitic flatworms was lacking until the first record by Fuhrmann et Baer in 1925. Parasitic organisms are recognized as prime targets for speciation research, in addition to their importance in terms of biodiversity, ecosystem functioning and economic impact. Despite their potential as tags for their hosts' ecological relationships, population structure and historical demography, parasites remain vastly overlooked group in most of these lakes. Lake Tanganyika, an emblematic biodiversity hotspot, harbours the morphologically, genetically and ecologically most diverse cichlid assemblage on the earth. Moreover, the lake figures as an irreplaceable source of livelihood for local communities especially in terms of fisheries. The aggregate demand for marine as well as freshwater fish production has been increasing on a global scale. Even though the pelagic zones are generally less diverse in terms of species richness compared to the littoral habitats, their importance lies in a high level of productivity maintaining the overall balance in the ecosystems. Lake Tanganyika has a distinct open water (pelagic and to some extent deepwater) environment to which a characteristic fauna adapted over time. Although some cichlid species such as representatives of Bathybatini are considered as eu- or bathypelagic, in biomass, the lake's pelagic realm is dominated by two species of clupeids and their latid predators. Importantly, the two clupeid species (*Limnothrissa miodon and Stolothrissa tanganicae*) together with *Lates stappersii* constitute 95% of fisheries catches. Overall, the pelagic fish stocks in Lake Tanganyika contribute to a maximal annual fisheries production of up to ca. 200 000 tonnes. The knowledge about the parasite fauna in Lake Tanganyika is incomplete and fragmentary and mainly limited to cichlid hosts in littoral habitats. There are many reports about cichlid radiation events causing remarkable species diversity, and parasites with direct lifecycles, such as monogenean flatworms, may mirror this diversity. The observed decrease of parasite species-richness in the pelagic community has been explained by, among others, lower host density or higher distance to the substrate, which could hinder parasite transmission. Do parasites follow the same pattern in the pelagic zone in one of the most diverse freshwater ecosystems worldwide? The diversification in the lake is further known

to be shaped by repeated lake level changes and the presence of three subbasins. Therefore, is there any geographically dependent diversification in the parasite fauna infecting pelagic fish hosts with a lake-wide distribution?

The existence of pelagic (and other "marine-like") animals in a freshwater lake caused early researchers to consider the possibility of an ancient connection of Lake Tanganyika with the sea. Monogenean parasites are widely accepted to be useful in fish research and have been proposed and successfully utilized as markers in the biogeography of their hosts including cichlid fishes. Since a different geographic origin of the economically important fish groups in the lake was suggested, comparison of the parasite taxa infecting these fisheries targets with records on an African-wide scale could provide an additional view on their biogeographical history. Moreover, as *L. miodon* was introduced as a fisheries target to other waterbodies in Africa, the potential of parasite co-introduction to non-native areas is considered as a threat for local ecosystem stability.

Based on recent reports, sardine stocks are declining, and local fishermen are pushed to illegally cross borders and/or catch juveniles. In Lake Tanganyika, research on economically important fishes has been mostly overlooked. Knowledge of the stock structure of fisheries target species is indispensable for fisheries management. Currently, detailed stock structure information is missing for *L. miodon* or *L. stappersii*, while it has recently been confirmed that geographically dependent structure in *S. tanganicae* is weak. Helminths were proposed as an alternative to identify stock structure and origin of fishes not easily traceable by classic techniques such as migratory and skin fragile fishes. A parasitological approach was specifically advocated for clupeids as a way to complement elusive patterns in sardine genetics. What is more, parasite genetics may potentially offer a higher resolution than can be reached through host genetics. Their phylogeography can hence reflect historical events that are too recent to be inferred from host genetics: parasites as a "magnifying glass". Moreover, sharing of parasite haplotypes is a clear signature of host connectivity or migration, definitely in parasites with short-lived free-living larval stages such as monogeneans. This PhD thesis presents a detailed investigation of intraspecific evolutionary processes in parasites infecting three economically important fish groups, for the first time in African pelagic freshwater systems.

2 AIMS

1) Inventory of the parasitic flatworms infecting three of the economically important pelagic fish groups in Lake Tanganyika (Clupeidae, Latidae and bathybatine cichlids) based on museum collections and fresh samples and map their host-specificity.

2) Reconstruction of the history and population structure of the most abundant parasite.

3) Exploration of the gill parasite fauna of the clupeids introduced from Lake Tanganyika to Lakes Kivu and Kariba to test for parasite co-introduction and for the enemy release hypothesis.

4) Link parasite population structure to the stock structure of its hosts(s) and make a first estimate of the use of parasites as tags for fisheries stocks in Lake Tanganyika.

3 LITERATURE OVERVIEW

3.1 The African Great Lakes as a study system

3.1.1 Biodiversity hotspot

Biodiversity was defined by the Biodiversity Convention in 1992 as "The variability among living organisms from all sources including inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems" (United Nations, 1992). The main components of biodiversity were set as ecological, genetic and organismal diversity, combined with cultural and human interaction at all levels (Heywood et al., 1995). Originally, regions with particularly high levels of diversity, assessed by species richness and levels of endemism were referred as biodiversity hotspots in order to prioritise limited funding available for conservation (Myers, 1988). The hotspots' boundaries have been determined by 'biological commonalities' each consisting of a particular fauna and flora that act as a biogeographic unit (Myers, 1990, 1988; Reid, 1998). For practical purposes, in areas with lack of available data, indicator groups such as birds, mammals or insects have been established for status assessment (Lombard, 1995). Biodiversity hotspots were delineated in various places worldwide (Mittermeier et al., 2002; Myers, 1990, 1988). Additionally, the term has been used in the figurative sense for areas characterised as an evolutionary hotspot of diversification or with high level of non-native species invasion in a smaller scale not uniquely set for conservation purposes (Brown et al., 2010; Semmens et al., 2004; Trape, 2016).

3.1.2 Diversification in the East African Great Lakes

Although freshwater ecosystems cover only 0.8% of the Earth's surface, they constitute a valuable natural resource of exceptional species richness (one quarter of global vertebrate diversity) in scientific as well as economic terms (Gleick, 1996). Yet, they are among the most endangered ecosystems worldwide (Sala et al., 2000) suffering from over-exploitation, water pollution, flow modification, species invasions and habitat degradation (Dudgeon et al., 2006). The East African Great Lakes figure as irreplaceable freshwater natural riches of high global and local importance. These waterbodies are part of Eastern Afromontane hotspot region (Bibby et al., 1992) and are often described as containing ongoing natural experiments offering a unique opportunity to witness and record evolution

in real-time, especially in fishes (Stiassny and Meyer, 1999). Their origin is linked to tectonic and climatological processes that started over 300 million years ago. The three largest lakes, Malawi, Tanganyika and Victoria, constitute more than 90% of Africa's surface freshwater sources (Tiercelin and Lezzar, 2002). The first phase of European exploration of these lakes can be dated back to the second half of the 19th century (Boulenger, 1909; Woodward, 1859). Since then, African Great Lakes got the attention particularly of ichthyologists, malacologists and evolutionary biologists. Cichlid fishes (Cichlidae, Actinopterygii) represents the most studied group characterised by an extraordinary species diversity. To date, almost 2,000 species of cichlids have been recognised in the African Great Lakes, many of them not yet formally described, of which 99% endemic to the particular lake (Coulter, 1991a; Kocher, 2004; Lowe-McConnell, 2009; Snoeks, 2000; Turner et al., 2008). Over the past decades, fish and especially cichlids have served as models for investigating basic evolutionary processes accompanying such an extraordinary level of diversity in behaviour and life strategies (Kornfield and Smith, 2000). The outstanding cichlid diversity in the African Great Lakes has evolved through a range of different evolutionary processes including adaptive radiation and explosive speciation (Kocher, 2004; Salzburger et al., 2014; Shaw et al., 2000). The main mechanism was found to lie in the biotic co-interactions characterised by local adaptations driven by ecological speciation and natural selection (Schluter, 2000). Interspecific boundaries between cichlid species were further maintained by assortative mating, depending on the visual recognition abilities of cichlids (Wagner et al., 2012) and thus enabling sympatric speciation (speciation without apparent physical/geographical barrier in gene flow) (Seehausen et al., 1999). Only recently, parts of the genome called "genomic islands" were identified to be associated with divergent mating preferences (Malinsky et al., 2015) supporting sexual selection and hence sympatric speciation as important evolutionary mechanism in the area (Turner and Burrows, 1995). Diversity in the three biggest African lakes is concentrated mainly in the littoral habitat where each rocky shore has its own specific cichlid community through the processes of ecological niche segregation connected with restricted gene flow and sexual selection as mentioned above (Salzburger et al., 2014; Salzburger and Meyer, 2004). Hybridisation has also influenced the evolutionary history of a number of species in the present-day cichlid species flock; this phenomenon is nowadays promoted by changes in water chemical composition (e.g. eutrophication) and turbidity (Seehausen, 1997). Because of their high diversity in colour patterns, cichlids have become extremely popular in the ornamental fish trade. Except for cichlids, other groups are known to have experienced

rapid and multiple radiations in African Great Lakes, e.g., gastropods (Wilson et al., 2004), spiny eels (Brown et al., 2010), sponges (Erpenbeck et al., 2011), atyid prawns (Fryer, 2006) and ostracods (Martens et al., 2007). There is a unique flock of clariid catfishes as well as mormyrids and lungfish in lakes Malawi and Victoria (Lowe-McConnell, 1993). Relatively recent but very rapid diversification was suggested for catfish of *Synodontis* (Cuvier, 1816) (Day et al., 2009) dated to about the similar time as the one estimated for the platythelphusid crab radiation (Marijnissen et al., 2006) in Lake Tanganyika. The spiny eels of *Mastacembelus* (Scopoli, 1777) show a similar pattern of diversification as *Cyprichromis* Scheuermann, 1977 cichlids including a single colonisation event in Lake Tanganyika (Brown et al., 2010). The presence of four species of lates perches in Lake Tanganyika makes it the hotspot for this family on a worldwide scale (Otero,2004). This is suggested to be a result of niche partitioning.

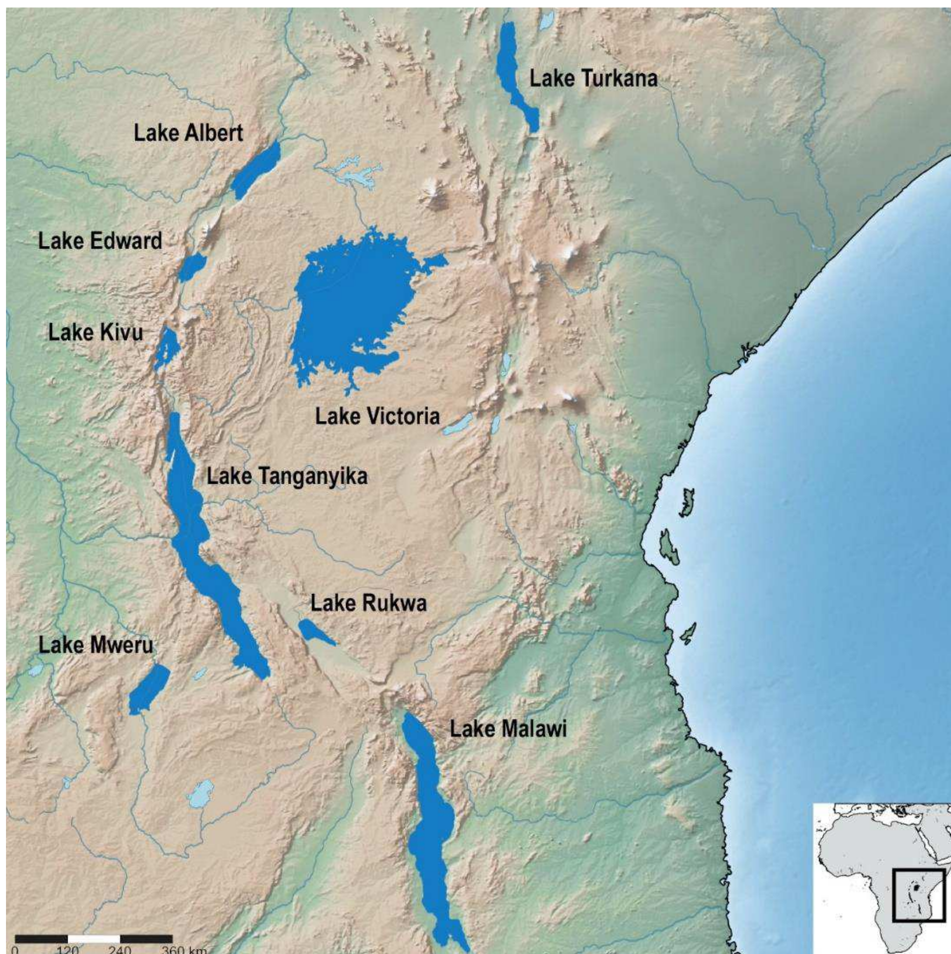


Fig. 1: East African Great Lakes. Map created using SimpleMappr software v7.0.0. (available at http://www.simplemappr.net. Accessed August 28, 2019).

3.1.3 Paleohydrological history of the African Great Lakes

Diversification in the African Great Lakes was further impacted by repeated lake level changes (Danley et al., 2012; Lezzar et al., 1996; Scholz et al., 2003; Tiercelin and Lezzar, 2002; Zohary and Ostrovsky, 2011). Substantial drops in lake levels of Lake Tanganyika and an almost complete dry up in Lake Malawi resulted from an extremely dry climate during the period between 1.6–0.57 MYA (Andrew S. Cohen et al., 1997; Delvaux, 1995; Lezzar et al., 1996). New regressions appeared repeatedly between 420,000–170,000 years ago, followed by draught periods in the late Pleistocene ice ages with the latest major lowstand estimated around 13,000 years ago and a gradual level rise in both lakes to the present states. A similar but less dramatic effect of global climate change on the other waterbodies in this part of the world was proposed (Owen et al., 1990). These water level fluctuations caused temporal barriers in gene flow among adjacent fish populations (Baric et al., 2003; Rossiter, 1995; Rüber et al., 2001; Sturmbauer et al., 2003; Sturmbauer and Meyer, 1992; Verheyen et al., 1996) and figures a key factor responsible for promoting speciation conditions also in non-cichlid groups (Brown et al., 2010; Day et al., 2009). Climate related lake level changes can explain the synchronised divergence of different cichlid flocks/lineages in African Lakes (Sturmbauer et al., 2001).

3.2 The overlooked importance of the pelagic realm

Pelagic zones in freshwater and marine habitats play a huge role in the ecological complexes which they are part of (Angel, 1993). The oceanic pelagic zone is by far the largest ecosystem on Earth with a similar latitudinal gradient in species diversity as in many terrestrial taxa (Lowe-McConnell, 2009). Overall, the global significance of pelagic ecosystems lies in the high level of productivity as well as the fixation of carbon dioxide; in turn, there is a lower level of species diversity (Berger et al., 1989). As the level of biodiversity and endemism is regarded as the main criterion for developing conservation strategies (Bibby et al., 1992), the importance of the relatively species poor pelagic zone is often underestimated or neglected (Angel, 1993). Even though the large areas of open water in the Pacific and Indian oceans are listed as biodiversity hotspots (Myers, 1990), the lack of comprehensive research and the high level of exploitation is reflected in the lack of pelagic protected areas worldwide (Game et al., 2009). Interestingly, by assessing the evolutionary history of Metazoa, it was proven that overall, lineage transition from the pelagic to the benthic zone has happened much more often than the other way around. This pattern further highlights the importance of the pelagic and deepwater habitat as a source

for global diversity. Generally, the pelagic and deepwater realm are often filled with migratory species. This makes the research challenging, requiring special techniques and equipment and a long-term sampling strategy. Moreover, the seasonal occurrence of migratory species complicates the studies. In the marine environment, classic tracking methods such as GPS failed to retrace the origin or migratory routes of pelagic species due to several reasons such as the small size, skin fragility or high mortality rate of these fishes. Migration by following prey or by moving to spawning sites supports gene flow over seas and distant geographic areas and usually prevents the identification of any clear population structure in highly mobile pelagic organisms (García-Rodríguez et al., 2011). Generally, a negative relationship between the life development with pelagic larvae stage and geographic population structure caused by isolation by distance was proven (Hellberg, 1996; Hellberg et al., 2002). Moreover, the population structure we infer from the present situation can be indeed affected by historical demography (Kyle and Boulding, 2000) which is not easily traceable in a habitat that nowadays lacks apparent physical barriers. In the East African Great Lakes, fish species richness drops substantially in open waters with sandy and muddy substrata offering fewer opportunities for resource-based diversification in comparison to the littoral zone. Evolutionary studies tend to focus mainly on littoral areas because of the easily measured level of biodiversity via species richness. They gave little attention to the pelagic zones where the processes including high level of productivity that support the maintenance of such a richness are occurring (Briscoe et al., 2016). Given the geographical limits, such lakes' pelagic zones could be model systems for the study of evolutionary processes in this open space habitat, and of conditions of upwelling mixture and horizontal transfer with the littoral zone.

3.2.1 Pelagic zone of African Great Lakes

Unlike the marine pelagic environment, which constitutes 99% of the biosphere (Angel, 1993), real freshwater pelagic conditions can be found only in big lakes of which the American and African Great Lakes are well studied ecosystems (Mills and Forney, 1988). In the deepest of these lakes, the water column is permanently stratified including an anoxic layer in lakes Malawi and Tanganyika. Nutrient distribution in these two lakes as the basic component of the food chain is provided mainly by upwelling flows, powered by persistent southerly winds combined with longitudinal temperature transects (Bootsma and Hecky, 2003). However, the levels of available nitrogen and phosphorus tend to be rather limited in comparison with the marine environment (Guildford and Hecky, 2000).

Additionally, plankton abundance and community composition are seasonally dependent (Cocquyt, 1999; Talling, 1986). The pelagic zones of African Great Lakes support some of the most productive inland fisheries in the world with crucial importance for livelihood in the surrounding areas (FAO, 2010; Tweddle, 1992). In the last decades, decreasing primary productivity was documented in Lake Tanganyika, correlated with overfishing (Cocquyt and Vyverman, 2005) or climate changes (O'Reilly et al., 2003). Conversely, eutrophication in lakes Malawi and Victoria was caused by intensive agriculture and was accelerated by soil erosion (Hecky, 1993; Otu et al., 2011; Seehausen, 1997). Unlike the littoral zone where, with only a little exaggeration, each piece of rock is occupied by a different fish species assemblage, the pelagic realm of the African Great Lakes is dominated by a few fish species. In this environment with by definition a lack of apparent physical barriers and a limited diversity of ecological niches, fish speciation in the pelagic zone has been driven mainly by resource partitioning (Koblmüller et al., 2005; Pereya et al., 2004), and spawning behaviour, as juveniles tend to stay in the littoral zone (Coulter, 1976; Hecky, 1991; Thompson et al., 1996). Fish diversification in the open water is seen as a possible demonstration of rarely documented sympatric speciation (Shaw et al., 2000). Alternatively, independent gradual adaptation of inshore generalists has been proposed (Fryer, 2006). In Lake Malawi, cichlid species of *Copadichromis* Eccles & Trewavas, 1989 filled the pelagic niche overcoming the lack of physical barriers most likely by sexual selection (Eccles and Trewavas, 1989), co-occurring with several other commercially-important zooplanktivorous cichlid species of *Diplotaxodon* Trewavas, 1935 (Thompson et al., 1996; Turner, 1994). The pelagic cyprinid *Engraulicypris sardella* (Günther, 1868), endemic to Lake Malawi, resembles sardines in schooling behaviour and high biomass and is one of the major sources of local fisheries (Lewis and Tweddle, 1990). Following the introduction of *Lates niloticus* L. to a fragile and highly isolated ecosystem of Lake Victoria, more than 300 native fish species are estimated to have vanished under the predation pressure and a changing environment including permanent stratification and eutrophication (Barel et al., 1985; Witte et al., 1992). The disrupted pelagic ecosystem of Lake Victoria is currently occupied almost exclusively by *L. niloticus* (Witte et al., 1995), its major prey species and plankton eater *Rastrineobola argentea* (Pellegrin, 1904) (Cypriniformes, Cyprinidae) (Wanink, 1999) and the recently recovered populations of two species of *Haplochromis* Hilgendorf, 1888 (Maeda et al., 2009). Unlike in lakes Malawi and Victoria, the pelagic zone of Lake Tanganyika is dominated by schooling freshwater species of sardines (Clupeiformes, Clupeidae), their latid predators (Perciformes, Latidae)

and rather deepwater and bathypelagic endemic cichlid lineages (Cichliformes, Cichlidae) belonging to the tribes Bathybatini, Boulengerochromini, Limnochromini and Perrisodini (Coulter, 1991b).

3.3 Lake Tanganyika

Lake Tanganyika is a unique study system that has acted as the finderscope of fish evolutionary biologists for decades (Boulenger, 1909; Coulter and Spigel, 1991; Koblmüller et al., 2005; Michel et al., 1992; Tiercelin and Mondeguer, 1991). Its geological age is estimated back between 9 and 12 million years ago. The lake consists of three subbasins with a maximum depth of 1,500 meters, which were separated and connected during its paleohydrological history (Andrew S. Cohen et al., 1997; Cohen et al., 1993). Lake Tanganyika is the second oldest and deepest lake in the world. Even though the lake remains stratified throughout the year with the deeper layer (below ~200 m) being permanently deoxygenated, seasonal tradewinds cause essential wind-induced mixing of nutrients (Hecky and Bugenyi, 1992) together with a thermally induced mixing in the southern part of the lake (Langenberg et al., 2003). The occurrence of benthic species in restricted by the oxygen concentration with anoxic layer ranges between 50 and 250 meters (Edmond et al., 1993). The lake is especially valuable from a scientific perspective in terms of the diversity in cichlids. It contains a mostly endemic cichlid assemblage comprising the highest diversity at tribe level of all African Great Lakes and count for more than 200 species (Salzburger et al., 2002; Snoeks, 2000). Moreover, it is assumed that the cichlid radiation in East Africa originated within Lake Tanganyika (Sturmbauer et al., 2011). Besides cichlids, Lake Tanganyika has been recognised as a hotspot for mastacembelid spiny eels (Brown et al., 2010), and as a place of remarkable species radiation of catfishes (Day and Wilkinson, 2006) and gastropods (Michel, 1995; Wilson et al., 2004). In total, representatives of 20 fish families occur in the lake and in rivers of its basin. Of the 115 non-cichlids, 53 species (46%) are endemic to the lake (Lowe-McConnell, 1993). Specific conditions caused by the upwelling of anoxic waters unable further diversification in the demersal community in Lake Tanganyika in contrast to Lake Victoria and Lake Malawi (Eccles 1986) accounting for 80 species of which 78 are endemic (Coulter, 1991). Different mechanisms were proposed to be involved in the diversification of fish lineages in Lake Tanganyika including rapid radiation driven mainly by niche segregation and speciation dependent on mating preferences and behavioural and prey differentiation (Day et al., 2009; Sturmbauer and Meyer, 1992; Wagner and McCune, 2009). To just name one example: the

composition of the benthic fauna strongly depends on the substrate type, which in Lake Tanganyika ranges from sandy patches in bays over pebbles and rocky shores in the littoral zone (Coulter, 1991). The ecological diversity is reflected by the number of cichlids' life strategies and modes of parental care (Sefc, 2011) Cichlid fishes have also been determined as models to study the effect of a particular breeding strategy on sexual selection (Amundsen, 2003). So far, small scale geographical structure driven by restricted gene flow over a north-south axis along the lake's coastline was reported among allopatric populations for many fish species (Duftner et al., 2006; Sefc et al., 2007; Taylor et al., 2001; Verheyen et al., 1996; Wagner and McCune, 2009). The pattern of population subdivision seems to be related to ecological and behavioural traits (Wagner and McCune, 2009) with different extent among stenotopic cichlid species (Sefc et al., 2007). However, interpopulation phenotypic variation is not always accompanied by genetic divergence (Sturmbauer et al., 2005) or *vice versa* (Duftner et al., 2006; Koblmüller et al., 2009). Moreover, the level of genetic differentiation among the current populations of animals in the lake might have been influenced by habitat structure and instability, e.g. lake level changes, seen in their demographic history (Egger et al., 2007; Koblmüller et al., 2011, 2007). Unfortunately, the outstanding diversity in the lake is threatened by an increased sedimentation, a localized urban and agricultural pollution, overfishing and the impact of climate change (Cocquyt and Vyverman, 2005; O'Reilly et al., 2003; Thieme et al., 2005). Recently, the depth of upwelling mixing has been decreasing and thus the oxygenated zone is reduced, leading to a loss of habitat for demersal species (Cohen et al., 2000).

3.3.1 Economically important fish species

Fisheries are important source of livelihood comprising at least 15% of the average animal protein consumption worldwide. There has been an increasing trend over the last decades with overall 47.5 million tonnes of fish being harvested every year according to (FAO, 2010). A decline in recovery of fisheries stocks has been recently experienced, and the overexploitation of stocks, climate change and increasing agriculture and intensive land use are proposed as potential causes hereof (FAO, 2016; Marshall, 2012; Naithani et al., 2011; Print et al., 2015). The transfer efficiency from primary to fish production in the pelagic zone of Lake Tanganyika is comparable with that of a marine environment (Hecky et al., 1981) and it is strongly dependent on upwelling mixing. The highest rates of vertical circulation occur in the dry season (three to ten times higher than in the rainy season). The pelagic food web in the lake is highly dependent on the primary production assemblage in

the pelagic zone, which is composed mainly by chlorophytes and cyanobacteria (Descy et al., 2010). The zooplankton community composition shows north-south differences in relative abundance of taxa (Kurki et al., 1999). The depth of mixing water varies (dry versus rainy season) influencing the vertical distribution of plankton (Descy et al., 2010, 2005). The pelagic zone of Lake Tanganyika has been colonised by freshwater lineages of clupeids and latids. This restricted most of the cichlid tribes to the littoral or benthic zones (Hecky, 1991; Lowe-McConnell, 2009). The lake's pelagic zone is dominated by two clupeid species (*Limnothrissa miodon* and *Stolothrissa tanganicae*) and their four latid predators (*Lates angustifrons, Lates mariae, Lates microlepis* and *Lates stappersii*). These species poor non-cichlid taxa comprise most of the overall biomass in Lake Tanganyika and up to 95% of lake's fisheries industry (Coulter, 1991c). Additionally, representatives of two predatory deepwater and bathypelagic cichlid tribes (Bathybatini, Boulengerochromini) together with large catfishes belonging to Clariidae figure as valuable catch for fishermen. In contrast to the monotypic lineage of *Boulengerochromis microlepis* (Boulenger, 1899), the Bathybatini have diversified into eight ecologically divergent species (Kirchberger et al., 2012). Recently, an increased occurrence of the littoral and the benthic cichlid communities was captured in local fish markets. In total, 47 species from 30 different cichlid genera were recognised as commercially important just in the north part of the lake (Mushagalusa et al., 2014). The need for catching small cichlid species occurring in the littoral zone is probably correlated with reduced unit catch rates in the traditional fisheries target species such as *S. tanganicae*. Management of littoral fisheries is challenging and complicated due to the variable number of fish species in relation to specific sites and habitats (Mushagalusa et al. 2014). Annual fisheries production at Lake Tanganyika has been estimated to vary in the range from 165,000 to 200,000 tons (Mölsä et al., 1999). The relative abundance of the target pelagic species differed between the northern and southern parts of the lake calculated over the same period (2004–2006) from the sampled units. It showed an overall predominance (83.1% by weight) of *S. tanganicae*, in the north compared to *L. stappersii* (15.7%), while inversely, in the south, *L. stappersii* dominated in the catches (82.1%) compared to sardines (10.7%) (Plisnier et al., 2009).

3.3.2 The clupeid species of the Lake Tanganyika

In total, 27 representatives of clupeids are known from African mostly riverine freshwater ecosystems (Lavoué et al., 2014). However, clupeids are important components of large lakes worldwide including Lake Volta and North American lakes (Whitehead et al., 1985).

Lake Tanganyika harbours two endemic and monotypic clupeid genera, *Stolothrissa* and *Limnothrissa*. These two pelagic sardines species are believed to have evolved from a common ancestor in the proto-Tanganyikan region about 8 MYA (Coulter, 1991b; Poll, 1953; Wilson et al., 2008). Clupeids in Lake Tanganyika are short-lived species with a mostly one-year lifespan (max. three years). They reproduce in inshore spawning grounds and exhibit schooling behaviour. School migration is driven by plankton migration on a lake-wide scale (Mulimbwa and Shirakihara, 1994). Both species mostly feed during the night and form large compact schools in deep-water related to predator avoidance during daytime (Mulimbwa and Shirakihara, 1994). Clupeids have an important function in the lake's food chain, being the link between the planktonic and the piscivorous level (Coulter, 1991b) and the main food source for 16 species piscivorous fish species (Brichard, 1978). Of the entire Lake Tanganyika fisheries, the two sardines combined make up for 65% of total catches (in mass), making them indispensable for the local fisheries and regionwide food security (Reynolds et al., 1999). They show several biomass cohorts through the year corresponding with the occurrence of zooplankton and spawning (Mushagalusa Cirhuza and Plisnier, 2016; Shirakihar et al., 1992). However, some of the cohorts can arise in the dry season under sub-ideal conditions (Mulimbwa et al., 2014). The unusual survival of larvae in the dry season indicates that the system is below its carrying capacity, which could be caused by overfishing of larvae (Mulimbwa, 2006). There are ecological differences between the two species of sardines. *Stolothrissa tanganicae* Regan, 1917 (Lake Tanganyika sprat) is the most abundant fish in the pelagic zone of Lake Tanganyika. The species forms very large schools and live at a depth range of 8–60 meters. Two major spawning peaks of *S. tanganicae* were documented, from May to June and between December and January (Mulimbwa et al., 2014; Poll, 1953). It shows a lake-wide distribution with a higher abundance in the southern part with seasonal variation (Kimirei and Mgaya, 2007) and it has been reported from up to the Lukuga River and Kisimba-Kilia Falls (Kullander and Roberts, 2011). Adults of *S. tanganicae* are strictly pelagic and spawn marginally closer to the shoreline. The breeding pattern of *S. tanganicae* geographically follow peaks in the abundance of phyto- and zooplankton, which is the main prey for both youngs and adults (Mulimbwa and Shirakihara, 1994; Phiri and Shirakihara, 1999). Juveniles tend to stay closer to shore before they reach standard length of 50 mm and become pelagic. The maximum size of *S. tanganicae* is around 100 mm (maturity in 75 mm in females and 64 mm in males) and its estimated longevity is 1.5 years (Eccles, 1992; Mulimbwa and Shirakihara, 1994). Juveniles of Lake Tanganyika sprat feed mainly

on phytoplankton while the adults prefer copepods, particularly calanoids, and atyid shrimps (Coulter, 1991). *Limnothrissa miodon* (Lake Tanganyika sardine) is the second most important fisheries target species in Lake Tanganyika (after *Stolothrissa tanganicae*) in the North of the lake and the third (after *S. tanganicae* and *L. stappersii*) in the southern and central part (Kimirei and Mgaya, 2007). Spawning occurs inshore throughout the year (Mulimbwa & Shirakihara, 1994), but mostly in the rainy season (between November and May) (Mulimbwa & Shirakihara, 1994) with peaks near the end of it (Coulter, 1991b). Larvae and fry remain in the coastal area until the fish reach a length of approximately 100 mm, around the age of 1 year, after which they move to the pelagic realm (Coulter 1991, Mulimbwa & Shirakihara, 1994). Maximum reported size ranges from 120 to 170 mm standard length (Mulimbwa & Shirakihara, 1994). Young *L. miodon* (< 42 mm) feed mainly on zooplankton (copepods and cladocerans) and some plant remnants. Adult *L. miodon* (> 85 mm) have more diverse feeding preferences. While they continue to feed on zooplankton and with plant material now taking up a bigger part of the daily food ratio, they also add insects, phytoplankton and fish to their diets via preying on *S. tanganicae*. Adults of *L. miodon* also display cannibalism, feeding on juveniles and larvae (Mulimbwa and Shirakihara, 1994). Schools of *L. miodon* tend to stay close to the bottom during the day and migrate upwards at dusk, remaining just below the surface at night (Coulter, 1991b; De Vos et al., 1996).

Introductions of* L. miodon *as a fisheries target species

Recently, 44 fish species were listed as non-native only in South Africa (Ellender and Weyl, 2014). However, the non-native fish fauna for freshwater aquaculture in Africa is dominated by a Nile tilapia (*Oreochromis niloticus* (L.)) being native to Nile River basin, West Africa and parts of the Congo Basin. Its high tolerance to external conditions and flexibility in spawning, feeding behaviour and maturation size make this species easy to culture, but render it into a threat for local ecosystems as well. Consequently, hybridisation, various forms of competition and predation on native species cause an often underestimated and overlooked threat for local ecosystems (Alcaraz et al., 2015; Canonico et al., 2005; Deines et al., 2014; Wise et al., 2007) and give Nile tilapia a high potential for invasiveness. Generally, biological invasions are classified into several categories based on the level of danger they pose to the natural stability of a particular ecosystem; invasive, self-sustaining population, self-sustaining reproduction and survival at a significant distance from the original point of reproduction, introduced but without stable reproduction (Blackburn et al.,

2011). Recently, several authors point to the problem of invasive species via trafficking or intended translocations and advised that research focusing on biological invasions should build on high-quality taxonomy, otherwise there is no way to precisely evaluate introduction pathways (Didham et al., 2007; Kelly et al., 2009; Sakai et al., 2001; Simberloff et al., 2013). Preliminary studies and empirical evaluation of the impact of an introduced species are rarely conducted.

Following the global trend of increasing fisheries pressure, non-native fish species have been introduced as targets for freshwater fisheries and aquaculture worldwide, amounting to 2,904 species (translocated between countries) reported in FishBase. However, this number is only an estimation since many fish translocations went unreported (Casal, 2006). In this context, even though *S. tanganicae* was intentionally targeted because of the high population densities and recovery rate, *Limnothrissa miodon* has been successfully introduced into different water bodies in Africa outside of Lake Tanganyika instead. This includes man-made Lake Kariba in the 1960s (Bell-Cross & Bell-Cross, 1971) and Lake Kivu in the 1950s (Guillard et al., 2012). The species has later also subsequently invaded Cahora Bassa (Marshall, 1993). More recently, *L. miodon* has established as a fisheries target species in the second largest water body in Zambia, man-made Lake Itezhi-Tezhi (Mubamba, 1993). *Limnothrissa miodon* has generally a low population food intake, suggesting very efficient grazing (Mandima, 1999). This, together with their generalist feeding habits, could explain why this species has been so successful at colonising other great lakes (Mandima et al., 2016).

3.3.3 Lates perches in Lake Tanganyika

Despite their primary marine origin, most of the lates perches, 7 out of 13 occur in Africa, are currently restricted to freshwater conditions, (Otero, 2004). The native range of *Lates niloticus* covers the Nilo-Sudan region including lakes Albert, Turkana and Tana. Other representatives can be found in lakes Rudolph and Turkana (*Lates longispinis* Worthington, 1932) and one species is endemic to Lake Albert (*Lates macrophthalmus* Worthington, 1929) (Harrison, 1991). The earliest occurrence of a common ancestor of the latid lineage in Africa has been dated back to the Early Miocene (23–15 MYA). The presence of this lineage was confirmed already in the proto-Tanganyikan region (Otero, 2004). Currently, four endemic species of *Lates* are present in Lake Tanganyika. They constitute one of the most abundant and ecologically important fish groups in the lake, with their biomass almost equal to that of clupeids (Plisnier et al., 2009). All four species have a lake-wide

distribution, a pattern seen also in other pelagic fish species in the lake such as sardines and pelagic cichlids (Coulter, 1991b; Koblmüller et al., 2015). This seems to be a global phenomenon indicating the high biomass of predators in unspoiled freshwater ecosystems (Chapman and van Well, 1978; Coulter, 1976; Hecky, 1991; Mulimbwa and Shirakihara, 1994). All four species of *Lates* are predators in the lake's open water. Their juveniles are commonly found in the plankton. However, differences in habitat preferences exist (Coulter, 1976; Poll, 1953). *Lates stappersii* (Boulenger, 1914) (sleek lates) is the most important latid species for the commercial fisheries in Lake Tanganyika. Unlike its congeners in the lake, *L. stappersii* (Boulenger, 1914) exhibits a truly pelagic lifestyle forming large groups preying upon *S. tanganicae*. Clupeids and shrimps alternate in the diet of young *L. stappersii* depending on their relative abundance in the environment (Mannini et al., 1999). Screening of the stomach contents of captured *L. stappersii* indicated that the feeding ecology was related to the area of origin. The species reaches a maximum size of 450 mm standard length and has a life span of about six years. They become piscivorous when they attain about 130 mm (Ellis, 1978) (Coulter, 1991b; Hecky, 1991). Its fragmentary distribution in the lake is probably correlated with only a few nursery areas being observed as well as by their preference of transparent waters allowing them to catch their prey more efficiently (Plisnier et al., 2009). *Lates mariae* Steindachner, 1909 (bigeye lates) and *L. microlepis* Boulenger, 1898 (forktail lates), are known to switch from a littoral juvenile form to exclusively pelagic top predators. They show night migration following their prey with the two species differing mainly by their preferred depth of occurrence: *L. mariae* tends to be found in higher depths (Coulter, 1976; Mölsä et al., 1999). *Lates mariae* exploits both fish and invertebrate prey (Hecky, 1991). Juveniles occur in the tall grass in deeper waters than *Lates angustifrons* Boulenger, 1906 (Tanganyika lates) until they reach 180 mm. Thereafter, they adopt a benthic habitat moving to deeper water (Kondo and Abe, 1995), with temporary occurrence in the anoxic area (Coulter, 1976). Adults migrate diurnally to the surface to feed on clupeids. *Lates microlepis* feeds on clupeids and on its congeners *L. stappersii* and *L. angustifrons*. It reaches a max. size of 930 mm (Poll, 1953). Breeding occurs in pelagic waters in areas with plants and drifting weed and shows distinct pairing (Breder and Rosen, 1966). Juveniles stay inshore until they reach 180 mm. Thereafter they move to the superficial pelagic zone and move upon their most frequent prey being clupeids (Coulter, 1976). *Lates angustifrons*, finally, has a predominantly solitary and more sedentary lifestyle compared to the above-mentioned species. It is a solitary lurking predator, favouring rocky bottoms. It has an unspecialised fish diet, but

feeds largely on benthic cichlids (Eccles, 1992; Poll, 1953) and can aggregate upon clupeid prey (Poll, 1953). It reaches a max. size of 2000 mm. Juveniles of *L. angustifrons* live in specific inshore habitats with grass beds of short grass in wadable depths and are also found in slow flowing affluents, until they reach 180 mm and migrate to the pelagic zone (Coulter, 1976; Poll, 1953). Adults are found far from the littoral occupying deeper water with increasing size, near the limit of the oxygenated zone (Coulter, 1976).

3.3.4 Bathybatine cichlids

As mentioned above, deepwater eu- and benthopelagic cichlids are frequently available in fish markets and are considered as valuable catch for local fishermen. Bathybatine cichlids form an ancient lineage within Lake Tanganyika's cichlid species assemblage (Koblmüller et al., 2005; Meyer et al., 2015; Salzburger et al., 2002). Ecological differentiation according to the type of preferred habitat and prey is proposed to be among the drivers of cichlid speciation in the open water habitat (Konings, 1998; Koblmüller et al., 2005; Kirchberger et al., 2012). Members of Bathybatini are large (maximum size between 300 and 400 mm) and highly mobile piscivorous predators of pelagic clupeids (*Bathybates fasciatus* Boulenger, 1901; *Bathybates leo* Poll, 1956), benthic cichlids (*Bathybates graueri* Steindachner, 1911; *Bathybates vittatus* Boulenger, 1914; *B. ferox* Boulenger, 1898, *Hemibates stenosoma* (Boulenger, 1901)) and small-sized clupeids (*Bathybates minor* Boulenger, 1906). For one species, *Bathybates horni* Steindachner, 1911 the prey is unknown. *Bathybates minor* lives and preys on huge shoals of sardines and it is characterized by performing surprise attacks on its victims. *Bathybates fasciatus* and *B. leo* are usually found in the open water together with their prey (sardines). All species show a lake-wide distribution and occur mainly in the deepwater pelagic and eu-pelagic habitat following their prey. They usually descend into depths of up to 150–200 m (Kirchberger et al., 2012; Konings, 1998; Maréchal and Poll, 1991). Two colour variants recognised in *H. stenosoma* were recently raised to species status followed by a description of *Hemibates koningsi* Schedel & Schliewen, 2017 (Schedel and Schliewen, 2017).

3.3.5 Fisheries practice and management

Local fishermen in Lake Tanganyika use traditional or artisanal methods such as gill nets, beach seine or lift nets (Roest, 1992). Industrial fisheries is mainly oriented on purse seine vessels, which are much more effective (30–140×) than traditional methods (Coulter, 1991c). Recently, the number of industrial fishing units and companies has increased but further expansion is limited by the lack of inland infrastructure. Most of the fishing on the

lake appears during the night and with methods relying on attracting clupeids using fishing lamps (Van der Knaap et al., 2014). Consequently, this practice is negatively correlated with moonlight as well as with the presence of breezes, waves, phytoplankton and medusae. The fishing effort is very unevenly distributed due to a lack of infrastructure in the area between big centres of human population with transportation over water playing an important role in the trade (Mölsä et al., 1999). Fishes are eaten mostly fresh at the markets in local population centres in the area (Uvira, Bujumbura, Kalemie, Mpulungu) or transported dried preferably by boat to other places.

Management of the fisheries of Lake Tanganyika has in the past been carried out by the four riparian countries individually. The integration of management strategies over the entire lake has been hindered by many factors such as the different ways of collecting fisheries data, legal frameworks regarding the methodology being used (Mölsä et al., 1999; Reynolds et al., 1999). Recently, an impact of overfishing, usage of destructive techniques and other aquatic and terrestrial resource exploitation practices, was observed on the catch per unit effort of *L. miodon* in Lake Tanganyika (Alin et al., 1999; Cohen et al., 1993; Jorgensen et al., 2006; Reynolds et al., 1999; Sarvala et al., 2017). Overfishing correlates with the introduction of more efficient industrial fishing methods and gears. Itresults in the decline of even larger predators such as lates perches (Coulter, 1976; Stone, 2007). Although Shirakihara et al. (2002) refused to mention overfishing as the primary cause of sardine population decrease in the northern part of the lake, illegal fishing, which is thought to form a significant proportion of annual catches focusing on littoral areas should be considered (Mushagalusa et al., 2014; Petit and Shipton, 2012). Distinct fisheries strategies in different parts of the lake have resulted in contrasting exploitation pressures. While the catches in the northern part are composed of young *L. miodon*, mostly adults are caught in the South (Mannini et al., 1996). Therefore, the decline in fisheries catches of targeted species could be related to the methodology rather than only to overexploitation (Pearce, 1995). A significant decline of *S. tanganicae* production was observed in the 1980s in the southern part of the lake together with a downward trend of 1,6% between 1956−92 reported from the Burundese part of the lake. However, this situation has probably been influenced by other environmental conditions affecting stock recruitment rate, which is still poorly understood (Mannini et al., 1996). In general, limnological cycles in Lake Tanganyika are linked with climate conditions as a cascade process derived from wind speed through upwelling mixing, primary production, and recruitment of planktivorous and

piscivorous fishes (O'Reilly et al., 2003; Plisnier et al., 1999; Verburg et al., 2003). A debate on the effect of climate warming on reduced fish yields in Lake Tanganyika has been ongoing since the beginning of the 20th century. To date, climate change is believed to be one of the causes of the decrease in lake mixing connected with the reduction of primary production and consequently also of production at higher trophic levels (Verburg et al., 2003; Verburga and Hecky, 2009). This could threaten ecosystem stability as a whole (Cohen et al., 2016; O'Reilly et al., 2003).

Science-based management is a key factor for the sustainable exploitation of natural resources. Fundamental knowledge of population structure is often overlooked and understudied in freshwater and tropical fisheries target species (Stephenson, 1999). One of the important unresolved questions about the economically relevant species inhabiting the lake's pelagic zone is their geographic population structure. Such information is crucial as the fisheries are managed by four different countries (Ovenden et al., 2015). Globally, in marine habitats, fish with large home ranges and large effective population sizes inhabiting coherent environments with few physical barriers usually display a lack of genetic differentiation (Grant and Bowen, 1998). Studies conducted on marine herring and sardine species showed philopatric spawning behaviour and local larval retention, believed to promote geographic separation in pelagic fish populations but without structure at spatial scale (Limborg et al., 2009; Mariani et al., 2005). With the lake being almost 700 km in length and with the observed migration patterns of sardine species (Plisnier et al., 1999), it is to be expected that there will be multiple populations present, rather than one stock for each species. Though there are studies pointing in this direction (Hauser et al., 1998; Kuusipalo, 1999; Sako et al., 2005), a nearly panmictic population with undetectable purely geographical structure has recently been proposed for *S. tanganicae* (De Keyzer et al., 2019). Population structure remains to be defined for *L. miodon* and lates perches. Both scenarios including extensive movement (Johannesson, 1974) or rather home range fidelity were concluded from fluctuating abundance of sardines' schools in the lake under predator pressure (Hermann, 1977) have been proposed. Moreover, the periodicity and abundance of *S. tanganicae* seem to be the key factors that regulate the annual cycles of its predators. Community interactions in the lake's pelagic ecosystem dependent on each food web component are evident, but a lot still remains to be discovered.

3.4 The parasitic part of the story in African Great Lakes

Research bias with respect to ecosystem type and to specific taxa keeps some groups understudied. Parasitism is a very frequent life-strategy on Earth (10−20% of all existing kinds of organisms known to science (Hammond, 1992; May, 1986)) with a significant effect on the evolution of their hosts (Thomas et al., 2010). With respect to their expected species richness and diversification abilities (Darwall et al., 2011; Poulin et al., 2019; Troudet et al., 2017), the level of exploration and species described in cichlid and other fish taxa in the African Great Lakes contrasts with the knowledge of their respective parasite fauna (Kuchta et al., 2018; Poulin et al., 2019). Theoretically, each free-living metazoan species is assumed to be infected by at least one parasite species (Poulin and Morand, 2000). Moreover, several parasitic lineages such as flatworms and nematodes have diversified exceeding the species richness of other parasitic taxa (Poulin, 2014; Poulin and Morand, 2004, 2000). The total number of parasitic flatworms being described from fish species in the African Great Lakes therefore confirms their overall understudied status in this biodiversity hotspot area (120 *versus* 1,800 fish species) (Kuchta et al., 2018).

3.4.1 Parasitic flatworms (Platyhelminthes, Neodermata)

Parasitic flatworms (Neodermata (Nordmann, 1832)) form a monophyletic lineage in Platyhelminthes (Platyzoa, Lophotrochozoa) and are classified into three classes: obligatory heteroxenous (two or more host species are included in the life-cycle) and mostly endoparasitic tapeworms (Cestoda (Chabert, 1787)) and flukes (Trematoda (Rudolphi, 1808)) in contrast to monoxenous (development restricted to a single host species) and ectoparasitic (with some exceptions) monogeneans (Monogenea (van Beneden, 1858) (Littlewood et al., 1999). The lineage is characterised by various ways of adaptation to a parasitic life-style including the existence of external body parts responsible for attachment (suckers and hooks in cestodes and trematodes, sclerotised structures in monogeneans) and a particularly high diversity in life-history strategies (Ehlers, 1985). The ciliated epidermis of the larval stage is replaced by a syncytial non-ciliated tegument, the neodermis, playing a crucial role in host-parasite interaction (Tyler and Hooge, 2004). Parasitic flatworms are mostly hermaphrodites, with reported cases of progenetic polyembryony (Monogenea, Gyrodactylidae), a portion of self-fertilisation (Cestoda, Eucestoda) and, rarely, dioecy (Trematoda, Schistosomatidae and Didymozoidae). Asexual reproduction is limited to the larval stages in intermediate hosts. The monophyletic status of monogeneans has been the subject of many studies and remains uncertain (Perkins et al., 2010). Their easily

distinguishable synapomorphy lies in the presence of the opisthaptor at the posterior part of the body. This opisthaptor is specialised in attachment to the host, possessing discs and various sclerotised structures such as clamps and hooks. They are mostly parasites of fish and cephalopods, but some species have adopted an endoparasitic lifestyle in amphibians, turtles and one species infects hippopotamus. They display a relatively high host-specificity. Monogenean flatworms are subdivided into two subclasses of which the classification remains controversial. Whereas the system proposed by Boeger and Kritsky, (1993) recognises three subclasses, which were mostly based on haptoral morphology (Polyonchoinea, Polystomatoinea and Oligonchoinea), Justine, (1991) classified monogeneans into the subclasses Monopisthocotylea and Polyopisthocotylea referring to their autapomorphies in spermiogenesis and spermatozoa. Monogeneans are important pathogenic agents in aquaculture and are often accompanied by secondary infections (Buchmann and Bresciani, 2006). In Africa, research on monogeneans mostly focused on economically important species and on cichlids, with 482 species from 4 families described so far in freshwaters, of which 91 occur in the Great Lakes (Kuchta et al., 2018). Unlike in monogeneans, the life cycle of cestodes and trematodes includes at least one intermediate host. Their specialised attachment organs develop on the anterior body part. Two subclasses are recognised in Trematoda, of which members of Aspidogastrea parasitise on marine cartilaginous fishes and turtles, while representatives of Digenea have strictly incorporated snails as their first intermediate hosts. Digenea is an extremely diverse group with representatives parasitizing on all major vertebrate groups as definitive hosts (Cribb, T.H. et al., 2001). In total, 100 species of trematodes from 27 families were reported infecting African freshwater fish so far of which 13 species in the Great Lakes (Kuchta et al., 2018). Members of Cestoda differ from other neodermatan groups by the secondary absence of a digestive tract, the structure of the protonephridial system and the presence of microtriches. They are currently classified in 19 orders defined mainly based on the morphology of their scolex (Caira et al., 2017) combined with mitochondrial phylogenetic reconstruction (Waeschenbach et al., 2012), with the basal lineage being Gyrocotylidea, parasites of chimaeras (A. E. Lockyer et al., 2003; Poddubnaya et al., 2006). The cestode diversity known from African freshwater fishes contains 55 species from 6 orders of which 16 species occur in the Great Lakes (Kuchta et al., 2018). Traditionally, species delineation in parasitic flatworms is based on a detailed morphological characterisation of external and/or internal organs. More recently, a combination with molecular characterisation has been adopted. The species richness of parasites is quantified in combination with ecological

infection parameters. These are routinely measured by the relative abundance, prevalence and infection intensity (Poulin, 2015). Parasitism has been widely accepted as an intrinsic factor driving host diversification as an adaptation to changing conditions promoted by the strong ecological and evolutionary association between parasite and host. As mentioned above, the role of parasitic flatworms in the evolution and diversification of cichlid species flocks was largely overlooked in the African Great Lakes. Only few direct observations of parasite communities (e.g. Maan et al., 2008; Raeymaekers et al., 2013) and some indirect quantifications of difference in immune response were conducted so far (Blais et al., 2007; Ono et al., 1993). Fundamental taxonomic research is necessary before any assumptions regarding host-parasite associations can be made.

3.4.2 Parasites' host-specificity and co-evolutionary scenarios

Host-specificity, also called host range, has been used as one of the fundamental characteristics of parasitic organisms. It is generally defined by the number of host species the parasite species infects and successfully reproduces in/on (Poulin, 2007). The basic division of host-specificity categorises parasites in two groups: specialists (infecting a single host species) and generalists (infecting and reproducing in/on two or more host species). The classification of host range might be further studied by considering geographic, structural and phylogenetic relationships among a parasite's host species (Poulin et al., 2011) and the ecological infection parameters (Poulin et al., 2011; Poulin and Mouillot, 2003; Rohde, 1980a). For the purpose of this study, the following categories of parasite's host-specificity were used (based on Desdevises et al., 2002 and Mendlová and Šimková, 2014): (i) strict specialist − infecting only a single host species; (ii) intermediate specialist − infecting two or more congeneric host species; (iii) intermediate generalist − infecting non-congeneric host species from the same lineage (level of tribe/subfamily) and (iv) generalist − infecting two or more host species from different lineages (level of tribe/family and higher). As a relatively high level phenotypic plasticity related to host species identity was observed several times in parasites, morphological and molecular characterisation should be used to verify species identity and host-specificity (Bueno-Silva et al., 2011; Huyse and Volckaert, 2002).

In general, evolution of parasites is believed to be tightly associated with that of the host organisms given their close ecological and resource-based relationships. Due to the shorter generation time and larger population sizes of parasites compared to the hosts, parasite evolution is believed to show a higher level of flexibility and adaptation. Such an

advantage derives from the discrepancy in selection pressures: all of the parasites giving offspring are exposed to the host, whereas only a portion of the host's population usually experiences the infection (Combes, 2001). However, traditional concepts of strict correlations between host and parasite phylogenies and geographical distributions (Inglis, 1971) have been questioned (Brooks, 1979) with the level of local adaptations being influenced by both intrapopulation and metapopulation dynamics (Gandon, 2002; Thompson, 1994). As a result, different scenarios rather than strict co-speciation of parasites and hosts were suggested (Johnson et al., 2003; Page and Charleston, 1998; Ronquist, 1997) and are widely used to explain historical evolutionary relationships in various host-parasite systems (De Vienne et al., 2007; Lauron et al., 2015; Page, 1993) including parasitic flatworms (Clayton et al., 2003; Desdevises et al., 2002; Huyse et al., 2005; Huyse and Volckaert, 2005; Johnson et al., 2003). Even though straightforward host and parasite co-speciation has been suggested considering a complete congruence of phylogenies (Demastes and Hafner, 1993), in some host-parasite systems such tree topologies are believed to rather result from phylogenetically constrained host switch events (Huyse and Volckaert, 2005; Jackson, 1999; Ziętara and Lumme, 2002). Further, recent host-switches related to lateral or ecological transfer seen as a decrease in host-specificity might indicate incomplete lineage sorting and possible ongoing parasite speciation (Bueno-Silva et al., 2011) rather than phylogenetic congruence. Parasite evolution does not always follow the diversification of its host lineage, a process called failure to diverge (Johnson et al., 2003) resulting in an increase in host range. Incongruence between the parasite and host phylogeny could also have resulted from several other reasons including the extinction of a parasitic lineage. Such a "sorting event" in principle might have happened after the co-speciation (i.e. lost overboard) possibly due to external changes, or parasites may have been absent on the host founder population (i.e. missed the boat) (Paterson and Gray, 1997). These two scenarios are not easily distinguishable. Lastly, an incongruent pattern might be caused by a duplication of a parasite lineage or by within-host allopatric speciation (Huyse et al., 2005; Šimková et al., 2004) intensified by competition (Guilhem et al., 2012). Co-phylogenetic reconstruction might also be hampered by low infection intensity or by seasonality in the incidence of a particular parasite lineage leading to an incomplete inventory of parasite lineage (Poulin and Morand, 2000). Overall, a pattern of complete co-speciation at macroevolutionary level of parasitic flatworms is nowadays considered as very rare. It seems to be restricted to higher taxonomical levels and is believed to have resulted from geographical isolation of particular

host lineages and their parasites (Boeger and Kritsky, 1997; de Vienne et al., 2013). The evolutionary relationships in host-parasites systems are complex and more than one of the above-mentioned scenarios is usually being reported (Garamszegi, 2009; Littlewood, 2006). Moreover, the congruence between parasites' phylogeographic patterns and their hosts' historical biogeography depends on transmission dynamics being more complex in heteroxenous parasites (Nieberding et al., 2004).

3.5 State of the art of parasitic flatworms in Lake Tanganyika

As mentioned above, the parasitic part of the story of the evolutionary history of the East African Great Lakes has been overlooked for many years. Currently, a total of 51 species of parasitic flatworms were described in Lake Tanganyika (see overview in Kuchta et al., 2018) including only eigth species of cestodes (*Lytocestoides tanganyikae* Baylis, 1928 (Caryophyllidea, Lytocestoidae) from an unidentified cichlid; *Lytocestoides* sp. from *Xenotilapia* sp., *Marsypocephalus tanganyikae* (Fuhrmann et Baer, 1925) (Onchoproteocephalidea, Proteocephalidae) from *Clarias gariepinus* (Burchell, 1822), *Monobothrioides cunningtoni* Fuhrmann et Baer, 1925 (Onchoproteocephalidea, Proteocephalidae) from *Auchenoglanis occidentalis* (Valenciennes, 1840), *Proteocephalus beauchampi* from *Chrysichthys* sp., *P. cunningtoni* Fuhrmann et Baer, 1925 from *Dinotopterus cunningtoni* Boulenger, 1906; *P. dinotopteri* Fuhrmann et Baer, 1925 (Onchoproteocephalidea, Proteocephalidae) from *Dinotopterus cunningtoni*) and a single trematode species (*Neocladocystis tanganyikae* (Prudhoe, 1951) (Opisthorchioidea, Cryptogonimidae) originally described by Prudhoe (1951) as *Cladocystis tanganyikae* (Prudhoe, 1951) possibly from the poecilid *Lamprichthys tanganicanus* (Boulenger, 1898). However, given the uncertainty on the identity of the host species, this record has to be revalidated (Prudhoe, 1951). So far, monogenean flatworms clearly surpass other taxa of parasitic flatworms in terms of known diversity in the lake measured by species richness. Except for three species of *Gyrodactylus* described from the cichlid *Simochromis diagramma* (Günther, 1894) (Vanhove et al., 2011a), all of the reported monogenean species (29) belong to the family Dactylogyridae. Overall, diversification in *Cichlidogyrus*, a genus infecting almost exclusively cichlid fish, mirrors the remarkable species richness of the host lineages with a high level of host-specificity and cases of within-host speciation being proposed (Gillardin et al., 2012; Kmentová et al., 2016a; Muterezi Bukinga et al., 2012; Pariselle et al., 2015b, 2015a; Vanhove et al., 2015)). Interestingly, phylogenetic congruence and geographically-dependent diversification was documented between the

species of *Cichlidogyrus* and their tropheine cichlid hosts (Vanhove et al., 2015). From a morphological point of view, haptoral and genital structures seem to mirror the cichlid hosts' phylogenetic affinities (Pariselle et al., 2015b; Rahmouni et al., 2018, 2017) including a potential geographically dependent variation (Rahmouni et al., 2018). Of only few cichlid species, monogenean communities have been compared at species level between different host populations. On a lake-wide scale, monogenean community structure of *Ophthalmotilapia nasuta* (Poll & Matthes, 1962) and *Interochromis loocki* (Poll, 1949) differs between the lake's northern and southern tip (Pariselle et al., 2015b; Rahmouni et al., 2018, 2017; Vanhove et al., 2011b) and concurs with the limited dispersal of these host species (Vanhove, 2012). Monogenean fauna could be therefore considered as a tag for their host's dispersal abilities. Suggested within-host speciation or host switch in the case of multiple species of *Cichlidogyrus* infecting *Ophthalmotilapia ventralis* (Vanhove et al., 2011a) and *I. loocki* (Pariselle et al., 2015b) might be related to the generalist life style of these fish species (Sefc et al., 2007). Co-evolutionary dynamics and the molecular rate of parasites in Lake Tanganyika was suggested to be accelerated by the fast cichlid radiation in the lake (Koblmüller et al., 2006). Moreover, monogenean parasites reflect the historic relationships between riverine and lacustrine African cichlid lineages (Muterezi Bukinga et al., 2012). Only a few studies investigating the role of parasitic flatworms in the lake in the evolutionary ecology of their hosts were conducted so far. The existence of contrasting parasite communities among allopatric cichlid populations belonging to the highly diverse *Tropheus* may suggest ongoing speciation facilitated by parasites (Raeymaekers et al., 2013). Moreover, an influence of the hosts' behaviour and dispersal on parasite community composition was reported (Grégoir et al., 2015). On the other hand, a weak link between the dispersal capacity and parasite community differentiation suggests evolution within Tropheini not to be dependent on parasitic infection (Hablützel et al., 2016). Only few pieces of the puzzle were uncovered regarding the role of parasites in cichlid diversification in this natural laboratory so far and this topic certainly deserves more attention (Vanhove et al., 2016).

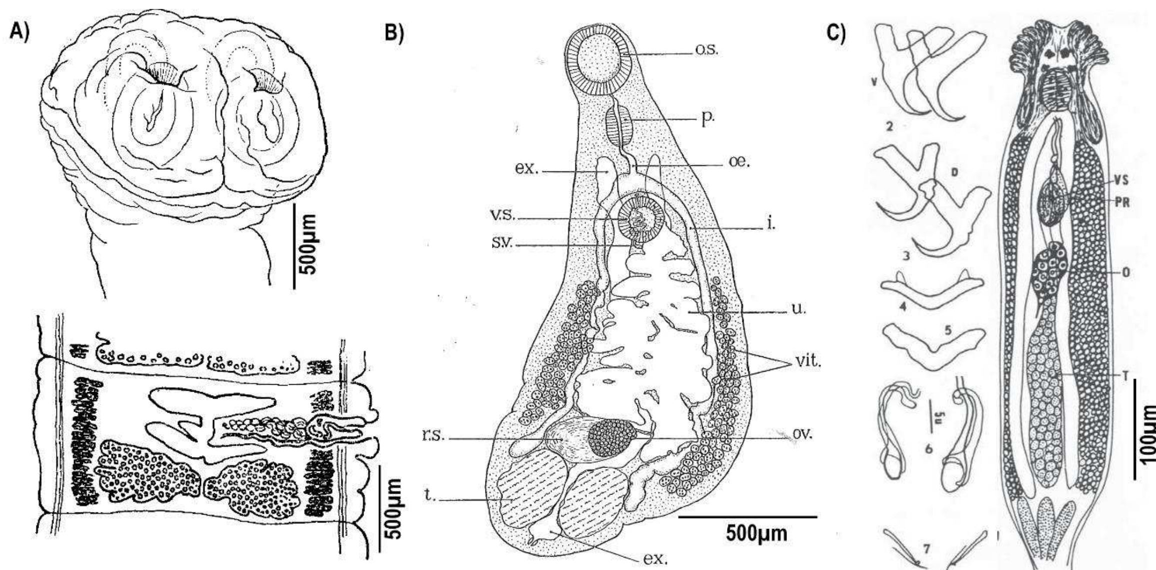


Fig. 2: Examples of parasitic flatworms described in Lake Tanganyika. A. *Marsypocephalus tanganyikae* (Fuhrmann et Baer, 1925) (Onchoproteocephalidea, Proteocephalidae) infecting *Clarias gariepinus* (Burchell, 1822) adapted from (Fuhrmann and Baer, 1925); B. *Neocladocystis tanganyikae* (Prudhoe, 1951) (Opisthorchioidea, Cryptogonimidae) most likely described from *Lamprichthys tanganicanus* (Boulenger, 1898) adapted from (Prudhoe, 1951); C. *Ancyrocephalus limnotrissae* Paperna, 1973 (Monogenea, Dactylogyridae) infecting *Limnothrissa miodon* adapted from (Paperna, 1973).

3.6 Parasitic flatworms infecting fisheries targets in the pelagic zone of Lake Tanganyika

Given the research bias towards cichlids, it is more than surprising that the first monogenean species ever described in the lake was *Ancyrocephalus limnotrissae* Paperna, 1973 from *L. miodon* (Paperna, 1973). However, that was the last record of a parasite from the lake's pelagic realm and the lake in general for many years. The knowledge gap has lasted for more than 38 years and was interrupted by the first description of gyrodactylid monogeneans in the lake in a study of Vanhove et al. (2011a). The first record of an endoparasitic flatworm dates back to 1951 (Prudhoe, 1951); pelagic hosts have never been examined for these helminths. To date, only three species of parasitic flatworms have been described from the lake's pelagic hosts and fisheries targets. Following *A. limnotrissae*, the world's largest cichlid *Boulengerochromis microlepis* was found to be infected by *Cichlidogyrus nshomboi* Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle, 2012. More recently, *Cichlidogyrus casuarinus* Pariselle, Muterezi Bukinga & Vanhove, 2015,

infecting six species of Bathybatini, was the first dactylogyrid species with a lower host-specificity in the lake (Kmentová et al., 2016c; Pariselle et al., 2015a).

3.6.1 Diversification and population structure of parasites in pelagic zones

Low species richness in the pelagic zones of the Great Lakes (Lowe-McConnell, 1996; Vadeboncoeur et al., 2011), is a common phenomenon, also in marine areas (Angel, 1993). Knowledge about the habitat preferences and the geographic distribution and, by extension, the stock structure of fisheries targets species, remains scarce and fragmentary, for want of the data needed for a clear consensus.

Similarly, lower levels of parasite diversification and host-specificity have been observed in the open water and in deep sea ecosystems/regions worldwide (Boxshall, 1998; Bray et al., 1999; Campbell et al., 1980; Klimpel et al., 2010, 2009, 2006; Mauchline and Gordon, 1984). Decreased levels of parasite species richness are often connected with lower host-specificity. This phenomenon is explained by the lower density of host individuals in most pelagic and deep-water zones. Consequently, migratory and schooling hosts such as sardines often harbour a lower parasite diversity in comparison to fishes with high philopatry and a solitary behaviour (Luque et al., 2004). Host traits such as habitat, trophic level (Luque and Poulin, 2008) or fish body length influence parasite diversity (Luque et al., 2004). However, the species richness of particular parasite taxa is expected to reflect differences in life cycle that are also connected with the availability of host taxa. Heteroxenous parasitic flatworms generally show a narrower host-specificity to their intermediate, compared to their definitive host taxa. Therefore, the decrease in host-specificity promoted by low densities of definitive hosts in trematodes and cestodes as seen in pelagic environment could be compensated by the presence of intermediate hosts functioning as evolutionary reservoirs (Gibson and Bray, 1994). Unlike in monogeneans and cestodes, digenean species richness has been further promoted by different mechanisms including possible adoption of new sites within hosts connected with new diets and feeding mechanisms, adaptations relating to the exploitation of ecologically similar groups of fishes and second intermediate hosts, and adaptations relating to the exploitation of various phylogenetic lineages of molluscs (Cribb et al., 2002). Moreover, species richness of marine digenean taxa shows a correlation with a latitudinal gradient, as often described for terrestrial free-living organisms (Rohde, 1978) but this correlation might be underestimated in particular lineages (Blasco-Costa et al., 2014).

As mentioned in chapter 3.5, species richness of monogeneans in the lake has been characterised by a relatively high level of host-specificity (Kmentová et al., 2016b, 2016a; Muterezi Bukinga et al., 2012; Rahmouni et al., 2017; Vanhove et al., 2011b). Phylogenetic congruence with the hosts, a community structure dependent on the host dispersal abilities, and rare host-switching events were mostly documented so far. The documented cases of within-host speciation would suggest that the monogenean diversity in the lake could surpass that of their hosts (Vanhove et al., 2015). Other factors such as the type of brood care in African cichlids were proposed to affect the level of host-specificity of monogeneans infecting them (Mendlová and Šimková, 2014). However, some of the African representatives of *Cichlidogyrus* show a broad host range and can be considered as intermediate generalist (le Roux and Avenant-Oldewage, 2010). In Lake Tanganyika, *C. casuarinus* was shown to hitherto be the only species of parasitic flatworms showing a generalist life-style infecting up to six species of Bathybatini cichlids (Kmentová et al., 2016c). This finding corroborates with the proposed negative correlation between the parasites' host-specificity and host dispersion availability. However, as a high level of morphological diversity was described in this parasite species, the conspecificity of *C. casuarinus* collected from different geographic areas should be further confirmed by intensive sampling and genetic characterisation. Water level fluctuations in Lake Tanganyika are known to have had a dramatic effect on the evolutionary history and diversification of littoral cichlid assemblages (Sturmbauer et al., 2001). However, they are expected to have less influence on highly mobile pelagic fish species (Pereya et al., 2004). Although signs of lake level fluctuations can be tracked in the demographic history of pelagic predators, the relatively fast reconnection of isolated populations promoted by long-distance migration most likely prevented divergence of temporarily isolated populations (Koblmüller et al., 2015, 2019).

3.6.2 Parasites' population structure and their potential as tags of host' biogeography

In the absence of barriers, species with high dispersal ability are expected to show little, if any, (phylo)geographic structure. In the African Great Lakes, no clear geographic population structure was detected for pelagic species of *Diplotaxodon* (Cichlidae, Haplochromini) in Lake Malawi (Shaw et al., 2000). Similarly, a true pelagic mix with lack of phylogeographic structure was detected in *B. microlepis* (Koblmüller et al., 2015) and other bentho- and eupelagic species of Bathybatini in Lake Tanganyika (Koblmüller et

al., 2019). In non-cichlids, a near-panmictic lake-wide population was detected for the clupeid *S. tanganicae* (De Keyzer et al., 2019). Since parasitic flatworms lack free living stages, host agility figures as the major determinant of their population structure (Nadler, 1995). However, significant differentiation in parasite populations can result from other factors apart from host population structure. Geographically dependent parasite differentiation might be related to clonal reproduction or host factors that select against certain parasite genotypes (Prugnolle et al., 2005a, 2005b). In general, parasite populations are dependent on various factors including (i) host mobility, (ii) mode of reproduction of the parasite, (iii) complexity of the parasite life cycle, (iv) parasite infrapopulation size and (v) host-specificity (Huyse et al., 2005). Comparative studies related digenean species with contrasting levels of geographic population differentiation to their host's dispersal capabilities (Criscione and Blouin, 2004) and to the level of digenean host-specificity (Nadler, 1995). The complexity of the life-cycle in heteroxenous parasites contributes to a stratified pattern of local differentiation but homogenisation across areas (Štefka et al., 2009) determined by the most mobile host (Bouzid et al., 2008; Prugnolle et al., 2005b). The lack of studies focusing on geographic population structure of parasites in the pelagic realm prevents any general conclusions (Baldwin, 2010).

In general, parasites are believed to have higher intraspecific mitochondrial diversity than free living animals (Criscione and Blouin, 2004). Therefore, as suggested in previous studies, parasites can be potentially used as tags for host species characteristics such as population structure, migration patterns and historical distribution (Barson et al., 2010; Criscione et al., 2006; Criscione and Blouin, 2006; Hoberg, 1997). However, using heteroxenous parasites as tags for stock structure or historical distribution (Jirsová et al., 2017) proved to be problematic due to a simultaneous influence of often contradicting patterns in the various hosts involved in the life-cycle. On the other hand, given their direct transmission, shorter generation time, fast mutation rate combined with usually a high host-specificity, monogeneans were proposed as potential tags of host population structure and biogeography (see Fig. 3). The parasite's ability to show more structure than can be seen in host genetics is referred to as the magnifying glass effect (Catalano et al., 2014; Nieberding et al., 2004; Nieberding and Olivieri, 2007; Poulin, 2007). Migrating fish hosts such as sardines may cause homogenisation of the parasite population. Contrasting parasite community composition over large geographic areas with seasonal differences has been recognised as a potential tool for stock identification of a highly mobile fish species

(Baldwin et al., 2012; Baldwin, 2010; Campbell et al., 2007; Costa-Pereira et al., 2014; MacKenzie, 2002; Weston et al., 2015). Pettersen et al. (2015) used a portion of the cytochrome *c* oxidase subunit 1 gene of *Gyrodactylus thymalli* Žitňan, 1960 combined with dehydrogenase subunit 5 to indirectly infer barriers in gene flow of grayling (*Thymallus thymallus* L.). Monogenean genetics was also used to track the historical distribution of clariid catfishes in Africa (Barson et al., 2010) and to reconstruct introduction pathways in *Perccottus glenii* Dybowski, 1877 (Ondračková et al., 2012). However, the absence of consistently higher population differentiation in *Gyrodactylus gondae* Huyse, Malmberg & Volckaert, 2004 compared to its host, the sand goby *Pomatoschistus minutus* (Pallas, 1770), lead Huyse et al. (2017) to reject the magnifying glass hypothesis in this case. In Lake Tanganyika, the cases of the monogenean fauna infecting species of *Tropheus* or *O. nasuta* (see chapter 3.5) indicate the potential of parasites to reflect host dispersal capacities and potentially population structure. However, the lack of generally accepted and traceable patterns and processes determining community structure of fish parasites remains and certainly deserves more attention and research (Timi, 2007; Timi and Poulin, 2003).

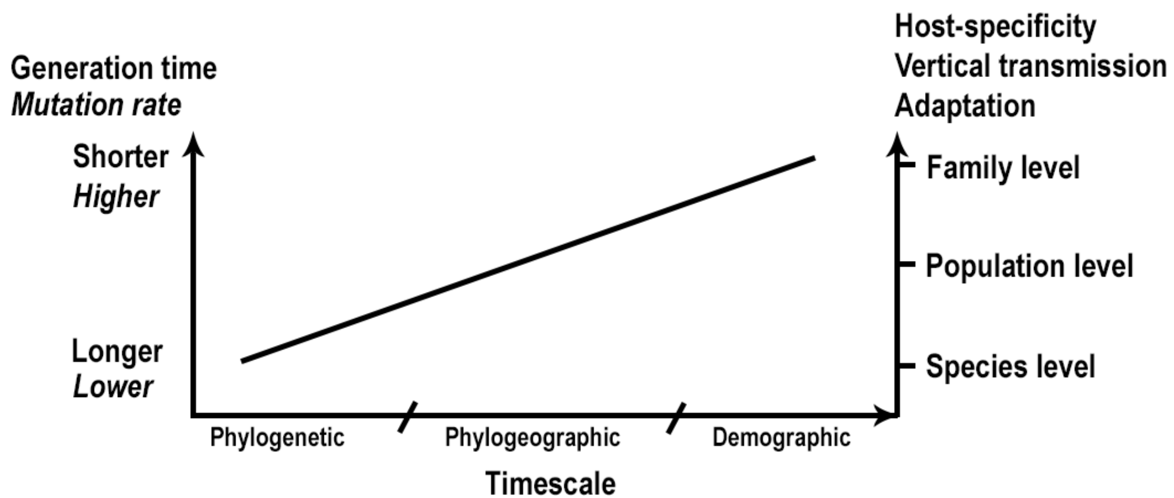


Fig. 3: Relationship between selected parasite characteristics and different study timescales important to assess the resolution of a host-parasite interaction. Adapted from Nieberding & Olivieri, (2007).

As mentioned above, phylogeographical or population genetic data can be further useful to trace the introduction of invasive parasite or host species (Jiménez-García, Vidal-Martínez & López-Jiménez, 2001; Ondračková, Matějusová & Grabowska, 2012; Huyse et al., 2015). In the context of fish introductions, parasites, mostly overlooked in invasion biology, can cross the host species barrier and become more dangerous for non-

adapted host populations or species (Kelly et al., 2009). Such a spillover effect was already reported in susceptible populations of Atlantic salmon devastated by infection of introduced populations of *Gyrodactylus salaris* Malmberg, 1957 (Mo, 1994) and as a consequence of the introduction of *Oreochromis niloticus* in Madagascar (Šimková et al., 2019). Globally, one of the most co-introduced and invasive parasite so far reported is *Schyzocotyle acheilognathi* (Yamaguti, 1934), the Asian fish tapeworm now reported to colonise all continents expect Antarctica (see overview in Pérez-Ponce de León et al., 2018). In Africa, the overall helminthological diversity including possible co-invasions is understudied and geographically biased towards certain countries (Poulin et al., 2019).

The link between panmixia and pelagic habitat use is poorly understood. Lake Tanganyika and its diverse fish fauna provide opportunities to test this hypothesis in pelagic fishes, which are presumed to be highly mobile and unrestricted in their movement by physical barriers. Niche partitioning according to food preferences or water depth has been suggested to have played a crucial role in the diversification of pelagic cichlid groups in the lake (Coulter, 1991b). As a freshwater lake, and therefore a closed ecosystem, Lake Tanganyika enables us to study general mechanisms in pelagic fisheries targets not only from the perspective of evolutionary processes but also ecological principles so difficult to tackle in the marine pelagic environment. By focusing on the parasite fauna of the lake's pelagic fish groups (see chapter 3.3.1), we create a unique opportunity to 1) compare parasite diversification patterns with the highly species-rich littoral zone, 2) investigate the link between host population density and parasite host-specificity, 3) study the differences in diversification between monoxenous and heteroxenous parasitic taxa and 4) map the parasites' population structure on a lake-wide scale. Moreover, as one of the sardine species has been introduced as a fisheries target to several non-native areas, the potential co-introduction of parasites should be verified. Given the high ecological and economic importance of pelagic fish groups in Lake Tanganyika, information about their population structure is crucial for fisheries management. To consider the applicability of parasitological investigations, the potential of parasite fauna as a tag for stock identification of fisheries targets in the lake should be evaluated.

4 MATERIAL & METHODS

4.1 Data collection

4.1.1 Sampling design

Samples of 7 host species, namely one representative of the cichlid tribe Bathybatini (*Hemibates stenosoma*), two species of clupeids (*Limnothrissa miodon and Stolothrissa tanganicae*) and four species of latid predators (*Lates angustifrons, L. mariae, L. microlepis and L. stappersii*), originated from localities throughout Lake Tanganyika including all three subbasins of the lake. Apart from these Lake Tanganyika endemics, specimens of *L. niloticus* were examined from several localities across Africa. Moreover, introduced populations of *L. miodon* in Lake Kivu and the hand-made dams Kariba and Ithezi-Tezhi were checked for potential parasite co-introduction. Fish specimens were either bought at fish markets or caught with gills nets during experimental fishing. To complete the taxon coverage and include geographical variation, fishes from the ichthyology collection of the Royal Museum for Central Africa (Tervuren, Belgium) were also dissected and included in the study. Fish specimens were identified to the species level *in situ* in combination with molecular characterisation in problematic cases. Host species examination followed the standard protocol of Ergens and Lom (1970). Infection parameters such as prevalence (percentage of infected hosts), infection intensity (mean number of parasite individuals per infected host) and abundance (mean number of parasites individuals per total number of hosts examined) were calculated following Ergens and Lom (1970).

4.1.2 Staining procedure

Since the host specimens from some localities were fixed prior to dissection, two different fixation methods were used for the monogeneans. While individuals collected from fresh fish specimens were placed on a slide in a drop of glycerine ammonium picrate (GAP) solution in 1:1 ratio, ethanol and formaldehyde-preserved samples were cleaned of host tissue in a drop of water followed by adding Hoyer's solution. In both cases, the procedure was followed by fixation under a cover slip. Part of the monogenean individuals were cut into three parts with the anterior and posterior parts mounted on slides and the rest used for genetic characterisation. Fish specimens examined for the presence of parasitic flatworms in the framework of this PhD are summarised in Table 1.

Endoparasitic flatworms were rinsed and cleaned alive in a Petri dish with saline solution. Subsequently, most of the saline water was gently removed using a pipet and

specimens were killed by pouring near-boiling water. Larvae of tapeworms were mounted between slide and cover slip in a drop of GAP and cover slip. Individuals of digeneans were preserved either in 4% formalin, 70% or 96% ethanol. Specimens preserved in 4% formalin or 70% ethanol were stained with acetocarmine dehydrated through a graded ethanol series, cleared in dimethyl phthalate and examined as permanent mounts in Canada balsam. Specimens of digeneans to be used for genetic characterisation were mounted on a slide with a little drop of water under a cover slip after which pictures of the details of internal organs were taken for further morphological analysis. Subsequently, a piece from the posterior part of specimens preserved in 96% (the post-testicular region) was excised and used for DNA isolation and the remainder kept as a molecular voucher. Specimens of lates perches examined for the presence of endoparasitic flatworms in the framework of this PhD are summarised in Paper V, Table 2.

Table 1: Host specimens examined in the framework of this PhD.

Host species	**Locality**	**Locality – basins in Lake Tanganyika** (Danley et al., 2012) **or other localities**	**Date of sampling**	**Number of fish specimens (accession number in RMCA or HU)**
Hemibates stenosoma	Mpulungu (8°46'S-31°07'E)	The southern basin	26.09.2018	4 (-)
Limnothrissa miodon	Baraka (4°05'S-29°06'E)	The northern basin	29.07.2017	24 (-)
	Bujumbura (3°23'S-29°22'E)	The northern basin	1.7.– 31.7.1928	2 (MRAC 23567-68)*
	Bujumbura	The northern basin	1.2.– 28.2.1935	11 (MRAC 43554-64)*
	Bujumbura	The northern basin	12.04.2018	30 (-)
	Chituta Bay (8°43'S-31°09'E)	The southern basin	26.8.2017	80 (-)
	Ilagala (5°14'S-29°47'E)	The northern basin	24.02.1947	8 (MRAC 89211-18,41)*
	Kalemie (5°56'S-29°12'E)	The central basin	22.10.1946	8 (MRAC 88891-89098)*
	Kalemie	The central basin	1.1.–31.1.1946	1 (MRAC 89151)*
	Kalemie	The central basin	22.10.1946	8 (MRAC 89137-144)*
	Kalemie	The central basin	11.08.2016	10 (-)
	Kalemie	The central basin	12.04.2018	20 (-)
	Kasaba Bay (8°31'S-30°42'E)	The southern basin	05.03.1947	1 (MRAC 89353)*
	Kasaba Bay	The southern basin	01.01.1967	2 (MRAC 190150-151)*
	Kigoma Bay (4°88'S-29°61'E)	The northern basin	12.4.–13.4.1947	4 (MRAC 89367-70)*
	Kiranda (7°25'S-30°36'E)	The southern basin	11.03.1947	2 (MRAC 89311-12)*
	Kivugwe (3°80'S-29°34'E)	The northern basin	22.02.1994	7 (MRAC 94069.0369-70)*
	Luhanga (3°52'S-29°15'E)	The northern basin	26.04.1994	2 (MRAC 94069.2375-76)*
	Moba Bay (7°03'S-29°47'E)	The central basin	21.03.1947	2 (MRAC 89335-36)*
	Mpulungu (8°46’S-31°07’E)	The southern basin	14.03.1966	3 (MRAC 189612-14)*
	Mpulungu	The southern basin	19.08.2016	2 (-)
	Mpulungu	The southern basin	7.4.– 21.4.2018	81 (-)
	Mvugo (4°18'S-29°34'E)	The northern basin	04.08.2016	6 (-)
	Mvuna Island (7°26'S-30°32'E)	The southern basin	18.08.2015	6 (-)
	Near Ruzizi (2°50'S-29°02'E)	The northern basin	02.12.1954	2 (MRAC 99633-34)*

	Near Ruzizi	The northern basin	26.10.1954	4 (MRAC 99623-32)*
	Rumonge (3°58'S-29°25'E)	The northern basin	1.2.1–28.2.1935	12 (MRAC 43763-72)*
	Uvira (3°22′S-29°09′E)	The northern basin	12.08.2016	41 (-)
	Uvira	The northern basin	12.04.2018	30 (-)
Stolothrissa tanganicace	Bujumbura	The northern basin	04.08.2016	29 (-)
	Bujumbura	The northern basin	12.04.2018	30 (-)
	Kalambo Lodge (8°59'S-31°18'E)	The southern basin	20.08.2016	48 (-)
	Kalemie	The central basin	9.2.–10.2.1947	6 (MRAC 89428-33)*
	Kalemie	The central basin	12.08.2016	33 (-)
	Kalemie	The central basin	12.04.2018	30 (-)
	Kigoma Bay	The northern basin	12.4.–13.4.1947	4 (MRAC 89494-98)*
	Kigoma Bay	The northern basin	13.05.1947	6 (MRAC 89462-65)*
	Mpulungu	The southern basin	4.7-5.7.1965	7 (MRAC 189618-19)*
	Mpulungu	The southern basin	03.10.1966	2 (MRAC 189595-601)*
	Mpulungu	The southern basin	19.08.2016	18 (-)
	Mpulungu	The southern basin	7.4.–21.4.2018	84 (-)
	Musende Bay (8°46'S-31°06'E)	The southern basin	07.04.1967	5 (MRAC 190171-74)*
	Mvugo	The northern basin	15.08.2015	6 (-)
	Rumonge	The northern basin	1.1.–31.12.1935	18 (MRAC 43763-72)*
	Utinta Bay (7°10'S-30°53'E)	The southern basin	17.02.1947	1 (MRAC 89442)*
	Uvira	The northern basin	01.01.1935	4 (MRAC 43787-88,90,98)*
	Uvira	The northern basin	01.01.1954	2 (MRAC 99603-4)*
	Uvira	The northern basin	12.08.2016	27 (-)
	Uvira	The northern basin	12.08.2016	25 (-)
	Uvira	The northern basin	12.04.2018	25 (-)
Lates angustifrons	Mpulungu	The southern basin	27.07.1967	1 (MRAC 190480)*
	Mpulungu	The southern basin	12.04.2018	7 (-)
	Rumonge	The northern basin	30.06.1967	2 (MRAC 94069.0052-53)*
	Sumbu Bay (08°31'S-30°29'E)	The southern basin	31.03.1947	1 (MRAC 90850)*
Lates mariae	Bujumbura	The northern basin	05.05.1947	5 (MRAC 90908-912)*
	Ilagala (05°12'S-29°50'E)	The northern basin	20.08.1993	3 (MRAC 93152.0318-20)*
	Kilomoni (04°20′S-29°09′E)	The northern basin	12.08.2016	2 (-)
	Mpulungu	The southern basin	27.07.1967	2 (MRAC 190493-94)*
	Mpulungu	The southern basin	16.04.2018	11 (-)
	Mulembwe (06°07′S-29°16′E)	The central basin	09.04.2010	7 (-)
	Nyanza Lac (04°20'S-29°35'E)	The northern basin	01.01.1997	1 (MRAC 53738)*
	Rumonge	The southern basin	30.06.1994	1 (MRAC 94069.0067)*
	Sumbu Bay	The southern basin	31.03.1947	2 (MRAC 90878-79)*
	Uvira	The northern basin	12.08.2016	2 (-)
Lates microlepis	Bujumbura	The northern basin	04.05.1947	5 (MRAC 90805-9)*
	Mutondwe Island (08°42'S-31°07'E)	The southern basin	16.04.2018	8 (-)
	Edith Bay (06°30'S-29°55'E)	The southern basin	30.05.1947	3 (MRAC 90833-35)*
	Katukula (08°35'S-31°10'E)	The southern basin	14.04.2018	5 (-)
	Moba Bay (07°03'S-29°47'E)	The central basin	30.12.1995	2 (MRAC 90725-6)*
	Mpulungu	The southern basin	13.4.–17.4.2018	14 (-)
	Nyanza Lac	The northern basin	01.01.1937	11 (MRAC 53698-703; 53725-29)*
	Sumbu Bay	The southern basin	09.04.1995	3 (MRAC 95096.1192,98,99)*

	Uvira	The northern basin	12.08.2016	7 (-)
Lates stappersii	Karala (05°33'S-29°28'E)	The northern basin	10.04.1947	1 (MRAC P90928)*
	Kasasa (08°31'S-30°42'E)	The southern basin	06.09.1967	3 (MRAC 190126;35-6)*
	Mpulungu	The southern basin	12.04.2008	3 (-)
	Mpulungu	The southern basin	06.04.2018	3 (-)
	Uvira	The northern basin	12.08.2016	28 (-)
		Other localities		
Limnothrissa miodon	Sanyati East Basin (16°59'S-28°82'E)	Lake Kariba	14.04.2016	19 (HU 49 - 67)
	Sanyati East Basin	Lake Kariba	05.05.2016	23 (HU 81 - 104)
	Sanyati East Basin	Lake Kariba	07.06.2016	23 (HU 105 - 121)
	Sanyati East Basin	Lake Kariba	05.07.2016	19 (HU 68-80)
	Sanyati East Basin	Lake Kariba	05.01.2017	22 (HU 151 - 170)
	Sanyati East Basin (16°60'S-28°87'E)	Lake Kariba	06.12.2017	9 (HU 171-179)
	Bukavu (2°29'S-28°51'E)	Lake Kivu	11.08.2016	42 (MRAC P.2016.20)*
	Cyangugu (2°47'S-28°90'E)	Lake Kivu	04.09.1979	13 (MRAC 79031.0197-200)*
	Gissenyi (1°70'S-29°25'E)	Lake Kivu	15.08.1979	2 (MRAC 79031.0071,72)*
	Kamiranzovu (2°25'S-29°13'E)	Lake Kivu	07.09.1979	34 (MRAC 79031.0284-88)*
	Kamiranzovu (2°25'S-29°13'E)	Lake Kivu	26.12.1979	2 (MRAC P80029.1164,65)*
	Kibuye (2°06'S-29°34'E)	Lake Kivu	17.08.1979	21(MRAC 79031.0086-88, 111, 112)*
	Kigufi (1°75'S-29°28'E)	Lake Kivu	08.08.1979	12 (MRAC 79031.0010-11, 28, 29,30)*
	Nyabahanga (2°04'S-29°22'E)	Lake Kivu	27.08.1981	10 (MRAC 81055.8524, 54-57, 90228,29)*
	Itezhi Tezhi (15°45'S-26°00'E)	Itezhi Tezhi Dam	17.5.2018	24 (-)
Lates niloticus	Bahr-Sara (08°56'N-17°58'E)	Tchad	1.–31.3.1965	1 (MRAC 154006)*
	Kisumu (00°06'S-34°45'E)	Lake Victoria	17.12.1991	3 (MRAC 91104.37-39)*
	Kossou (07°10'N-05°20'E)	Ivory Coast	17.12.1973	5 (MRAC 74014.328-29; 2755-56)*
	Lake Nasser (24°05'N-33°00'E)	Egypt	26.2.–11.3.1984	3 (MRAC 84006.0116-18)*
	Lake Nasser	Egypt	1.9.–30.9.1983	2 (MRAC 83030.0114-15)*
	Luxor market (25°42'N 32°38'E)	Egypt	24.11.2000	1 (MRAC 190480)*
	Njala, riv. Taja (08°06'N-12°04'E)	Sierra Leone	12.04.1969	5 (MRAC 73010.7057-61)*
	Nyawiega (01°28'N-30°56'E)	Lake Albert	21.11.–6.12.1989	3 (MRAC 89059.0279)*
	Nzunzu (01°19'N-30°72'E)	Lake Albert	5.4.–6.4.2017	1 (MRAC 2016.036.P)*
	Nzunzu	Lake Albert	5.4.–6.4.2017	11 (MRAC 2016.036.P)*

* Only one gill arch examined in the case of specimens retrieved from the RMCA. Internal organs for the presence of endoparasitic flatworms were examined only during own field work off Uvira in 2016 and at the southern part of the lake (off Katukula, Mutondwe Island and Mpulungu) in 2018.

4.1.3 Morphometrics and geomorphometrics

Since the vast majority of parasite specimens collected during this PhD are representatives of Monogenea and Digenea, further analyses were restricted to these two groups. Morphological species identification and description of monogeneans were based on the

sclerotised haptoral and genital structures. Measurements and photos were taken using an Olympus BX51 microscope with incorporated phase contrast at a magnification of 1000x (objective x100 immersion, ocular x10) with MicroImage 3.1. In total, 25−29 different features, respectively, were measured on each individual dependent on genus classification. To characterize the internal anatomy of newly described genus, some specimens were stained using the Carmine method described by Justine (2005) without the initial step of putting a live parasite under a cover slip. The terminology in monogeneans combined Řehulková et al., (2013), Pariselle et al., (2015a) and Justine & Henry (2010). Stained specimens of digeneans were photographed using Leica Application Suite v.4.3.0. analysis software (magnification of 100–1000×), drawn at a high magnification and digitalised using Adobe Illustrator. In total, 33 different parameters were measured in both immature and mature individuals (see Table 1 in Paper V). Terminology was based on (Miller and Cribb, 2008).

Type and voucher material was deposited in several curated collections in Europe and in Africa. To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN) (International Commission on Zoological Nomenclature, 2012), details of each species have been submitted to ZooBank.

Phenotypic variation of monogenean species infecting sardines related to geographic origin was further studied by complex shape analysis to provide a view additional to classical morphometrics (linear measurements). Geomorphometric data were obtained by digitising the shape of dorsal and ventral anchor, respectively, using landmarks and semi-landmarks. For this, Dig v2.30 (Rohlf, 2006) from the thin-plate spline (TPS) packages (Rohlf, 2006) was used. Anchors were chosen because their shape was successfully used in intraspecific studies on members of *Ligophorus* Euzet & Suriano, 1977 (Monogenea, Dactylogyridae) (Rodríguez-González et al., 2015). The shape of other monogenean sclerotised structures, such as bars and marginal hooks, was shown to be highly related to the method of sample preparation (Vignon et al., 2011).

4.1.4 DNA extraction and genetic characterisation

Species delimitation based on morphological characters was combined with genetic characterisation conducted using a range of nuclear and mitochondrial markers with different rates of molecular evolution. To genetically verify parasite species delineation, we

opted for nuclear gene portions of ribosomal DNA regions frequently used in monogenean and digenean taxonomy from the small and large ribosomal subunit gene (18 and 28 rDNA) and the first internal transcribed spacer region (ITS-1). To assess intraspecific genetic diversity, part of the mitochondrial COI gene was used.

Whole genomic DNA was extracted using several different methodologies and isolation kits (for more details see Material & Method section of Papers I−V). Partial 18S rDNA together with ITS-1 were amplified using the S1 primer (5-ATT CCG ATA ACG AAC GAG ACT-3´) (Sinnappah et al., 2001) combined with the Lig5.8R primer (5´-GAT ACT CGA GCC GAG TGA TCC-3´) (Blasco-Costa et al., 2012) for monogeneans. Primers C1 (5´-ACC CGC TGA ATT TAA GCA T-3´) and D2 (5´-TGG TCC GTG TTT CAA GAC-3´) (Hassouna et al., 1984) were used for amplification of the partial 28S rDNA gene in monogeneans. Part of the mitochondrial COI gene in the case of monogenean parasites was amplified using ASmit1 (5'-TTT TTT GGG CAT CCT GAG GTT TAT-3') (Littlewood et al., 1997) combined with Schisto3 (5'-TAA TGC ATM GGA AAA AAA CA-3') (A E Lockyer et al., 2003), and with ASmit2 (5'-TAA AGA AAG AAC ATA ATG AAA ATG-3') in a nested PCR (Littlewood et al., 1997). To increase amplification success another set of COI primers was used. The first PCR reaction was performed with the primer combination Mono5 (5'-TAA TWG GTG GKT TTG GTA A-3') and Mono3 (5'-TAA TGCA TMG GAA AAA AAC A-3') followed by a nested PCR reaction with Mono5 and Mono3-int (5'-ACA TAA TGA AAR TGA GC-3') (Plaisance et al., 2008). Species distinction of digeneans was verified using a portion of 28 rDNA amplified with primer combinations digl2 (forward: 5'-AAG CAT ATC ACT AAG CGG-3') or LSU5 (forward: 5'-TAG GTC GAC CCG CTG AAY TTA AGC A-3') and 1500R (reverse: 5'-GCT ATC CTG AGG GAA ACT TCG-3') (Tkach et al., 2003). A COI gene portion was amplified using JB3 (forward: 5'-TTT TTT GGG CAT CCT GAG GTT TAT-3'; Bowles et al. 1992) and CO1-R (reverse: 5'-CAA CAA AAT CAT GAT GCA AAA GG-3'; Miura et al. 2005). Details of amplification protocols are given in the M&M parts in Papers I – V.

The PCR products were visualized using horizontal gel electrophoresis using a GoldView stained agarose gel (1%) followed by enzymatic purification using either the High Pure PCR Product Purification Kit (Roche), ExoSAP-IT reagent or QIAquick PCR purification kit (Qiagen Ltd., Hilden, Germany) according to the manufacturer's instructions. Identical primers as in the amplification reactions were used for sequencing with a Big Dye Chemistry Cycle Sequencing Kit 3.1, following the manufacturer's

recommendations. Fragments were cleaned using the BigDye XTerminator Purification Kit and visualized on an ABI3130 capillary sequencer or outsourced to Macrogen Sequencing Core in Amsterdam.

4.2 Data analysis

4.2.1 Morphometrics

To analyse interspecific morphological differences and check for within-species variation in haptor morphology of monogeneans, a principal component analysis (PCA) was performed on linear haptoral measurements of monogenean parasite species in the R package Adegenet (Jombart, 2008). Missing data were replaced by the average value, while morphological characters with more than 50% of missing data were omitted from the analyses. Outliers were visually checked or identified and removed using Mahalanobis distances in the package mvoutlier (Filzmoser and Gschwandtner, 2017). To take possible geographical intraspecific variation into consideration, samples were also grouped into three basins according to (Danley et al., 2012). The effect of host species, season (dry period from May to September, wet period from October to April), geographic origin, sampling size and host body size on haptoral morphometrics of monogenean species infecting clupeids was tested using ANOVA in STATISTICA v12 or in R, package stats (R Core Team, 2013), with Pillai's test of significance. Geographic structure and overall significance of the particular morphological characters of monogenean species infecting clupeids was further analysed in relation to sampling site origin (see M&M in Paper III) using a linear or generalised linear model approach corrected for host size differences followed by F-statistics and a generalised linear model approach followed by Chi Square statistics, respectively, conducted in the R package stats (R Core Team, 2013). Significance between the groups was assessed post-hoc by ANOVA and Kruskall-Wallis tests, respectively, with Bonferroni correction. To visualise the variance in the total size of the ventral anchor of monogeneans species infecting lates perches, a density plot using uncorrected measurements was drawn using ggplot2 (Wickham, 2009) and factoextra (Kassambara and Mundt, 2017). The assumption of homogeneous variance within our sample groups was verified by Levene's test in the R package stats (R Core Team, 2013). Normality of the data was checked with the Shapiro-Wilks test in the R packages stats or onewaytests (Dag et al., 2018). Additionally, Two-sample T tests with Bonferroni correction were performed to provide information about intraspecific variability in copulatory organ morphometric parameters of species infecting sardines related to host

species, host size, season and geographic origin. Non-parametric variants, namely Kruskal-Wallis ANOVA or Mann-Whitney U were performed where the assumptions were not met.

4.2.2 Geomorphometrics

The degree of shape deformation of anchors within-species was quantified by estimating the minimal shape parameters (relative warps) needed to deform the consensus configuration to the anchor of each specimen computed from partial warps using TPSRelw v1.6 (Rohlf, 1993). To visualize mean shape anchor differences of monogenean species, thin-plate spline deformation grids were depicted in TPSSpline. The overall shape of dorsal and ventral anchors, captured using fixed landmarks and semi-landmarks, was analysed using tps Relw v1.49. A Relative Warp Analysis (RWA) (Rohlf, 1993) was performed with the Procrustes coordinates. Configurations of fixed landmarks were superimposed using Generalized Full Procrustes Analysis (Cox and Cox, 1989; Zelditch et al., 2012) under the Least Squares criterion to minimize bending energy with respect to a mean reference form. Additionally, canonical variate analysis (Klingenberg and Monteiro, 2005) and PCA using fixed landmarks only were performed on the anchor shape of monogenean species infecting sardines in MorphoJ v2.0 (Klingenberg, 2011). A permutation test with 10,000 iterations was used to statistically validate pairwise differences between pre-defined groups.

In the analyses including monogenean species collected from clupeids, relationships between the individual scores inferred with PCA and RWA analyses, respectively, and the host size were checked via linear regression analyses. Further, t and F-statistics were calculated in the R package stats (R Core Team, 2013). Potential correlation between the host size and each of the tested morphological or shape characters was tested via Pearson's and Kendall's correlation coefficient, respectively. Additionally, t and Z statistics, respectively, were calculated in the R package ggpubr (Kassambara, 2018).

4.2.3 Population dynamics

Population dynamics of monogenean species infecting clupeids in relation to host standard length were analysed by parameters of intensity of infection and observed incidence (infected/not infected). Fish specimens collected during recent field works in Bujumbura (April 2018), Kalambo Lodge (August 2016), Kalemie (August 2016, April 2018), Mpulungu (April 2018, August 2016) and Uvira (August 2016, April 2018) were included in this preliminary study. Generalised linear model approach with Chi Square post hoc test of significance in the R package lme4 (Bates et al., 2015) was applied to analyse potential effect of host size on infection intensity and incidence of parasite species. Because of

possible non-independence in our dataset, intercept of both locality and month of origin were treated as random factors.

4.2.4 Phylogenetic reconstruction

Phylogenetic analyses were based on the above-mentioned ribosomal DNA regions and were performed at the family level for each taxon of parasites analysed. Additionally, a restricted dataset of 10 species of *Acanthostomum* together with newly obtained sequences of digeneans was analysed separately (see M&M sections in Papers I, IV and V). In the case of monogeneans, newly obtained sequences from all three ribosomal gene portions that were sequenced (28S, 18S and ITS-1) were combined with previously published sequences of representatives of freshwater and marine species of the respective family. Alignment matrices of both ribosomal regions used for reconstruction were concatenated using Mesquite v3.2 (Maddison and Maddison, 2017) or analysed separately in the case of lack of sequences for which both regions were available. Topali v2.5 (Milne et al., 2004) or jModelTest v.2 (Darriba et al., 2012), were used to select the 'best-fitting' model of sequence evolution under the Bayesian information criterion.

Electropherograms were visually inspected and sequences of monogeneans were aligned using MUSCLE (Edgar, 2004) under default distance measures as implemented in MEGA v7 (Kumar et al., 2016) or in MAFFT (Katoh and Standley, 2013) on the EMBL-EBL bioinformatics web platform (http://www.ebi.ac.uk/Tools/msa/mafft/) in the case of digenean parasites. Pairwise distances were calculated in MEGA v.7 (Kumar et al., 2016). The consistency of each alignment was checked and corrected using a heuristic search method implemented in trimAl v1.2 (Capella-Gutiérrez et al., 2009). Highly variable parts of the alignments including sequences of digeneans were identified and excluded by Gblocks (Castresana, 2000) as implemented in SeaView v4 (Gouy et al., 2010) under less stringent parameters and refined by eye.

Phylogenetic analyses were carried out using maximum likelihood (ML) and Bayesian inference (BI) in RAxML v8 (Stamatakis, 2014) and MrBayes v3.2.0 (Ronquist et al., 2012), respectively (for details see M&M of papers II, V and VI). As Dactylogyridae and Diplectanidae were shown to be sister taxa (Šimková et al., 2003), *Dactylogyrus extensus* (Mueller and Van Cleave, 1932) and *Diplectanum aequans* (Wagener, 1857) were used as outgroups in the respective phylogenetic reconstructions. The outgroup choices for

digenean phylogenetic reconstruction were informed by broader phylogenies of the subclass (Olson et al., 2003).

4.2.5 Population genetic structure and demographic history

Intraspecific genetic diversity and population structure of monogenean species were assessed by sequences of the mitochondrial COI gene. The number of haplotypes and polymorphic sites, haplotype diversity and nucleotide diversity were calculated using Arlequin v3.5 (Excoffier and Lischer, 2010). The genealogy of COI haplotypes of monogeneans was inferred by means of a Median Joining network in PopART v1.71 (Leigh and Bryant, 2015). Differentiation among pre-defined populations was estimated by Fst statistics in Arlequin v3.5 (Excoffier and Lischer, 2010). Geographically complex genetic structure among pre-defined monogenean populations considering subbasin origin was analysed by AMOVA as incorporated in Arlequin v3.5 (Excoffier and Lischer, 2010).

Demographic history was investigated in monogenean species infecting clupeids. Population size changes were inferred by mismatch distribution analyses of haplotypes and subsequently tested by means of two different neutrality test statistics, Tajima's D (Tajima, 1989) and Fu's FS (Fu, 1997) in Arlequin v3.5 (Excoffier and Lischer, 2010). Past population size trajectories were assessed by employing a Bayesian coalescent approach (Bayesian skyline plot) (Drummond et al., 2005) as implemented in BEAST v1.8.2 (Suchard et al., 2018). The substitution rate used was decreased to 10% compared to 13.7–20.0% estimated for representatives of *Gyrodactylus* (Meinilä et al., 2004) given the expected longer generation time of species of *Kapentagyrus* as members of Dactylogyridae. Detailed info about the number of independent MCMC runs, generations, sampling frequency and burn-in phase is provided in the methodological section of each manuscript.

5 SUMMARY OF RESULTS

This chapter is divided into three main subchapters based on the host groups examined for the presence of parasitic flatworms. The results presented in this chapter are based on manuscripts (already published, accepted or under revision in scientific journals) and results in preparation. The manuscripts in each subchapter are listed chronologically.

5.1 Clupeids

Paper I

Kmentová N., Van Steenberge M., Raeymaekers J.A.M., Koblmüller S., Hablützel P. I., Muterezi Bukinga F., Mulimbwa N'sibula T., Masilya Mulungula P., Nzigidahera B., Ntakimazi G., Gelnar M., Vanhove M. P. M. 2018. Monogenean parasites of sardines in Lake Tanganyika: diversity, origin and intra-specific variability. *Contributions to Zoology* 87(2): 105-132 [Q1, IF (2018) = 2.139].

Whereas the littoral zone of Lake Tanganyika harbours a rich aggregation of mainly cichlids, the pelagic zone is relatively species poor. It is dominated mainly by two clupeid species, endemic to the lake and the basis of a vital fishery. These fishes, like many invertebrate lifeforms in the lake, were historically often considered typical to marine or brackish waters. Our study is the first comprehensive report on the monogenean fauna of the economically most important fish species in Lake Tanganyika. Based on our findings, two monogenean species of *Kapentagyrus* Kmentová, Gelnar and Vanhove, gen. nov. were identified combining morphological and molecular characterisation. One of the species, *Kapentagyrus limnotrissae* comb. nov., is host-specific to *L. miodon* while its congener, which is new to science and described as *Kapentagyrus tanganicanus* Kmentová, Gelnar and Vanhove, sp. nov., infects both clupeid species. The erection of *Kapentagyrus* n. gen. was further supported by phylogenetic reconstruction at the family level using three ribosomal markers, with the two species from Lake Tanganyika representing a quite distinct lineage. Interestingly, two different morphotypes of *K. tanganicanus* sp. nov. were recognised. This situation is probably correlated with an adaptation of haptoral structures to the host identity as suggested in previous studies. Moreover, significant differences in the genital parts between *K. tanganicanus* collected from different host species could indicate an incipient reproductive barrier. Significant intra-specific differences in haptor morphometrics between the northern and southern end of Lake Tanganyika were found and

support a potential geographically driven structure and the use of monogeneans as tags for host population structure. Moreover, an influence of season and host size on the haptoral sclerites was reported. So far, this African freshwater sardine lineage is the only clupeid lineage known to be infected by dactylogyrid monogeneans.

Paper II

Kmentová N., Van Steenberge M., van den Audenaerde D.R., Nhiwatiwa T., Muterezi Bukinga F., Mulimbwa N'sibula T, Masilya Mulungula P., Gelnar M., Vanhove M. P. M. 2019. Co-introduction success of monogeneans infecting the fisheries target *Limnothrissa miodon* differs between two non-native areas: the potential of parasites as tag for introduction pathway. *Biological Invasions* 21(3): 757-773. DOI: https://doi.org/10.1007/s10530-018-1856-3 [Q1, IF (2018) =2.897].

The introduction pathways and source populations of species translocations are often unknown. Unintended co-introduction of parasites to non-native areas might pose often-underestimated threats to ecosystems. However, in many cases, parasites are neglected 'hitch-hikers' without any effect on the native fauna being noticed. Lake Tanganyika sardine, *L. miodon*, was introduced to several natural and artificial African lakes, among which lakes Kivu and Kariba, in 1959 and 1968, respectively. Combining historical collections and recent samples, a difference in co-introduction success of monogenean parasites infecting *Limnothrissa miodon* between Lake Kivu and the man-made Kariba dam was revealed. Out of two species of *Kapentagyrus* infecting *L. miodon* in Lake Tanganyika, only *K. limnotrissae* was collected in the Sanyati East Basin of Kariba dam so far. In contrast, not a single monogenean individual was retrieved from this fish in Lake Kivu. As juveniles of *L. miodon* are suggested to be free of monogenean infection, the size of introduced sardine specimens is proposed as an important factor of co-introduction success. Moreover, as specimens from Kariba are morphologically more like those from the southern part compared to other places in Lake Tanganyika, the potential of using monogeneans as indicators for the introduction pathway and the region of origin should be further investigated.

Paper III

Kmentová N., Koblmüller S., Van Steenberge M., Artois T., De Keyzer E., Muterezi Bukinga F., Mulimbwa N'sibula T, Masilya Mulungula P., Gelnar M., Vanhove M. P. M.

Population structure and demographic history of *Kapentagyrus* spp. (Monogenea: Dactylogyridae) infecting sardine species (Teleostei: Clupeidae) in Lake Tanganyika: an indication of near-panmictic lake-wide occurrence. Under review in *International Journal for Parasitology* (submitted in August 2019).

Recently, helminths were proposed as alternative tags to identify the stock structure and origin of fishes that are not easily traceable by classical techniques, such as migratory and skin-fragile fishes. Given their direct lifecycle, mostly high host-specificity, shorter generation time and higher mutation rate (leading to a so-called "magnifying glass effect") compared to their fish host, monogenean flatworms were proposed as candidates for fish stock structure identification. Fisheries in Lake Tanganyika, the oldest African Great Lake, are mostly dependent on two endemic species of sardines, *L. miodon* and *S. tanganicae*. They are parasitized by two species of *Kapentagyrus* (Monogenea, Dactylogyridae), *Kapentagyrus limnotrissae* and *Kapentagyrus tanganicanus*. Even though these sardines' lake-wide population structure has been studied, no clear pattern has been determined so far. Considering the potential "magnifying glass effect", we investigated the population structure of *Kapentagyrus* spp. as indirect tags for sardine stock identification via a combined morphological and molecular approach. Secondly, as evolution and demographic history of various cichlid species in the lake were reported to relate to historical lake level changes, we investigated the effect of such events on past population changes of *Kapentagyrus* spp. living in the understudied pelagic zone of the lake. Although significant differences in some of the morphological parameters may indicate limited parasite migration, no clear geographical pattern was identified. Population structure of both species of *Kapentagyrus* was analysed to assess its link with their sardine hosts' size and origin. A high frequency of one central haplotype supports a rather unlimited gene flow at a lake-wide scale in both species of *Kapentagyrus*. We hence suggest an overall lack of geographic structure, which corresponds with the results reported for one of the host species in a recent next-generation sequencing-based study. Additionally, we might be witnessing, for the first time for any parasite species in the lake, incipient speciation of *K. tanganicanus* related to host species identity. There is also evidence of historical introgression via hybridisation between the two monogenean species. This is the first time hybridisation is reported in dactylogyrid monogeneans. Furthermore, our findings provide additional support for the impact of lake level changes also on organisms inhabiting the lake's pelagic zone. The suggested magnifying effect was not observed in this host-parasite system as genetic

diversity indices in the COI region show more intraspecific variation in the hosts compared to their monogenean parasites. Our study highlights the overall monogenean morphological plasticity and the limitations of the magnifying glass hypothesis. These should be considered in future studies investigating parasites' potential for host stock identification.

Preliminary results on population dynamics of *Kapentagyrus* spp.

The incidence of parasitic infection can be influenced by many factors related to the host's or the environment. Using an available data set from the recent field expeditions monitoring infection parameters of both species of *Kapentagyrus*, we tested the influence of host standard body length on the level of parasitic infection possibly affected by other factors

In *K. limnotrissae*, both parasite's intensity of infection and incidence seem to be related to the host size (see Fig. 4a&b, Table 2). Based on the plotted results, the highest level of infection intensity occurs on medium-sized host specimens with a peak between 6 – 9 cm of standard length. In *K. tanganicanus* collected from *L. miodon*, a positive relationship between the host body size and both intensity of infection and incidence (Fig. 5a&b, Table 2). Contrary to *K. limnotrissae*, the highest level of infection intensity was reported in fish size >10 cm. Unlike in the case of *L. miodon*, no clear relationship between host size and infection intensity or incidence of *K. tanganicanus* on *S. tanganicae* was documented (Fig. 6a&b). Nevertheless, infection intensity was reported to be significantly influenced by standard length of *S. tanganicae* (Table 2). The result is assumed to be affected by a low prevalence and therefore high number of negative incidences.

The results provided in this chapter are preliminary. Population dynamics of *Kapentagyrus* spp. will be further analysed by adding infection parameters from sampling campaigns in April 2019 as well as August 2018 and 2019.

Table 2: Results of generalised model approach with Chi Square post hoc statistics analysing the effect of host size (SL – standard length) on the parasites' infection intensity and incidence. Only significant factors or interactions are listed.

Parasite species	Host species	Factor	Intensity of infection	Incidence
K. limnotrissae	*L. miodon*	SL	$\chi^2_{(3, 249)}$ =102.64, $P < 0.001$	$\chi^2_{(3, 249)}$ =30.45, $P < 0.001$
K. tanganicanus	*L. miodon*	SL	$\chi^2_{(3, 249)}$ =742.4, $P < 0.001$	$\chi^2_{(1, 249)}$=30.14, $P < 0.001$
K. tanganicanus	*S. tanganicae*	SL	$\chi^2_{(1, 391)}$=6.67, $P=0.009$	-

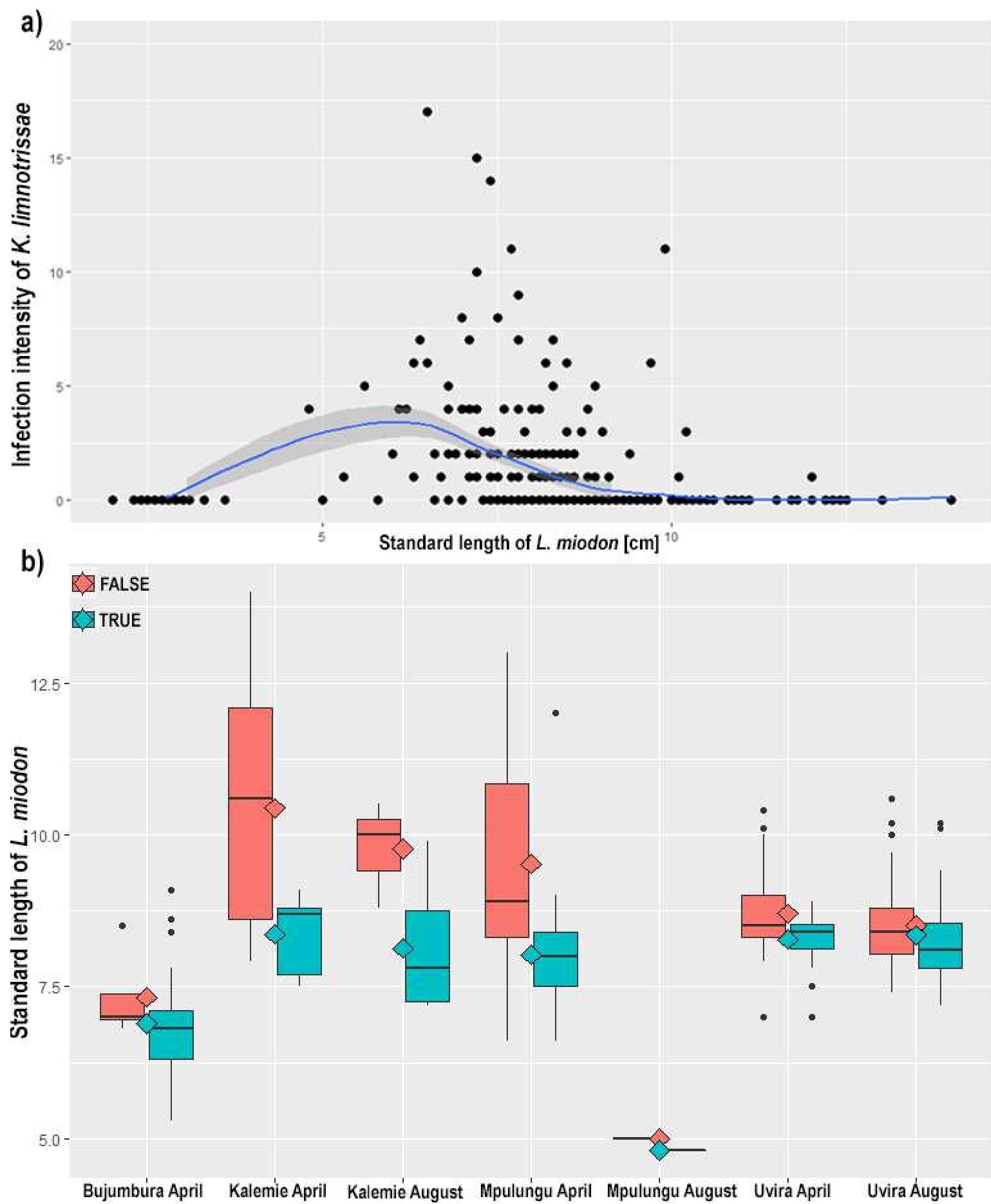


Fig. 4: Relationship between the standard length of *L. miodon* and a) the infection intensity and b) the incidence of *K. limnotrissae*.

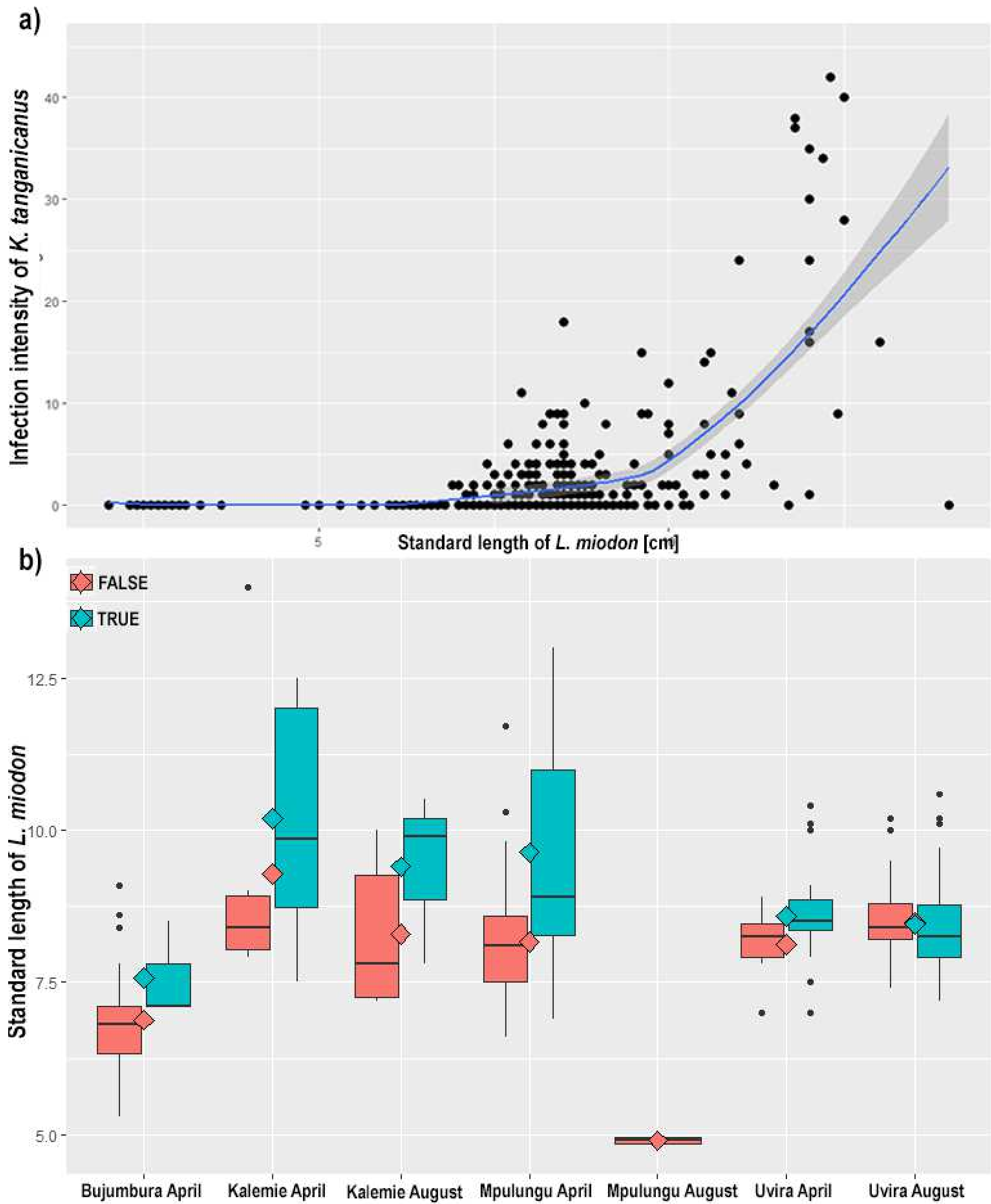


Fig. 5: Relationship between the standard length of *L. miodon* and a) the infection intensity and b) the incidence of *K. tanganicanus*.

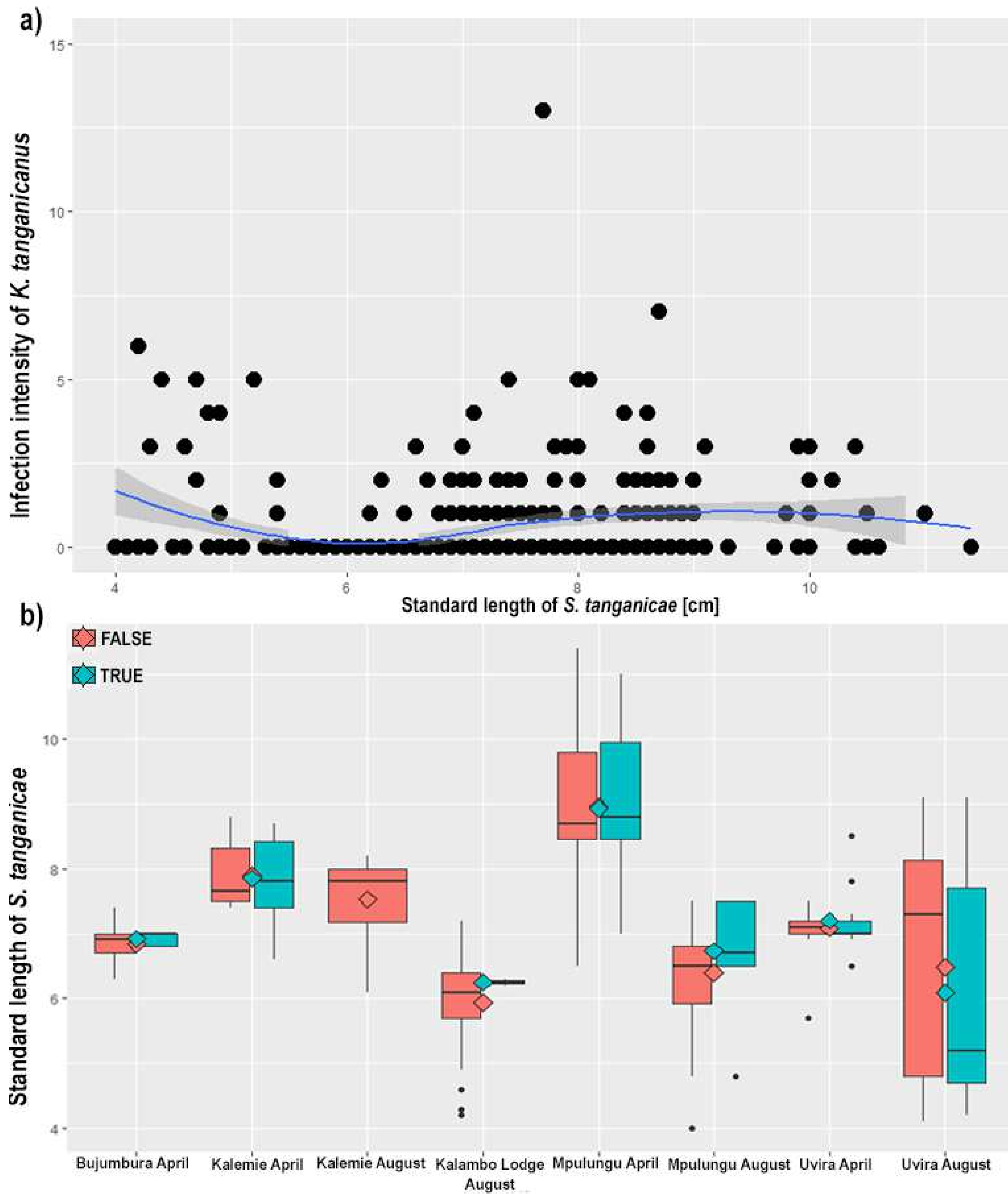


Fig. 6: Relationship between the standard length of *S. tanganicae* and a) the infection intensity and b) the incidence of *K. tanganicanus*.

5.2 Latidae

Paper IV

Kmentová N., Koblmüller S., Van Steenberge M., Artois T., Muterezi Bukinga F., Mulimbwa N'sibula T, Masilya Mulungula P., Muzumani Risasi D., Gelnar M., Vanhove M. P. M. Failure to diverge in African Great Lakes: the case of *Dolicirroplectanum lacustre* comb. nov. (Monogenea, Diplectanidae) infecting latid hosts. Accepted in *Journal of Great Lakes Research* [Q1, IF (2018) = 2.175].

Despite their primary marine origin, *Lates* perches have diversified in African freshwaters including several Great Lakes. While seven diplectanid monogenean species from three different genera were documented from *Lates calcarifer* (Bloch, 1790) from the Indo-Pacific region (Tingbao et al., 2006), only one species was described from African *Lates* spp. so far: *Diplectanum lacustre* Thurston & Paperna, 1969 infecting *Lates niloticus* L. In our study, we asked whether monogenean parasites infecting species of *Lates* underwent similar diversification as their hosts in African freshwaters including intralacustrine speciation in Lake Tanganyika. In total, monogenean individuals from 20 different localities were collected including 12 different locations in Lake Tanganyika. As there was no consistent morphological difference related to host species nor geographical origin, a single species assigned to a newly described diplectanid genus *Dolicirroplectanum* gen. nov., named *D. lacustre* comb. nov. was reported. The genetic distance over the COI region between parasites of geographically isolated host species indicated failure to diverge of *D. lacustre* comb. nov. as it did not reach the level typically associated with distinct diplectanid species. The erection of the new genus was confirmed by phylogenetic reconstruction at the family level with *D. lacustre* comb. nov. placed in a monophyletic clade sister to *Dolicirroplectanum penangi* comb. nov. collected from *Lates calcarifer* in Asia. Futher, a decrease in host-specificity in Lake Tanganyika mirrors the phenomenon seen in other species infecting pelagic hosts in the lake, with no sign of geographical structure at a lake-wide scale. Moreover, as a high portion of identical COI haplotypes were shared among individuals of *D. lacustre* comb. nov. collected from *L. mariae* originating from the central subbasin and *L. microlepis* collected from the northern and southern subbasins of Lake Tanganyika, no geographically dependent restriction in gene flow was detected. Using a maximum of 9 MYA as estimated date of the lakes' formation, we calculated a particularly low mutation rate over COI mtDNA in *D. lacustre* comb. nov. in

comparison to other monogenean species. However, apart from a relatively slow rate of diplectanid molecular evolution, there are several scenarios which could explain this situation.

Paper V

Kmentová N., Bray R., Koblmüller S., Artois T., De Keyzer E., Gelnar M., Vanhove M. P. M., Georgieva S. Uncharted digenean diversity in Lake Tanganyika: cryptogonimids infecting endemic lates perches. Under review in *Parasites & Vectors* (submitted in August 2019).

Despite their high importance for local fisheries, the parasite fauna in the lates perches has been vastly ignored. There is only a single record of a parasite species. In this chapter, three of four species of *Lates* were found infected with at least one digenean species. Detailed morphological descriptions of a total of six cryptogonimid trematodes are provided, all new to science. Two out of three reported digenean genera, *Tanganyikatrema* n. gen. and *Grandifundilamena* n. gen., are new to science. The presence of *Neocladocystis* in the lake, first reported by Prudhoe, 1951, was confirmed, with three new species being recovered (*N. bemba* n. sp., *N. biliaris* n. sp. and *Neocladocystis* sp.). *Neocladocystis biliaris* n. sp. is the first record for cryptogonimids in the gallbladder. Considering their small morphological and genetic differences, we suggest recent diversification of the three reported species of *Neocladocystis* infecting two species of latid hosts in Lake Tanganyika. *Grandifundilamena* n. gen. is a currently monotypic genus with the uncommon character of multiple testes. *Grandifundilamena novemtestes* n. sp is described as the type species. Phylogenetic reconstruction at the level of Cryptogonimidae placed *Neocladocystis* and *Tanganyikatrema* n. gen. in the same clade with species of *Acanthostomum* forming an exclusively freshwater clade in Cryptogonimidae. Our study provides the first molecular data for trematode parasites in lates perches in the lake. The results suggest a higher species diversity compared to a single monogenean species described from this host family in the lake. Moreover, high intraspecific genetic divergence (COI) was documented in *N. bemba* n. sp. This study serves not only as a baseline for future studies on the digeneans of this biodiversity hotspot, but also offers the first molecular data of cryptogonimid digeneans available from Africa.

There are still interesting unpublished results regarding other endoparasite taxa. Regarding digeneans, except for the cryptogonimid digeneans infecting three species of lates perches,

metacercariae were found forming cysts in the gill cartilage of *L. miodon* in some of the museum specimens. This infection site is known only for representatives of Heterophyidae and Didymozoidae. Considering cestodes, procercoid larvae of Proteocephalidae gen. sp. possessing five suckers were found infecting both sardine species and *L. microlepis* in Mpulungu, 2018, always in intestinal mesenteric tissue (identification by prof. T. Scholz). As the overall morphology of proteocephalid procercoids is rather uniform, molecular characterisation is needed for species level identification (Scholz, 1999). Interestingly, the presence of *Schizocotyle acheilognathi* (Yamaguti, 1934), an invasive cestode occurring worldwide, was documented for the first time in an African Great Lake, found attached to the intestinal mesenteric tissue of *L. miodon* in Mpulungu, 2018. So far, establishment of non-native organisms in Lake Tanganyika appears to be limited (Van Steenberge et al., 2011) and the presence of *S. acheilognathi* figures as an important finding to be reported. However, only molecular evidence without any morphological voucher is currently available. Overall, given the lack of genetic data of parasitic flatworms from the lake and from Africa in general, molecular species level identification of the collected larval stages is not very promising. Future studies are needed for life cycle reconstruction of endoparasitic flatworms and to enable formal description.

5.3 Bathybatine cichlids

Preliminary results on geographic structure of *C. casuarinus* infecting *H. stenosoma*

In general, in the African Great Lakes, a lack of phylogeographic structure is presumed for highly mobile species inhabiting pelagic zones, just like in the marine environment. In bathybatine cichlids, pelagic predators inhabiting the eupelagic and bathypelagic zone of Lake Tanganyika, the pattern of lake-wide population differentiation differs among species. This was proposed to be related to their dispersal propensity. To assess geographic structure of *C. casuarinus* infecting bathybatine cichlids in Lake Tanganyika, results of *C. casuarinus* ex *H. stenosoma* as a part of Kmentová et al., (2016) were combined with newly obtained parasite specimens of this host species from the opposite side of the lake off Mpulungu (see Table 1). Collected individuals of *C. casuarinus* were morphologically and molecularly characterised. The total dataset consisted of parasite specimens from the northern basin (off Bujumbura, Uvira and near the Malagarasi river delta) and the southern basin (off Mpulungu). Phenotypic variation was evaluated by a PCA of morphometric data from the haptoral region and by a two-sample t-test using morphometric data from the male

copulatory organ. The population genetic structure was analysed by constructing a median joining haplotype network using a COI mtDNA gene portion of 392 bp fragment size. Population differentiation between parasite individuals originating from Bujumbura and Mpulungu was further tested using Fst statistics in Arlequin (for further details see M&M chapter of this thesis).

Based on the observed mutual position of parasitic individuals in the PCA scatterplot, phenotypic variation related to geographic origin was visible. Moreover, separation to some extent was detected among the individuals from three sampling sites in the northern subbasin. The pattern was influenced mainly by parameters of the anchors and the dorsal bar (Fig. 1). Conversely, no significant differences in the morphology of the parasite's male copulatory organ related to subbasin were detected (see Fig 2). No clear genetic structure related to the geographic origin of samples was visible in the haplotype network (Fig. 3). This is supported by non-significant differentiation between populations from the northern subbasin and Mpulungu (the southern subbasin) (Fst = 0.01030, P = 0.18256 ± 0.0040). Phenotypic variation of *C. casuarinus* was therefore suggested to be influenced by environmental factors rather than by geographically dependent genetic differentiation. Interestingly, the lack of a clear phylogeographic structure in *C. casuarinus* contrasted with the reported north-south gradient seen in the host species, *H. stenosoma* (Koblmüller et al., 2019). Overall, the lower level, compared to the host, of geographically dependent differentiation in *C. casuarinus* ex *H. stenosoma* is proposed to be explained by its generalist lifestyle. Other host species of *C. casuarinus*, such as *B. fasciatus* and *B. leo*, showed no restriction of gene flow, resulting from migration on a lake wide scale, and may hence transport *C. casuarinus* across Lake Tanganyika. However, increased sample size, host species coverage and specimens from the central subbasin are needed to further unravel the geographically dependent morphological and genetic differentiation of *C. casuarinus*.

This study will be completed by applying a geomorphometric approach on the specimens of *C. casuarinus* collected from *H. stenosoma* in Mpulungu, September 2018.

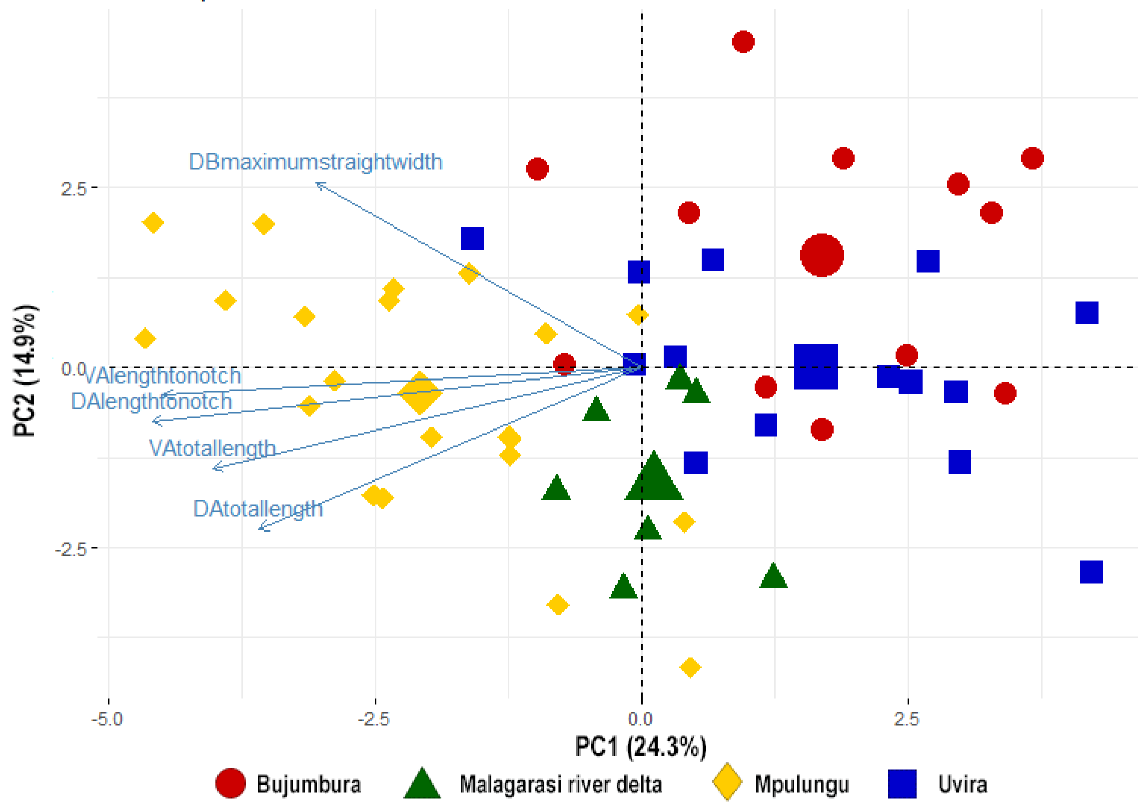


Fig. 1: PCA of haptoral measurements showing the variation in haptoral structures of *C. casuarinus* with the five best contributing variables indicates by arrows

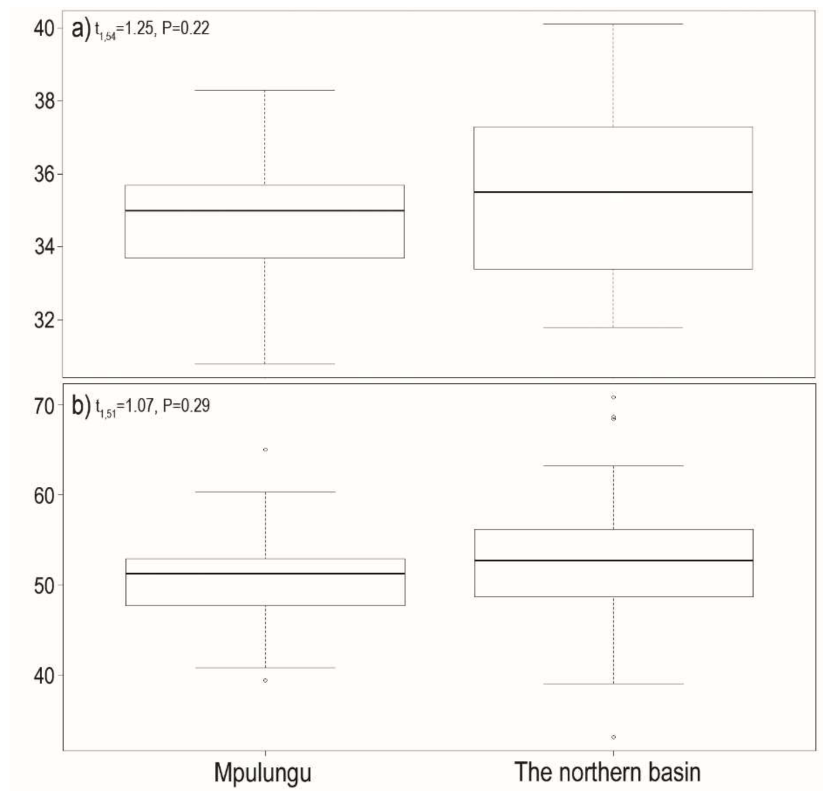


Fig. 2: Box-plots depicting morphometric variation of *C. casuarinus* related to subbasin origin with the specimens and the results of the t-test. a) length of copulatory tube; b) heel length.

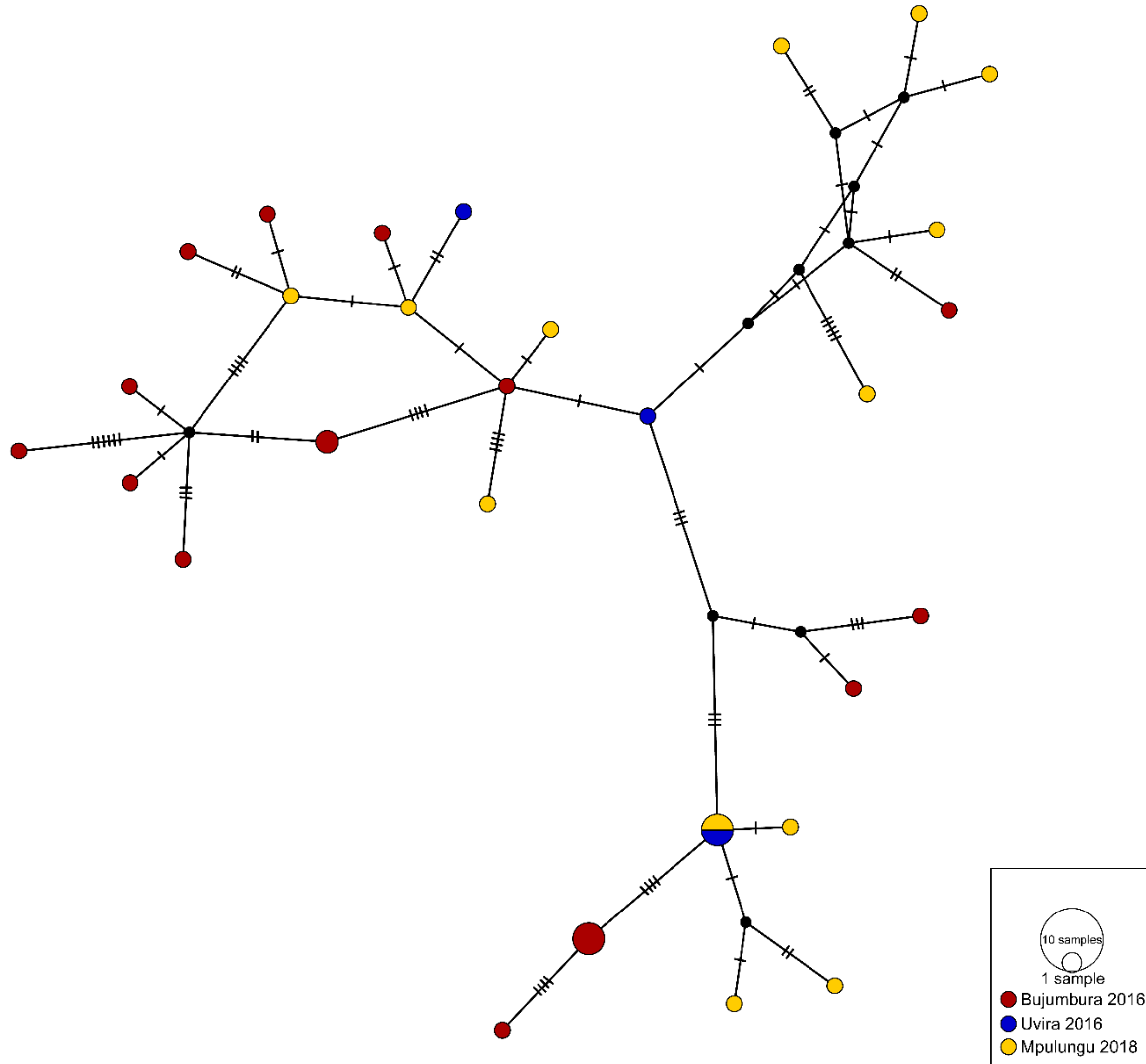


Fig. 3: Median joining haplotype network of *C. casuarinus* ex *H. stenosoma*. The circles represent different haplotypes with their size proportional to the number of individuals represented. Haplotypes are connected with lines, indicating the number of mutations. Small black circles indicate hypothetical haplotypes, predicted by the model. Colours represent geographic origin as mentioned in the legend.

6 DISCUSSION

Parasitic flatworms infecting pelagic fishes in Lake Tanganyika

Within this PhD, three of the economically important fish groups inhabiting the pelagic zone of Lake Tanganyika have been examined for the presence of parasitic flatworms. While bathybatine cichlids and lates perches have been found infected by a single species of monogenean, respectively, the presence of two species of *Kapentagyrus* infecting clupeids was detected. In contrast to this low monogenean diversity, six species from three different digenean genera were recovered from lates perches. In total, the inventory of parasitic flatworms known from the lake was enriched by two monogenean and six digenean species, linked with the erection of four new genera.

Decrease of parasite host-specificity compared to the current state of knowledge in the littoral zone was detected, with *Cichlidogyrus casuarinus* (Kmentová et al., 2016c; Pariselle et al., 2015a) and *Kapentagyrus tanganicanus* listed as the only intermediate generalist parasitic flatworms in the lake so far. Moreover, *Dolicirroplectanum lacustre* appears an intermediate specialist, just like *C. vandekerkhovei, C. makasai, C. centesimus* and *C. sturmbaueri* Vanhove, Volckaert & Pariselle, 2011 recorded from two to three species of *Ophthalmotilapia* (Vanhove et al., 2011b) and *C. franswittei* Pariselle & Vanhove, 2015 infecting two species of *Pseudosimochromis* (Van Steenberge et al., 2015). The predictability and temporal stability of resources influenced by factors such as fish longevity, abundance or position at the food chain play an important role in the evolution of monogenean host-specificity (Rohde, 1989, 1979; Šimková and Morand, 2008). Contrasting patterns in the speciation processes of monogeneans in Lake Tanganyika correspond with different levels of host availability and therefore predictability of resources. Host availability is here approximated by density and population size in the lake's pelagic zone compared to the littoral zone. This phenomenon was previously proposed in the marine environment (Bagge et al., 2004; Justine et al., 2012; Poulin, 1992; Rohde, 1980a, 1980b; Schoelinck et al., 2012). In general, parasite host-specificity is determined by a combination of various factors such as phylogenetic constraints, host hybridisation and complex ecological and evolutionary interactions (Poulin and Mouillot, 2005). Substantial phenotypic variation related to host species identity was recovered, even reaching the interspecific level in the case of *K. tanganicanus*. Low host-specificity of this species was supported by the lack of genetic intraspecific variation within the respective

parasite species across the two different host species. As the nuclear rDNA regions used are considered reliable for assisting with species delineation in parasitic flatworms, our data therefore showed true parasite conspecificity, regardless of the morphological differences. Interestingly, *K. limnotrissae* appeared to be strictly host specific to *L. miodon*. Monogenean species coexistence is believed to be facilitated by niche segregation or morphological differentiation in their copulatory organs enabling and maintaining reproductive segregation (Jarkovský et al., 2004; Kadlec et al., 2003; Koskivaara et al., 1992; Morand et al., 2002; Šimková et al., 2002; Yang et al., 2006). The presence of multiple monogenean species on a single host species was further suggested to be enabled by host size preferences or temporal variation in their presence and abundance (Knipes and Janovy, 2009; Koskivaara et al., 1992; Özer, 2002; A. Šimková et al., 2001a). However, a positive correlation between the prevalence of sympatric monogenean species was also recorded in previous studies (Luo and Yang, 2010). The differentiation of the two species of *Kapentagyrus* is suggested to have followed the speciation of their clupeid hosts, assumed to have taken place in the proto-Tanganyikan area (Wilson et al., 2008). The presence on both clupeid species of *K. tanganicanus* could then be explained by host-switch after a secondary contact of clupeid species in the lake's pelagic zone. This was possibly facilitated by predator-prey transmission (see Strona 2015) as *Limnothrissa miodon* is known to feed on *Stolothrissa tanganicanus* (Coulter, 1991b). This scenario corresponds with proposed strict host-specificity as an ancestral state for *Dactylogyrus* (Šimková et al., 2006, 2004). Further, interspecific interactions as a selective factor determining niche size was suggested as a mechanism promoting species coexistence (Mouillot et al., 2005). This evolutionary scenario is further supported by the noticeable morphological similarity of the two monogenean species, especially in the male copulatory organ. Indeed, host-switches of monogeneans have been documented to be accompanied by morphological changes in haptoral region rather than in their copulatory organs (Benovics et al., 2018; Messu Mandeng et al., 2015). Alternatively, intra-host speciation facilitated by niche segregation followed by colonisation of *S. tanganicae* by *K. tanganicanus* is another scenario. However, duplication events seem to be connected with strict host-specificity (Šimková et al., 2004) which is not the case in *K. tanganicanus*. Coexistence of *Kapentagyrus* spp. on *L. miodon* seems to be facilitated by temporal resource partitioning as contrasting patterns between the infection parameters and the length of *L. miodon* between these two monogenean species were reported (sympatric occurrence reported in 49 out of 355 examined specimens). This scenario is further supported by the fact that the positive relationship

between the infection parameters of *K. tanganicanus* and the length of *S. tanganicae*, hosting only one species of *Kapentagyrus*, is weaker compared to *L. miodon*, which harbours both parasite species. Moreover, the population dynamics of both species of *Kapentagyrus* on a lake-wide scale can be further influenced by locality and month of sampling. These factors should be included in future studies focusing on the coexistence of these two monogenean species. The highest infection intensity of *C. casuarinus* was reported in larger bathybatine species, suggesting a positive correlation between host size and monogenean infection. However, the small number of the respective hosts found during recent field campaigns prevented any further investigation of population dynamics of *D. lacustre* or *C. casuarinus*.

Interestingly, the digenean diversity recovered from three species of lates perches clearly surpassed the single monogenean species shared by these host species. The known diversity of cryptogonimids in Africa was almost doubled. Rather small morphological differences were visible between *Neocladocystis bemba* and *Neocladocystis biliaris* with a single nucleotide substitution in a large subunit ribosomal gene portion. Such recent speciation was most likely facilitated by the reported differences in host species, infection site and/or geographic origin. Unlike in *Neocladocystis* spp., no niche partitioning was detected in two species of *Tanganyikatrema* as specimens sharing the same host specimen and localisation were collected. However, the overall abundance and prevalence of *T. fusiforma* clearly surpassed *T. elongataeforma*. Therefore, temporal variation in infection intensity could be a driver maintaining interspecific boundaries as reported for other digenean communities (Désilets et al., 2013). However, as microhabitat localisation within the intestine was not documented in our study, within-host niche segregation could also be a reproductive barrier. The fauna of adult parasites is believed to reflect differences in host diet in the study area (Muňoz et al., 2006). The three species of lates perches infected by digeneans as juveniles indeed share a common prey of mainly copepod and fish larvae at least to some extent (Coulter, 1976; Jessen et al., n.d.). In general, digeneans tend to be specific rather to their snail hosts, with often distantly related definitive hosts involved in the life-cycle (Wright, 1973). Such a fundamental difference in comparison to monogeneans, together with an incorporated highly reproductively productive asexual stage probably enabled to overcome the relatively low densities of lates perches acting as definitive hosts (Cribb et al., 2002). Overall, the digenean fauna in the lake is vastly understudied, preventing any further conclusions regarding the level of host-specificity or

geographically dependent diversification of species infecting lates perches. Even though infection by digenean metacercariae and larval cestodes was reported in examined specimens of sardines, no cryptogonimid metacercariae were recovered from these fishes. We can therefore not yet suggest the predator-prey relationship between latids and clupeids as an infection pathway for the digenean species described from lates perches.

So far, *Lates stappersii* was found to be free of any infection caused by parasitic flatworms. We proposed this observation to be connected with its truly pelagic life style (see Roest 1988; Mulimbwa and Mannini 1993; Mannini et al. 1999). Indeed, the short-lived and slow swimming monogenean oncomiracidia, or the digenean species depending on mollusc hosts restricted to inshore habitats, have less possibilities for infecting this species than its congeners (see Rohde 1980a; Rohde et al. 1995). Host biology figures as a key determinant of the structure of parasite communities (González and Poulin, 2005). Since *S. tanganicae* figures as the exclusive fish species in the diet of *L. stappersii* (Ellis, 1978; Mannini et al., 1999), the lack of cryptogonimid metacercariae recovered from this sardine species so far may explain the absence of adults in *L. stappersii*. However, more certainty on this absence requires a more extended sampling covering a large geographic and seasonal scale over a couple of years.

Population-level analyses using the partial mitochondrial COI gene were performed to check whether host species identity is driving speciation as it has been suggested that a broad host range of morphometrically similar monogeneans can result from cryptic speciation processes (Huyse et al., 2005; Kuusela et al., 2008; Pouyaud et al., 2006; Ziętara and Lumme, 2002). No host preference possibly reflected in restriction of gene flow among the individuals, and therefore no sign of recent or incipient host species dependent speciation processes, were recorded for *C. casuarinus* (Kmentová et al., 2016c). Similarly, no evident clustering related to host species of which *D. lacustre* was collected for genetic analysis *(L. mariae* and *L. microlepis)* was reported. The situation therefore resembles that of *Pseudorhabdosynochus cyanopodus* Sigura & Justine, 2008 infecting two deep-sea grouper species in New Caledonia (Schoelinck et al., 2012). Its maximum intraspecific distance of 1.2% over COI compares to that of 0.7% in *D. lacustre* in Lake Tanganyika. As only a small sample of the mitochondrial intraspecific diversity was recovered and mostly of specimens collected from *L. microlepis*, the level of host preference in digeneans infecting lates perches in the lake needs to be tackled in future studies. A combination of host related characteristics, such as identity and size, and environmental factors rather than

restriction of gene flow were therefore proposed as drivers of the observed phenotypic plasticity, developed during parasite ontogeny, as suggested in previous experimental studies (Beaumont, 1997; Bueno-Silva et al., 2011; Dávidová et al., 2005; Ergens, 1976; Ergens and Gelnar, 1985; Jarkovský et al., 2004; Rohde and Watson, 1985; Šebelová et al., 2002; A Šimková et al., 2001). Conversely, the morphological differentiation of *K. tanganicanus* was reflected in significant host-induced population genetic distinction estimated by the Fst index. According to Rice (1987), the presence of distinct phenotypes driven by habitat preferences is a sign of selection and habitat-based assortative mating. In addition, Rueffler et al., (2006) proposed that increased phenotypic variation leads to an increase in genetic variation within a species. This corresponds with our findings as nucleotide diversity as well as max. divergence was reported to be higher in *K. tanganicanus* compared to *K. limnotrissae*. However, the uniformity in three nuclear gene fragments, the low Fst value and the high proportion of common haplotypes suggest an ongoing gene flow in *K. tanganicanus* between the two host species. Speciation is then hampered by the hosts forming mixed schools or might have started just recently. Overall, even though the COI region showed to be a suitable mtDNA marker to reflect host preferences in previous studies (Bueno-Silva et al., 2011; Meinilä et al., 2004; Wu et al., 2005), future studies employing mtDNA markers with faster mutation rate and/or a large number of unlinked nuclear loci (i.e. generated by next-generation sequencing approaches) might reveal some host related preferences (Falush et al., 2007; Spinks et al., 2014). Moreover, the nuclear–mitochondrial discordance seen in the representatives of *Kapentagyrus* suggested potential introgression of *K. tanganicanus* into *K. limnotrissae*. Morphologically, all reported hybrid individuals fit within the nuclear species and phenotype of *K. limnotrissae*: no deviation was observed in the morphology of haptoral nor copulatory organ structures. Unlike in *Macrogyrodactylus* in which historical hybridisation has resulted in a new species (Barson et al., 2010), the reported case of nuclear–mitochondrial discordance in *Kapentagyrus* spp. rather indicates occasional introgression events followed by backcrossing into the paternal species and eventual dilution and loss of alleles inherited from the maternal species, as no intraspecific variation in neither of the ribosomal gene portions was reported (Okamoto et al., 2010). This hybridisation in the past was probably promoted by the similar morphology of the copulatory organs, with overlapping ranges of the length of the copulatory tube and accessory piece. This corresponds with the suggested host switch scenario promoted by sympatric occurrence of both species of sardines in the lake, followed by demographic expansion of *K.*

tanganicanus (see Rieseberg et al. 2007; Seixas et al. 2018). Moreover, long-term data indicate differentiation of infection on a temporal scale related to host species size and therefore could explain the rarity of such a hybridisation event.

Population structure on a geographical scale

Host preference is not the only mechanism recognised as a driver of diversification in parasite populations. Geographical differentiation over the host's distribution is one of the other factors (Brazenor et al., 2018; Plaisance et al., 2008). Moreover, lake level fluctuations played an important role in the evolutionary history of fish assemblages throughout the paleohydrological history of African Great Lakes. In Lake Tanganyika, the climate at times induced desiccation into three subbasins present already during the lake's creation (Danley et al., 2012; Tiercelin and Mondeguer, 1991). Such a physical barrier followed by periods of secondary admixis across the north-south gradient had a dramatic effect on the geographic differentiation of the lake's fauna (Nevado et al., 2013; Sefc et al., 2017; Sturmbauer et al., 2017). On the other hand, a pelagic environment, promoting dispersal, and large effective population sizes, are known to limit genetic drift and differentiation in fishes (Gonzalez and Zardoya, 2007; Kinsey et al., 1994; Koblmüller et al., 2019; Martínez et al., 2006; Waples, 1987). Even though the morphology of *C. casuarinus* ex *H. stenosoma* studied using morphometric techniques showed variation related to subbasin origin, no sign of incipient speciation or restriction in gene flow was detected. This result therefore indicated a discrepancy with the phylogeographic structure reported for *H. stenosoma* (Koblmüller et al., 2019). We suggested that decreased parasite host-specificity combined with high dispersal abilities of at least some of the host species, figuring as "stepping-stone", lead to the lake wide distribution. Therefore, *C. casuarinus* is an intermediate generalist monogenean species with no sign of host or geographically induced speciation. In *D. lacustre*, a lack of replicates prevented differentiation between host and geographically induced phenotypic and genetic variation in the lake. The overall difference in genetic diversity among the studied monogenean species might be related to differences in demographic parameters. Indeed, larger effective population size, host range and intensity of infection are able to maintain a higher level of genetic diversity in parasite populations (Keeney et al., 2009). The core-satellite structure in the haplotype network documented for both species of *Kapentagyrus* might be further caused by recent bottleneck events as the reduction of sardines' population size and yearly fluctuations have been suggested (Chikhi et al., 1998). The difference between two species of *Kapentagyrus* could

be further enhanced by host size related population dynamics and possibly contrasting dispersion capacity between life stages of *L. miodon* as well as between two sardine hosts.

So far, stock identification of important pelagic fish stocks proved to be problematic (Emmett et al., 2005). Generally, the genetic structure of parasites is heavily influenced by the dispersal ability of their hosts (Miura et al., 2006). Given the general characteristics connected with monogenean infection such as high host-specificity (Catalano et al., 2014), shorter generation time (Poulin, 2007) and faster mutation rate in comparison to their fish hosts (Nieberding et al., 2004), monogenean parasites have been proposed as additional tags to reconstruct host population structure (Baldwin et al., 2012; Baldwin, 2010; Catalano et al., 2014). In general, such a potential ability to reflect historical events that are too recent to be inferred from host genetics has been called a "magnifying glass effect" (Nieberding and Olivieri, 2007). The potential of both species of *Kapentagyrus* as tags for population structure of their sardine hosts was tested via morphological analysis and genetic characterisation based on the COI mtDNA region. Our sampling design enabled us to analyse the spatial population structure of these monogenean populations without the potential effect of school migration. Moreover, a combined spatio-temporal pattern was considered. In our study, in some cases, morphometrics of the haptoral and male copulatory organ structures showed significant intraspecific shape variation with respect to sampling site origin. An overall high proportion of one central haplotype supported the existence of a near panmictic population of *Kapentagyrus* spp. with an indication of temporally restricted gene flow between some of the populations on a lake-wide scale. Low temporally stable replicability of morphological differentiation suggests dependency of phenotypic differentiation on actual environmental conditions rather than fidelity to a certain geographic location in *Kapentagyrus* spp. and consequently of their sardine host species. Moreover, the lower genetic diversity in the COI region of *Kapentagyrus* in comparison to *L. miodon* led us to doubt the magnifying ability of parasites in the study system. The limitations may be caused by the host biology. The generally reported short generation time of less than a month in dactylogyrids (Harris, 1983; Scott and Nokes, 1984; Tomnatik and others, 1990; Xiaoqin et al., 2000) accelerated by the effect of latitudinal gradient (Rohde, 1999), might be simply similar in frequency to sardine population dynamics. Indeed, multiple spawning events per year were reported in both species. Together with their short life span, this may erase the expected effect of faster molecular evolution in parasites. Alternatively, even counting on a shorter generation time in *Kapentagyrus* spp. compared

to their hosts, the existence of common breeding sites of sardines would most likely erase any pattern of population structuring in the next spawning season. The magnifying glass effect has been already rejected in *Gyrodactylus gondae* Huyse, Malmberg & Volckaert, 2004 infecting *Potamoschistus minutus* (Pallas, 1770). In the latter host-parasite system, other factors have been listed as potential causes of the lack of magnification such as sampling bias, size fluctuations in the parasite populations resulting in frequent extinctions and genetic drift, and the relatively young age of the host-parasite association (Huyse et al., 2017). It is suggested that host induced speciation has a stronger effect on species differentiation than temporal school induced barriers in species of *Kapentagyrus*.

Finally, the paleohydrological history of the study area is characterised by sequential periods of draught and rainfalls (Danley et al., 2012; McGlue et al., 2007; Tiercelin and Lezzar, 2002). As mentioned above, Lake Tanganyika is divided in three different subbasins which had an impact on evolutionary processes of population differentiation and demographic history in various fish species (Sturmbauer et al., 2001). In the pelagic realm, this phenomenon was documented to effect population size of bathybatine cichlids, possibly leading to the geographical differentiation seen nowadays in *H. stenosoma* (Koblmüller et al., 2019). Population increase related to different lake level changes resulted in demographic expansion. Lake level drops reduced its inhabitable area considerably, even for pelagic and benthopelagic deepwater fish species. The subsequent lake level rise resulted in an expansion of the available habitat and might have triggered population expansion, a pattern reported for other pelagic and benthopelagic cichlid species from lakes Malawi and Tanganyika. Processes that structure the populations of hosts often have a direct impact on those of their parasites. Our findings provide additional support for the impact of lake level changes on organisms inhabiting the lake's pelagic zone: population expansion was reported for *C. casuarinus*, dating back to around 100 KYA, and for *K. tanganicanus* estimated at 18 KYA. The recent and ongoing population expansion documented for *C. casuarinus* and *K. tanganicanus* can further reflect the availability of a new host species. Worldwide, studies on parasites' demographic history are scarce and fragmentary. However, the implications of biogeographical history for parasite diversification and speciation have been already recovered for *Gyrodactylus thymalli* (Meinilä et al., 2004) with population increase correlated with the end of the last Ice Age in Europe (Pettersen et al., 2015). Analyses of demographic history helped to the reveal the mechanism behind the ongoing speciation in *Gyrodactylus corydori* Bueno-Silva and

Boeger, 2009 supporting sympatric incipient speciation driven by host species preference (Bueno-Silva et al., 2011). Overall, parasite genealogies and population genetic patterns are proposed as an underexplored resource to investigate the demographic history of their hosts (Whiteman and Parker, 2005).

Parasite fauna of* Limnothrissa miodon *in non-native areas

With the high global importance of freshwater fisheries for human consumption, the number of species introduced as fisheries targets has increased in the last decades. Unintended co-introduction of parasites to non-native areas might pose often-underestimated threats to ecosystems. Helminths are the most commonly detected parasite group co-introduced with non-native species, with fish as the most common alien hosts (Gozlan, 2008; Lymbery et al., 2014a; Zholdasova, 1997). Recently, a parasite spillover of several species of *Cichlidogyrus* translocated from Africa infecting native fishes in Madagascar has been detected (Šimková et al., 2019). However, in many cases, parasites are neglected 'hitch-hikers' without any effect on the native fauna being noticed (Lymbery et al., 2014a; Peeler et al., 2004). In general, host translocation can be a true challenge for associated parasitic organisms, especially for heteroxenous taxa with complex life cycles. These are expected to show divergent evolutionary patterns at local versus regional scale influenced mainly by the dispersal abilities of the definitive hosts (Jarne and Théron, 2001; Prugnolle et al., 2005a). Parasite establishment in non-native area is further influenced by the size of the founder host population (Anderson and May, 1991; Dlugosch and Parker, 2008; Sakai et al., 2001) and by biotic and abiotic environmental conditions (Lymbery et al., 2014b; Taraschewski, 2006). Alternatively, a decrease in parasitic infections can increase the host's chances for successful establishment in non-native area as predicted by the enemy release hypothesis (Colautti et al., 2004; Mitchell and Power, 2003). In the case of *L. miodon*, the contrasting success of parasite co-introduction between the lakes Kariba and Kivu is proposed to reflect differences in introduction pathway. In Lake Tanganyika, fry and small juveniles (up to 3,6 cm) of *L. miodon* were reported as monogenean-free. Sardine fry was preferred during translocation towards both lakes. The presence of bigger individuals reported only in a batch on its way to Lake Kariba was proposed as a crucial factor for monogenean co-introduction and consequently the reason of the parasites' presence in Lake Kariba rather than Lake Kivu. Additionally, the different host size preferences, based on their infection intensities, between *K. limnotrissae* and *K. tanganicanus* observed for *L. miodon* could explain the absence of the second parasite

species in Lake Kariba. Release from infection of *K. tanganicanus* supports the enemy release hypothesis (Prenter et al., 2004). However, as *L. miodon* in Lake Kariba was reported to be infected by various endohelminths (a spill back event) (Douëllou, 1991), the evolutionary advantage derived from enemy release may be compensated by additional parasite infections. Release from interspecific competition might be the cause behind the higher observed abundance and prevalence of *K. limnotrissae* in Lake Kariba compared to the infection parameters reported in its native Lake Tanganyika. Differences in parasite infection parameters might be related to the population dynamics of *L. miodon* or to abiotic factors (Coche, 1974; Edmond et al., 1993). In Lake Kariba, a relatively faster growth but smaller size of *L. miodon* was reported compared to natural lakes (Lake Tanganyika and Lake Kivu), probably as a result of the unstable conditions and high predation pressure (Marshall, 1987). Environmental conditions such as water temperature differences might cause the observed seasonality in infection as reported in monogenean species in a temporal climate (Marchiori et al., 2015; A. Šimková et al., 2001b). Moreover, because of morphological similarities of *K. limnotrissae* observed between specimens from Lake Kariba and the southern subbasin of Lake Tanganyika, the area of which the host individuals where introduced from, the parasite was proposed as a tag for the origin of introduction. However, as the pattern of morphological geographic differentiation of *K. limnotrissae* was not found to be temporally stable (see Paper III), similarities and differences between the two lakes could therefore be rather related to environmental conditions than to a founder effect. The question remains whether the release from monogenean infection has affected the introduced population of *L. miodon* in Lake Kivu. In contrast to Lake Kariba and Cahora Bassa, the average length and life-span of *L. miodon* in Lake Kivu is similar to the native population. Contrasting patterns in life strategies between natural and artificial lakes were proposed to reflect differences in predation pressure and ecosystem stability (Marshall, 1993). As there is no data about the endohelminth fauna of *L. miodon* in Lake Kivu, no conclusions about the existence of spill-back events can be made. So far, *L. miodon* has been introduced to other places in Africa such as Itezhi-Tezhi dam in Zambia (Mubamba, 1993); the species has also invaded Cahora Bassa. Although preliminary data do not show co-introduction of any species of *Kapentagyrus* (own results in preparation), a year-long study is needed to confirm their absence as temporal variation in prevalence of *K. limnotrissae* in Lake Kariba was reported. Recent species translocations to non-native areas might reveal the parasites' ability to survive and successfully establish in different environmental conditions. Introduced

populations are usually subjected to an extreme genetic bottleneck and consequently lower genetic diversity compared to native populations (Grosberg and Cunningham, 2001). However, the importance of such a founder effect is directly correlated to the population size and diversity present in the translocated population, and to the frequency of introduction events (Darling et al., 2008; Roman, 2006; Voisin et al., 2005). More samples and genomic data are needed to investigate the potential genetic signature of a bottleneck caused by the small size of the introduced population of *K. limnotrissae* in Lake Kariba.

Evolutionary history of parasitic lineages in the pelagic zone of Lake Tanganyika

The evolutionary history and origin of the recovered parasite lineages in Lake Tanganyika was studied via phylogenetic reconstruction. The results supported the previously suggested multiple origin of *Cichlidogyrus* species in Lake Tanganyika, which corresponds to the idea that several Tanganyikan cichlid tribes predate the extant Lake Tanganyika basin, supporting the independent colonisation of the lake by several cichlid lineages (Genner et al., 2007; Schedel et al., 2019). The lake was considered as a source of cichlid radiations in Central and East African rivers and lakes (Salzburger et al., 2008, 2002). Therefore, the reported non-monophyly of *Cichlidogyrus* in Lake Tanganyika might be simply an artefact of low taxon coverage. The evolutionary history of this species rich genus in African freshwaters should be reconstructed including the many recently newly described species. The suggested congruence with the phylogenetic and phylogeographic history of their cichlid hosts should be tested (Messu Mandeng et al., 2015; Pariselle et al., 2015b; Vanhove et al., 2016, 2013). The close morphological similarity of *C. casuarinus* with *C. centesimus* and *C. nshomboi* infecting cichlids classified in other tribes (Boulengerochromini and Ectodini) (Muterezi Bukinga et al., 2012; Vanhove et al., 2011b) further indicates the existence of host switching events as Bathybatini and Ectodini are not sister tribes (Meyer et al., 2015).

Despite the poor overall resolution, phylogenetic inference of dactylogyrids supported the separate position of *Kapentagyrus*. Species of *Kapentagyrus* are the only dactylogyrid lineage known to infect sardine species worldwide so far; not a single dactylogyrid monogenean was ever reported from marine clupeids. Based on the observed difference, a release of typically marine monogenean taxa followed by secondary infection by this dactylogyrid lineage is proposed as a consequence of the incursion to African freshwater systems dated back to 25–50 MYA. A similar process has been suggested for

the monogeneans of cichlids in South America and Africa (Pariselle et al., 2011). The existence of *Kapentagyrus pellonulae* (Paperna, 1969) infecting *Pellonula leonensis* Boulenger, 1916 in Lake Volta and at the Black and White Volta confluence supports the origin of *Kapentagyrus* outside Lake Tanganyika. The colonisation of African freshwater systems by this sardine lineage most likely started at the western Atlantic coast, with fossils known from the Congo river system and the proto-Tanganyikan area. Ancestral state reconstruction of *Kapentagyrus* spp. following the sardines' biogeographical distribution could help to identify the sister monogenean lineage of the genus, and the origin of sardine host expansion in Africa. Repeated marine incursion into African freshwaters through the Atlantic coast was already suggested by Moore, (1903) with other candidate taxa such as Tanganyika gastropods (Van Damme and Pickford, 2003; Wilson et al., 2004) and fish fossils in the Congo river system being subjected to investigation (Giresse, 2005). Monogenean parasites have been already used to track the historical biogeography of sciaenid fishes subjected to marine transgression about 20 MYA (Boeger and Kritsky, 2003). The historical biogeography of Balkan cyprinids is reflected in the phylogeny of their monogenean parasites (Benovics et al., 2018). On a global scale, parasites have been proposed to resolve a long-standing debate on the origin and worldwide distributional pattern of cichlid fishes (Friedman et al., 2013; Pariselle et al., 2011; Vanhove et al., 2016). However, no study focusing on this phenomenon within Africa via a comparative host-parasite approach was conducted so far.

Unlike for African freshwater sardines, the infection by diplectanid monogeneans is assumed to be directly associated with the primary marine origin of lates perches. This marine origin of the hosts is supported by several juvenile characters of *Lates* that are observed in species of marine fish families (Coulter, 1991a). The latid ancestors occurred in Europe and on the Afro-Arabian plate in the Early Miocene (Greenwood, 1987; Otero and Gayet, 2001) following by the separation of the African and Arabian lineages of *Lates* (Bosworth et al., 2005; Otero, 2004). An African ancestral representative of *Lates* was most likely present in the proto-Tanganyikan region since the Mio-Pliocene (23–15 MYA) (Otero, 2004). Given the sister relationship of *D. lacustre* with *Dolicirroplectanum penangi* (Liang & Leong, 1991) infecting *L. calcarifer* (Bloch, 1790) in Asia, latid ancestors were most likely already infected by the same diplectanid lineage as the extant species. Despite the persistent geographic separation between Lakes Albert and Tanganyika for 9 MYA (A. S. Cohen et al., 1997; Cohen et al., 1993; Girdler et al., 1969), and the speciation of the

hosts, the respective populations of *D. lacustre* have not reached the level of morphological and genetic differentiation typically associated with distinct diplectanid species. Hence, we concluded that this is an example of a lineage that failed to diverge. Interestingly, specimens originating from Taja River showed morphological distinction from the other localities, which correlated with the long-term hydrological isolation of the Upper Guinean province. Unfinished speciation is assumed to be correlated with a lower level of genetic diversity documented for *D. lacustre*. Overall, lower genetic diversity reported for both species of *Kapentagyrus* and *D. lacustre* suggest their more recent diversification compared to *C. casuarinus*. Moreover, the difference might be related to the age of the host lineage estimated at 15.9–2.1 MYA and 18–7 MYA in clupeids and bathybatine cichlids, respectively, and recent colonisation and subsequent diversification assumed for lates perches (personal communication S. Koblmüller). Alternatively, other scenarios could explain the reported situation including: 1) the rate of evolution of latids and their parasites is slower in comparison to other fish (Bermingham et al., 1997; Bowen et al., 2009; Muss et al., 2001) and other monogenean taxa, and 2) latid origin in the proto-Tanganyikan region with more recent admixture of populations via lacustrine and riverine connections resulting in the polyphyly of latid species in Lake Tanganyika. There often is a discrepancy between morphological and genetic differentiation of diplectanids (Poisot et al., 2011; Schoelinck et al., 2012; Vilas et al., 2005; Wu et al., 2005). Similar often conflicting results between morphological and molecular characterisation are puzzling taxonomists not only in the field of parasitic flatworms. The lack of consensus about a reliable barcoding gap is directly connected with the plastic nature of morphological characters used in species delineation in parasitic flatworms. Moreover, genetic data are lacking for a vast majority of parasitic flatworms with only a small portion of species that have been subjected to population level investigation. Moreover, the taxonomical designation might be influenced by the manner of staining as the shape is modified by muscle contraction and different techniques are used for these delicate organisms. Last but not least, only a neglectable number of parasitic flatworms are currently being kept in experimental conditions, because of complicated establishment procedures, especially for heteroxenous taxa. Therefore, there is an overall impossibility to directly and adequately test interspecific reproduction boundaries recognised under the biological species concept.

Unlike in *D. lacustre*, cryptogonimid digenean species infecting lates perches in the lake form an exclusively freshwater clade in a family level reconstruction. The presence of

species of *Acanthostomum* in a clade reported from three different continents and their relation to the digenean fauna in Lake Tanganyika could be correlated with little phylogenetic congruence of digeneans with fishes as definitive hosts (Cribb et al., 2002) and a high frequency of host switches during the evolution of this group of parasitic flatworms (Cribb et al., 2001). As an alternative, given the fact that three species of *Acanthostomum* were described from *L. niloticus*, and that *L. calcarifer* is also infected by cryptogonimid digeneans, the infection of lates perches by this cryptogonimid lineage could be caused by a host switch in African freshwater systems or its actual presence before the split of latids into an African and Asian branch. One of the well-known cases of digeneans' spectacular ability for host replacement within their life cycle is the recent translocation of *Schistosoma mansoni* Sambon, 1907 into the Americas (Després et al., 1993). A successful host switch was already reported in the phylogeographic history at the level of intermediate and definitive host in digeneans (Kvach et al., 2017) and tapeworms (Hoberg et al., 2001) coupled with geographical colonisation and/or ecological diversification (Hoberg and Brooks, 2008). Tracking the historical distribution of parasite taxa already proved to provide an additional view not only on host biogeography and diversification, but also geological processes and climate reconstruction in a local as well as global scale (Morand and Krasnov, 2010). Our data provide the first molecular information on cryptogonimids in Africa and the first COI data of this family worldwide. Overall, the general knowledge of digenean communities at a molecular level is geographically biased due to historical reasons driving the concentration of taxonomists and funding sources (Poulin et al., 2019).

7 CONCLUSIONS AND FUTURE PERSPECTIVES

Overall, the suggested decrease of parasite host-specificity was present in all three studied pelagic and deepwater fish groups in Lake Tanganyika. Such a general pattern is believed to be correlated with the sympatric occurrence of host species and, in the case of cichlid and latid predators, small population densities in an open water realm. The reported lack of clear geographic structure is proposed to mirror the lake wide distribution of a particular host lineage involved in the life cycle related to the lack of behavioural and physical restrictions of gene flow. The observed geographically dependent community structure of digeneans needs to be verified by increased sample size. The results of this PhD thesis further highlight the phenotypic plasticity and high ability for morphological adaptation in monogenean sclerotised structures, depending of both host and external environments, and the close morphological similarities in genetically different species of cryptogonimid digeneans. Importantly, it seems that the environment has a significant influence on monogenean morphology, not necessarily under the extreme conditions that were tested in previous studies. As suggested for fish taxa inhabiting the African Great Lakes, the demographic history and population size of parasites have been shaped by lake level changes. Nevertheless, parasite species diversification in the pelagic zone seems to have been free of the influence of such fluctuations, probably as a consequence of unrestricted lake wide distribution and other biological characteristics of their hosts. This scenario correlates with the decrease of host-specificity and smaller species richness compared to the lake's littoral zone.

Given the contrasting co-introduction success reported for monogeneans in this PhD thesis, parasitological surveys should be carried out before potential introduction of any organism to the non-native area, to avoid co-introduction of potentially dangerous parasites and to enable enemy release for targeted species. Our study confirmed that the occurrence of parasites is related to host life stage and highlighted the importance of introduction conditions for parasites' co-introduction. The suggested release from parasitic infection of *L. miodon* in Lake Kivu should be subjected to future studies in the framework of the enemy release hypothesis. Moreover, given the successful establishment of *K. limnotrissae* in Lake Kariba, there is a unique opportunity to compare genetic diversity between native and non-native areas as bottleneck and founder effects are expected. Interspecific boundaries of the two species of *Kapentagyrus* are suggested to be maintained by competition and/or

observed host size preferences. The presence of interspecific competition could be tested in Lake Kariba as *K. tanganicanus* seems to be absent in this area.

Parasites are widely overlooked organisms in the field of evolutionary biology in the African Great Lakes study systems. The marine origin of some taxa in Lake Tanganyika was reflected in their parasite fauna, and the reported ongoing speciation and decrease of host-specificity are patterns suggested to be directly connected with host characteristics especially in monoxenous parasite taxa. Therefore, there is a potential of parasites as tags of their hosts' biogeographical history and of their general biology and behaviour, which certainly deserves more attention. Ancestral state reconstruction of *Kapentagyrus* could be used as an additional view to track the historical freshwater invasion by sardine hosts and indicate the ancestral morphology of this monogenean lineage. The contrasting origins of monogenean genera infecting the primary marine host taxa in Lake Tanganyika indicate lineage dependent survival of parasites to environmental changes. Moreover, this thesis underlines that the African Great Lakes are an excellent natural laboratory to study the general patterns and processes at micro- and macroevolutionary level of parasitic flatworms. Host preferences and possible ongoing speciation of monogenean and digenean parasites should be further investigated. A genome wide scan and comparison of *Kapentagyrus tanganicanus* collected from different host species could give us an opportunity to identify regions under selection and therefore responsible for high monogenean host-specificity. Speciation is a time related process driven by natural selection. Phylogenetically distinct lineages, as in the case of *D. lacustre*, can be either classified as species, subspecies or populations under ongoing diversification, depending on the species concept and overall philosophy of each taxonomist (Zachos et al, 2016). Subspecies status is considered as an interstate during a geographically dependent speciation process (Mayr, 1942) most likely under natural selection driven by environmental differences (Pyron et al., 2015). The continuum between reproductive isolation and complete panmixia is defined by the amount of interpopulation breeding in a particular ecosystem. However, the implication is dependent on the methodology and the overall minimum level of distinction seen among different genetic lineages (Patten, 2015; Zink, 2004). The concept of subspecies has proven to be highly valuable especially in the conservation of endangered species (Gippoliti and Amori, 2007) and the preservation of genetic diversity known as adaptive evolutionary conservation (Fraser and Bernatchez, 2001). Species conservation including genetic diversity as a predictor of adaptiveness to

the changing environment has been highlighted (Whitlock, 2014). Nobody is considering protection of parasitic organisms as people traditionally always have intended to get rid of them for obvious reasons (Dougherty et al., 2016). The balance in ecosystem has been created together with parasites representing a major regulatory force in ecosystems and more than 50% of Earth's biodiversity (Poulin, 2010). In parasitology, the concept of subspecies is usually not applied. Microevolutionary processes mostly studied by genetic divergence are lacking extensive morphological evaluation. Conversely, detailed morphological studies often lack the genetic part of the story. Moreover, the biological species concept defined by reproductive compatibility where the species consists of variably isolated populations which can reproduce, is often difficult to follow in most parasitic organisms. Indeed, there is no direct possibility to experimentally test mating success and the production of fertile offspring. Under the phylogenetic species concept, the existence of two distinct lineages proves the occurrence of species in nature. In parasitic flatworms, this concept is often used for species delineation and erection based on several mostly ribosomal gene portions with host identity as one of the main environmental factors considered. However, a purely phylogenetic species concept is usually unable to tackle ongoing diversification. Therefore, rather than a classification in a purely discrete manner, more complex recent and contemporary dynamics should be taken into consideration together with the potential of genetic and demographic exchangeability. Given the observed intraspecific phenotypic variation in monogeneans, a combination of morphological and molecular techniques is proposed to provide evidence of ongoing speciation processes versus existence of gene flow and phenotypic plasticity most likely caused by environmental differences. Moreover, providing genetic data of traditionally used markers is necessary to discern between the presence of cryptic species versus intraspecific morphological plasticity, and for life cycle reconstruction in cases where experimental infection is not possible. Alternatively, a high number of parasite samples covering extensively the geographic range of a host species should be subjected to detailed morphological evaluation to 1) avoid mistaking a gradient of phenotypic plasticity for species-level differences by looking only at populations at the geographical extremes as happened for example in the case of certain Lake Tanganyika cichlids 2) prevent overlooking cryptic diversity as limited morphological characters in some groups of parasitic flatworms have probably resulted in an underestimation of the real species richness.

The results of this PhD thesis also pointed out limitations of the magnifying glass theory proposed to be the consequence of a similar generation time and population size of representatives of *Kapentagyrus* and their sardine hosts. Therefore, even though monogeneans are theoretically good candidates as tags for their host population structure, a broad implementation relying on the magnifying effect could be problematic. Host-parasite interactions and general biology should be considered in future studies investigating parasites' potential for host stock identification.

8 REFERENCES

Alcaraz, G., López-Portela, X., Robles-Mendoza, C., 2015. Response of a native endangered axolotl, *Ambystoma mexicanum* (Amphibia), to exotic fish predator. Hydrobiologia 753, 73–80. https://doi.org/10.1007/s10750-015-2194-4

Alin, S.R., Cohen, A.S., Bills, R., Gashagaza, M.M., Michel, E., Tiercelin, J.J., Martens, K., Coveliers, P., Mboko, S.K., West, K., Soreghan, M., Kimbadi, S., Ntakimazi, G., 1999. Effects of landscape disturbance on animal communities in Lake Tanganyika, East Africa. Conserv. Biol. 13, 1017–1033. https://doi.org/10.1046/j.1523-1739.1999.96476.x

Amundsen, T., 2003. Fishes as models in studies of sexual selection and parental care. J. Fish Biol. 63, 17–52. https://doi.org/10.1111/j.1095-8649.2003.00219.x

Anderson, R.M., May, R.M., 1991. Infectious diseases of humans: dynamics and control. Oxford University Press.

Angel, M. V., 1993. Biodiversity of the pelagic ocean. Conserv. Biol. 7, 760–772. https://doi.org/10.1046/j.1523-1739.1993.740760.x

Bagge, A.M., Poulin, R., Valtonen, E.T., 2004. Fish population size, and not density, as the determining factor of parasite infection: a case study. Parasitology 128, 305–313. https://doi.org/10.1017/S0031182003004566

Baldwin, R.E., Banks, M.A., Jacobson, K.C., 2012. Integrating fish and parasite data as a holistic solution for identifying the elusive stock structure of Pacific sardines (*Sardinops sagax*). Rev. Fish Biol. Fish. 22, 137–156. https://doi.org/10.1007/s11160-011-9227-5

Baldwin, R.E.B., 2010. Using parasite community data and population genetics for assessing Pacific sardine (*Sardinops sagax*) population structure along the west coast of North America.

Barel, C.D.N., Dorit, R., Greenwood, P.H., Fryer, G., Hughes, N., Jackson, P.B.N., Kawanabe, H., Lowe-McConnell, R.H., Nagoshi, M., Ribbink, A.J., Trewavas, E., Witte, F., Yamaoka, K., 1985. Destruction of fisheries in Africa's lakes. Nature 315, 19–20. https://doi.org/10.1038/315019a0

Baric, S., Salzburger, W., Sturmbauer, C., 2003. Phylogeography and evolution of the Tanganyikan cichlid genus *Tropheus* based upon mitochondrial DNA sequences. J. Mol. Evol. 56, 54–68. https://doi.org/10.1007/s00239-002-2380-7

Barson, M., Přikrylová, I., Vanhove, M.P.M., Huyse, T., 2010. Parasite hybridization in African *Macrogyrodactylus* spp. (Monogenea, Platyhelminthes) signals historical host distribution. Parasitology 137, 1585–1595. https://doi.org/10.1017/S0031182010000302

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using {lme4}. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Beaumont, M., 1997. Book review: Sublethal and chronic effects of pollutants on freshwater fish, edited by R. Müller and R. Lloyd. Regul. Rivers Res. Manag. 13, 95–96. https://doi.org/10.1002/(SICI)1099-1646(199701)13:1<95::AID-RRR428>3.0.CO;2-V

Benovics, M., Desdevises, Y., Vukić, J., Šanda, R., Šimková, A., 2018. The phylogenetic relationships and species richness of host-specific *Dactylogyrus* parasites shaped by the biogeography of Balkan cyprinids. Sci. Rep. 8, 13006. https://doi.org/10.1038/s41598-018-31382-w

Berger, W.H., Smetacek, V., Wefer, G., 1989. Ocean productivity and paleoproductivity-An overview, in: Berger, W.H., Smetacek, V., Wefer, G. (Eds.), Productivity of the Ocean: Present and Past. John Wiley & Sons Limited, New York, pp. 1–34.

Bermingham, E., McCafferty, S., Martin, A., 1997. Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus., in: Kocher, T., Stepien, C. (Eds.), Molecular Systematics of Fishes. Academic Press, pp. 113–128.

Bibby, C.J., Collar, N.J., Crosby, M.J., Heath, M.F., Imboden, C., Johnson, T.H., Long, A.J., Stattersfield, A.J., Thirgood, S.J., 1992. Putting biodiversity on the map: priority areas for global conservation. International council for bird preservation. Trends Ecol Evol. 8, 1–37.

Blackburn, T.M., Pyšek, P., Bacher, S., Carlton, J.T., Duncan, R.P., Jarošík, V., Wilson, J.R.U., Richardson, D.M., 2011. A proposed unified framework for biological invasions. Trends Ecol. Evol. 26, 333–339. https://doi.org/10.1016/j.tree.2011.03.023

Blais, J., Rico, C., Van Oosterhout, C., Cable, J., Turner, G.F., Bernatchez, L., 2007. MHC adaptive divergence between closely related and sympatric African cichlids. PLoS One 2. https://doi.org/10.1371/journal.pone.0000734

Blasco-Costa, I., Faltýnková, A., Georgieva, S., Skírnisson, K., Scholz, T., Kostadinova, A., 2014. Fish pathogens near the Arctic Circle: molecular, morphological and ecological evidence for unexpected diversity of *Diplostomum* (Digenea: diplostomidae) in Iceland. Int. J. Parasitol. 44, 703–715. https://doi.org/10.1016/j.ijpara.2014.04.009

Blasco-Costa, I., Míguez-Lozano, R., Sarabeev, V., Balbuena, J.A., 2012. Molecular phylogeny of species of *Ligophorus* (Monogenea: Dactylogyridae) and their affinities within the Dactylogyridae. Parasitol. Int. 61, 619–627. https://doi.org/10.1016/j.parint.2012.06.004

Boeger, W.A., Kritsky, D.C., 2003. Parasites, fossils and geologic history: Historical biogeography of the South American freshwater croakers, *Plagioscion* spp. (Teleostei, Sciaenidae). Zool. Scr. 32, 3–11. https://doi.org/10.1046/j.1463-6409.2003.00109.x

Boeger, W.A., Kritsky, D.C., 1997. Coevolution of the monogenoides (Platyhelminthes) based on a revised hypothesis of parasite phylogeny. Int. J. Parasitol. 27, 1495–1511. https://doi.org/10.1016/S0020-7519(97)00140-9

Boeger, W.A., Kritsky, D.C., 1993. Phylogeny and a revised classification of the Monogenoidea Bychowsky, 1937 (Platyhelminthes). Syst. Parasitol. 26, 1–32. https://doi.org/10.1007/BF00009644

Bootsma, H.A., Hecky, R.E., 2003. A comparative introduction to the biology and limnology of the African Great Lakes. J. Great Lakes Res. 29, 3–18. https://doi.org/10.1016/S0380-1330(03)70535-8

Boulenger, G.A., 1909. Catalogue of the fresh-water fishes of Africa in the British Museum (Natural history). Codicote (UK) Wheldon and Wesley.

Bouzid, W., Štefka, J., Hypša, V., Lek, S., Scholz, T., Legal, L., Hassine, O.K. Ben, Loot, G., 2008. Geography and host specificity: Two forces behind the genetic structure of the freshwater fish parasite *Ligula intestinalis* (Cestoda: Diphyllobothriidae). Int. J. Parasitol. 38, 1465–1479. https://doi.org/10.1016/J.IJPARA.2008.03.008

Bowen, B.W., Bass, A.L., Rocha, L.A., Grant, W.S., Robertson, D.R., 2009. Phylogeography of the trumpetfishes (*Aulostomus*): ring species complex on a global scale. Int. J. Org. Evol. 55, 1029–1039. https://doi.org/10.1554/0014-3820(2001)055[1029:POTTAR]2.0.CO;2

Bowles, J., Blair, D., McManus, D.P., 1992. Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. Mol. Biochem. Parasitol. 54, 165–173. https://doi.org/10.1016/0166-6851(92)90109-W

Boxshall, G.A., 1998. Host specificity in copepod parasites of deep-sea fishes. J. Mar. Syst. 15, 215–223. https://doi.org/10.1016/S0924-7963(97)00058-4

Bray, R.A., Littlewood, D.T.J., Herniou, E.A., Williams, B., Henderson, R.E., 1999. Digenean parasites of deep-sea teleosts: a review and case studies of intrageneric phylogenies. Parasitology 119.

Brazenor, A.K., Saunders, R.J., Miller, T.L., Hutson, K.S., 2018. Morphological variation in the cosmopolitan fish parasite *Neobenedenia girellae* (Capsalidae: Monogenea). Int. J. Parasitol. 48, 125–134. https://doi.org/10.1016/J.IJPARA.2017.07.009

Breder, C.M., Rosen, D.E., 1966. Modes of reproduction in fishes. T.F.H. Publications, Neptune City, New Jersey.

Brichard, P., 1978. Fishes of Lake Tanganyika. T.F.H. Publications, Inc., Neptune City, New Jersey.

Briscoe, D., Maxwell, S., Kudela, R., Crowder, L., Croll, D., 2016. Are we missing important areas in pelagic marine conservation? Redefining conservation hotspots in the ocean. Endanger. Species Res. 29, 229–237. https://doi.org/10.3354/esr00710

Brown, K.J., Rüber, L., Bills, R., Day, J.J., 2010. Mastacembelid eels support Lake Tanganyika as an evolutionary hotspot of diversification. BMC Evol. Biol. 10, 188. https://doi.org/10.1186/1471-2148-10-188

Buchmann, K., Bresciani, J., 2006. Monogenea (Phylum Platyhelminthes), in: Woo, P.T.K., Leatherland, J.F. (Eds.), Fish Diseases and Disorders, Volume 1: Protozoan and Metazoan Infections, Second Edition. CAB International, Oxford, pp. 297–344.

Bueno-Silva, M., Boeger, W. a., Pie, M.R., 2011. Choice matters: Incipient speciation in *Gyrodactylus corydori* (Monogenoidea: Gyrodactylidae). Int. J. Parasitol. 41, 657–667. https://doi.org/10.1016/j.ijpara.2011.01.002

Caira, J.N., Jensen, K., Bueno, V.M., Gallagher, K., 2017. Planetary biodiversity inventory (2008-2017): tapeworms from vertebrate bowels of the Earth. Natural History Museum, University of Kansas.

Campbell, N., Cross, M.A., Chubb, J.C., Cunningham, C.O., Hatfield, E.M.C., MacKenzie, K., 2007. Spatial and temporal variations in parasite prevalence and infracommunity structure in herring (*Clupea harengus* L.) caught to the west of the British Isles and in the North and Baltic Seas: implications for fisheries science. J. Helminthol. 81, 137. https://doi.org/10.1017/S0022149X07747454

Campbell, R.A., Headrich, R.L., Munroe, T.A., 1980. Parasitism and ecological relationships among deep-see benthic fishes. Mar. Biol. 57, 301–313. https://doi.org/https://doi.org/10.1007/BF00387573

Canonico, G.C., Arthington, A., McCrary, J.K., Thieme, M.L., 2005. The effects of introduced tilapias on native biodiversity. Aquat. Conserv. Mar. Freshw. Ecosyst. 15, 463–483. https://doi.org/10.1002/aqc.699

Capella-Gutiérrez, S., Silla-Martínez, J.M., Gabaldón, T., 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinforma. Appl. Note 25, 1972–1973. https://doi.org/10.1093/bioinformatics/btp348

Casal, C.M. V., 2006. Global documentation of fish introductions: the growing crisis and recommendations for action. Biol. Invasions 8, 3–11. https://doi.org/10.1007/s10530-005-0231-3

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17, 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Catalano, S.R., Whittington, I.D., Donnellan, S.C., Gillanders, B.M., 2014. Parasites as biological tags to assess host population structure: Guidelines, recent genetic advances and comments on a holistic approach. Int. J. Parasitol. Parasites Wildl. 3, 220–6. https://doi.org/10.1016/j.ijppaw.2013.11.001

Chapman, D.W., van Well, P., 1978. Growth and mortality of *Stolothrissa tanganicae*. Trans. Am. Fish. Soc. 107, 26–35. https://doi.org/10.1577/1548-8659(1978)107<26:GAMOST>2.0.CO;2

Chikhi, L., Bonhomme, F., Agnese, J.-F., 1998. Low genetic variability in a widely distributed and abundant clupeid species, *Sardinella aurita*. New empirical results and interpretations. J. Fish Biol. 52, 861–878. https://doi.org/10.1111/j.1095-8649.1998.tb00588.x

Clayton, D.H., Bush, S.E., Goates, B.M., Johnson, K.P., 2003. Host defense reinforces host-parasite cospeciation. Proc. Natl. Acad. Sci. U. S. A. 100, 15694–9. https://doi.org/10.1073/pnas.2533751100

Coche, A.G., 1974. Limnological study of a tropical reservoir, in: Balon, E.K., Cache, A.G. (Eds.), Lake Kariba: A Man-Made Tropical Ecosystem in Central Africa. Dr. W. Junk b.v., Publishers, Hague, p. 246. https://doi.org/13: 978-94-010-2336-8

Cocquyt, C., 1999. Seasonal variations of epilithic diatom communities in the northern basin of Lake Tanganyika. Syst. Geogr. Plants 69, 265. https://doi.org/10.2307/3668547

Cocquyt, C., Vyverman, W., 2005. Phytoplankton in Lake Tanganyika: a comparison of community composition and biomass off Kigoma with previous studies 27 years ago. J. Great Lakes Res. 31, 535–546. https://doi.org/10.1016/S0380-1330(05)70282-3

Cohen, A.S., Gergurich, E.L., Kraemer, B.M., McGlue, M.M., McIntyre, P.B., Russell, J.M., Simmons, J.D., Swarzenski, P.W., 2016. Climate warming reduces fish production and benthic habitat in Lake Tanganyika, one of the most biodiverse freshwater ecosystems. Proc. Natl. Acad. Sci. 113, 9563–9568. https://doi.org/10.1073/pnas.1603237113

Cohen, A. S., Lezzar, K.-E., Tiercelin, J.-J., Soreghan, M., 1997. New palaeogeographic and lake-level reconstructions of Lake Tanganyika: implications for tectonic, climatic and biological evolution in a rift lake. Basin Res. 9, 107–132. https://doi.org/10.1046/j.1365-2117.1997.00038.x

Cohen, A.S., Scholz, C.A., Johnson, T.C., 2000. The international decade of East African Lakes (IDEAL) drilling initiative for the african great lakes. J. Paleolimnol. 24, 231–235. https://doi.org/10.1023/A:1008158902422

Cohen, A.S., Soreghan, M.J., Scholz, C.A., 1993. Estimating the age of formation of lakes: An example from Lake Tanganyika, East African Rift system. Geology 21, 511–514. https://doi.org/10.1130/0091-7613(1993)021<0511:ETAOFO>2.3.CO;2

Cohen, Andrew S., Talbot, M.R., Awramik, S.M., Dettman, D.L., Abell, P., 1997. Lake level and paleoenvironmental history of Lake Tanganyika, Africa, as inferred from late Holocene and modern stromatolites. Geol. Soc. Am. Bull. 109, 444–460. https://doi.org/10.1130/0016-7606(1997)109<0444:LLAPHO>2.3.CO;2

Colautti, R.I., Ricciardi, A., Grigorovich, I.A., MacIsaac, H.J., 2004. Is invasion success explained by the enemy release hypothesis? Ecol. Lett. 7, 721–733. https://doi.org/10.1111/j.1461-0248.2004.00616.x

Combes, C., 2001. Parasitism: the ecology and evolution of intimate interactions. University of Chicago Press.

Costa-Pereira, R., Paiva, F., Tavares, L.E.R., 2014. Variation in the parasite community of the sardine fish *Triportheus nematurus* (Actinopterygii: Characidae) from the Medalha lagoon in

the Pantanal wetland, Brazil. J. Helminthol. 88, 272–277. https://doi.org/10.1017/S0022149X1300014X

Coulter, G.W., 1991a. Composition of the flora and fauna, in: Coulter, G.W. (Ed.), Lake Tanganyika and Its Life. Natural History Musem Publications & Oxford University Press, London Oxford & New York, pp. 200–274.

Coulter, G.W., 1991b. Pelagic Fish, in: Coulter, G.W. (Ed.), Lake Tanganyika and Its Life. Natural History Musem & Oxford University Press, London Oxford & New York, pp. 111–150.

Coulter, G.W., 1991c. Fisheries, in: Coulter, G.W. (Ed.), Lake Tanganyika and Its Life. Natural History Musem & Oxford University Press, London Oxford & New York, pp. 139–150.

Coulter, G.W., 1976. The biology of *Lates* species (Nile perch) in Lake Tanganyika, and the status of the pelagic fishery for *Lates* species and *Luciolates stappersii* (Blgr.). J. Fish Biol. 9, 235–259. https://doi.org/10.1111/j.1095-8649.1976.tb04676.x

Coulter, G.W., Spigel, R.H., 1991. Hydrodynamics, in: Lake Tanganyika and Its Life. Natural History Museum Publications & Oxford University Press, London Oxford & New York, pp. 49–75.

Cox, C.F., Cox, M.A.A., 1989. Procrustes analysis, in: Cox, C.F., Cox, M.A.A. (Eds.), Multidimentional Scaling. Second Edition. Chapman & Hall, London, pp. 123–139. https://doi.org/10.1137/1028043

Cribb, T.H., Bray, R.A., Littlewood, D.T.J., Pichelin, S.P., Herniou, E.., 2001. The Digenea, in: Littlewood, D.T.J., Bray, Rodney A. (Eds.), Interrelationships of the Platyhelminthes. Taylor and Francis, London, pp. 168–185.

Cribb, T.H., Bray, R.A., Littlewood, D.T.J., 2001. The nature and evolution of the association among digeneans, molluscs and fishes. Int. J. Parasitol. 31, 997–1011. https://doi.org/10.1016/S0020-7519(01)00204-1

Cribb, T.H., Chisholm, L.A., Bray, R.A., 2002. Diversity in the Monogenea and Digenea: does lifestyle matter? Int. J. Parasitol. 32, 321–328. https://doi.org/10.1016/S0020-7519(01)00333-2

Criscione, C.D., Blouin, M.S., 2006. Parasite phylogeographical congruence with salmon host evolutionarily significant units: implications for salmon conservation. Mol. Ecol. 16, 993–1005. https://doi.org/10.1111/j.1365-294X.2006.03220.x

Criscione, C.D., Blouin, M.S., 2004. Life cycles shape parasite evolution: comparative population genetics of salmon trematodes. Evolution (N. Y). 58, 198–202. https://doi.org/10.1554/03-359

Criscione, C.D., Cooper, B., Blouin, M.S., 2006. Parasite genotypes identify source populations of migratory fish more accurately than fish genotypes. Ecology 87, 823–828. https://doi.org/https://doi.org/10.1890/0012-9658(2006)87[823:PGISPO]2.0.CO;2

Dag, O., Dolgun, A., Konar, N.M., 2018. onewaytests: an R package for one-way tests in independent groups designs. R J. 10, 175–199. https://doi.org/doi.org/10.32614/RJ-2018-022

Danley, P.D., Husemann, M., Ding, B., Dipietro, L.M., Beverly, E.J., Peppe, D.J., 2012. The impact of the geologic history and paleoclimate on the diversification of East African cichlids. Int. J. Evol. Biol. 574851. https://doi.org/10.1155/2012/574851

Darling, J.A., Bagley, M.J., Roman, J., Tepolt, C.K., Geller, J.B., 2008. Genetic patterns across multiple introductions of the globally invasive crab genus *Carcinus*. Mol. Ecol. 17, 4992–5007. https://doi.org/10.1111/j.1365-294X.2008.03978.x

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772–772. https://doi.org/10.1038/nmeth.2109

Darwall, W., Smith, K., Allen, D., Holland, R., Harrison, I., Brooks, E., others, 2011. The diversity of life in African freshwaters: underwater, under threat: an analysis of the status and distribution of freshwater species throughout mainland Africa. Divers. life African freshwaters underwater, under Threat an Anal. status Distrib. Freshw. species throughout Mainl. Africa.

Dávidová, M., Jarkovský, J., Matějusová, I., Gelnar, M., 2005. Seasonal occurrence and metrical variability of *Gyrodactylus rhodei* Žitňan 1964 (Monogenea, Gyrodactylidae). Parasitol. Res. 95, 398–405. https://doi.org/10.1007/s00436-005-1311-0

Day, J.J., Bills, R., Friel, J.P., 2009. Lacustrine radiations in African *Synodontis* catfish. J. Evol. Biol. 22, 805–17. https://doi.org/10.1111/j.1420-9101.2009.01691.x

Day, J.J., Wilkinson, M., 2006. On the origin of the *Synodontis* catfish species flock from Lake Tanganyika. Biol. Lett. 2, 548–52. https://doi.org/10.1098/rsbl.2006.0532

De Keyzer, E.L.R., De Corte, Z., Van Steenberge, M., Raeymaekers, J.A.M., Calboli, F.C.F., Kmentová, N., N'Sibula Mulimbwa, T., Virgilio, M., Vangestel, C., Mulungula, P.M., Volckaert, F.A.M., Vanhove, M.P.M., 2019. First genomic study on Lake Tanganyika sprat *Stolothrissa tanganicae*: a lack of population structure calls for integrated management of this important fisheries target species. BMC Evol. Biol. 19, 6. https://doi.org/10.1186/s12862-018-1325-8

De Vienne, D.M., Giraud, T., Shykoff, J.A., 2007. When can host shifts produce congruent host and parasite phylogenies? A simulation approach. J. Evol. Biol. 20, 1428–1438. https://doi.org/10.1111/j.1420-9101.2007.01340.x

de Vienne, D.M., Refrégier, G., López-Villavicencio, M., Tellier, A., Hood, M.E., Giraud, T., 2013. Cospeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. New Phytol. 198, 347–385. https://doi.org/10.1111/nph.12150

De Vos, L., Nshombo, M., Thys van den Audenaerde, D., 1996. *Trematocara zebra* (Perciformes; Cichlidae), new species from the north-west of Tanganyika Lake (Zaire). Belgian J. Zool.

Deines, A., Bbole, I., Katongo, C., Feder, J., Lodge, D., 2014. Hybridisation between native *Oreochromis* species and introduced Nile tilapia *O. niloticus* in the Kafue River, Zambia. African J. Aquat. Sci. 39, 23–34. https://doi.org/10.2989/16085914.2013.864965

Delvaux, D., 1995. Age of Lake Malawi (Nyasa) and water level fluctuations. Royal Museum for Central Africa, Tervuren, Belgium.

Demastes, J.W., Hafner, M.S., 1993. Cospeciation of Pocket Gophers *(Geomys)* and their Chewing Lice *(Geomydoecus)*. J. Mammal. 74, 521–530. https://doi.org/10.2307/1382271

Descy, J.-P., Tarbe, A.-L., Stenuite, S., Pirlot, S., Stimart, J., Vanderheyden, J., Leporcq, B., Stoyneva, M.P., Kimirei, I., Sinyinza, D., Plisnier, P.-D., 2010. Drivers of phytoplankton diversity in Lake Tanganyika. Hydrobiologia 653, 29–44. https://doi.org/10.1007/s10750-010-0343-3

Descy, J.P., Hardy, M.A., Sténuite, S., Pirlot, S., Leporcq, B., Kimirei, I., Sekadende, B., Mwaitega, S.R., Sinyenza, D., 2005. Phytoplankton pigments and community composition in Lake Tanganyika. Freshw. Biol. 50, 668–684. https://doi.org/10.1111/j.1365-2427.2005.01358.x

Desdevises, Y., Morand, S., Legendre, P., 2002. Evolution and determinants of host specificity in

the genus *Lamellodiscus* (Monogenea). Biol. J. Linn. Soc. 77, 431–443. https://doi.org/10.1046/j.1095-8312.2002.00114.x

Désilets, H.D., Locke, S.A., McLaughlin, J.D., Marcogliese, D.J., 2013. Community structure of *Diplostomum* spp. (Digenea: Diplostomidae) in eyes of fish: Main determinants and potential interspecific interactions. Int. J. Parasitol. 43, 929–939. https://doi.org/10.1016/J.IJPARA.2013.07.002

Després, L., Imbert-Establet, D., Monnerot, M., 1993. Molecular characterization of mitochondrial DNA provides evidence for the recent introduction of *Schistosoma mansoni* into America. Mol. Biochem. Parasitol. 60, 221–229. https://doi.org/10.1016/0166-6851(93)90133-I

Didham, R.K., Tylianakis, J.M., Gemmell, N.J., Rand, T.A., Ewers, R.M., 2007. Interactive effects of habitat modification and species invasion on native species decline. Trends Ecol. Evol. 22, 489–496. https://doi.org/10.1016/J.TREE.2007.07.001

Dlugosch, K.M., Parker, I.M., 2008. Founding events in species invasions: Genetic variation, adaptive evolution, and the role of multiple introductions. Mol. Ecol. 17, 431–449. https://doi.org/10.1111/j.1365-294X.2007.03538.x

Douëllou, L., 1991. Survey of fish parasites in Lake Kariba. Highlights Res. Program. Univ. Zimbabwe 49–50.

Dougherty, E.R., Carlson, C.J., Bueno, V.M., Burgio, K.R., Cizauskas, C.A., Clements, C.F., Seidel, D.P., Harris, N.C., 2016. Paradigms for parasite conservation. Conserv. Biol. 30, 724–733.

Drummond, A.J., Rambaut, A., Shapiro, B., Pybus, O.G., 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. Mol. Biol. Evol. 22, 1185–1192. https://doi.org/10.1093/molbev/msi103

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.-I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.-H., Soto, D., Stiassny, M.L.J., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. 81, 163. https://doi.org/10.1017/S1464793105006950

Duftner, N., Sefc, K.M., Koblmüller, S., Nevado, B., Verheyen, E., Phiri, H., Sturmbauer, C., 2006. Distinct population structure in a phenotypically homogeneous rock-dwelling cichlid fish from Lake Tanganyika. Mol. Ecol. 15, 2381–2395. https://doi.org/10.1111/j.1365-294X.2006.02949.x

Eccles, D.H., 1992. Field guide to the freshwater fishes of Tanzania. FAO Species Identif. Sheets Fish. Purp.

Eccles, D.H., Trewavas, E., 1989. Malawian cichlid fishes: the classification of some haplochromine genera. Lake Fish Movies, Herten.

Edgar, R.C., 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797. https://doi.org/10.1093/nar/gkh340

Edmond, J.M., Stallard, R.F., Craig, H., Craig, V., Weiss, R.F., Coulter, G.W., 1993. Nutrient chemistry of the water column of Lake Tanganyika. Limnol. Oceanogr. 38, 725–738. https://doi.org/10.4319/lo.1993.38.4.0725

Egger, B., Koblmüller, S., Sturmbauer, C., Sefc, K.M., 2007. Nuclear and mitochondrial data reveal different evolutionary processes in the Lake Tanganyika cichlid genus *Tropheus*. BMC Evol. Biol. 7, 137. https://doi.org/10.1186/1471-2148-7-137

Ehlers, U., 1985. Das phylogenetische System der Plathelminthes. 1985, Stuttgart: Gustav Fisher.

Gustav Fischer, Stuttgart.

Ellender, B., Weyl, O., 2014. A review of current knowledge, risk and ecological impacts associated with non-native freshwater fish introductions in South Africa. Aquat. Invasions 9, 117–132. https://doi.org/10.3391/ai.2014.9.2.01

Ellis, C.M.A., 1978. Biology of *Luciolates stappersi* in Lake Tanganyika (Burundi). Trans. Am. Fish. Soc. 107, 557–566. https://doi.org/10.1577/1548-8659(1978)107<557:BOLSIL>2.0.CO;2

Emmett, R.L., Brodeur, R.D., Miller, T.D., Pool, S.S., Krutzikowsky, G.K., Bentley, P.J., Mccrae, J., 2005. Pacific sardine (*Sardinops sagax*) abundance, distribution, and ecological relationships in the Pacific Northwest. Calif. Coop. Ocean. Fish. Investig. Rep. 46, 122–143.

Ergens, R., 1976. Variability of hard parts of opisthaptor of two species of *Gyrodactylus* Nordmann, 1832 (Monogenoidea) from *Phoxinus phoxinus* (L.). Folia Parasitol. (Praha). 23, 111–126.

Ergens, R., Gelnar, M., 1985. Experimental verification of the effect of temperature on the size of the hard parts of haptor of *Gyrodactylus katharineri* Malberg 1964. Folia Parasitol. (Praha). 32, 377–380.

Erpenbeck, D., Weier, T., de Voogd, N.J., Wörheide, G., Sutcliffe, P., Todd, J.A., Michel, E., 2011. Insights into the evolution of freshwater sponges (Porifera: Demospongiae: Spongillina): Barcoding and phylogenetic data from Lake Tanganyika endemics indicate multiple invasions and unsettle existing taxonomy. Mol. Phylogenet. Evol. 61, 231–6. https://doi.org/10.1016/j.ympev.2011.05.021

Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Falush, D., Stephens, M., Pritchard, J.K., 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol. Ecol. Notes 7, 574–578. https://doi.org/10.1111/j.1471-8286.2007.01758.x

FAO, 2016. The state of world fisheries and aquaculture. Rome.

FAO, 2010. The state of world fisheries and aquaculture. Rome.

Filzmoser, P., Gschwandtner, M., 2017. mvoutlier: Multivariate outlier detection based on robust methods [WWW Document]. URL https://cran.r-project.org/package=mvoutlier (accessed 10.29.17).

Fraser, D.J., Bernatchez, L., 2001. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Mol. Ecol. 10, 2741–2752. https://doi.org/10.1046/j.0962-1083.2001.01411.x

Friedman, M., Keck, B.P., Dornburg, A., Eytan, R.I., Martin, C.H., Hulsey, C.D., Wainwright, P.C., Near, T.J., 2013. Molecular and fossil evidence place the origin of cichlid fishes long after Gondwanan rifting. Proc. R. Soc. B Biol. Sci. 280, 20131733. https://doi.org/10.1098/rspb.2013.1733

Fryer, G., 2006. Evolution in ancient lakes: radiation of Tanganyikan atyid prawns and speciation of pelagic cichlid fishes in Lake Malawi. Hydrobiologia 568, 131–142. https://doi.org/10.1007/s10750-006-0322-x

Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147, 915–925.

Fuhrmann, O., Baer, J.G., 1925. Report on the Cestoda. Zoological results of the Third

Tanganyika Expedition (1904-1905). Proc. Zool. Soc. London 1, 79–100.

Game, E.T., Grantham, H.S., Hobday, A.J., Pressey, R.L., Lombard, A.T., Beckley, L.E., Gjerde, K., Bustamante, R., Possingham, H.P., Richardson, A.J., 2009. Pelagic protected areas: the missing dimension in ocean conservation. Trends Ecol. Evol. 24, 360–369. https://doi.org/10.1016/J.TREE.2009.01.011

Gandon, S., 2002. Local adaptation and the geometry of host-parasite coevolution. Ecol. Lett. 5, 246–256. https://doi.org/10.1046/j.1461-0248.2002.00305.x

Garamszegi, L.Z., 2009. Patterns of co-speciation and host switching in primate malaria parasites. Malar. J. 8, 110. https://doi.org/10.1186/1475-2875-8-110

García-Rodríguez, F.J., García-Gasca, S.A., Cruz-Agüero, J.D. La, Cota-Gómez, V.M., 2011. A study of the population structure of the Pacific sardine *Sardinops sagax* (Jenyns, 1842) in Mexico based on morphometric and genetic analyses. Fish. Res. 107, 169–176. https://doi.org/10.1016/J.FISHRES.2010.11.002

Genner, M.J., Seehausen, O., Lunt, D.H., Joyce, D. a., Shaw, P.W., Carvalho, G.R., Turner, G.F., 2007. Age of cichlids: New dates for ancient lake fish radiations. Mol. Biol. Evol. 24, 1269–1282. https://doi.org/10.1093/molbev/msm050

Gibson, D.I., Bray, R.A., 1994. The evolutionary expansion and host-parasite relationships of the Digenea. Int. J. Parasitol. 24, 1213–1226. https://doi.org/10.1016/0020-7519(94)90192-9

Gillardin, C., Vanhove, M.P.M., Pariselle, A., Huyse, T., Volckaert, F.A.M., 2012. Ancyrocephalidae (Monogenea) of Lake Tanganyika: II: description of the first *Cichlidogyrus* spp. parasites from Tropheini fish hosts (Teleostei, Cichlidae). Parasitol. Res. 110, 305–313. https://doi.org/10.1007/s00436-011-2490-5

Gippoliti, S., Amori, G., 2007. The problem of subspecies and biased taxonomy in conservation lists: the case of mammals. Folia Zool. 56, 113–117.

Girdler, R.W., Fairhead, J.D., Searle, R.C., Sowerbutts, W.T.C., 1969. Evolution of rifting in Africa. Nature 224, 1178–1182. https://doi.org/10.1038/2241178a0

Giresse, P., 2005. Mesozoic-Cenozoic history of the Congo Basin. J. African Earth Sci. 43, 301–315.

Gleick, P.H., 1996. Water resources., in: Schneider, S.H. (Ed.), Encyclopedia of Climate and Weather. Oxford University Press, New York, USA, pp. 817–823.

Gonzalez, E.G., Zardoya, R., 2007. Relative role of life-history traits and historical factors in shaping genetic population structure of sardines (*Sardina pilchardus*). BMC Evol. Biol. 7, 197. https://doi.org/10.1186/1471-2148-7-197

González, M.T., Poulin, R., 2005. Spatial and temporal predictability of the parasite community structure of a benthic marine fish along its distributional range. Int. J. Parasitol. 35, 1369–1377. https://doi.org/10.1016/J.IJPARA.2005.07.016

Gouy, M., Guindon, S., Gascuel, O., 2010. SeaView Version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol. Biol. Evol. 27, 221–224. https://doi.org/10.1093/molbev/msp259

Gozlan, R.E., 2008. Introduction of non-naive freshwater fish: is it all bad? Fish Fish. 9, 106–115.

Grant, W., Bowen, B., 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. J. Hered. 89, 415–426. https://doi.org/10.1093/jhered/89.5.415

Grégoir, A.F., Hablützel, P.I., Vanhove, M.P.M., Pariselle, A., Bamps, J., Volckaert, F.A.M.,

Raeymaekers, J.A.M., 2015. A link between host dispersal and parasite diversity in two sympatric cichlids of Lake Tanganyika. Freshw. Biol. 60, 323–335. https://doi.org/10.1111/fwb.12492

Grosberg, R.K., Cunningham, C.W., 2001. Genetic structure in the sea from populations to communities, in: Bertness, M., Gaines, S., Hay, M. (Eds.), Marine Community Ecology. Sinauer Associates, Sunderland, Massachusetts, pp. 61–84.

Guildford, S.J., Hecky, R.E., 2000. Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? Limnol. Oceanogr. 45, 1213–1223. https://doi.org/10.4319/lo.2000.45.6.1213

Guilhem, R., Šimková, A., Morand, S., Gourbière, S., 2012. Within-host competition and diversification of macro-parasites. J. R. Soc. Interface 9, 2936–2946. https://doi.org/10.1098/rsif.2012.0358

Hablützel, P.I., Grégoir, A.F., Vanhove, M.P.M., Volckaert, F.A.M., Raeymaekers, J.A.M., 2016. Weak link between dispersal and parasite community differentiation or immunogenetic divergence in two sympatric cichlid fishes. Mol. Ecol. 25, 5451–5466. https://doi.org/10.1111/mec.13833

Hammond, P.M.., 1992. Species inventory, in: Groombridge, B. (Ed.), Global Diversity: Status of the Earth’s Living Resources. London: Chapman & Hall., pp. 17–39.

Harris, P.D., 1983. The morphology and life-cycle of the oviparous *Oögyrodactylus farlowellae* gen. et sp.nov. (Monogenea, Gyrodactylidea). Parasitology 87, 405–420. https://doi.org/10.1017/S0031182000082937

Harrison, K., 1991. The taxonomy of East African Nile perch, *Lates* spp. (Perciformes, Centropomidae). J. Fish Biol. 38, 175–186. https://doi.org/10.1111/j.1095-8649.1991.tb03104.x

Hassouna, N., Michot, B., Bachellerie, J.-P., Narbonne, D., 1984. The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher eukaryotes. Nucleic Acids Res. 12, 3563–3583. https://doi.org/10.1093/nar/12.8.3563

Hauser, L., Carvalho, G.R., Pitcher, T.J., 1998. Genetic population structure in the Lake Tanganyika sardine *Limnothrissa miodon*. J. Fish Biol. 53, 413–429. https://doi.org/10.1111/j.1095-8649.1998.tb01040.x

Hecky, R.E., 1993. The eutrophication of Lake Victoria. SIL Proc. 25, 39–48. https://doi.org/10.1080/03680770.1992.11900057

Hecky, R.E., 1991. The pelagic ecosystem, in: Coulter, G.W. (Ed.), Lake Tanganyika and Its Life. Natural History Musem & Oxford University Press, London Oxford & New York, pp. 91–110.

Hecky, R.E., Bugenyi, F.W.B., 1992. Hydrology and chemistry of the African Great Lakes and water quality issues: Problems and solutions. SIL Commun. 23, 45–54. https://doi.org/10.1080/05384680.1992.11904007

Hecky, R.E., Fee, E.J., Kling, H.J., Rudd, J.W.M., 1981. Relationship between primary production and fish production in Lake Tanganyika. Trans. Am. Fish. Soc. 110, 336–345. https://doi.org/10.1577/1548-8659(1981)110<336:RBPPAF>2.0.CO;2

Hellberg, M.E., 1996. Dependence of gene flow on geographic distance in two solitary colars with different larval dispersal capabilities. Evolution (N. Y). 50, 1167–1175. https://doi.org/10.1111/j.1558-5646.1996.tb02357.x

Hellberg, M.E., Burton, R.S., Neigel, J.E., Palumbi, S.R., 2002. Genetic assessment of connectivity among marine populations. Bull. Mar. Sci. 70, 273–290.

Hermann, C., 1977. Fishing in Lake Tanganyika. Present situation and prospects for development. CIFA/77/Symp. 20 Nov. 1977. Bujumbura, Burundi.

Heywood, V.H., Watson, R.T., others, 1995. Global biodiversity assessment. Cambridge University Press Cambridge.

Hoberg, E.P., 1997. Phylogeny and historical reconstruction: host-parasite systems as keystones in biogeography and ecology, Biodiversity II: understanding and protecting our biological resources. Joseph Henry Press Washington, DC.

Hoberg, E.P., Alkire, N.L., de Queiroz, A., Jones, A., 2001. Out of Africa: origins of the Taenia tapeworms in humans. Proceedings. Biol. Sci. 268, 781–7. https://doi.org/10.1098/rspb.2000.1579

Hoberg, E.P., Brooks, D.R., 2008. A macroevolutionary mosaic: episodic host-switching, geographical colonization and diversification in complex host-parasite systems. J. Biogeogr. 35, 1533–1550. https://doi.org/10.1111/j.1365-2699.2008.01951.x

Huyse, T., Oeyen, M., Larmuseau, M.H.D., Volckaert, F.A.M., 2017. Co-phylogeographic study of the flatworm *Gyrodactylus gondae* and its goby host *Pomatoschistus minutus*. Parasitol. Int. 66, 119–125. https://doi.org/10.1016/J.PARINT.2016.12.008

Huyse, T., Poulin, R., Théron, A., 2005. Speciation in parasites: A population genetics approach. Trends Parasitol. 21, 469–475. https://doi.org/10.1016/j.pt.2005.08.009

Huyse, T., Vanhove, M.P.M., Mombaerts, M., Volckaert, F.A.M., Verreycken, H., 2015. Parasite introduction with an invasive goby in Belgium: double trouble? Parasitol. Res. 114, 2789–2793. https://doi.org/10.1007/s00436-015-4544-6

Huyse, T., Volckaert, F.A.., 2002. Identification of a host-associated species complex using molecular and morphometric analyses, with the description of *Gyrodactylus rugiensoides* n. sp. (Gyrodactylidae, Monogenea). Int. J. Parasitol. 32, 907–919. https://doi.org/10.1016/S0020-7519(02)00026-7

Huyse, T., Volckaert, F.A.M., 2005. Comparing host and parasite phylogenies: *Gyrodactylus* flatworms jumping from goby to goby. Syst. Biol. 54, 710–718. https://doi.org/10.1080/10635150500221036

Inglis, W.G., 1971. Speciation in parasitic nematodes. Adv. Parasitol. 9, 185–223. https://doi.org/10.1016/S0065-308X(08)60162-1

International Commission on Zoological Nomenclature, 2012. Amendment of articles 8, 9, 10, 21 and 78 of the International Code of Zoological Nomenclature to expand and refine methods of publication. Zootaxa 3450, 1–7.

Jackson, J.A., 1999. Analysis of parasite host-switching: limitations on the use of phylogenies. Parasitology 119 Suppl, S111-23.

Jarkovský, J., Morand, S., Šimková, A., Gelnar, M., 2004. Reproductive barriers between congeneric monogenean parasites (*Dactylogyrus*: Monogenea): Attachment apparatus morphology or copulatory organ incompatibility? Parasitol. Res. 92, 95–105. https://doi.org/10.1007/s00436-003-0993-4

Jarne, P., Théron, A., 2001. Genetic structure in natural populations of flukes and snails: a practical approach and review. Parasitology 123, 27–40. https://doi.org/10.1017/S0031182001007715

Jessen, H., Mgana, M.F., Grønkjær, P., Lauridsen, T.L., n.d. Dietary differences among

commercially important fishes in Lake Tanganyika assessed using stable isotope analysis. J. Great Lakes Res. https://doi.org/https://doi.org/10.1016/j.jglr.2019.08.008

Jiménez-García, M.I., Vidal-Martínez, V.M., López-Jiménez, S., 2001. Monogeneans in introduced and native cichlids in México: evidence for transfer. J. Parasitol. 87, 907–9. https://doi.org/10.1645/0022-3395(2001)087[0907:MIIANC]2.0.CO;2

Jirsová, D., Štefka, J., Jirků, M., 2017. Discordant population histories of host and its parasite: A role for ecological permeability of extreme environment? PLoS One 12, e0175286. https://doi.org/10.1371/journal.pone.0175286

Johannesson, K.A., 1974. Preliminary quantitative estimates of pelagic fish stock in Lake Tanganyika by use of echo integration methods, EIFAC. Aviemore, Scotland.

Johnson, K.P., Adams, R.J., Page, R.D.M., Clayton, D.H., 2003. When do parasites fail to speciate in response to host speciation? Syst. Biol. 52, 37–47. https://doi.org/10.1080/10635150390132704

Jombart, T., 2008. Adegenet: A R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jorgensen, S.E., Ntakimazi, G., Kayombo, S., 2006. Lake Tanganyika: Experience and lessons learned brief 6, 363-375 p.

Justine, J.-L., 2005. Species of *Pseudorhabdosynochus* Yamaguti, 1958 (Monogenea: Diplectanidae) from *Epinephelus fasciatus* and *E. merra* (Perciformes: Serranidae) off New Caledonia and other parts of the Indo-Pacific Ocean, with a comparison of measurements. Syst. Parasitol. 62, 1–37. https://doi.org/10.1007/s11230-005-5480-0

Justine, J.-L., 1991. Cladistic study in the monogenea (Platyhelminthes), based upon a parsimony analysis of spermiogenetic and spermatozoal ultrastructural characters. Int. J. Parasitol. 21, 821–838. https://doi.org/10.1016/0020-7519(91)90151-V

Justine, J.-L., Beveridge, I., Boxshall, G. a, Bray, R. a, Miller, T.L., Moravec, F., Trilles, J.-P., Whittington, I.D., 2012. An annotated list of fish parasites (Isopoda, Copepoda, Monogenea, Digenea, Cestoda, Nematoda) collected from Snappers and Bream (Lutjanidae, Nemipteridae, Caesionidae) in New Caledonia confirms high parasite biodiversity on coral reef fish. Aquat. Biosyst. 8, 22, 10.1186/2046-9063-8–22. https://doi.org/10.1186/2046-9063-8-22

Justine, J.-L., Henry, É., 2010. Monogeneans from *Epinephelus chlorostigma* (Val.) (Perciformes: Serranidae) off New Caledonia, with the description of three new species of diplectanids. Syst. Parasitol. 77, 81–105. https://doi.org/10.1007/s11230-010-9263-x

Kadlec, D., Šimková, A., Gelnar, M., 2003. The microhabitat distribution of two *Dactylogyrus* species parasitizing the gills of the barbel, *Barbus barbus*. J. Helminthol. 77, 317–325. https://doi.org/10.1079/JOH2003183

Kassambara, A., 2018. ggpubr: “ggplot2” based publication ready plots.

Kassambara, A., Mundt, F., 2017. factoextra: extract and visualize the results of multivariate data analyses.

Katoh, K., Standley, D.M., 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010

Keeney, D.B., King, T.M., Rowe, D.L., Poulin, R., 2009. Contrasting mtDNA diversity and population structure in a direct-developing marine gastropod and its trematode parasites. Mol. Ecol. 18, 4591–4603. https://doi.org/10.1111/j.1365-294X.2009.04388.x

Kelly, D.W., Paterson, R.A., Townsend, C.R., Poulin, R., Tompkins, D.M., 2009. Parasite spillback: A neglected concept in invasion ecology? Ecology 90, 2047–2056. https://doi.org/10.1890/08-1085.1

Kimirei, I., Mgaya, Y., 2007. Influence of environmental factors on seasonal changes in clupeid catches in the Kigoma area of Lake Tanganyika. African J. Aquat. Sci. 32, 291–298. https://doi.org/10.2989/AJAS.2007.32.3.9.308

Kinsey, S.T., Orsoy, T., Bert, T.M., Mahmoudi, B., 1994. Population structure of the Spanish sardine *Sardinella aurita*: natural morphological variation in a genetically homogeneous population. Mar. Biol. 118, 309–317. https://doi.org/10.1007/BF00349798

Kirchberger, P.C., Sefc, K.M., Sturmbauer, C., Koblmüller, S., 2012. Evolutionary history of Lake Tanganyika's predatory deepwater cichlids. Int. J. Evol. Biol. 2012, 716209, 10.1155/2012/716209. https://doi.org/10.1155/2012/716209

Klimpel, S., Busch, M.W., Kellermans, E., Kleinertz, Palm, H.W., 2009. Metazoan deep-sea fish parasites. Acta Biol Benrodis Supplbd 11.

Klimpel, S., Busch, M.W., Sutton, T., Palm, H.W., 2010. Meso- and bathy-pelagic fish parasites at the Mid-Atlantic Ridge (MAR): Low host specificity and restricted parasite diversity. Deep. Res. Part I Oceanogr. Res. Pap. 57, 596–603. https://doi.org/10.1016/j.dsr.2010.01.002

Klimpel, S., Palm, H.W., Busch, M.W., Kellermanns, E., Rückert, S., 2006. Fish parasites in the Arctic deep-sea: Poor diversity in pelagic fish species vs. heavy parasite load in a demersal fish. Deep Sea Res. Part I Oceanogr. Res. Pap. 53, 1167–1181. https://doi.org/10.1016/J.DSR.2006.05.009

Klingenberg, C.P., 2011. MORPHOJ: an integrated software package for geometric morphometrics. Mol. Ecol. Resour. 11, 353–357. https://doi.org/10.1111/j.1755-0998.2010.02924.x

Klingenberg, C.P., Monteiro, L.R., 2005. Distances and directions in multidimensional shape spaces: implications for morphometric applications. Syst. Biol. 54, 678–688. https://doi.org/10.1080/10635150590947258

Kmentová, N., Gelnar, M., Koblmüller, S., Vanhove, M.P.M., 2016a. First insights into the diversity of gill monogeneans of "*Gnathochromis*" and *Limnochromis* (Teleostei, Cichlidae) in Burundi: do the parasites mirror host ecology and phylogenetic history? PeerJ 4, e1629, 10.7717/peerj.1629. https://doi.org/10.7717/peerj.1629

Kmentová, N., Gelnar, M., Koblmüller, S., Vanhove, M.P.M., 2016b. Deep-water parasite diversity in Lake Tanganyika: description of two new monogenean species from benthopelagic cichlid fishes. Parasit. Vectors 9, 426, 10.1186/s13071-016-1696–x. https://doi.org/10.1186/s13071-016-1696-x

Kmentová, N., Gelnar, M., Mendlová, M., Van Steenberge, M., Koblmüller, S., Vanhove, M.P.M., 2016c. Reduced host-specificity in a parasite infecting non-littoral Lake Tanganyika cichlids evidenced by intraspecific morphological and genetic diversity. Sci. Rep. 6, 39605. https://doi.org/10.1038/srep39605

Knipes, A.K., Janovy, J., 2009. Community structure and seasonal dynamics of *Dactylogyrus* Spp. (Monogenea) on the fathead minnow (*Pimephales promelas*) from the Salt Valley Watershed, Lancaster County, Nebraska. J. Parasitol. 95, 1295–1305. https://doi.org/10.1645/GE-2166.1

Koblmüller, S., Duftner, N., Katongo, C., Phiri, H., Sturmbauer, C., 2005. Ancient divergence in bathypelagic Lake Tanganyika deepwater cichlids: Mitochondrial phylogeny of the tribe

Bathybatini. J. Mol. Evol. 60, 297–314. https://doi.org/10.1007/s00239-004-0033-8

Koblmüller, S., Duftner, N., Sefc, K.M., Aigner, U., Rogetzer, M., Sturmbauer, C., 2009. Phylogeographic structure and gene flow in the scale-eating cichlid *Perissodus microlepis* (Teleostei, Perciformes, Cichlidae) in southern Lake Tanganyika. Zool. Scr. 38, 257–268. https://doi.org/10.1111/j.1463-6409.2008.00378.x

Koblmüller, S., Odhiambo, E.A., Sinyinza, D., Sturmbauer, C., Sefc, K.M., 2015. Big fish, little divergence: phylogeography of Lake Tanganyika's giant cichlid, *Boulengerochromis microlepis*. Hydrobiologia 748, 29–38. https://doi.org/10.1007/s10750-014-1863-z

Koblmüller, S., Salzburger, W., Obermüller, B., Eigner, E., Sturmbauer, C., Sefc, K.M., 2011. Separated by sand, fused by dropping water: habitat barriers and fluctuating water levels steer the evolution of rock-dwelling cichlid populations in Lake Tanganyika. Mol. Ecol. 20, 2272–2290. https://doi.org/10.1111/j.1365-294X.2011.05088.x

Koblmüller, S., Sefc, K.M., Duftner, N., Warum, M., Sturmbauer, C., 2007. Genetic population structure as indirect measure of dispersal ability in a Lake Tanganyika cichlid. Genetica 130, 121–131. https://doi.org/10.1007/s10709-006-0027-0

Koblmüller, S., Sturmbauer, C., Verheyen, E., Meyer, A., Salzburger, W., 2006. Mitochondrial phylogeny and phylogeography of East African squeaker catfishes (Siluriformes: *Synodontis*). BMC Evol. Biol. 6, 49. https://doi.org/10.1186/1471-2148-6-49

Koblmüller, S., Zangl, L., Börger, C., Daill, D., Vanhove, M.P.M., Sturmbauer, C., Sefc, K.M., 2019. Only true pelagics mix: comparative phylogeography of deepwater bathybatine cichlids from Lake Tanganyika. Hydrobiologia 832, 93–103. https://doi.org/10.1007/s10750-018-3752-3

Kocher, T.D., 2004. Adaptive evolution and explosive speciation: the cichlid fish model. Nat. Rev. Genet. 5, 288–298. https://doi.org/10.1038/nrg1316

Kondo, T., Abe, N., 1995. Habitat preference, food habits and growth of juveniles of *Lates angustifrons* and *Lates mariae* (Pisces: Centropomidae) in Lake Tanganyika. Ecol. Res. 10, 275–280. https://doi.org/10.1007/BF02347853

Konings, A., 1998. Guide to Tanganyika Cichlids. Cichlid Press, El Paso.

Kornfield, I., Smith, P.F., 2000. African Cichlid Fishes: Model Systems for Evolutionary Biology. Annu. Rev. Ecol. Syst. 31, 163–196. https://doi.org/10.1146/annurev.ecolsys.31.1.163

Koskivaara, M., Valtonen, E.T., Vuori, K.-M., 1992. Microhabitat distribution and coexistence of *Dactylogyrus* species (Monogenea) on the gills of roach. Parasitology 104, 273–281. https://doi.org/10.1017/S0031182000061710

Kuchta, R., Basson, L., Cook, C., Fiala, I., Bartošová-Sojková, P., Řehulková, E., 2018. A systematic survey of the parasites of freshwater fishes in Africa, in: Scholz, T., Vanhove, M.P.M., Jayasundera, Z., Gelnar, M. (Eds.), A Guide to the Parasites of African Freshwater Fishes. Abc Taxa, pp. 137–402.

Kullander, S.O., Roberts, T.R., 2011. Out of Lake Tanganyika: endemic lake fishes inhabit rapids of the Lukuga River. Ichthyol. Explor. Freshwaters 22, 355.

Kumar, S., Stecher, G., Tamura, K., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., Glockner, F., 2016. MEGA7: Molecular evolutionary genetics analysis Version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/molbev/msw054

Kurki, H., Vuorinen, I., Bosma, E., Bwebwa, D., 1999. Spatial and temporal changes in copepod zooplankton communities of Lake Tanganyika, in: From Limnology to Fisheries: Lake

Tanganyika and Other Large Lakes. Springer Netherlands, Dordrecht, pp. 105–114. https://doi.org/10.1007/978-94-017-1622-2_10

Kuusela, J., Ziętara, M., Lumme, J., 2008. Description of three new European cryptic species of *Gyrodactylus* Nordmann, 1832 supported by nuclear and mitochondrial phylogenetic characterization. Acta Parasitol. 53, 120–126. https://doi.org/10.2478/s11686-008-0015-x

Kuusipalo, L., 1999. Genetic differentiation of endemic nile perch *Lates stappersi* (Centropomidae, Pisces) populations in Lake Tanganyika suggested by RAPD markers, in: From Limnology to Fisheries: Lake Tanganyika and Other Large Lakes. Springer Netherlands, Dordrecht, pp. 141–148. https://doi.org/10.1007/978-94-017-1622-2_14

Kvach, Y., Jurajda, P., Bryjová, A., Trichkova, T., Ribeiro, F., Přikrylová, I., Ondračková, M., 2017. European distribution for metacercariae of the North American digenean *Posthodiplostomum* cf. *minimum centrarchi* (Strigeiformes: Diplostomidae). Parasitol. Int. 66, 635–642. https://doi.org/10.1016/J.PARINT.2017.06.003

Kyle, C.J., Boulding, E.G., 2000. Comparative population genetic structure of marine gastropods (*Littorina* spp.) with and without pelagic larval dispersal. Mar. Biol. 137, 835–845. https://doi.org/10.1007/s002270000412

Langenberg, V.T., Sarvala, J., Roijackers, R., 2003. Effect of wind induced water movements on nutrients, chlorophyll- a, and primary production in Lake Tanganyika. Aquat. Ecosyst. Health Manag. 6, 279–288. https://doi.org/10.1080/14634980301488

Lauron, E.J., Loiseau, C., Bowie, R.C.K., Spicer, G.S., Smith, T.B., Melo, M., Sehgal, R.N.M., 2015. Coevolutionary patterns and diversification of avian malaria parasites in African sunbirds (Family Nectariniidae). Parasitology 142, 635–647. https://doi.org/10.1017/S0031182014001681

Lavoué, S., Konstantinidis, P., Chen, W.-J., 2014. Progress in clupeiform systematics, in: Ganias, K. (Ed.), Biology and Ecology of Sardines and Anchovies. CRC Press, pp. 3–42.

le Roux, L.E., Avenant-Oldewage, A., 2010. Checklist of the fish parasitic genus *Cichlidogyrus* (Monogenea), including its cosmopolitan distribution and host species. African J. Aquat. Sci. 35, 21–36. https://doi.org/10.2989/16085914.2010.466632

Leigh, J.W., Bryant, D., 2015. PopART : full-feature software for haplotype network construction. Methods Ecol. Evol. 6, 1110–1116. https://doi.org/10.1111/2041-210X.12410

Lewis, D.S.C., Tweddle, D., 1990. The yield of usipa (*Engraulicypris sardella*) from the Nankumba Peninsula, Lake Malawi (1985-1986). Collect. Reports Fish. Res. Malawi, Occas. Pap. 1, 57–66.

Lezzar, K.E., Tiercelin, J.-J., Batist, M. DE, Cohen, A.S., Bandora, T., Rensbergen, P. VAN, Turdu, C. LE, Mifundu, W., Klerkx, J., 1996. New seismic stratigraphy and Late Tertiary history of the North Tanganyika Basin, East African Rift system, deduced from multichannel and high-resolution reflection seismic data and piston core evidence. Basin Res. 8, 1–28. https://doi.org/10.1111/j.1365-2117.1996.tb00112.x

Limborg, M., Pedersen, J., Hemmer-Hansen, J., Tomkiewicz, J., Bekkevold, D., 2009. Genetic population structure of European sprat *Sprattus sprattus*: differentiation across a steep environmental gradient in a small pelagic fish. Mar. Ecol. Prog. Ser. 379, 213–224. https://doi.org/10.3354/meps07889

Littlewood, D.T.J., 2006. The evolution of parasitism in flatworms. Parasit. flatworms Mol. Biol. Biochem. Immunol. Physiol. Wallingford (United Kingdom) Cabi Publ. a B Int 1–36.

Littlewood, D.T.J., Rohde, K., Clough, K.A., 1999. The interrelationships of all major groups of Platyhelminthes: phylogenetic evidence from morphology and molecules. Biol. J. Linn. Soc.

66, 75–114. https://doi.org/10.1111/j.1095-8312.1999.tb01918.x

Littlewood, D.T.J., Rohde, K., Clough, K.A., 1997. Parasite speciation within or between host species? - Phylogenetic evidence from site-specific polystome monogeneans. Int. J. Parasitol. 27, 1289–1297. https://doi.org/10.1016/S0020-7519(97)00086-6

Lockyer, A. E., Olson, P.D., Littlewood, D.T.J., 2003. Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. Biol. J. Linn. Soc. 78, 155–171. https://doi.org/10.1046/j.1095-8312.2003.00141.x

Lockyer, A E, Olson, P.D., Ostergaard, P., Rollinson, D., Johnston, D.A., Attwood, S.W., Southgate, V.R., Horak, P., Snyder, S.D., Le, T.H., Agatsuma, T., McManus, D.P., Carmichael, A.C., Naem, S., Littlewood, D.T.J., 2003. The phylogeny of the Schistosomatidae based on three genes with emphasis on the interrelationships of *Schistosoma* Weinland, 1858. Parasitology 126, 203–24. https://doi.org/https://doi.org/10.1017/S0031182002002792

Lombard, A.T., 1995. The problems with multi-species conservation: do hotspots, ideal reserves and existing reserves coincide? African Zool. 30, 145–163.

Lowe-McConnell, R., 2009. Fisheries and cichlid evolution in the African Great Lakes: progress and problems. Freshw. Rev. 2, 131–151. https://doi.org/10.1608/FRJ-2.2.2

Lowe-McConnell, R., 1996. Fish communities in the African Great Lakes. Environ. Biol. Fishes 45, 219–235. https://doi.org/10.1007/BF00003090

Lowe-McConnell, R.H., 1993. Fish faunas of the African Great Lakes: origins, diversity, and vulnerability. Conserv. Biol. 7, 634–643. https://doi.org/10.1046/j.1523-1739.1993.07030634.x

Luo, Y.F., Yang, T.B., 2010. Seasonal population dynamics of the monogeneans *Pseudorhabdosynochus coioidesis* and *P. serrani* on wild versus cultured groupers in Daya Bay, South China Sea. Helminthologia 47, 241–250. https://doi.org/10.2478/s11687-010-0038-3

Luque, J.L., Mouillot, D., Poulin, R., 2004. Parasite biodiversity and its determinants in coastal marine teleost fishes of Brazil. Parasitology 128, 671–682. https://doi.org/10.1017/S0031182004005050

Luque, J.L., Poulin, R., 2008. Linking ecology with parasite diversity in Neotropical fishes. J. Fish Biol. 72, 189–204. https://doi.org/10.1111/j.1095-8649.2007.01695.x

Lymbery, A.J., Morine, M., Kanani, H.G., Beatty, S.J., Morgan, D.L., 2014a. Co-invaders: The effects of alien parasites on native hosts. Int. J. Parasitol. Parasites Wildl. 3, 171–177. https://doi.org/10.1016/j.ijppaw.2014.04.002

Lymbery, A.J., Morine, M., Kanani, H.G., Beatty, S.J., Morgan, D.L., 2014b. Co-invaders: The effects of alien parasites on native hosts. Int. J. Parasitol. Parasites Wildl. 3, 171–177. https://doi.org/10.1016/j.ijppaw.2014.04.002

Maan, M.E., Van Rooijen, A.M.C., Van Alphen, J.J.M., Seehausen, O., 2008. Parasite-mediated sexual selection and species divergence in Lake Victoria cichlid fish. Biol. J. Linn. Soc. 94, 53–60. https://doi.org/10.1111/j.1095-8312.2008.00989.x

MacKenzie, K., 2002. Parasites as biological tags in population studies of marine organisms: an update. Parasitology 124, 153–163. https://doi.org/10.1017/S0031182002001518

Maddison, W.P., Maddison, D.R., 2017. Mesquite: a modular system for evolutionary analysis. Version 3.2 http://mesquiteproject.org [WWW Document].

Maeda, K., Takeda, M., Kamiya, K., Aibara, M., Mzighani, S.I., Nishida, M., Mizoiri, S., Sato, T., Terai, Y., Tachida, H., 2009. Population structure of two closely related pelagic cichlids in Lake Victoria, *Haplochromis pyrrhocephalus* and *H. laparogramma*. Gene 441, 67–73. https://doi.org/10.1016/J.GENE.2008.11.008

Malinsky, M., Challis, R.J., Tyers, A.M., Schiffels, S., Terai, Y., Ngatunga, B.P., Miska, E.A., Durbin, R., Genner, M.J., Turner, G.F., 2015. Genomic islands of speciation separate cichlid ecomorphs in an East African crater lake. Science (80-.). 350, 1493–1498. https://doi.org/10.1126/science.aac9927

Mandima, J., Kortet, R., Sarvala, J., 2016. *Limnothrissa miodon* (Boulenger, 1906) in Lake Kariba: daily ration and population food consumption estimates, and potential application to predict the fish stock biomass from prey abundance. Hydrobiologia 780, 99–111. https://doi.org/10.1007/s10750-016-2732-8

Mandima, J.J., 1999. The food and feeding behaviour of *Limnothrissa miodon* (Boulenger, 1906) in Lake Kariba, Zimbabwe, in: From Limnology to Fisheries: Lake Tanganyika and Other Large Lakes. Springer Netherlands, Dordrecht, pp. 175–182. https://doi.org/10.1007/978-94-017-1622-2_16

Mannini, P., Aro, E., Katonda, K.I., Kassaka, B., Mambona, C., Milindi, G., Paffen, P., Verburg, P., 1996. Pelagic fish stocks of Lake Tanganyika: biology and exploitation. FAO/FINNIDA research for the management of the fisheries of Lake Tanganyika., GCP/RAF/271/FIN—TD/53 (En). Bujumbura.

Mannini, P., Katonda, I., Kissaka, B., Verburg, P., 1999. Feeding ecology of *Lates stappersii* in Lake Tanganyika. Hydrobiologia 407, 131–139. https://doi.org/10.1023/A:1003714203943

Marchiori, N.C., Gonçalves, E.L.T., Tancredo, K.R., Pereira-Junior, J., Garcia, J.R.E., Martins, M.L., Marchiori, N.C., Gonçalves, E.L.T., Tancredo, K.R., Pereira-Junior, J., Garcia, J.R.E., Martins, M.L., 2015. Effect of water temperature and salinity in oviposition, hatching success and infestation of *Aphanoblastella mastigatus* (Monogenea, Dactylogyridae) on Rhamdia quelen. Brazilian J. Biol. 75, 245–252. https://doi.org/10.1590/1519-6984.14014

Maréchal, C., Poll, M., 1991. *Bathybates*, *Hemibates*, in: Daget, J., Gosse, J.-P., Teugels, G.G., Thys van den Audenaerde, D.F.E. (Eds.), Checklist of the Freshwater Fishes of Africa (CLOFFA). Royal Museum for Central Africa, Tervuren, pp. 21–24, 186.

Mariani, S., Hutchinson, W., Hatfield, E., Ruzzante, D., Simmonds, E., Dahlgren, T., Andre, C., Brigham, J., Torstensen, E., Carvalho, G., 2005. North Sea herring population structure revealed by microsatellite analysis. Mar. Ecol. Prog. Ser. 303, 245–257. https://doi.org/10.3354/meps303245

Marijnissen, S.A.E., Michel, E., Daniels, S.R., Erpenbeck, D., Menken, S.B.J., Schram, F.R., 2006. Molecular evidence for recent divergence of Lake Tanganyika endemic crabs (Decapoda: Platythelphusidae). Mol. Phylogenet. Evol. 40, 628–34. https://doi.org/10.1016/j.ympev.2006.03.025

Marshall, B.E., 2012. Does climate change really explain changes in the fisheries productivity of Lake Kariba (Zambia-Zimbabwe)? Trans. R. Soc. South Africa 67, 45–51. https://doi.org/10.1080/0035919X.2012.694083

Marshall, B.E., 1993. Biology of the African clupeid *Limnothrissa miodon* with reference to its small size in artificial lakes. Rev. Fish Biol. Fish. 3, 17–38. https://doi.org/10.1007/BF00043296

Marshall, B.E., 1987. Growth and mortality of the introduced Lake Tanganyika clupeid, *Limnothrissa miodon*, in Lake Kariba. J. Fish Biol. 31, 603–615. https://doi.org/10.1111/j.1095-8649.1987.tb05265.x

Martens, K., Schön, I., Meisch, C., Horne, D.J., 2007. Global diversity of ostracods (Ostracoda, Crustacea) in freshwater, in: Freshwater Animal Diversity Assessment. Springer Netherlands, Dordrecht, pp. 185–193. https://doi.org/10.1007/978-1-4020-8259-7_20

Martínez, P., González, E.G., Castilho, R., Zardoya, R., 2006. Genetic diversity and historical demography of Atlantic bigeye tuna (*Thunnus obesus*). Mol. Phylogenet. Evol. 39, 404–416. https://doi.org/10.1016/j.ympev.2005.07.022

Mauchline, J., Gordon, J.D.M., 1984. Incidence of parasitic worms in stomachs of pelagic and demersal fish of the Rockall Trough, northeastern Atlantic Ocean. J. Fish Biol. 24, 281–285. https://doi.org/10.1111/j.1095-8649.1984.tb04799.x

May, R.M., 1986. Biological diversity: How many species are there? Nature 324, 514–515.

Mayr, E., 1942. Systematics and the Origin of Species, Columbia University Press. New York. https://doi.org/10.2307/4079684

McGlue, M.M., Lezzar, K.E., Cohen, A.S., Russell, J.M., Tiercelin, J.-J., Felton, A.A., Mbede, E., Nkotagu, H.H., 2007. Seismic records of late Pleistocene aridity in Lake Tanganyika, tropical East Africa. J. Paleolimnol. 40, 635–653. https://doi.org/10.1007/s10933-007-9187-x

Meinilä, M., Kuusela, J., Ziętara, M.S., Lumme, J., 2004. Initial steps of speciation by geographic isolation and host switch in salmonid pathogen *Gyrodactylus salaris* (Monogenea: Gyrodactylidae). Int. J. Parasitol. 34, 515–526. https://doi.org/10.1016/j.ijpara.2003.12.002

Mendlová, M., Šimková, A., 2014. Evolution of host specificity in monogeneans parasitizing African cichlid fish. Parasit. Vectors 7, 69. https://doi.org/10.1186/1756-3305-7-69

Messu Mandeng, F.D., Bilong Bilong, C.F., Pariselle, A., Vanhove, M.P.M., Bitja Nyom, A.R., Agnèse, J., 2015. A phylogeny of *Cichlidogyrus* spp. (Monogenea, Dactylogyridea) clarifies a host-switch between fish families and reveals an adaptive component to attachment organ morphology of this parasite genus. Parasit. Vectors 8, 582. https://doi.org/10.1186/s13071-015-1181-y

Meyer, B.S., Matschiner, M., Salzburger, W., 2015. A tribal level phylogeny of Lake Tanganyika cichlid fishes based on a genomic multi-marker approach. Mol. Phylogenet. Evol. 83, 56–71. https://doi.org/10.1016/j.ympev.2014.10.009

Michel, A.E., Cohen, A.S., West, K., Johnston, M.R., Kat, P.W., 1992. Large African Lakes as natural laboratories for evolution: Examples from the endemic gastropod fauna of Lake Tanganyika. SIL Commun. 23, 85–99. https://doi.org/10.1080/05384680.1992.11904012

Michel, E., 1995. Evolutionary diversification of rift lake gastropods: Morphology, anatomy, genetics and biogeography of *Lavigeria* (Mollusca: Thiaridae) in Lake Tanganyika. The University of Arizona.

Miller, T.L., Cribb, T.H., 2008. Family Cryptogonimidae Ward, 1917, in: Bray, R.A., Gibson, D.I.,]ones, A. (Eds.), Keys to Trematoda Volume 3. CAB International and Natural History Museum, London, pp. 51–112.

Mills, E.L., Forney, J.L., 1988. Trophic dynamics and development of freshwater pelagic food webs, in: Complex Interactions in Lake Communities. Springer New York, New York, NY, pp. 11–30. https://doi.org/10.1007/978-1-4612-3838-6_2

Milne, I., Wright, F., Rowe, G., Marshall, D.F., Husmeier, D., McGuire, G., 2004. TOPALi: software for automatic identification of recombinant sequences within DNA multiple alignments. Bioinformatics 20, 1806–1807. https://doi.org/10.1093/bioinformatics/bth155

Mitchell, C.E., Power, A.G., 2003. Release of invasive plants from fungal and viral pathogens.

Nature 421, 625–627. https://doi.org/10.1038/nature01317

Mittermeier, R.A., Myers, N., Gill, P.C., Mittermeier, C.G., 2002. Hotspots: Earth's biologically richest and most endangered terrestrial ecoregions. J. Mammal. 83, 630–633. https://doi.org/10.1644/1545-1542(2002)083<0630:>2.0.CO;2

Miura, O., Kuris, A.M., Torchin, M.E., Hechinger, R.F., Dunham, E.J., Chiba, S., 2005. Molecular-genetic analyses reveal cryptic species of trematodes in the intertidal gastropod, *Batillaria cumingi* (Crosse). Int. J. Parasitol. 35, 793–801. https://doi.org/10.1016/J.IJPARA.2005.02.014

Miura, O., Torchin, M.E., Kuris, A.M., Hechinger, R.F., Chiba, S., 2006. Introduced cryptic species of parasites exhibit different invasion pathways. Proc. Natl. Acad. Sci. U. S. A. 103, 19818–23. https://doi.org/10.1073/pnas.0609603103

Mo, T.A., 1994. Status of *Gyrodactylus salaris* problems and research in Norway, in: Lewis, J.W. (Ed.), Parasitic Diseases of Fish. Samara Publishing, Tresaith, Dyfed, Wales, pp. 43–48.

Mölsä, H., Reynolds, J.E., Coenen, E.J., Lindqvist, O.V., 1999. Fisheries research towards resource management on Lake Tanganyika. Hydrobiologia 407, 1–24. https://doi.org/10.1023/A:1003712708969

Moore, J.E.S., 1903. The Tanganyika Problem. Hurst and Blackett, London.

Morand, S., Krasnov, B.R., 2010. The biogeography of host-parasite interactions. Oxford University Press.

Morand, S., Simková, A., Matejusová, I., Plaisance, L., Verneau, O., Desdevises, Y., 2002. Investigating patterns may reveal processes: evolutionary ecology of ectoparasitic monogeneans. Int. J. Parasitol. 32, 111–119. https://doi.org/10.1016/S0020-7519(01)00347-2

Mouillot, D., Šimková, A., Morand, S., Poulin, R., 2005. Parasite species coexistence and limiting similarity: a multiscale look at phylogenetic, functional and reproductive distances. Oecologia 146, 269–278. https://doi.org/10.1007/s00442-005-0194-1

Mubamba, R. (Department of F.C. (Zambia)), 1993. Introduction of Lake Tanganyika sardines into Itezhi-Tezhi reservoir, Zambia. CIFA Occas. Pap.

Mulimbwa, 2006. Assessment of the commercial artisanal fishing impact on three endemic pelagic fish stocks, *Stolothrissa tanganicae*, *Limnothrissa miodon* and *Lates stappersi*, in Bujumbura and Kigoma sub-basins of Lake Tanganyika. Int. Assoc. Theor. Appl. Limnol. 29, 1189–1193. https://doi.org/https://doi.org/10.1080/03680770.2005.11902872

Mulimbwa, N., Mannini, P., 1993. Demographic characteristics of *Stolothrissa tanganicae*, *Limnothrissa miodon* and *Lates stappersii* in the Northwestern (Zairean) waters of Lake Tanganyika. CIFA Occas. Pap.

Mulimbwa, N., Raeymaekers, J.A.M., Sarvala, J., 2014. Seasonal changes in the pelagic catch of two clupeid zooplanktivores in relation to the abundance of copepod zooplankton in the northern end of Lake Tanganyika. Aquat. Ecosyst. Heal. Manag. 17, 25–33. https://doi.org/10.1080/14634988.2014.883896

Mulimbwa, N., Shirakihara, K., 1994. Growth, recruitment and reproduction of sardines (*Stolothrissa tanganicae* and *Limnothrissa miodon*) in northwestern Lake Tanganyika. Tropics 4, 57–67. https://doi.org/10.3759/tropics.4.57

Muňoz, G., Grutter, A.S., Cribb, T.H., 2006. Endoparasite communities of five fish species (Labridae: Cheilininae) from Lizard Island: how important is the ecology and phylogeny of the hosts? Parasitology 132, 363–374. https://doi.org/10.1017/S0031182005009133

Mushagalusa, C.D., Nshombo, M., Lushombo, M., 2014. Littoral fisheries on Cichlidae (Pisces) from the northwestern part of Lake Tanganyika, East Africa. Aquat. Ecosyst. Health Manag. 17, 41–51. https://doi.org/10.1080/14634988.2014.883893

Mushagalusa Cirhuza, D., Plisnier, P.-D., 2016. Composition and seasonal variations in abundance of Copepod (Crustacea) populations from the northern part of Lake Tanganyika. Aquat. Ecosyst. Health Manag. 19, 401–410. https://doi.org/10.1080/14634988.2016.1251277

Muss, A., Robertson, D.R., Stepien, C.A., Wirtz, P., Bowen, B.W., 2001. Phylogeography of *Ophioblennius*: the role of ocean currents and geography in reef fish evolution. Evolution (N. Y). 55, 561–572.

Muterezi Bukinga, F., Vanhove, M.P.M., Van Steenberge, M., Pariselle, A., 2012. Ancyrocephalidae (Monogenea) of Lake Tanganyika: III: *Cichlidogyrus* infecting the world's biggest cichlid and the non-endemic tribes Haplochromini, Oreochromini and Tylochromini (Teleostei, Cichlidae). Parasitol. Res. 111, 2049–2061. https://doi.org/10.1007/s00436-012-3052-1

Myers, N., 1990. The biodiversity challenge: expanded hot-spots analysis. Environmentalist 10, 243–256. https://doi.org/https://doi.org/10.1007/BF02239720

Myers, N., 1988. Threatened biotas: "Hot spots" in tropical forests. Environmentalist 8, 187–208. https://doi.org/10.1007/BF02240252

Nadler, S.A., 1995. Microevolution and the genetic structure of parasite populations. J. Parasitol. 81, 395. https://doi.org/10.2307/3283821

Naithani, J., Plisnier, P.-D., Deleersnijder, E., 2011. Possible effects of global climate change on the ecosystem of Lake Tanganyika. Hydrobiologia 671, 147–163. https://doi.org/10.1007/s10750-011-0713-5

Nevado, B., Mautner, S., Sturmbauer, C., Verheyen, E., 2013. Water-level fluctuations and metapopulation dynamics as drivers of genetic diversity in populations of three Tanganyikan cichlid fish species. Mol. Ecol. 22, 3933–3948. https://doi.org/10.1111/mec.12374

Nieberding, C., Morand, S., Libois, R., Michaux, J.R., 2004. A parasite reveals cryptic phylogeographic history of its host. Proceedings. Biol. Sci. 271, 2559–68. https://doi.org/10.1098/rspb.2004.2930

Nieberding, C.M., Olivieri, I., 2007. Parasites: proxies for host genealogy and ecology? Trends Ecol. Evol. 22, 156–165. https://doi.org/10.1016/j.tree.2006.11.012

O'Reilly, C.M., Alin, S.R., Plisnier, P.-D., Cohen, A.S., McKee, B.A., 2003. Climate change decreases aquatic ecosystem productivity of Lake Tanganyika, Africa. Nature 424, 766–8. https://doi.org/10.1038/nature01833

Okamoto, M., Nakao, M., Blair, D., Anantaphruti, M.T., Waikagul, J., Ito, A., 2010. Evidence of hybridization between *Taenia saginata* and *Taenia asiatica*. Parasitol. Int. 59, 70–74. https://doi.org/10.1016/j.parint.2009.10.007

Olson, P.D., Cribb, T.H., Tkach, V.V., Bray, R.A., Littlewood, D.T.J., 2003. Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). Int. J. Parasitol. 33, 733–755. https://doi.org/10.1016/S0020-7519(03)00049-3

Ondračková, M., Matějusová, I., Grabowska, J., 2012. Introduction of *Gyrodactylus perccotti* (Monogenea) into Europe on its invasive fish host, Amur sleeper (*Perccottus glenii*, Dybowski 1877). Helminthologia 49, 21–26. https://doi.org/10.2478/s11687-012-0004-3

Ono, H., O'hUigin, C., Tichy, H., Klein, J., 1993. Major-histocompatibility-complex variation in

two species of cichlid fishes from Lake Malawi. Mol. Biol. Evol. 10, 1060–1072. https://doi.org/10.1093/oxfordjournals.molbev.a040055

Otero, O., 2004. Anatomy, systematics and phylogeny of both recent and fossil latid fishes (Teleostei, Perciformes, Latidae). Zool. J. Linn. Soc. 81–133. https://doi.org/10.1111/j.1096-3642.2004.00111.x

Otu, M.K., Ramlal, P., Wilkinson, P., Hall, R.I., Hecky, R.E., 2011. Paleolimnological evidence of the effects of recent cultural eutrophication during the last 200 years in Lake Malawi, East Africa. J. Great Lakes Res. 37, 61–74. https://doi.org/10.1016/J.JGLR.2010.09.009

Ovenden, J.R., Berry, O., Welch, D.J., Buckworth, R.C., Dichmont, C.M., 2015. Ocean's eleven: a critical evaluation of the role of population, evolutionary and molecular genetics in the management of wild fisheries. Fish Fish. 16, 125–159. https://doi.org/10.1111/faf.12052

Owen, R.B., Crossley, R., Johnson, T.C., Tweddle, D., Kornfield, I., Davison, S., Eccles, D.H., Engstrom, D.E., 1990. Major low levels of Lake Malawi and their implications for speciation rates in cichlid fishes. Proc. R. Soc. B Biol. Sci. 240, 519–553. https://doi.org/10.1098/rspb.1990.0052

Özer, A., 2002. Co-existence of *Dactylogyrus anchoratus* Dujardin, 1845 and *D. extensus* Mueller & Van Cleave, 1932 (Monogenea), parasites of common carp (*Cyprinus carpio*). Helmintologia 39, 45–50.

Page, R.D.M., 1993. Parasites, phylogeny and cospeciation. Int. J. Parasitol. 23, 499–506. https://doi.org/10.1016/0020-7519(93)90039-2

Page, R.D.M., Charleston, M.A., 1998. Trees within trees: phylogeny and historical associations. Trends Ecol. Evol. 13, 356–359. https://doi.org/10.1016/S0169-5347(98)01438-4

Paperna, I., 1973. New species of Monogenea (Vermes) from African freshwater fish. A preliminary report. Rev. Zool. Bot. Africaines 87, 505–518.

Pariselle, A., Boeger, W.A., Snoeks, J., Bilong Bilong, C.F., Morand, S., Vanhove, M.P.M., 2011. The monogenean parasite fauna of cichlids: a potential tool for host biogeography. Int. J. Evol. Biol. 2011, 471480, 10.4061/2011/471480. https://doi.org/10.4061/2011/471480

Pariselle, A., Muterezi Bukinga, F., Van Steenberge, M., Vanhove, M.P.M., 2015a. Ancyrocephalidae (Monogenea) of Lake Tanganyika: IV: *Cichlidogyrus* parasitizing species of Bathybatini (Teleostei, Cichlidae): reduced host-specificity in the deepwater realm? Hydrobiologia 748, 99–119. https://doi.org/10.1007/s10750-014-1975-5

Pariselle, A., Van Steenberge, M., Snoeks, J., Volckaert, F.A.M., Huyse, T., Vanhove, M.P.M., 2015b. Ancyrocephalidae (Monogenea) of Lake Tanganyika: Does the *Cichlidogyrus* parasite fauna of *Interochromis loocki* (Teleostei, Cichlidae) reflect its host's phylogenetic affinities? Contrib. to Zool. 84, 25–38.

Paterson, A.M., Gray, R.D., 1997. Host-parasite co-speciation, host switching, and missing the boat, in: Clazyton, D.H., Moore, J. (Eds.), Host--Parasite Evolution: General Principles and Avian Models. Oxford University Press Oxford, pp. 236–250.

Patten, M.A., 2015. Subspecies and the philosophy of science. Auk 132, 481–485. https://doi.org/10.1642/AUK-15-1.1

Pearce, M.J., 1995. Effects of exploitation on the pelagic fish community in the south of Lake Tanganyika, in: Pitcher, T.J., Hart, P.J.B. (Eds.), The Impact of Species Changes in African Lakes. Springer Netherlands, Dordrecht, pp. 425–441. https://doi.org/10.1007/978-94-011-0563-7_20

Peeler, E.J., Gardiner, R., Thrush, M.A., 2004. Qualitative risk assessment of routes of

transmission of the exotic fish parasite *Gyrodactylus salaris* between river catchments in England and Wales. Prev. Vet. Med. 64, 175–189. https://doi.org/10.1016/j.prevetmed.2004.05.005

Pereya, R., Taylor, M.I., Turner, G.F., Rico, C., 2004. Variation in habitat preference and population structure among three species of the Lake Malawi cichlid genus *Protomelas*. Mol. Ecol. 13, 2691–2697. https://doi.org/10.1111/j.1365-294X.2004.02224.x

Pérez-Ponce de León, G., Lagunas-Calvo, O., García-Prieto, L., Briosio-Aguilar, R., Aguilar-Aguilar, R., 2018. Update on the distribution of the co-invasive *Schyzocotyle acheilognathi* (=*Bothriocephalus acheilognathi*), the Asian fish tapeworm, in freshwater fishes of Mexico. J. Helminthol. 92, 279–290. https://doi.org/10.1017/S0022149X17000438

Perkins, E.M., Donnellan, S.C., Bertozzi, T., Whittington, I.D., 2010. Closing the mitochondrial circle on paraphyly of the Monogenea (Platyhelminthes) infers evolution in the diet of parasitic flatworms. Int. J. Parasitol. 40, 1237–1245. https://doi.org/10.1016/J.IJPARA.2010.02.017

Petit, P., Shipton, T., 2012. IUU Fishing on Lake Tanganyika. Smart Fish 61.

Pettersen, R.A., Mo, T.A., Hansen, H., Vøllestad, L.A., 2015. Genetic population structure of *Gyrodactylus thymalli* (Monogenea) in a large Norwegian river system. Parasitology 142, 1693–1702. https://doi.org/10.1017/S003118201500133X

Phiri, H., Shirakihara, K., 1999. Distribution and seasonal movement of pelagic fish in southern Lake Tanganyika. Fish. Res. 41, 63–71. https://doi.org/10.1016/S0165-7836(99)00008-9

Plaisance, L., Rousset, V., Morand, S., Littlewood, D.T.J., 2008. Colonization of Pacific islands by parasites of low dispersal ability: Phylogeography of two monogenean species parasitizing butterflyfishes in the South Pacific Ocean. J. Biogeogr. 35, 76–87. https://doi.org/10.1111/j.1365-2699.2007.01794.x

Plisnier, P.-D., Chitamwebwa, D., Mwape, L., Tshibangu, K., Langenberg, V., Coenen, E., 1999. Limnological annual cycle inferred from physical-chemical fluctuations at three stations of Lake Tanganyika, in: Lindqvist, O.V., Mölsä, H., Salonen, K., Sarvala, J. (Eds.), From Limnology to Fisheries: Lake Tanganyika and Other Large Lakes. Springer Netherlands, Dordrecht, pp. 45–58. https://doi.org/10.1007/978-94-017-1622-2_4

Plisnier, P.D., Mgana, H., Kimirei, I., Chande, A., Makasa, L., Chimanga, J., Zulu, F., Cocquyt, C., Horion, S., Bergamino, N., Naithani, J., Deleersnijder, E., André, L., Descy, J.P., Cornet, Y., 2009. Limnological variability and pelagic fish abundance (*Stolothrissa tanganicae* and *Lates stappersii*) in Lake Tanganyika. Hydrobiologia 625, 117–134. https://doi.org/10.1007/s10750-009-9701-4

Poddubnaya, L., Bruňanská, M., Kuchta, R., Scholz, T., 2006. First evidence of the presence of microtriches in the Gyrocotylidea. J. Parasitol. 92, 703–707. https://doi.org/10.1645/GE-755R.1

Poisot, T., Verneau, O., Desdevises, Y., Kitchener, A., Daniels, M., 2011. Morphological and molecular evolution are not linked in *Lamellodiscus* (Plathyhelminthes, Monogenea). PLoS One 6, e26252. https://doi.org/10.1371/journal.pone.0026252

Poll, M., 1953. Exploration hydrobiologique du lac Tanganika, in: Poissons Non Cichlidae. Institut Royal des Sciences Naturelles de Belgique, Brussels, p. 251.

Poulin, R., 2015. Quantifying parasite diversity. Parasite Divers. Diversif. Evol. Ecol. meets Phylogenetics 9–26.

Poulin, R., 2014. Parasite biodiversity revisited: frontiers and constraints. Int. J. Parasitol. 44, 581–589. https://doi.org/10.1016/J.IJPARA.2014.02.003

Poulin, R., 2010. Parasite manipulation of host behavior: an update and frequently asked questions, in: Advances in the Study of Behavior. Elsevier, pp. 151–186.

Poulin, R., 2007. Evolutionary ecology of parasites. 2nd ed. Princeton University Press, Princeton, New Jersey.

Poulin, R., 1992. Determinants of host-specificity in parasites of freshwater fishes. Int. J. Parasitol. 22, 753–758. https://doi.org/10.1016/0020-7519(92)90124-4

Poulin, R., Hay, E., Jorge, F., 2019. Taxonomic and geographic bias in the genetic study of helminth parasites. Int. J. Parasitol. 49, 429–435. https://doi.org/10.1016/J.IJPARA.2018.12.005

Poulin, R., Krasnov, B.R., Mouillot, D., 2011. Host specificity in phylogenetic and geographic space. Trends Parasitol. 27, 355–361. https://doi.org/10.1016/j.pt.2011.05.003

Poulin, R., Morand, S., 2004. The Parasite Biodiversity. Smithsonian Institution Press, Washington, D.C.

Poulin, R., Morand, S., 2000. The diversity of parasites. Q. Rev. Biol. 75, 277–293. https://doi.org/10.1086/393500

Poulin, R., Mouillot, D., 2005. Combining phylogenetic and ecological information into a new index of host specificity. J. Parasitol. 91, 511–514. https://doi.org/10.1645/GE-398R

Poulin, R., Mouillot, D., 2003. Parasite specialization from a phylogenetic perspective: a new index of host specificity. Parasitology 126, 473–480. https://doi.org/10.1017/S0031182003002993

Pouyaud, L., Desmarais, E., Deveney, M., Pariselle, A., 2006. Phylogenetic relationships among monogenean gill parasites (Dactylogyridea, Ancyrocephalidae) infesting tilapiine hosts (Cichlidae): Systematic and evolutionary implications. Mol. Phylogenet. Evol. 38, 241–249. https://doi.org/10.1016/j.ympev.2005.08.013

Prenter, J., MacNeil, C., Dick, J.T.., Dunn, A.M., 2004. Roles of parasites in animal invasions. Trends Ecol. Evol. 19, 385–390. https://doi.org/10.1016/j.tree.2004.05.002

Print, I., Akonkwa, B., Muhigwa, B., Montcho, S.A., Laleye, P., Biologie, D. De, De, U.O., Recherche, C. De, Biologie, D. De, Uvira, B.P., Congo, R.D., Abomey, U., Cotonou, B.P., Bukavu, B.P., Congo, R.D., 2015. Climate change and its impact on the fisheries in Lake Kivu, East Africa Bénin 6, 312–327.

Prudhoe, S., 1951. Trematoda, Cestoda and Acanthocephala. Résultats Sci. l'Exploration Hydrobiol. du Lac Tanganika 3, 1–10.

Prugnolle, F., Liu, H., de Meeûs, T., Balloux, F., 2005a. Population genetics of complex life-cycle parasites: an illustration with trematodes. Int. J. Parasitol. 35, 255–263. https://doi.org/10.1016/j.ijpara.2004.10.027

Prugnolle, F., Théron, A., Pointier, J.P., Jabbour-Zahab, R., Jarne, P., Durand, P., de Meeûs, T., 2005b. Dispersal in a parasitic worm and its two hosts: consequence for local adaptation. Evolution (N. Y). 59, 296–303.

Pyron, R.A., Costa, G.C., Patten, M.A., Burbrink, F.T., 2015. Phylogenetic niche conservatism and the evolutionary basis of ecological speciation. Biol. Rev. 90, 1248–1262. https://doi.org/10.1111/brv.12154

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raeymaekers, J.A.M., Hablützel, P.I., Grégoir, A.F., Bamps, J., Roose, A.K., Vanhove, M.P.M.,

Van Steenberge, M., Pariselle, A., Huyse, T., Snoeks, J., Volckaert, F. a M., 2013. Contrasting parasite communities among allopatric colour morphs of the Lake Tanganyika cichlid *Tropheus*. BMC Evol. Biol. 13, 41. https://doi.org/10.1186/1471-2148-13-41

Rahmouni, C., Vanhove, M.P.M., Šimková, A., 2018. Seven new species of *Cichlidogyrus* Paperna, 1960 (Monogenea: Dactylogyridae) parasitizing the gills of Congolese cichlids from northern Lake Tanganyika. PeerJ 6, e5604. https://doi.org/10.7717/peerj.5604

Rahmouni, C., Vanhove, M.P.M., Šimková, A., 2017. Underexplored diversity of gill monogeneans in cichlids from Lake Tanganyika: eight new species of *Cichlidogyrus* Paperna, 1960 (Monogenea: Dactylogyridae) from the northern basin of the lake, with remarks on the vagina and the heel of the male copu. Parasit. Vectors 10, 591. https://doi.org/10.1186/s13071-017-2460-6

Řehulková, E., Mendlová, M., Šimková, A., 2013. Two new species of *Cichlidogyrus* (Monogenea: Dactylogyridae) parasitizing the gills of African cichlid fishes (Perciformes) from Senegal: Morphometric and molecular characterization. Parasitol. Res. 112, 1399–1410. https://doi.org/10.1007/s00436-013-3291-9

Reid, W. V, 1998. Biodiversity hotspots. Trends Ecol. Evol. 13, 275–280. https://doi.org/10.1016/S0169-5347(98)01363-9

Reynolds, J.E., Hanek, G., Mölsä, H., Lindqvist, O.V., 1999. Lake Tanganyika Framework Fisheries Management Plan: Background, policy considerations, and main elements. FAO/FINNIDA Research for the Management of the Fisheries on Lake Tanganyika. GCP/RAF/271/FIN/97 (En).

Rice, W.R., 1987. Speciation via habitat specialization: the evolution of reproductive isolation as a correlated character. Evol. Ecol. 1, 301–314. https://doi.org/10.1007/BF02071555

Rieseberg, L.H., Kim, S.-C., Randell, R.A., Whitney, K.D., Gross, B.L., Lexer, C., Clay, K., 2007. Hybridization and the colonization of novel habitats by annual sunflowers. Genetica 129, 149–165. https://doi.org/10.1007/s10709-006-9011-y

Rodríguez-González, A., Míguez-Lozano, R., Llopis-Belenguer, C., Balbuena, J.A., 2015. Phenotypic plasticity in haptoral structures of *Ligophorus cephali* (Monogenea: Dactylogyridae) on the flathead mullet (*Mugil cephalus*): a geometric morphometric approach. Int. J. Parasitol. 45, 295–303. https://doi.org/10.1016/j.ijpara.2015.01.005

Roest, F.C., 1992. The pelagic fisheries resources of Lake Tanganyika. SIL Commun. 23, 11–15. https://doi.org/10.1080/05384680.1992.11904003

Roest, F.C., 1988. Predator–prey relations in northern Lake Tanganyika and fluctuations in the pelagic fish stocks. CIFA Occas. Pap. 15, 104–129.

Rohde, K., 1999. Latitudinal gradients in species diversity and Rapoport's rule revisited: a review of recent work and what can parasites teach us about the causes of the gradients? Ecography (Cop.). 22, 593–613. https://doi.org/10.1111/j.1600-0587.1999.tb00509.x

Rohde, K., 1989. Simple ecological systems, simple solutions to complex problems. Evol. Theory 8, 305–350.

Rohde, K., 1980a. Host specificity indices of parasites and their application. Experientia 36, 1369–1371. https://doi.org/10.1007/BF01960103

Rohde, K., 1980b. Diversity gradients of marine Monogenea in the Atlantic and Pacific Oceans. Experientia 36, 1368–1369. https://doi.org/10.1007/BF01960102

Rohde, K., 1979. A critical evaluation of intrinsic and extrinsic factors responsible for niche restriction in parasites. Am. Nat. 114, 648–671. https://doi.org/10.1086/283514

Rohde, K., 1978. Latitudinal differences in host-specificity of marine Monogenea and digenea. Mar. Biol. 47, 125–134. https://doi.org/10.1007/BF00395633

Rohde, K., Hayward, C., Heap, M., 1995. Aspects of the ecology of metazoan ectoparasites of marine fishes. Int. J. Parasitol. 25, 945–970. https://doi.org/10.1016/0020-7519(95)00015-T

Rohde, K., Watson, N., 1985. Morphology, microhabitats and geographical variation of *Kuhnia* spp. (Monogenea: Polyopisthocotylea). Int. J. Parasitol. 15, 569–586. https://doi.org/10.1016/0020-7519(85)90054-2

Rohlf, F.J., 1993. Relative warp analysis and an example of its application to mosquito wings, in: Marcus, L., Bello, E., Garcia-Valdecasas, A. (Eds.), Contributions to Morphometrics. Stony Brook, NY: State University, New York, pp. 132–158.

Roman, J., 2006. Diluting the founder effect: cryptic invasions expand a marine invader's range. Proc. R. Soc. B Biol. Sci. 273, 2453. https://doi.org/10.1098/RSPB.2006.3597

Ronquist, F., 1997. Phylogenetic approaches in coevolution and biogeography, Zoologica Scripta.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542. https://doi.org/10.1093/sysbio/sys029

Rossiter, A., 1995. The cichlid fish assemblages of Lake Tanganyika: ecology, behaviour and evolution of its species flocks. Adv. Ecol. Res. 26, 187–252. https://doi.org/10.1016/S0065-2504(08)60066-5

Rüber, L., Meyer, A., Sturmbauer, C., Verheyen, E., 2001. Population structure in two sympatric species of the Lake Tanganyika cichlid tribe Eretmodini: evidence for introgression. Mol. Ecol. 10, 1207–25.

Rueffler, C., Van Dooren, T.J.M., Leimar, O., Abrams, P.A., 2006. Disruptive selection and then what? Trends Ecol. Evol. 21, 238–245. https://doi.org/10.1016/J.TREE.2006.03.003

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N., Weller, S.G., 2001. The population biology of invasive species. Annu. Rev. Ecol. Syst. 32, 305–332. https://doi.org/10.1146/annurev.ecolsys.32.081501.114037

Sako, A., O'Reilly, C.M., Hannigan, R., Bickford, N., Johnson, R.L., 2005. Variations in otolith elemental compositions of two clupeid species, *Stolothrissa tanganicae* and *Limnothrissa miodon* in Lake Tanganyika. Geochemistry Explor. Environ. Anal. 5, 91–97. https://doi.org/10.1144/1467-7873/03-039

Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H., 2000. Global biodiversity scenarios for the year 2100. Science (80-.). 287, 1770–1774. https://doi.org/10.1126/science.287.5459.1770

Salzburger, W., Meyer, A., 2004. The species flocks of East African cichlid fishes: recent advances in molecular phylogenetics and population genetics. Naturwissenschaften 91, 277–290. https://doi.org/10.1007/s00114-004-0528-6

Salzburger, W., Meyer, A., Baric, S., Verheyen, E., Sturmbauer, C., 2002. Phylogeny of the Lake Tanganyika cichlid species flock and its relationship to the Central and East African haplochromine cichlid fish faunas. Syst. Biol. 51, 113–135. https://doi.org/10.1080/106351502753475907

Salzburger, W., Meyer, A., Baric, S., Verheyen, E., Sturmbauer, C., Salzburger, W., Meyer, A., Baric, S., Verheyen, E., Sturmbauer, C., 2008. Phylogeny of the Lake Tanganyika cichlid species flock and its relationship to the Central and East African haplochromine cichlid fish faunas. Society 51, 113–135.

Salzburger, W., Van Bocxlaer, B., Cohen, A.S., 2014. Ecology and evolution of the African Great Lakes and their faunas. Annu. Rev. Ecol. Evol. Syst. 45, 519–545. https://doi.org/10.1146/annurev-ecolsys-120213-091804

Sarvala, J., Langenberg, V., Salonen, K., Chitamwebwa, D., Coulter, G.W., Huttula, T., Kanyaru, R., Kotilainen, P., Makasa, L., Mulimbwa, N., Mölsä, H., 2017. Fish catches from Lake Tanganyika mainly reflect changes in fishery practices, not climate. SIL Proceedings, 1922-2010 29, 1182–1188. https://doi.org/10.1080/03680770.2005.11902871

Schedel, F.D.B., Musilova, Z., Schliewen, U.K., 2019. East African cichlid lineages (Teleostei: Cichlidae) might be older than their ancient host lakes: new divergence estimates for the east African cichlid radiation. BMC Evol. Biol. 19, 94. https://doi.org/10.1186/s12862-019-1417-0

Schedel, F.D.B., Schliewen, U.K., 2017. *Hemibates koningsi* spec. nov: a new deep-water cichlid (Teleostei: Cichlidae) from Lake Tanganyika. Zootaxa 4312, 92. https://doi.org/10.11646/zootaxa.4312.1.4

Schluter, D., 2000. The ecology of adaptive radiation. OUP Oxford.

Schoelinck, C., Cruaud, C., Justine, J.-L., 2012. Are all species of *Pseudorhabdosynochus* strictly host specific? - A molecular study. Parasitol. Int. 61, 356–359. https://doi.org/10.1016/j.parint.2012.01.009

Scholz, C.A., King, J.W., Ellis, G.S., Swart, P.K., Stager, J.C., Colman, S.M., 2003. Paleolimnology of Lake Tanganyika, East Africa, over the past 100 kyr. J. Paleolimnol. 30, 139–150. https://doi.org/10.1023/A:1025522116249

Scholz, T., 1999. Life cycles of species of *Proteocephalus*, parasites of fishes in the Palearctic Region: a review. J. Helminthol. 73, 1–19. https://doi.org/10.1017/s0022149x99000013

Scott, M.E., Nokes, D.J., 1984. Temperature-dependent reproduction and survival of *Gyrodactylus bullatarudis* (Monogenea) on guppies (*Poecilia reticulata*). Parasitology 89, 221–228. https://doi.org/10.1017/S0031182000001256

Šebelová, S., Kuperman, B., Gelnar, M., 2002. Abnormalities of the attachment clamps of representatives of the family Diplozoidae. J. Helminthol. 76, 249–259. https://doi.org/10.1079/JOH2002133

Seehausen, O., 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. Science (80-.). 277, 1808–1811. https://doi.org/10.1126/science.277.5333.1808

Seehausen, O., Van Alphen, J.J.M., Lande, R., 1999. Color polymorphism and sex ratio distortion in a cichlid fish as an incipient stage in sympatric speciation by sexual selection. Ecol. Lett. 2, 367–378. https://doi.org/10.1046/j.1461-0248.1999.00098.x

Sefc, K., Mattersdorfe, K., Ziegelbecker, A., Neuhüttler, N., Steiner, O., Goessler, W., Koblmüller, S., 2017. Shifting barriers and phenotypic diversification by hybridization. Ecol. Lett. 20, 651–662.

Sefc, K.M., 2011. Mating and parental care in Lake Tanganyika's cichlids. Int. J. Evol. Biol. 2011, 470875. https://doi.org/10.4061/2011/470875

Sefc, K.M., Baric, S., Salzburger, W., Sturmbauer, C., 2007. Species-specific population structure in rock-specialized sympatric cichlid species in Lake Tanganyika, East Africa. J. Mol. Evol.

64, 33–49. https://doi.org/10.1007/s00239-006-0011-4

Seixas, F.A., Boursot, P., Melo-Ferreira, J., 2018. The genomic impact of historical hybridization with massive mitochondrial DNA introgression. Genome Biol. 19, 91. https://doi.org/10.1186/s13059-018-1471-8

Semmens, B., Buhle, E., Salomon, A., Pattengill-Semmens, C., 2004. A hotspot of non-native marine fishes: evidence for the aquarium trade as an invasion pathway. Mar. Ecol. Prog. Ser. 266, 239–244. https://doi.org/10.3354/meps266239

Shaw, P.W., Turner, G.F., Idid, M.R., Robinson, R.L., Carvalho, G.R., 2000. Genetic population structure indicates sympatric speciation of Lake Malawi pelagic cichlids. Proc. R. Soc. B Biol. Sci. 267, 2273–2280. https://doi.org/10.1098/rspb.2000.1279

Shirakihar, K., Kamikawa, S., Mambona, W.B., 1992. Population changes of sardines in northern Lake Tanganyika. Afr. Study Monogr. 13, 57–67.

Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E., Vilà, M., 2013. Impacts of biological invasions: what's what and the way forward. Trends Ecol. Evol. 28, 58–66. https://doi.org/10.1016/J.TREE.2012.07.013

Šimková, A, Desdevise, Y., Gelnar, M., Morand, S., 2001. Morphometric correlates of host specificity in *Dactylogyrus* species (Monogenea) parasites of European Cyprinid fish. Parasitology 123, 169–77.

Šimková, A., Gelnar, M., Sasal, P., 2001a. Aggregation of congeneric parasites (Monogenea: *Dactylogyrus*) among gill microhabitats within one host species (*Rutilus rutilus* L.). Parasitology 123, 599–607. https://doi.org/10.1017/S0031182001008782

Šimková, A., Morand, S., 2008. Co-evolutionary patterns in congeneric monogeneans: a review of *Dactylogyrus* species and their cyprinid hosts. J. Fish Biol. 73, 2210–2227. https://doi.org/10.1111/j.1095-8649.2008.02064.x

Šimková, A., Morand, S., Jobet, E., Gelnar, M., Verneau, O., 2004. Molecular phylogeny of congeneric monogenean parasites (*Dactylogyrus*): a case of intrahost speciation. Evolution 58, 1001–1018. https://doi.org/10.1111/j.0014-3820.2004.tb00434.x

Šimková, A., Ondráčková, M., Gelnar, M., Morand, S., 2002. Morphology and coexistence of congeneric ectoparasite species: reinforcement of reproductive isolation? Biol. J. Linn. Soc. 76, 125–135. https://doi.org/10.1111/j.1095-8312.2002.tb01719.x

Šimková, A., Plaisance, L., Matějusová, I., Morand, S., Verneau, O., 2003. Phylogenetic relationships of the Dactylogyridae Bychowsky, 1933 (Monogenea: Dactylogyridea): The need for the systematic revision of the Ancyrocephalinae Bychowsky, 1937. Syst. Parasitol. 54, 1–11. https://doi.org/10.1023/A:1022133608662

Šimková, A., Řehulková, E., Rasoloariniaina, J.R., Jorissen, M.W.P., Scholz, T., Faltýnková, A., Mašová, Š., Vanhove, M.P.M., 2019. Transmission of parasites from introduced tilapias: a new threat to endemic Malagasy ichthyofauna. Biol. Invasions 21, 803–819. https://doi.org/10.1007/s10530-018-1859-0

Šimková, A., Sasal, P., Kadlec, D., Gelnar, M., 2001b. Water temperature influencing dactylogyrid species communities in roach, *Rutilus rutilus*, in the Czech Republic. J. Helminthol. 75, 373–383. https://doi.org/10.1017/S0022149X01000580

Šimková, A., Verneau, O., Gelnar, M., Morand, S., 2006. Specificity and specialization of congeneric monogeneans parasitizing cyprinid fish. Evolution (N. Y). 60, 1023–1037. https://doi.org/10.1554/05-521.1

Sinnappah, N.D., Lim, L.-H.S., Rohde, K., Tinsley, R., Combes, C., Verneau, O., 2001. A paedomorphic parasite associated with a neotenic amphibian host: phylogenetic evidence suggests a revised systematic position for sphyranuridae within anuran and turtle polystomatoineans. Mol. Phylogenet. Evol. 18, 189–201. https://doi.org/10.1006/mpev.2000.0877

Snoeks, J., 2000. Ancient lakes: biodiversity, ecology and evolution. Adv. Ecol. Res. 31, 17–38. https://doi.org/10.1016/S0065-2504(00)31005-4

Spinks, P.Q., Thomson, R.C., Shaffer, H.B., 2014. The advantages of going large: genome-wide SNPs clarify the complex population history and systematics of the threatened western pond turtle. Mol. Ecol. 23, 2228–2241. https://doi.org/10.1111/mec.12736

Stamatakis, A., 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Štefka, J., Hypša, V., Scholz, T., 2009. Interplay of host specificity and biogeography in the population structure of a cosmopolitan endoparasite: microsatellite study of *Ligula intestinalis* (Cestoda). Mol. Ecol. 18, 1187–1206. https://doi.org/10.1111/j.1365-294X.2008.04074.x

Stephenson, R.L., 1999. Stock complexity in fisheries management: a perspective of emerging issues related to population sub-units. Fish. Res. 43, 247–249. https://doi.org/10.1016/S0165-7836(99)00076-4

Stiassny, M.L.J., Meyer, A., 1999. Cichlids of the Rift Lakes: the extraordinary diversity of cichlid fishes challenges entrenched ideas of how quickly new species can arise. Sci. Am. February, 64–69.

Stone, R., 2007. The last of the Leviathans. Science (80-.). 316, 1684–1688. https://doi.org/10.1126/science.316.5832.1684

Strona, G., 2015. The underrated importance of predation in transmission ecology of direct lifecycle parasites. Oikos 124, 685–690. https://doi.org/doi: 10.1111/oik.01850

Sturmbauer, C., Baric, S., Salzburger, W., Rüber, L., Verheyen, E., 2001. Lake level fluctuations synchronize genetic divergences of cichlid fishes in African lakes. Mol. Biol. Evol 18, 144–154.

Sturmbauer, C., Börger, C., Van Steenberge, M., Koblmüller, S., 2017. A separate lowstand lake at the northern edge of Lake Tanganyika? Evidence from phylogeographic patterns in the cichlid genus *Tropheus*. Hydrobiologia 791, 51–68. https://doi.org/10.1007/s10750-016-2939-8

Sturmbauer, C., Hainz, U., Baric, S., Verheyen, E., Salzburger, W., 2003. Evolution of the tribe Tropheini from Lake Tanganyika: synchronized explosive speciation producing multiple evolutionary parallelism. Hydrobiologia 500, 51–64. https://doi.org/10.1023/A:1024680201436

Sturmbauer, C., Husemann, M., Danley, P., 2011. Explosive speciation and adaptive radiation of East African cichlid fishes, in: Zachos, F.E., Habel, J.C. (Eds.), Biodiversity Hotspots. Springer Berlin Heidelberg, Berlin, pp. 333–362.

Sturmbauer, C., Koblmüller, S., Sefc, K.M., Duftner, N., 2005. Phylogeographic history of the genus *Tropheus*, a lineage of rock-dwelling cichlid fishes endemic to Lake Tanganyika, in: Aquatic Biodiversity II. Springer-Verlag, Berlin/Heidelberg, pp. 335–366. https://doi.org/10.1007/1-4020-4111-X_30

Sturmbauer, C., Meyer, A., 1992. Genetic divergence, speciation and morphological stasis in a

lineage of African cichlid fishes. Nature. https://doi.org/10.1038/358578a0

Suchard, M.A., Lemey, P., Baele, G., Ayres, D.L., Drummond, A.J., Rambaut, A., 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evol. 4. https://doi.org/10.1093/ve/vey016

Tajima, F., 1989. Statistical methods to test for nucleotide mutation hypothesis by DNA polymorphism. Genetics 123, 585–595. https://doi.org/PMC1203831

Talling, J.F., 1986. The seasonality of phytoplankton in African lakes, in: Seasonality of Freshwater Phytoplankton. Springer Netherlands, Dordrecht, pp. 139–160. https://doi.org/10.1007/978-94-009-4818-1_10

Taraschewski, H., 2006. Hosts and parasites as aliens. J. Helminthol. 80, 99–128. https://doi.org/10.1079/JOH2006364

Taylor, M.I., Rüber, L., Verheyen, E., 2001. Microsatellites reveal high levels of population substructuring in the species–poor Eretmodine cichlid lineage from Lake Tanganyika. Proc. R. Soc. London. Ser. B Biol. Sci. 268, 803–808. https://doi.org/10.1098/rspb.2000.1580

Thieme, M.L., Abell, R., Burgess, N., Lehner, B., Dinerstein, E., Olson, D., 2005. Freshwater ecoregions of Africa and Madagascar: a conservation assessment. Island Press.

Thomas, F., Poulin, R., Brodeur, J., 2010. Host manipulation by parasites: a multidimensional phenomenon. Oikos 119, 1217–1223. https://doi.org/10.1111/j.1600-0706.2009.18077.x

Thompson, A.B., Allison, E.H., Ngatunga, B.P., 1996. Distribution and breeding biology of offshore cichlids in Lake Malawi/ Niassa. Environ. Biol. Fishes 47, 235–254. https://doi.org/10.1007/BF00000496

Thompson, J.N., 1994. The coevolutionary process. University of Chicago Press.

Tiercelin, J.-J., Lezzar, K.-E., 2002. A 300 million years history of Rift Lakes in Central and East Africa: An updated broad review, in: Odada, E.O., Olago, D.O. (Eds.), The East African Great Lakes: Limnology, Palaeolimnology and Biodiversity. Advances in Global Change Research, Vol. 12. Springer, Dordrecht, pp. 3–60. https://doi.org/10.1007/0-306-48201-0_1

Tiercelin, J., Mondeguer, A., 1991. The geology of the Tanganyika Through, in: Coulter, G.W. (Ed.), Lake Tanganyika and Its Life. Natural History Museum Publications & Oxford University Press, London Oxford & New York, pp. 7–48.

Timi, J.T., 2007. Parasites as biological tags for stock discrimination in marine fish from South American Atlantic waters. J. Helminthol. 81, 107. https://doi.org/10.1017/S0022149X07726561

Timi, J.T., Poulin, R., 2003. Parasite community structure within and across host populations of a marine pelagic fish: how repeatable is it? Int. J. Parasitol. 33, 1353–1362. https://doi.org/10.1016/S0020-7519(03)00203-0

Tkach, V. V., Littlewood, D.T.J., Olson, P.D., Kinsella, J.M., Swiderski, Z., 2003. Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). Syst. Parasitol. 56, 1–15. https://doi.org/10.1023/A:1025546001611

Tomnatik, V.E., others, 1990. The influence of water temperature on the sexual maturation of Dactylogyrus vastator. Parazitologiya 24, 235–238.

Trape, S., 2016. A new cichlid fish in the Sahara: The Ounianga Serir lakes (Chad), a biodiversity hotspot in the desert. C. R. Biol. 339, 529–536. https://doi.org/10.1016/J.CRVI.2016.08.003

Troudet, J., Grandcolas, P., Blin, A., Vignes-Lebbe, R., Legendre, F., 2017. Taxonomic bias in biodiversity data and societal preferences. Sci. Rep. 7, 9132. https://doi.org/10.1038/s41598-

017-09084-6

Turner, G.F., 1994. Description of a commercially important pelagic species of the genus *Diplotaxodon* (Pisces: Cichlidae) from Lake Malawi, Africa. J. Fish Biol. 44, 799–807. https://doi.org/10.1111/j.1095-8649.1994.tb01256.x

Turner, G.F., Burrows, M.T., 1995. A model of sympatric speciation by sexual selection. Proc. R. Soc. London. Ser. B Biol. Sci. 260, 287–292. https://doi.org/10.1098/rspb.1995.0093

Turner, G.F., Seehausen, O., Knight, M.E., Allender, C.J., Robinson, R.L., 2008. How many species of cichlid fishes are there in African lakes? Mol. Ecol. 10, 793–806. https://doi.org/10.1046/j.1365-294x.2001.01200.x

Tweddle, D., 1992. Conservation and threats to the resources of Lake Malawi. SIL Commun. 1953-1996 23, 17–24. https://doi.org/10.1080/05384680.1992.11904004

Tyler, S., Hooge, M., 2004. Comparative morphology of the body wall in flatworms (Platyhelminthes). Can. J. Zool. 82, 194–210. https://doi.org/10.1139/z03-222

United Nations, 1992. Convention on biological diversity.

Vadeboncoeur, Y., Mcintyre, P.B., Jake Vander Zanden, M., 2011. Borders of biodiversity: Life at the edge of the World's large lakes. Bioscience 61, 527–537. https://doi.org/10.1525/bio.2011.61.7.7

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. https://doi.org/10.1023/A:1026298512117

Van der Knaap, M., Kamitenga, D.M., Many, L.N., Tambwe, A.E., De Graaf, G.J., 2014. Lake Tanganyika fisheries in post-conflict Democratic Republic of Congo. Aquat. Ecosyst. Health Manag. 17, 34–40. https://doi.org/10.1080/14634988.2014.882722

Van Steenberge, M., Pariselle, A., Huyse, T., Volckaert, F.A.M., Snoeks, J., Vanhove, M.P.M., 2015. Morphology, molecules, and monogenean parasites: an example of an integrative approach to cichlid biodiversity. PLoS One 10, e0124474. https://doi.org/10.1371/journal.pone.0124474

Van Steenberge, M., Vanhove, M.P.M., Muzumani Risasi, D., Mulimbwa N'Sibula, T., Muterezi Bukinga, F., Pariselle, A., Gillardin, C., Vreven, E., Raeymaekers, J. a M., Huyse, T., Volckaert, F. a M., Nshombo Muderhwa, V., Snoeks, J., 2011. A recent inventory of the fishes of the north-western and central western coast of Lake Tanganyika (Democratic Republic Congo). Acta Ichthyol. Piscat. 41, 201–214. https://doi.org/10.3750/AIP2011.41.3.08

Vanhove, M.P.M., 2012. Species flocks and parasite evolution towards a co-phylogenetic analysis of monogenean flatworms of cichlids and gobies. University of Leuven, Belgium.

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 Parasitol. 10.1016/j.pt.2016.07.002. https://doi.org/10.1016/j.pt.2016.07.002

Vanhove, M.P.M., Pariselle, A., Van Steenberge, M., Raeymaekers, J.A.M., Hablützel, P.I., Gillardin, C., Hellemans, B., Breman, F.C., Koblmüller, S., Sturmbauer, C., Snoeks, J., Volckaert, F.A.M., Huyse, T., 2015. Hidden biodiversity in an ancient lake: phylogenetic congruence between Lake Tanganyika tropheine cichlids and their monogenean flatworm parasites. Sci. Rep. 5, 13669, 10.1038/srep13669. https://doi.org/10.1038/srep13669

Vanhove, M.P.M., Snoeks, J., Volckaert, F.A.M., Huyse, T., 2011a. First description of

monogenean parasites in Lake Tanganyika: the cichlid *Simochromis diagramma* (Teleostei, Cichlidae) harbours a high diversity of *Gyrodactylus* species (Platyhelminthes, Monogenea). Parasitology 138, 364–80. https://doi.org/10.1017/S0031182010001356

Vanhove, M.P.M., Van Steenberge, M., Dessein, S., Volckaert, F.A.M., Snoeks, J., Huyse, T., Pariselle, A., 2013. Biogeographical implications of Zambezian *Cichlidogyrus* species (Platyhelminthes: Monogenea: Ancyrocephalidae) parasitizing Congolian cichlids. Zootaxa 3608, 398–400.

Vanhove, M.P.M., Volckaert, F.A.M., Pariselle, A., 2011b. Ancyrocephalidae (Monogenea) of Lake Tanganyika: I: Four new species of *Cichlidogyrus* from *Ophthalmotilapia ventralis* (Teleostei: Cichlidae), the first record of this parasite family in the basin. Zool. 28, 253–263. https://doi.org/10.1590/S1984-46702011000200016

Verburg, P., Hecky, R.E., Kling, H., 2003. Ecological consequences of a century of warming in Lake Tanganyika. Science (80-.). 301, 505–507. https://doi.org/10.1126/science.1084846

Verburga, P., Hecky, R.E., 2009. The physics of the warming of Lake Tanganyika by climate change. Limnol. Oceanogr. 54, 2418–2430. https://doi.org/10.4319/lo.2009.54.6_part_2.2418

Verheyen, E., Rüber, L., Snoeks, J., Meyer, A., 1996. Mitochondrial phylogeography of rock-dwelling cichlid fishes reveals evolutionary influence of historical lake level fluctuations of Lake Tanganyika, Africa. Philos. Trans. R. Soc. London. Ser. B Biol. Sci. 351, 797–805. https://doi.org/10.1098/rstb.1996.0074

Vignon, M., Pariselle, A., Vanhove, M.P.M., 2011. Modularity in attachment organs of African *Cichlidogyrus* (Platyhelminthes: Monogenea: Ancyrocephalidae) reflects phylogeny rather than host specificity or geographic distribution. Biol. J. Linn. Soc. 102, 694–706. https://doi.org/10.1111/j.1095-8312.2010.01607.x

Vilas, R., Criscione, C.D., Blouin, M.S., 2005. A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. Parasitology 131, 839. https://doi.org/10.1017/S0031182005008437

Voisin, M., Engel, C.R., Viard, F., 2005. Differential shuffling of native genetic diversity across introduced regions in a brown alga: aquaculture vs. maritime traffic effects. Proc. Natl. Acad. Sci. U. S. A. 102, 5432–7. https://doi.org/10.1073/pnas.0501754102

Waeschenbach, A., Webster, B.L., Littlewood, D.T.J., 2012. Adding resolution to ordinal level relationships of tapeworms (Platyhelminthes: Cestoda) with large fragments of mtDNA. Mol. Phylogenet. Evol. 63, 834–847. https://doi.org/10.1016/J.YMPEV.2012.02.020

Wagner, C.E., Harmon, L.J., Seehausen, O., 2012. Ecological opportunity and sexual selection together predict adaptive radiation. Nature 487, 366–369. https://doi.org/10.1038/nature11144

Wagner, C.E., McCune, A.R., 2009. Contrasting patterns of spatial genetic structure in sympatric rock-dwelling cichlid fishes. Evolution (N. Y). 63, 1312–1326. https://doi.org/10.1111/j.1558-5646.2009.00612.x

Wanink, J.H., 1999. Prospects for the fishery on the small pelagic *Rastrineobola argentea* in Lake Victoria, in: From Limnology to Fisheries: Lake Tanganyika and Other Large Lakes. Springer Netherlands, Dordrecht, pp. 183–189. https://doi.org/10.1007/978-94-017-1622-2_17

Waples, R.S., 1987. A multispecies approach to the analysis of gen flow in marine shore fishes. Evolution (N. Y). 41, 385. https://doi.org/10.2307/2409146

Weston, L.F., Reed, C.C., Hendricks, M., Winker, H., van der Lingen, C.D., 2015. Stock

discrimination of South African sardine (*Sardinops sagax*) using a digenean parasite biological tag. Fish. Res. 164, 120–129. https://doi.org/10.1016/J.FISHRES.2014.11.002

Whitehead, P.J.P., Nelson, G.J., Thosaporn Wongratana., 1985. FAO species catalogue. Vol. 7: Clupeoid fishes of the world (suborder Clupeoidei): an annotated and illustrated catalogue of the herrings, sardines, pilchards, sprats, shads, anchovies, and wolfherrings. United Nations Development Programme.

Whiteman, N.K., Parker, P.G., 2005. Using parasites to infer host population history: a new rationale for parasite conservation. Anim. Conserv. 8, 175–181. https://doi.org/10.1017/S1367943005001915

Whitlock, R., 2014. Relationships between adaptive and neutral genetic diversity and ecological structure and functioning: a meta-analysis. J. Ecol. 102, 857–872.

Wickham, H., 2009. Ggplot2 : elegant graphics for data analysis. Springer.

Wilson, A.B., Glaubrecht, M., Meyer, A., 2004. Ancient lakes as evolutionary reservoirs: evidence from the thalassoid gastropods of Lake Tanganyika. Proc. R. Soc. London. Ser. B Biol. Sci. 271, 529–536. https://doi.org/10.1098/rspb.2003.2624

Wilson, A.B., Teugels, G.G., Meyer, A., 2008. Marine incursion: the freshwater herring of Lake Tanganyika are the product of a marine invasion into west Africa. PLoS One 3, e1979. https://doi.org/10.1371/journal.pone.0001979

Wise, R.M., Van Wilgen, B.W., Hill, M.P., Schulthess, F., Tweddle, D., Chabi-Olay, A., Zimmermann, H.G., 2007. The economic impact and appropriate management of selected invasive alien species on the African continent.

Witte, F., Goldschmidt, T., Wanink, J., van Oijen, M., Goudswaard, K., Witte-Maas, E., Bouton, N., 1992. The destruction of an endemic species flock: quantitative data on the decline of the haplochromine cichlids of Lake Victoria. Environ. Biol. Fishes 34, 1–28. https://doi.org/10.1007/BF00004782

Witte, F., Goldschmidt, T., Wanink, J.H., 1995. Dynamics of the haplochromine cichlid fauna and other ecological changes in the Mwanza Gulf of Lake Victoria, in: The Impact of Species Changes in African Lakes. Springer Netherlands, Dordrecht, pp. 83–110. https://doi.org/10.1007/978-94-011-0563-7_6

Woodward, S.P., 1859. On some new freshwater shells from Central Africa, in: Proceedings of the Zoological Society of London. pp. 348–351.

Wright, C.A., 1973. Flukes and snails. Macmillan, New York.

Wu, X.Y., Chilton, N.B., Zhu, X.Q., Xie, M.Q., Li, A.X., 2005. Molecular and morphological evidence indicates that *Pseudorhabdosynochus lantauensis* (Monogenea: Diplectanidae) represents two species. Parasitology 130, 669–677. https://doi.org/10.1017/S0031182004007152

Xiaoqin, X., Weijun, W., Weijian, Y., 2000. The life span of *Dactylogyrus vaginulatus* on *Hypophthalmichthys molitrix*. Jounrnal Nanjing Agric. Univ. 22, 95–98.

Yang, T., Liu, J., Gibson, D.I., Dong, A., 2006. Spatial distributions of two species of monogeneans on the gills of *Siganus fuscescens* (houttuyn) and their seasonal dynamics in caged versus wild-caught. J. Parasitol. 92, 933–940. https://doi.org/10.1645/GE-796R1.1

Zelditch, M., Swiderski, D.L., Sheets, H.D., 2012. Geometric morphometrics for biologists : a primer. Elsevier, London.

Zholdasova, I., 1997. Sturgeons and the Aral Sea ecological catastrophe. Environ. Biol. Fishes 48, 373–380. https://doi.org/10.1023/A:1007329401494

Ziętara, M.S., Lumme, J., 2002. Speciation by host switch and adaptive radiation in a fish parasite genus *Gyrodactylus* (Monogenea, Gyrodactylidae). Evolution 56, 2445–58.

Zink, R.M., 2004. The role of subspecies in obscuring avian biological diversity and misleading conservation policy. Proc. R. Soc. London. Ser. B Biol. Sci. 271, 561–564. https://doi.org/10.1098/rspb.2003.2617

Zohary, T., Ostrovsky, I., 2011. Ecological impacts of excessive water level fluctuations in stratified freshwater lakes. Inl. Waters 1, 47–59. https://doi.org/10.5268/IW-1.1.406

9 LIST OF PUBLICATIONS

Presented Ph.D. thesis is compiled of five manuscripts co-authored by Nikol Kmentová. As the original papers listed below are protected by copyright or not published yet, they are presented in full version in the printed thesis only. The electronic (publicly available) version of the thesis contains links to the papers available online.

Paper I

Kmentová N., Van Steenberge M., Raeymaekers J.A.M., Koblmüller S., Hablützel P. I., Muterezi Bukinga F., Mulimbwa N'sibula T., Masilya Mulungula P., Nzigidahera B., Ntakimazi G., Gelnar M., Vanhove M. P. M. 2018 Monogenean parasites of sardines in Lake Tanganyika: diversity, origin and intra-specific variability. *Contributions to Zoology* 87(2): 105-132 [Q1, IF (2018) = 2.139].

Publication is available at: https://brill.com/view/journals/ctoz/87/2/article-p105_4.xml

NK performed morphological description and molecular characterisation of newly described species, obtained morphometric data, conducted phylogenetic reconstruction, contributed to data analyses and interpretation and wrote the manuscript. Overall contribution: c. 70%

Monogenean parasites of sardines in Lake Tanganyika: diversity, origin and intra-specific variability

Nikol Kmentová[1, 15], Maarten Van Steenberge[2,3,4,5], Joost A.M. Raeymaekers[5,6,7], Stephan Koblmüller[4], Pascal I. Hablützel[5,8], Fidel Muterezi Bukinga[9], Théophile Mulimbwa N'sibula[9], Pascal Masilya Mulungula[9], Benoît Nzigidahera†[10], Gaspard Ntakimazi[11], Milan Gelnar[1], Maarten P.M. Vanhove[1,5,12,13,14]

[1] *Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic*
[2] *Biology Department, Royal Museum for Central Africa, Leuvensesteenweg 13, 3080, Tervuren, Belgium*
[3] *Operational Directorate Taxonomy and Phylogeny, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium*
[4] *Institute of Biology, University of Graz, Universitätsplatz 2, A-8010 Graz, Austria*
[5] *Laboratory of Biodiversity and Evolutionary Genomics, Department of Biology, University of Leuven, Ch. Deberiotstraat 32, B-3000 Leuven, Belgium*
[6] *Centre for Biodiversity Dynamics, Department of Biology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway*
[7] *Faculty of Biosciences and Aquaculture, Nord University, N-8049 Bodø, Norway*
[8] *Flanders Marine Institute, Wandelaarkaai 7, 8400 Oostende, Belgium*
[9] *Centre de Recherche en Hydrobiologie*, Département de Biologie, *B.P. 73 Uvira, Democratic Republic of Congo*
[10] *Office Burundais pour la Protection de l'Environnement, Centre de Recherche en Biodiversité, Avenue de l'Imprimerie Jabe 12, B.P. 2757, Bujumbura, Burundi*
[11] *Département de Biologie, Université du Burundi, Campus Mutanga, B.P. 2700, Bujumbura, Burundi*
[12] *Capacities for Biodiversity and Sustainable Development, Operational Directorate Natural Environment, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium*
[13] *Centre for Environmental Sciences, Research Group Zoology: Biodiversity and Toxicology, Hasselt University, Agoralaan Gebouw D, B-3590 Diepenbeek, Belgium*
[14] *Zoology Unit, Finnish Museum of Natural History, University of Helsinki, P.O.Box 17, Helsinki FI-00014, Finland*
[15] *E-mail: kmentovan@mail.muni.cz*

Keywords: Clupeidae, Dactylogyridae, fisheries targets, phenotypic variability, phylogeny

Abstract

Whereas Lake Tanganyika's littoral and benthic zones are famous for their diverse fish communities, its pelagic zone is dominated by few species, of which two representatives of Clupeidae (*Limnothrissa miodon* and *Stolothrissa tanganicae*) take a pivotal role. We investigated the monogenean fauna infecting these freshwater clupeids to explore the link between parasite morphology and host species identity, or seasonal and geographical origin, which may reveal host population structure. Furthermore, we conducted phylogenetic analyses to test whether these parasitic flatworms mirror their host species' marine origin. Based on 406 parasite specimens infecting 385 host specimens, two monogenean species of *Kapentagyrus* Kmentová, Gelnar and Vanhove, gen. nov. were morphologically identified and placed in the phylogeny of Dactylogyridae using three molecular markers. One of the species, *Kapentagyrus limnotrissae* comb. nov., is host-specific to *L. miodon* while its congener, which is new to science and described as *Kapentagyrus tanganicanus* Kmentová, Gelnar and Vanhove, sp. nov., is infecting both clupeid species. Morphometrics of the parasites' hard parts showed intra-specific variability, related to host species identity and seasonality in *K. tanganicanus*. Significant intra-specific differences in haptor morphometrics between the northern and southern end of Lake Tanganyika were found, and support the potential use of monogeneans as tags for host population structure. Based on phylogenetic inference, we suggest a freshwater origin of the currently known monogenean species infecting clupeids in Africa, with the two species from Lake Tanganyika representing a quite distinct lineage.

Contents

Introduction 106
Materials and methods 107
Sampling 107
Morphometrics.. 111
Molecular characterisation 112

Phylogeny 112
Results 114
Monogenean species records and description 114
Morphometrics 114
Discussion 117
Intra-specific morphological variability 119
Monogenean host-specificity in the lake's pelagic zone 120
Phylogenetic position and origin of Kapentagyrus *in Lake Tanganyika* 121
Conclusions 121
Acknowledgements 122
References 122
Appendix 128

Introduction

Lake Tanganyika is a unique freshwater ecosystem that is famous for its remarkable species richness and high levels of endemism (Moore, 1897; Coulter, 1991a). The lake was formed by tectonic rifting in East Africa between 9 and 12 million years ago (MYA) (Cohen *et al.,* 1993). In the past, geological processes and recurrent cycles of droughts and increased humidity caused lake level fluctuations with an extent of several hundreds of meters, potentially leading to recurrent separation of the lake's three sub-basins (Danley *et al*., 2012). It is suggested that those processes played an important role in shaping the lake's biodiversity (Sturmbauer *et al*., 2001; Sefc *et al*., 2017) and productivity (Cohen *et al*., 2006). Lake Tanganyika has attracted scientific interest for decades, mainly because of its diverse cichlid species assemblage, which comprises over 200 endemic species (Koblmüller *et al.,* 2008). Together with the endemic cichlid radiations of Lake Malawi and the region of Lake Victoria, it forms one of the prime model systems for studying adaptive radiation and speciation in vertebrates (e.g. Turner, 2007; Santos and Salzburger, 2012; Muschick *et al*., 2014). However, besides cichlids, numerous other fish and invertebrate taxa also radiated into flocks of largely endemic species in Lake Tanganyika (e.g. Fryer, 1991; Michel, 1995; Koblmüller *et al*., 2006; Marijnissen *et al*., 2006; Day and Wilkinson, 2006; Meixner *et al*., 2007; Glaubrecht, 2008; Brown *et al*., 2010; Erpenbeck *et al*., 2011; Peart *et al*., 2014; Vanhove *et al*., 2015). This makes Lake Tanganyika an ideal study system for studying patterns of intra-lacustrine radiation across a variety of taxa.

Contrary to the lake's littoral, the pelagic zone is relatively poor in terms of fish species richness. It is dominated by two endemic clupeids, *Limnothrissa miodon* (Boulenger, 1906) and *Stolothrissa tanganicae* Regan, 1917 and four endemic latid predators, *Lates angustifrons* Boulenger, 1906, *L. mariae* Steindachner, 1909, *L. microlepis* Boulenger, 1898, and *L. stappersii* (Boulenger, 1914) (Hecky, 1991). Worldwide, Clupeidae comprises 197 species (Nelson, 2006; Eschmeyer and Fong, 2017), of which 27 strictly riverine species of Dorosomatinae are found in Africa (Lavoué *et al.,* 2014). Originating from a marine environment, clupeids have expanded across Africa starting from the north-western coast (Wilson *et al.,* 2008). The ancestors of the two present-day Lake Tanganyika endemics, *L. miodon* and *S. tanganicae* reached the area of the Congo Basin around 27 MYA and diverged 8 MYA in the emerging Lake Tanganyika (Wilson *et al*., 2008). Both species have a lake-wide distribution, a short lifespan, a nocturnal vertical migration to feed on plankton, and schooling behaviour. Two nominal species have been recognised previously in *Limnothrissa*: *L. miodon* and *L. stappersii* (Poll, 1948). However, we consider *L. stappersii* a synonym of *L. miodon*, in view of the detailed study performed by Gourène and Teugels (1993), which demonstrated that the differences between the two nominal species could be interpreted as juvenile traits, and in view of the fact that no motivation was given for the revalidation of *L. stappersii* by Poll and Gosse (1995). Hence, *Limnothrissa* and *Stolothrissa* are monotypic genera.

Clupeids are an important part of the food web as they link the planktonic and piscivorous trophic levels (Hecky *et al*., 1981). Fluctuations of clupeid populations in Lake Tanganyika on an annual and a seasonal basis (dry and wet season) are related to environmental changes (Coulter, 1976; Hecky, 1991; Marshall, 1993; Plisnier *et al*., 2009). *Stolothrissa tanganicae* is the most abundant fish species in the lake and is the principal food source for large-sized pelagic fish. Juveniles of *L. miodon* feed on plankton and their adults also prey on juvenile representatives of *Limnothrissa* and on adult and juvenile specimens of *Stolothrissa* that mostly inhabit the pelagic zone. Reproducing populations and juveniles of *L. miodon* are found in bays, inshore waters and river deltas (Coulter, 1970; Marshall, 1993; Mulimbwa and Shirakihara, 1994). In contrast to *L. miodon*, *S. tanganicae* tends to stay more offshore from its early life stages onwards, with adults feeding only on plankton (Chapman and van Well, 1978; Plisnier *et al*., 2009). While significant geographical morphological variability was found in *L. miodon*, there is thus far no evidence for significant population genetic structure on neither small nor large geographical scales (Hauser *et al.,* 1995, 1998). Interestingly, previous studies in other systems already

demonstrated the potential use of parasites as tags for fish population structure on the level of parasite community composition (Oliva and Gonzalez, 2004; Criscione *et al.,* 2006; Poulin and Kamiya, 2015).

Although clupeids are important components of the food web and form the main fisheries in Lake Tanganyika, almost nothing is known about their parasite fauna. Only one helminth species has been described: the monogenean flatworm *Ancyrocephalus limnotrissae* Paperna, 1973, which infects the gills of *L. miodon* (Paperna, 1973). Monogenea is a class of parasitic flatworms (Platyhelminthes) characterised by a one-host life cycle with fish as their main hosts, a worldwide distribution and usually a high level of host-specificity (Pugachev *et al*., 2009). Despite their important role in all levels of ecosystem productivity (Kuris *et al*., 2008; Preston *et al*., 2013), parasites have been almost ignored in Lake Tanganyika for many decades. Systematic studies on the lake's parasite fauna are still fragmentary and only a tiny fraction of potential hosts has been investigated. Hitherto, such surveys have led to species descriptions of parasites from 25 host fish species, 19 of which were cichlids (Coulter, 1991b; Kmentová *et al*., 2016). Nevertheless, over the last decade, an increasing number of surveys has been conducted, including studies on ecological and evolutionary mechanisms behind parasite diversity and on the potential interplay with host evolution (Raeymaekers *et al*., 2013; Hablützel *et al*., 2014, 2016, 2017; Grégoir *et al*., 2015). Most recent taxonomic studies focused on monogeneans infecting cichlids in the species-rich littoral zone (see overview in Kmentová *et al*., 2016). Interestingly, while a high host-specificity was observed in monogeneans infecting cichlids in Lake Tanganyika's littoral zone (Vanhove *et al*., 2015), the observed lower level of host-specificity reported on bathypelagic cichlids of the tribe Bathybatini (Pariselle *et al*., 2015; Kmentová *et al*., 2016) resembles the situation in pelagic and deep water marine environments (Dogiel and Bogolepova, 1957; Rohde, 1980; Justine *et al*., 2012; Schoelinck *et al.,* 2012). This phenomenon has been explained by lower host species densities and increased home range compared to the littoral habitat and hence a decreasing speciation rate of parasites (Rohde, 1988).

Lake Tanganyika consists of three different sub-basins. Although the lake never desiccated, it is very likely that these sub-basins were at times separated due to fluctuations in water level (Danley *et al*., 2012). Moreover, latitudinal differences in mixing due to prevailing winds (Langenberg *et al*., 2002) resulted in different depths of the oxygenated layer (Coenen *et al.,* 1993). These factors have contributed to limnological differences among sub-basins with consequences for fish communities and hence, potentially, also their parasites. The effect of geographic variation (Kmentová *et al*., 2016), seasonality (Mo, 1991; Dávidová *et al*., 2005) and host species (Šimková *et al*., 2001a; Kmentová *et al*., 2016) on parasite morphology at the intra-specific level has been extensively documented.

As mentioned above, almost nothing is known about the geographical population structure of the two clupeid species from Lake Tanganyika, even though they are of great economic importance. As monogeneans are parasites with a direct life cycle and low pathogenicity, they have already been used as markers for host population structure in other fish (MacKenzie, 1983; Williams *et al.,* 1992). Clupeidae have a primary marine origin and the majority of its representatives in oceans worldwide are known to be infected by monogenean species, mainly from Mazocraeidae (Gérard *et al*., 2015), but also from Microcotylidae (Mendoza-Garfias and Pérez-Ponce de Léon, 1998) and Gyrodactylidae (Huyse and Malmberg, 2004). The origin of the monogeneans infecting African freshwater clupeids, which were described as representatives of Dactylogyridae, has never been investigated.

Here, we provide the first comprehensive study on the monogenean parasite diversity of the two economically important clupeids from Lake Tanganyika to answer four questions. (1) Which monogenean parasites infect clupeids in Lake Tanganyika? (2) Do monogeneans on these clupeids follow the pattern of low host-specificity that was already observed in other parasites from the lake's pelagic zone? (3) Is the morphology of monogeneans affected by seasonality, host species identity or geographic origin, and can monogenean parasites, therefore, be used for host stock identification? (4) Can the origin of these monogeneans be inferred from phylogenetic data?

Materials and methods

Sampling

Specimens of the two species of clupeids (*Limnothrissa miodon*, *Stolothrissa tanganicae*) were sampled from 19 localities in Lake Tanganyika (see Table 1, Fig. 1). Samples included specimens from the ichthyology

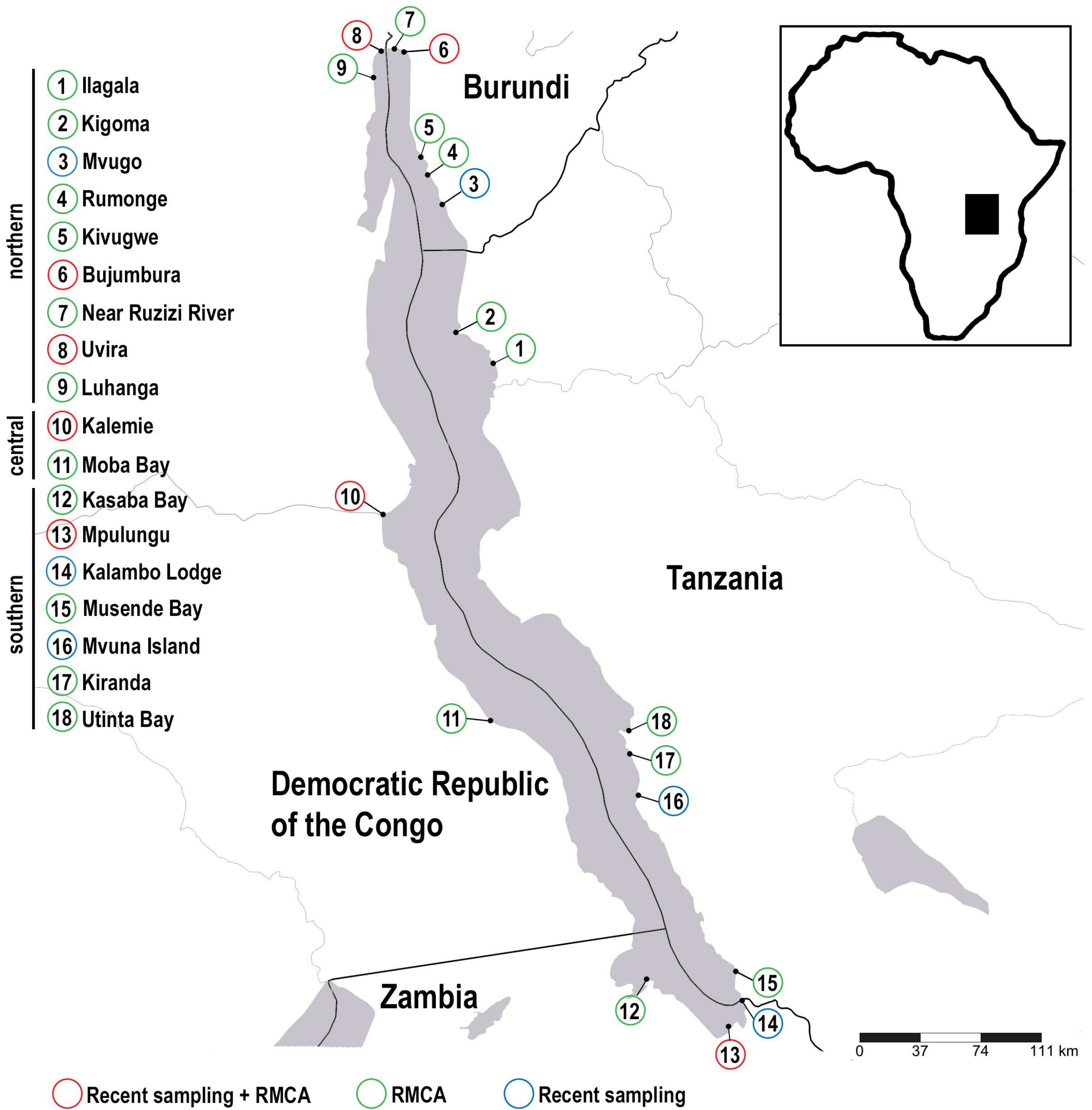


Figure 1. Sampling localities in Lake Tanganyika including sub-basin specification. Map created using SimpleMappr software v7.0.0. (available at http://www.simplemappr.net. Accessed April 20, 2017).

collection of the Royal Museum for Central Africa (RMCA) (Tervuren, Belgium) and fresh specimens that were either obtained from local fishermen or caught with gill nets from the experimental fishing unit of the Centre de Recherche en Hydrobiologie - Uvira (CRH) (Uvira, Democratic Republic of the Congo, August 2016) (see Fig. 1). In total, gills and fins of 385 fish specimens were examined following the standard protocol of Ergens and Lom (1970). Infection parameters such as prevalence (percentage of infected hosts) and infection intensity (mean number of monogenean individuals per infected host) were calculated following Ergens and Lom (1970). Monogeneans were mounted on slides using a solution of glycerine ammonium picrate (GAP) or, in the case of specimens retrieved from hosts from

Table 1. An overview of host species examined for monogenean parasites with localities and infection parameters (*Kapentagyrus limnotrissae* before and *K. tanganicanus* behind slashes).

Host species	Locality (geographic coordinates, year)	Locality – basins (Danley *et al.*, 2012)	Number of fish specimens (accession number in RMCA)	Number of monogenean individuals	Prevalence (%)	Infection intensity/one gill chamber	Abundance/one gill chamber (range)
Limnothrissa miodon	Bujumbura (3°23'S-29°22'E, 1.7.-31.7.1928)	The northern basin	2 (MRAC 23567-68)	0/0	0/0	0/0	0/0
	Bujumbura (1.2.-28.2.1935)	The northern basin	11 (MRAC 43554-64)	1/0	9/0	1/0	0.09 (0-1)/0
	Kalemie (5°56'S-29°12'E, 22.10.1946)	The central basin	8 (MRAC 88891-89098)	4/0	25/0	2/0	0.5 (0-3)/0
	Kalemie (1.1.-31.1.1946)	The central basin	1 (MRAC 89151)	2/0	100/0	2/0	2/0
	Kalemie (20.11.1946)	The central basin	8 (MRAC 89137-144)	22/0	25/0	5.5/0	2.75 (0-9)/0
	Kalemie (11.8.2016)	The central basin	10 (-)	55/5	80/33	6.9/1.7	5.5 (0-15)/0.5(0-2)
	Kasaba Bay (8°31'S-30°42'E, 1.1.1967)	The southern basin	2 (MRAC 190150-151)	0/49	0/100	0/29.5	0/29.5 (12-37)
	Kigoma Bay (4°88'S-29°61'E, 12.4.-13.4.1947)	The northern basin	4 (MRAC 89367-70)	2/0	25/0	2/0	0.5 (0-4)/0
	Kiranda (07°25'S-30°36'E, 11.3.1947)	The southern basin	2 (MRAC 89311-12)	9/0	50/0	9/0	4.5 (0-9)/0
	Kivugwe (3°80'S-29°34'E, 22.2.1994)	The northern basin	7 (MRAC 94069.0369-70)	2/0	28.6/0	1/0	0.28 (0-1)/0
	Kasaba Bay (5.3.1947)	The southern basin	1 (MRAC 89353)	1/2	100/100	1/2	1/2
	Luhanga (3°52'S-29°15'E, 26.4.1994)	The northern basin	2 (MRAC 94069.2375-76)	0/1	0/50	0/1	0/0.5 (0-1)
	Moba Bay (7°03'S-29°47'E, 21.3.1947)	The central basin	2 (MRAC 89335-36)	9/0	50/0	11/0	5.5 (0-11)/0
	Mpulungu (8°46'S-31°07'E, 14.3.1966)	The southern basin	3 (MRAC 189612-14)	1/0	33.3/0	1/0	0.33 (0-1)/0
	Mpulungu (19.8.2016)	The southern basin	2 (-)	4/0	50/0	4/0	2 (0-4)/0
	Mvugo (4°18'S-29°34'E, 4.8.2016)	The northern basin	6 (-)	9/25	50/100	3/4.2	1.5/4.2(1-10)

Host species	Locality (geographic coordinates, year)	Locality – basins (Danley *et al*., 2012)	Number of fish specimens (accession number in RMCA)	Number of monogenean individuals	Prevalence (%)	Infection intensity/one gill chamber	Abundance/one gill chamber (range)
	Mvuna Island (7°26'S 30°32'E, 18.8.2015)	The southern basin	6 (-)	11/5	50/50	3.7/1.7	1.8 (0-8)/0.83(0-3)
	Near Ruzizi (2°50'S-29°02'E, 2.12.1954)	The northern basin	2 (MRAC 99633-34)	6/0	50/0	6/0	3 (0-6)/0
	Near Ruzizi (26.10.1954)	The northern basin	4 (MRAC 99623-32)	7/0	50/0	7/0	3.5 (0-7)/0
	Rumonge (3°58'S-29°25'E, 1.2. - 28.2.1935)	The northern basin	12 (MRAC 43763-72)	10/4	25/16.7	3.3/2	0.8 (0-6)/0.2(0-2)
	Ilagala (05°14'S-29°47'E, 24.2.1947)	The northern basin	8 (MRAC 89211-18,41)	14/0	62.5/0	2.8/0	1.75 (0-6)/0
	Uvira (3°22′ S 29°09′E, 12.8.2016)	The northern basin	41 (-)	12/28	35/40	1.7/3.5	0.6 (0-3)/1.4(0-9)
Stolothrissa tanganicae	Bujumbura (4.8.2016)	The northern basin	29 (-)	7	13.3	1.5	0.14 (0-2)
	Kalambo Lodge (8°59'S-31°18'E, 20.8.2016)	The southern basin	48 (-)	6	4.2	1.5	0.06 (0-2)
	Kalemie (9.2.-10.2.1947)	The central basin	6 (MRAC 89428-33)	0	0	0	0
	Kalemie (12.8.2016)	The central basin	33 (-)	0	0	0	0
	Kigoma Bay (12.4.-.13.4.1947)	The northern basin	4 (MRAC 89494-98)	0	0	0	0
	Kigoma Bay (13.5.1947)	The northern basin	6 (MRAC 89462-65)	5	33.3	2.5	2.5 (1-4)
	Mpulungu (4.7-5.7.1965)	The southern basin	7 (MRAC 189618-19)	0	0	0	0
	Mpulungu (3.10.1966)	The southern basin	2 (MRAC 189595-601)	3	28.6	1.5	0.4 (0-2)
	Mpulungu (19.8.2016)	The southern basin	18 (-)	2	11.1	1	0.11 (0-1)
	Musende Bay (8°46'S-31°06'E, 7.4.1967)	The southern basin	5 (MRAC 190171-74)	4	20	3.5	0.8 (0-4)
	Mvugo (15.8.2015)	The northern basin	6 (-)	7	33.3	4	1.2 (0-6)
	Rumonge (1.1.-31.12.1935)	The northern basin	18 (MRAC 43763-72)	28	61	3.5	1.55 (0-7)
	Utinta Bay (7°10'S-30°53'E, 17.2.1947)	The southern basin	1 (MRAC 89442)	1	100	1	1
	Uvira (1.1.1935)	The northern basin	4 (MRAC 43787-88,90,98)	0	0	0	0
	Uvira (1.1.1954)	The northern basin	2 (MRAC 99603-4)	0	0	0	0
	Uvira (12.8.2016)	The northern basin	27 (-)	31	44	2.6	1.1 (0-6)
	Uvira (12.8.2016)	The northern basin	25 (-)	12	28	1.7	0.5 (0-3)

the museum collection, Hoyer's medium. Some of the individuals were cut into three parts with the anterior and posterior parts mounted on slides and the rest used for genetic identification. Monogeneans selected for molecular analyses were transferred into Eppendorf tubes containing 99% ethanol. Parasite identification and description were carried out using an Olympus BX51 microscope equipped with a drawing tube and OLYMPUS KL 1500 LED illumination. Fish tissue samples were deposited in the ichthyology collection of the RMCA under collection number MRAC P. 2016.20 and parasite voucher and type specimens are available in the invertebrate collection of the RMCA; the Finnish Museum of Natural History (MZH), Helsinki, Finland; the Iziko South African Museum (SAMC), Cape Town, Republic of South Africa; the Muséum national d'Histoire naturelle (MNHN), Paris, France; and the Natural History Museum (NHMUK), London, United Kingdom. Collected monogenean species were also compared to type material (MRAC MT. 35572 and 35711).

Morphometrics

All monogeneans found in this study were identified as representatives of Dactylogyridae. Since the taxonomy of dactylogyrids at species level is principally based on the morphology of their sclerotised structures (Pugachev *et al*., 2009; García-Varela *et al*., 2016), and since monogenean specimens could not be collected alive because the sardine hosts invariably die immediately upon capture (rendering staining of monogenean fresh specimens impossible), differential diagnoses focused on details of the parasites' hard parts. Measurements of sclerotized structures were taken at a magnification of 1000× using an Olympus BX51 microscope with incorporated phase contrast and the software Digital Image Analysis v4. In total, 25 different parameters regarding the total body size, the hard parts of haptoral and male copulatory organs (MCOs) were measured (see Fig. 2). Terminology was based on Řehulková *et al*. (2013). To check for inter-specific and intra-specific parasite phenotypic variability in haptor morphology, measurements were analysed by multivariate statistical techniques in the R package Adegenet (Jombart, 2008; R development core team, 2011), where principal component analysis (PCA) was conducted with standardised variables on the co-variance matrix of 21 morphological characters (total length and width of body, and the size of the sixth and seventh pair of marginal hooks were discarded because of the low number of observations). Outliers were identified and removed using Mahalanobis distances in the package mvoutlier (Filzmoser and Gschwandtner, 2017). To take possible geographical intra-specific variation into account, samples were grouped according to the three sub-basins, following Danley *et al*. (2012). The effect of season (dry period from May to September, wet period from October to April), geography and host body size on haptoral morphometrics was tested using MANOVA, package stats (R Core Team, 2013), with Pillai's test of significance. To test the possible effect of host body size, fish specimens were assigned to three groups as follows (one group of sub-adults and one of adults for *L. miodon* (B and C) and *S. tanganicae* (A and B), respectively): A (4-6 cm), B (>6-9 cm), C (>9cm) (Eccles, 1992). To avoid correlation between host size and any other parameter, specimens from one locality (Uvira and Kalemie, respectively) and

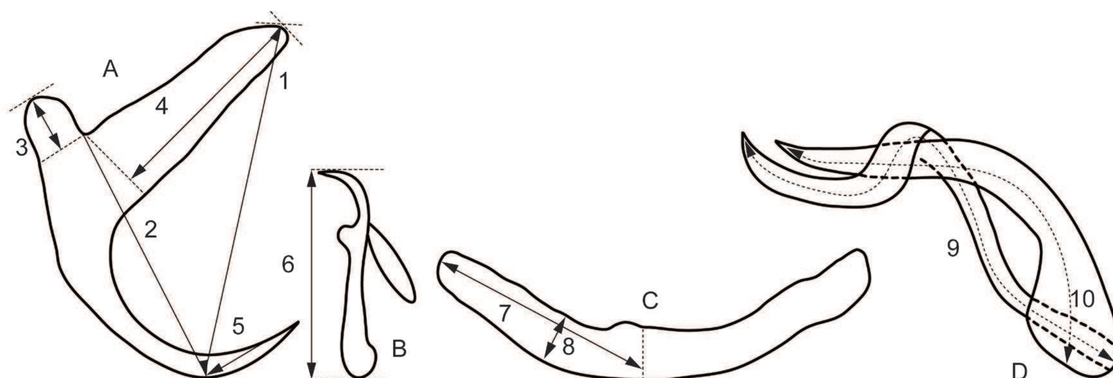


Figure 2. Measurements for sclerotized structures of haptor and reproductive organs of *Kapentagyrus* spp. A Anchor: 1—Total length, 2—Length to notch, 3—Outer root length, 4—Inner root length, 5—Point length; B Hook: 6—length; C Bar: 7—Branch length, 8—Branch width; D Male copulatory organ: 9—Copulatory tube length, 10—Accessory piece length.

from the dry season were used for these analyses. The assumption of homogeneous variance within sample groups was verified by Levene's test. Two-sample T tests or non-parametric Mann-Whitney U tests (when the assumption of homogenous variance was not met) were performed to provide information about intra-specific variability in copulatory organ morphometric parameters related to host species, host size, season and geographic origin of all collected monogenean species.

Molecular characterisation

Species delimitation based on morphological characters was combined with genetic characterisation using tissue samples of a subset of the parasite individuals mentioned above and ribosomal DNA markers commonly used for dactylogyrid species delimitation. Specimens from all three sub-basins and both host species were included to investigate potential intra-specific genetic variation. Whole genomic DNA was extracted using the Qiagen Blood and Tissue Isolation Kit following the manufacturer's instructions with some modifications (samples in ATL buffer (180 ml) with protein kinase (20 ml) were kept in 1.5 ml Eppendorf tubes overnight at room temperature). The DNA extract was concentrated to a volume of 80 ml in 1.5 ml Eppendorf tubes using a vacuum centrifuge and stored at a temperature of -20 °C. To confirm parasite species delineation genetically, we used three different nuclear sequence fragments, from the small and large ribosomal subunit gene (18 and 28 rDNA) and the internal transcribed spacer 1 (ITS-1). Partial 18S rDNA together with ITS-1 were amplified using the S1 (5´-ATTCCGATAACGAACGAGACT-3´) (Sinnappah *et al*., 2001) and Lig5.8R (5´-GATACTCGAGCCGAGTGATCC-3´) (Blasco-Costa *et al*., 2012) primers. Each reaction mix contained 1.5 unit of *Taq* Polymerase, 1X buffer containing 0.1 mg/ml BSA, 1.5 mM $MgCl_2$, 200 mM dNTPs, 0.8 mM of each primer and 3 μl of isolated DNA (concentration was not measured) in a total reaction volume of 30 μl under the following conditions: 2 min at 95 ºC, 39 cycles of 1 min at 95 ºC, 1 min at 55 ºC and 1 min and 30 s at 72 ºC, and finally 10 min at 72 ºC. Primers C1 (5´-ACCCGCTGAATTTAAGCAT-3´) and D2 (5´-TGGTCCGTGTTTCAAGAC-3´) (Hassouna *et al*., 1984) were used for amplification of the partial 28S rDNA gene. Each PCR reaction contained 1.5 unit of *Taq* Polymerase, 1X buffer containing 0.1 mg/ml BSA, 1.5 mM $MgCl_2$, 200 mM dNTPs, 0.5 mM of each primer and 50 ng of genomic DNA in a total reaction volume of 30 μl under the following conditions: 2 min at 94 ºC, 39 cycles of 20 seconds at 94 ºC, 30 seconds at 58 ºC and 1 min and 30 s at 72 ºC, and finally 10 min at 72 ºC. Amplification success was checked by agarose gel electrophoresis and positive samples were enzymatically cleaned up using 1 μl of ExoSAP-IT reagent and 2.5 μl of PCR product under the following conditions: 15 min at 37 ºC and 15 min at 80 ºC. After cycle sequencing of purified PCR products using the BigDye protocol v3.1, following the manufacturer's recommendations, fragments were cleaned up using the BigDye XTerminator® Purification Kit and visualized on an ABI3130 capillary sequencer. Electropherograms were visually inspected, corrected and sequences were aligned using MUSCLE (Edgar, 2004) under default distance measures as implemented in MEGA v7 (Kumar *et al*., 2016), together with previously published sequences of representative freshwater and marine dactylogyrid species (see Supplementary file 1: Table S1). The newly obtained sequences were deposited in NCBI GenBank under the accession numbers MH071782-83 and MH071807-8. For all sequenced loci, pairwise genetic distances (uncorrected p-distances) among all dactylogyrid species included in the phylogenetic reconstruction were calculated in MEGA v7.

Phylogeny

Phylogenetic analyses were based on three loci: 18S, 28S and ITS-1 rDNA. The consistency of all alignments was checked and corrected under the "automated 1" option in trimAL v1.2, which uses a heuristic search to find the best method for trimming the alignment (Capella-Gutiérrez *et al.,* 2009). Alignment matrices of both ribosomal regions used for reconstruction of the dactylogyrid phylogeny were concatenated using Mesquite v3.2 (Maddison and Maddison, 2017).

Topali v2.5 (Milne *et al*., 2004) was used to select the most appropriate evolutionary model (based on the Bayesian information criterion) to be used in subsequent phylogenetic analyses. The GTR (Rodríguez *et al*., 1990) + Γ + I model with a gamma shape parameter of 0.952 and a proportion of invariable sites I of 0.276 was used for the 28S rDNA region, the GTR + Γ model with a gamma shape parameter of 0.222 was used for 18S rDNA, and the HKY + Γ model with a gamma shape parameter of 3.539 was used for the ITS-1 region. Phylogenetic analyses employed maximum likelihood (ML) and Bayesian inference (BI) in RAxML v8 (Stamatakis, 2014) and MrBayes v3.2.0 (Ronquist *et al*., 2012), respectively,

Table 2. Results of MANOVA tests performed on haptoral measurements of *Kapentagyrus limnotrissae* and *K. tanganicanus* (haptoral morphologies from *L. miodon* and *S. tanganicae*, respectively). Only significant parameters are listed. Abbreviation of sub-basin in brackets: N – northern, C – central, S – southern.

	K. limnotrissae			*K. tanganicanus* (from L. *miodon*)		*K. tanganicanus* (from *S. tanganicae*)	
Parameter	Subbasin	Season	Host body size	Subbasin	Season	Season	Host body size
Dorsal bar							
Branch length	$F_{1,90}$=8.10; p<0.01 (N, C) $F_{1,42}$=4.79; p<0.05 (N, S) $F_{1,59}$=18.18; p<0.001 (S, C)	-	$F_{1,59}$=18.19; <0.001	$F_{1,67}$=10.10; p<0.01	$F_{1,73}$=6.2; p<0.05	$F_{1,61}$=6.72; p<0.05	$F_{1,39}$=6.54; p<0.05
Thickness at midlength	-	-	-	-	-	-	$F_{1,39}$=17.56; p<0.001
Ventral bar							
Branch length	$F_{1,59}$=12.69; p<0.001 (S, C)		$F_{1,39}$=12.69; <0.001	-	-	-	$F_{1,39}$=12.50; p<0.01
Branch maximum width	$F_{1,59}$=5.65; p<0.05 (S, C)	$F_{1,100}$=20.13; p<0.001	$F_{1,59}$=5.64; p<0.05	-	-	$F_{1,61}$=10.02; p<0.05	$F_{1,39}$=21.14; p< 0.001
Hooks							
Pair I	-	$F_{1,100}$=17.18; p<0.001	-	$F_{1,67}$=5.58; p>0.05	$F_{1,73}$=3.97; p<0.05	-	-
Pair II	-	-	-	$F_{1,67}$=5.82; p<0.05	-		$F_{1,39}$=6.36; p<0.05
Pair III	-	-	-	-	-	$F_{1,61}$=4.03; p<0.05	$F_{1,39}$=5.76; p<0.05
Pair V	-	-	-	-	-	$F_{1,61}$=5.43; p<0.05	$F_{1,39}$=8.28; p<0.01
Pair VI	-	-	-	$F_{1,67}$=8.77; p<0.01	$F_{1,73}$=5.41; p<0.05	-	-
Pair VII	-	-	-	-	-	-	$F_{1,39}$=6.25; p<0.05
Dorsal anchor							
Inner root length	-	-	-	$F_{1,67}$=10.10; p<0.01	$F_{1,73}$=10.5; p>0.01	-	-
Length to notch	$F_{1,90}$=6.43; p<0.05 (N, C)	-	-	-	-	-	-
Ventral anchor							
Inner root length	-	$F_{1,100}$=4.8; p<0.05	-	$F_{1,67}$=9.98; p<0.01	$F_{1,73}$=10.1; p>0.01	-	-
Outer root length	-	-	-	$F_{1,67}$=6.00; p<0.05	$F_{1,73}$=5.2; p>0.05	-	-
Length to notch	$F_{1,59}$=7.24; p<0.01 (S, C)	-	$F_{1,59}$=7.24; p<0.01	-	-	-	-

with data partitioned per marker. The ML tree search was conducted using RAxML's standard tree search algorithm and bootstrap support was calculated using the option with an automated number of replicates to obtain stable support values under the frequency stopping criterion (Stamatakis, 2014). Bayesian inference in MrBayes was based on two independent runs (10^7 generations, sampled every 1,000th generation and with a burn-in of 10%). Parameter convergence and run stationarity were assessed in Tracer v1.6 (Rambaut *et al.,* 2014). As Dactylogyridae and Diplectanidae were shown to be sister taxa (Šimková *et al*., 2003), sequences of *Diplectanum aequans* (Wagener, 1857), 1991 were used as outgroup. To compare the

genetic diversity within dactylogyrid genera in Lake Tanganyika, sequences of *Cichlidogyrus* Paperna, 1960 species infecting cichlids from the lake's pelagic zone (*C. attenboroughi*, *C. brunnensis* Kmentová, Gelnar, Koblmüller and Vanhove, 2016 and *C. casuarinus* Pariselle, Muterezi Bukinga and Vanhove, 2015) as well as a sequence of a littoral species of *Cichlidogyrus* (*C. irenae* Gillardin, Vanhove, Pariselle, Huyse and Volckaert, 2012) were included. Phylogenetic trees were edited in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree).

Results

Monogenean species records and description

In total, 406 monogenean specimens were recorded from two host species, namely *L. miodon* (300) and *S. tanganicae* (106) (Table 1). Morphological characterisation revealed the presence of two monogenean species belonging to a newly described genus, *Kapentagyrus* Kmentová, Gelnar and Vanhove, gen. nov. The existence of *Kapentagyrus* as a new genus is supported by phylogenetic reconstruction at the family level (Fig. 4) and its description is provided in Appendix. The previously described species *Ancyrocephalus limnotrissae* is reassigned to this new genus. The two species of *Kapentagyrus* in Lake Tanganyika can be morphologically distinguished by the proportion of inner/outer root length of both ventral and dorsal anchors (around 3 in *K. limnotrissae* Kmentová, Gelnar and Vanhove comb. nov., whereas this proportion is close to 2 in *K. tanganicanus* Kmentová, Gelnar and Vanhove, sp. nov.). Only one species, described in this study as *K. tanganicanus*, was collected from *S. tanganicae*. *Limnothrissa miodon* was infected by both *K. tanganicanus* and by the host-specific monogenean species *K. limnotrissae*. Due to the bad state of the holotype, a redescription of this species is provided as the type species of the newly described genus in Appendix, together with the description of *K. tanganicanus*. Infection parameters are shown in Table 1. Considering morphological and morphometric differences, especially in the total length of both anchors and in the branch length of the dorsal bar (see Appendix, Table 3), phenotypic variability related to host species identity in *K. tanganicanus*. is described (see Table 3, Figs 7 and 8). Even though these intraspecific morphological and morphometric differences are of the same magnitude as the interspecific distances (Fig. 3 and Table 3), ribosomal DNA sequences in all three regions were identical for all individuals assigned to *K. tanganicanus*. Therefore, we consider the individuals infecting *L. miodon* and *S. tanganicae* as belonging to the same species.

Morphometrics

Interspecific and between-host level

Principal component analysis was performed to examine and visualise the morphometric differences between *K. limnotrissae* and the two haptor morphologies of *K. tanganicanus*. The analysis was done using haptoral morphometric parameters of 96 individuals of *K. limnotrissae*. including the holotype, and on 58 and 69 individuals of *K. tanganicanus* collected from *S. tanganicae* and *L. miodon*, respectively. The first PC explained 33.0 % and the second 16.3 % of the variation in the dataset. Results show a strong separation of three groups (Fig. 3). The combination of both PCA axes chiefly described host species identity of *K. tanganicanus*, resulting in two distinct haptoral morphologies. The first axis separated specimens of *K. limnotrissae* and *K. tanganicanus*, both parasitizing on *L. miodon*. The type specimen of *K. limnotrissae* clustered with the rest of the measured individuals of this species, hereby confirming species identification (Fig. 3). Mann-Whitney U tests showed a significantly larger copulatory tube and accessory piece in *K. limnotrissae* than in *K. tanganicanus* (copulatory tube - $Z_{1,80}$=-9.90; p<0.001; accessory piece - $Z_{1,78}$=-9.52; p<0.001), hereby confirming species delineation. There was no difference in copulatory tube length between specimens of *K. tanganicanus* collected from different host species, but the accessory piece was longer in individuals collected from *L. miodon* than in individuals from *S. tanganicae* (Mann Whitney U test; $Z_{1,77}$=-3.31; p<0.001).

Intra-specific level: influence of season, sub-basin and host size

Intraspecific morphometric variation was analysed by MANOVA. In *K. limnotrissae* two of the 21 parameters, namely inner root length of the dorsal anchor and length of the first marginal hook, were significantly larger in specimens collected during the dry compared to specimens collected during the rainy season, while the maximum straight width of the ventral bar was significantly smaller in the dry compared to the rainy

Table 3. Comparison of measurements performed on *Kapentagyrus limnotrissae* haptoral and genital hard parts described in Paperna (1973), *Kapentagyrus limnotrissae* redescribed in this study and *K. tanganicanus* (a – mean value±standard deviation, b – range).

Parameters (µm)	*K. limnotrissae* (original description)	*K. limnotrissae* (present study)	*K. tanganicanus (L. miodon)*	*K. tanganicanus (S. tanganicae)*
Total length	440-610	458.1±128.5 (n=21); (286.2-748.3)	214.2±39.6 (n=23); (121.7-285.8)	167.9±25.57 (n=20); (146.8-199.4)
Total width	90-130	143.9±26.2 (n=20); (97.8-220.3)	779.5±170.1 (n=25); (474.4-1171.3)	565.5±122.55 (n=4); (462.7-710.9)
Ventral anchor				
Total length	20-27	26.2±1.5 (n=99); (22.3-31.8)	31.6±2.4 (n=77); (25.3-36.7)	19.4±1.8 (n=42); (15.3-24.6)
Length to notch	19-20	18.8±1.6 (n=98); (15.5-28.3)	21.3±1.8 (n=72); (16.5-26.7)	24.3±2.4 (n=41); (19-32.8)
Inner root length	7-13	15.6±1.4 (n=99); (11.4-18.6)	18.6±2.5 (n=77); (10.0-22.9)	13.0±2.1 (n=41); (9.0-18.2)
Outer root length	5-8	5.0±0.8 (n=94); (3.6-7.9)	8.7±1.5 (n=75); (4.9-11.4)	6.6±1.1 (n=43); (4.3-9.8)
Point length	5-7	8.1±1.1 (n=90); (5.3-11.0)	8.7±1.1 (n=69); (6.7-12.3)	7.7±1.3 (n=39); (5.0-10.5)
Dorsal anchor				
Total length	23-26	21.48±1.55 (n=88); (18.7-26.1)	27.7±2.0 (n=73); (20.832.6)	21.5±1.96 (n=40); (17.5-26.2)
Length to notch	15-19	16.5±1.3 (n=87); (13.8-20.5)	19.9±2.1 (n=74); (13.4-28.2)	17.9±1.16 (40); (15.3-21.5)
Inner root length	10-13	11.2±1.3 (n=87); (7.8-14.6)	14.4±2.3 (n=72); (6.6-20.6)	10.5±1.42 (n=40); (7.7-14.8)
Outer root length	5-7	4.5±1.0 (n=84); (2.5-8.0)	8.1±1.6 (n=70); (4.9-16.1)	5.8±0.89 (n=39); (4.2-7.8)
Point length	5-7	7.8±1.1 (n=84); (5.1-10.9)	8.2±1.2 (n=67); (5.7-11.4)	7.5±0.98 (n=38); (5.9-9.6)
Ventral bar				
Branch length	27-35	16.7±3.0 (n=84); (12.5-33.3)	21.4±3.7 (n=66); (14.5-32.7)	18.9±3.10 (n=40); (14.2-27.8)
Branch maximum width	-	4.4±0.7 (n=86); (3.0-7.0)	7±1.5 (n=72); (3.9-11.4)	4.8±1.28 (n=40); (3.0-7.7)
Dorsal bar				
Branch length	22-35	17.4±3 (n=69); (12.0-27.1)	25.1±4.1 (n=72); (18.4-35.2)	20.8±3.23 (n=39); (14.9-28.7)
Thickness at midlength	-	4.2±0.7 (n=75); (2.9-6.3)	6.8±1.3 (n=75); (4-10)	4.9±4.94 (n=41); (3.7-7.5)
Hooks				
Pair I	-	14.4±1.4 (n=67); (11.4-17.9)	13.3±1.3 (n=63); (10.0-17.8)	12.8±1.17 (n=38); (9.7-15.7)
Pair II	-	15.4±1.3 (n=63); (12.1-18.2)	14.6±1.6 (n=47); (11.8-17.9)	13.5±1.22 (n=32); (10.8-15.8)
Pair III	-	15.9±1.2 (n=68); (13.3-19.3)	14.8±1.5 (n=53); (11.2-17.7)	13.7±1.08 (n=33); (12-16.1)
Pair IV	-	16.2±1.1 (n=59); (13.0-19.3)	15.3±1.6 (n=42); (10.8-19.4)	13.8±1.60 (n=31); (11.3-16.2)
Pair V	-	14.2±1.6 (n=35); (9.3-17)	13.5±1.4 (n=32); (10.4-16.2)	5.8±0.89 (n=39); (4.2-7.8)
Pair VI	-	16.3±1.1 (n=34); (13.0-18.8)	15.3±1.4 (n=32); (11.9-18.1)	14.6±0.92 (n=25); (12.0-15.9)
Pair VII	-	16,7±1,4 (n=24); (14,3-20,8)	15±1,3 (n=22); (12,9-17,5)	13,8±0,97 (n=16); (11,0-15,5)
Pair I, II, III, IV, VI, VII average size	13-15	15,5±1,5 (n=350); (9,3-20,8)	14,6±1,6 (n=259); (10,0-19,4)	13,6±1,2 (n=174); (9,7-16,2)
Copulatory tube curved length	21-23	30,4±2,5 (n=75); (25,1-38,3)	39,7±4,6 (n=63); (30,4-49,9)	38,1±2,27 (n=12); (33,7-42,2)
Accessory piece curved length	-	36,1±3,6 (n=69); (28,0-47,1)	52,5±6,0 (n=62); (33,9-62,6)	45,9±4,2 (n=12); (38,3-53,5)

season. Moreover, some of the parameters also showed significant differences in relation to the geographic origin of the specimens. An extended length from the point to the notch of the dorsal anchor and an extended branch length of the dorsal bar were seen in specimens from the northern compared to those from the central sub-basin, while the branch length of the dorsal bar of specimens from the northern sub-basin is shorter compared to specimens from the southern sub-basin. An extended length of four other parameters: branch length of the dorsal bar, branch length of the ventral bar, maximum width of the ventral bar and length to notch of the ventral anchor was documented in specimens from the southern compared to those from the central sub-basin. Significantly higher length of the branch of the dorsal bar, length to notch of the ventral anchor and branch length and maximum width of the ventral bar were reported in specimens stemming from the larger size class of host specimens of *L. miodon*. There were no significant differences in copulatory tube parameters between specimens of *K. limnotrissae* collected in different seasons or from different sub-basins.

In *K. tanganicanus*, phenotypic variability related to host species was found. Hence, the relation of morphometric parameters to season, sub-basin and host size was tested separately for specimens collected from *L. miodon* and *S. tanganicae*. Similar to the situation in *K. limnotrissae*, the inner root length of the dorsal anchor of *K. tanganicanus* collected from *L. miodon* was larger in the dry compared to the rainy season, as were the inner and outer root lengths of the ventral anchor. On the other hand, the length of the dorsal bar branch and of the first and sixth marginal hook was larger in the rainy compared to the dry season. An extended length of the dorsal's bar branch, the third and fifth marginal hook and a thicker ventral bar was reported in the rainy compared to the dry season in *K. tanganicanus* collected from *S. tanganicae*. Since there were not enough specimens from the central and southern sub-basin of *K. tanganicanus* collected from *S. tanganicae*, the influence of geographic origin could only be tested on specimens collected from *L. miodon*. Similar to *K. limnotrissae*, geographical differences were reflected in an extended branch length of the dorsal bar in the northern compared to the southern part of the lake for *K. tanganicanus*. Moreover, the length of the dorsal and ventral anchor inner root, the ventral anchor outer root and the first, second and sixth marginal hook significantly differed between sub-basins. While there was no indication of any morphometric parameter being influenced by host size in the case of parasites of *L. miodon*, significantly larger haptoral structures were found in parasites collected from larger specimens of *S. tanganicae*: branch length and width of the dorsal bar; branch length and maximum width of the ventral bar; length of the second, third, sixth and seventh marginal hook. The complete list of significant results of MANOVA tests is given in Table 2.

Analyses testing for the influence of season and geographic origin on the MCO of *K. tanganicanus* were conducted only on specimens collected from *L. miodon*. The copulatory tube was shorter in specimens collected in the dry season than in those collected in the rainy season (copulatory tube, t-test - $t_{1,46}=3.87$; $p<0.001$). Individuals from the central sub-basin were omitted from the analyses because of the small sample size. There was no significant difference between specimens from the northern and southern basin in measurements of the MCO.

Genetic characterisation

To study genetic diversity within and between the parasite species under consideration, markers with different rates of molecular evolution were used. Sequences of three nuclear rDNA regions were obtained from five sequenced individuals for each species, sub-basin and marker. The length of the successfully sequenced 28S rDNA fragment was 643 base pairs (bp). The 18S rDNA and ITS-1 fragments were 459 and 321 bp long, respectively. Uncorrected p-distances between the two species of *Kapentagyrus* amounted to 0.9%, 0.2% and 4% in 28S, 18S and ITS-1 rDNA fragments, respectively. The difference of 4% in the ITS-1 region is well above the proposed 1% cut-off between species for the best-studied monogenean genus, *Gyrodactylus* von Nordmann, 1832 (Ziętara and Lumme, 2002). Hence, the presence of two species, *K. limnotrissae* and *K. tanganicanus*, was also confirmed genetically. No genetic differences were found among individuals assigned to *K. tanganicanus*, suggesting that the two observed haptoral morphologies are the result of phenotypic variability among conspecifics.

Phylogenetic affinities of monogenean genera

Both ML and BI produced the same tree topologies. The alignment combined fragments of 28S, 18S and ITS-1 of 34 species (see Supplementary file 1: Table S1) for a total of 1388 bp. The species of *Kapentagyrus* reported in our study formed a well-supported monophyletic lineage within Dactylogyridae and did not show any

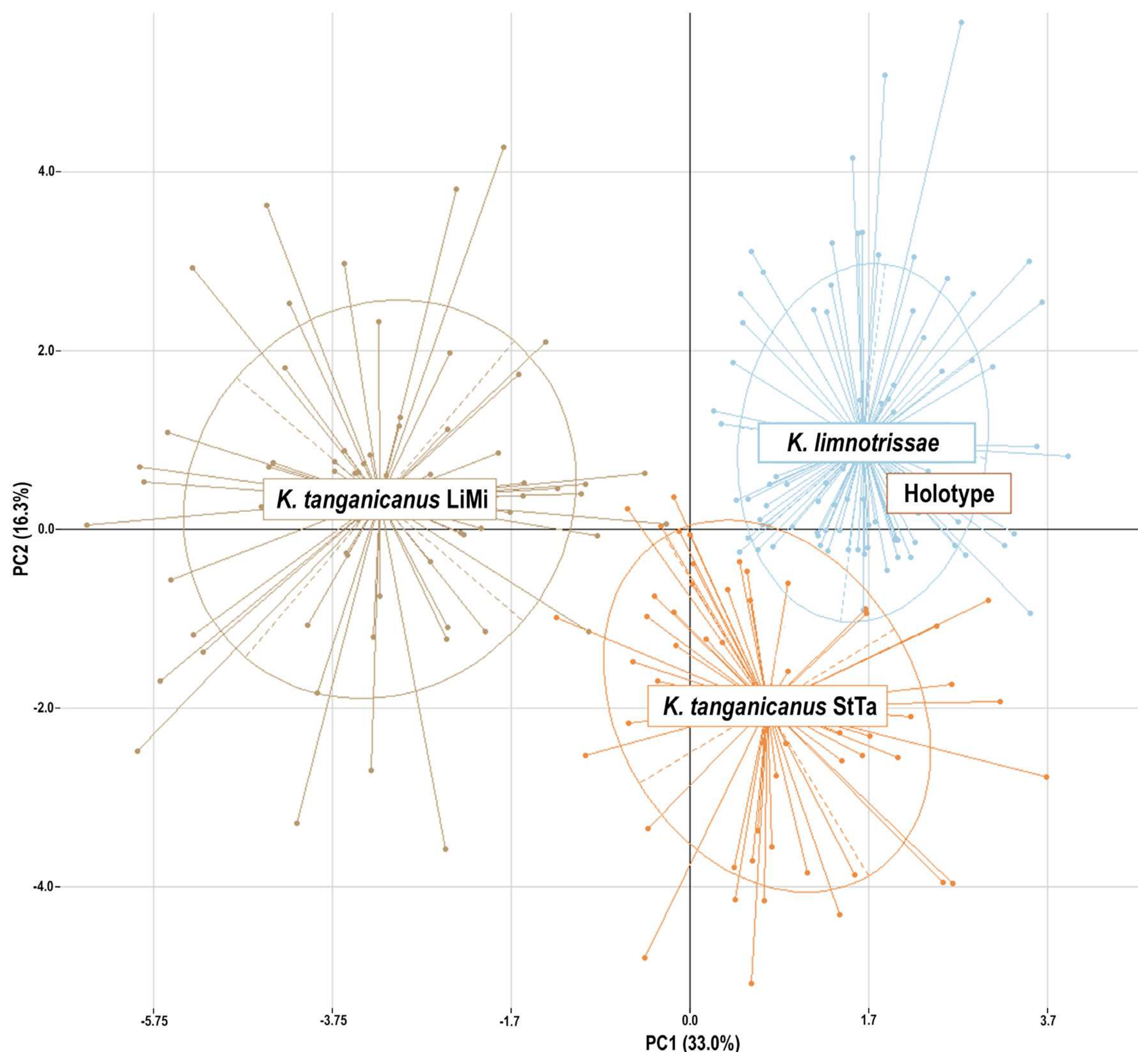


Figure 3. A biplot of PCA (first two axes) based on measurements of haptoral sclerotized structures of *Kapentagyrus limnotrissae* and *K. tanganicanus*. Ellipses indicate the distribution of the individuals from different groups centred by the mean value. The position of the holotype of *K. limnotrissae* and of two phenotypes of *K. tanganicanus* separated by host species, LiMi – *L. miodon*, StTa – *S. tanganicae* are indicated separately.

phylogenetic affinity to the type species of the genus *Ancyrocephalus*, *Ancyrocephalus paradoxus* Creplin, 1839, or any other monogenean lineage (Fig. 4). The phylogenetic analysis therefore supports *Kapentagyrus* as a new genus of Dactylogyridae. Low support values were observed at deeper phylogenetic levels.

Discussion

This is the first comprehensive study of the monogenean fauna of two of the most economically important fish species in Lake Tanganyika, using a combination of historical and recently collected host specimens. A new monogenean genus of Dactylogyridae is described as *Kapentagyrus* with two species: *Kapentagyrus limnotrissae* and *Kapentagyrus tanganicanus*, recorded in this study. The latter, newly discovered species, shows phenotypic variability in relation to its host species, but conspecificity of both phenotypes was confirmed by genetic data. Analyses on morphometric data were performed to test the effect of a range of factors on parasite morphology. The species' phylogenetic affinities were inferred at the family level.

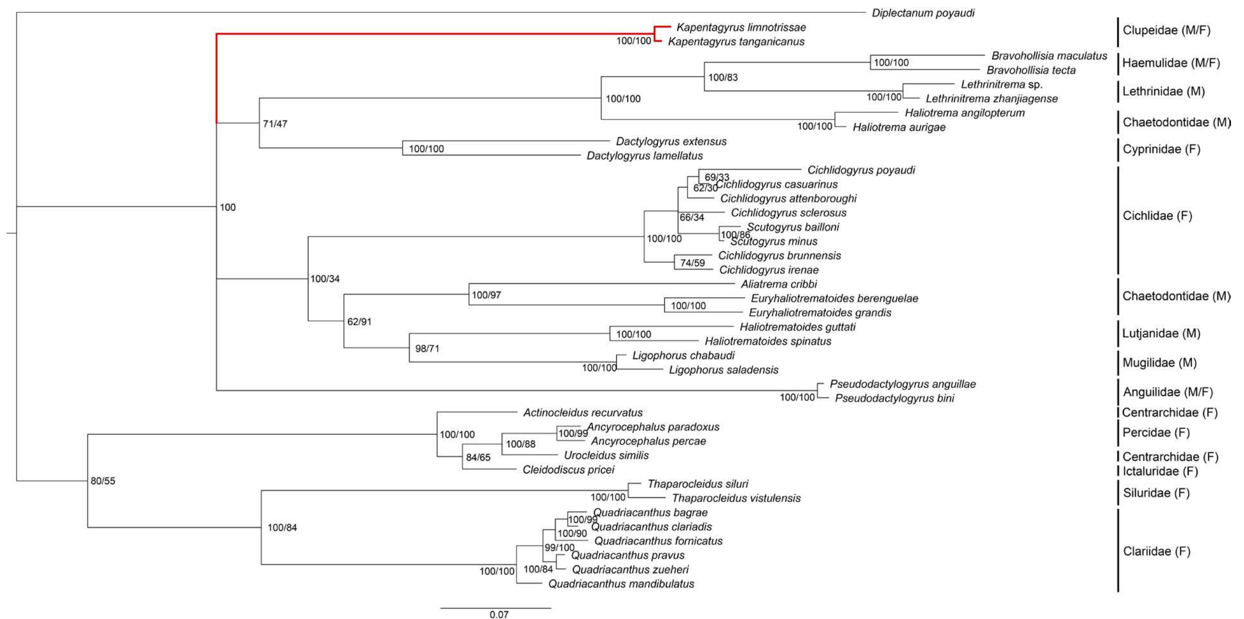


Figure 4. Bayesian inference phylogram based on 28S, 18S and ITS-1 rDNA fragments from 34 haplotypes of different dactylogyrid species. Bootstrap percentages for maximum likelihood (before slashes) and posterior probabilities for Bayesian inference (behind slashes) are shown. Host families together with their marine (M) or freshwater lifestyle (F), respectively, are specified behind vertical lines. The scale bar indicates the expected number of substitutions per site.

Monogenean species infecting sardines in Lake Tanganyika

The two dactylogyrid monogenean species infecting clupeids in Lake Tanganyika are placed in *Kapentagyrus*. The previously described species infecting *L. miodon*, *Ancyrocephalus limnotrissae* was reassigned to the new genus. The group of monogeneans referred to as *Ancyrocephalus* sensu lato with *A. paradoxus* as the type species is characterised by an S-shaped copulatory tube longer than 7 μm with a triangular accessory piece and anchors with a broad base and a short point (Pugachev *et al*., 2009). Although the copulatory tube of the species of *Kapentagyrus* infecting *L. miodon* and *S. tanganicae* corresponds to this characterisation, the shape of their anchors, their elongated accessory piece and the presence of a single seminal vesicle and prostatic gland, do not conform to this diagnosis (see Appendix). Moreover, our phylogenetic analysis of Dactylogyridae show that species belonging to *Ancyrocephalus* sensu stricto (*A. paradoxus* and *A. percae* Ergens, 1966) do not cluster with the species collected in this study (Fig. 4). Together with the apparent morphological differences with the type species of *Ancyrocephalus*, *A. paradoxus*, this shows polyphyly of *Ancyrocephalus* and justifies the proposal of a new genus of dactylogyrids infecting African freshwater clupeids, described as *Kapentagyrus*. Other species of *Ancyrocephalus* have been described from non-clupeid African fish: *A. barilli* Paperna, 1973 from the cyprinid *Raiamas senegalensis* (Steindachner, 1870) and *A. claveaui* Birgi, 1988 from the poeciliid *Poropanchax luxopthalmus* (Brüning, 1929). Based on their morphology neither of these species belong to *Kapentagyrus* or to *Ancyrocephalus* sensu stricto. *Ancyrocephalus* sensu lato can be considered as a catch-all genus and therefore a formal revision is needed (Pugachev *et al*., 2009). The monogenean described from the clupeid *Pellonula leonensis* Boulenger, 1916 is morphologically very similar to those infecting clupeids in Lake Tanganyika and is therefore reassigned to *Kapentagyrus* as *K. pellonulae* comb. nov. (Paperna, 1969). Interestingly, the three species of *Kapentagyrus* described from African clupeids (*K. limnotrissae*, *K. tanganicanus* and *K. pellonulae*) share a highly similar MCO that differs only slightly in size.

While the general morphology of monogenean haptoral sclerites is often believed to represent variation at the genus or family level, the shape and size of copulatory organs is considered to be species-specific (Pugachev *et al*., 2009). Although differences

in the shape and size of haptoral hard parts between the two Lake Tanganyika species of *Kapentagyrus* are evident and were also clearly visible in a PCA plot (see Fig. 3, Table 3), no structural morphological difference except a size difference was seen in the MCO. This discrepancy has been previously reported in other monogenean species belonging to *Pseudorhabdosynochus* Yamaguti, 1958 (Sigura and Justine, 2008) and *Cichlidogyrus* (Messu Mandeng *et al*., 2015). This is supposed to be influenced by the degree of host genetic differentiation correlated with the age of the parasite lineage (Poulin, 1992, 2007; Poulin and Morand, 2004). Hence this may be linked to the recent divergence of the two species of *Kapentagyrus* on Tanganyika clupeids, indicated by their low interspecific genetic distances. The morphology of copulatory organs therefore does not seem to be the only reproductive isolation mechanism in dactylogyrids; indeed, reproductive isolation of conspecific monogeneans was suggested to be a result of microhabitat specialisation (Šimková *et al*., 2006). Despite the lack of shape differences in the MCO between *K. limnotrissae* and *K. tanganicanus*, significant morphometric differences in both tested traits (copulatory tube and accessory piece length) point towards a reproductive barrier in these two sympatric monogenean species, as was confirmed by genetic characterisation.

The morphology-based delineation of the collected monogenean species was confirmed by all three analysed ribosomal DNA regions. No genetic intra-specific variability related to host species identity or geographical origin was detected. Recent divergence among the species of *Kapentagyrus* from Lake Tanganyika is indicated by the low genetic distances obtained for all three genetic markers analysed (0.9%, 0.2% and 4% in 28S, 18S and ITS-1 rDNA fragments, respectively) compared to species of *Cichlidogyrus* (5.6 %, 2.6% and 15.8% in 28S, 18S and ITS-1 rDNA fragments, respectively), the other dactylogyrid lineage present in the pelagic waters of Lake Tanganyika (sequences part of this study).

While a similar prevalence was observed for both monogenean species parasitizing on *L. miodon* (35% for *K. limnotrissae* and 40% for *K. tanganicanus*), a lower prevalence was found for *K. tanganicanus* on *S. tanganicae* (18%). This pattern might be explained by the more pelagic lifestyle and shorter lifespan of *S. tanganicae* (Mulimbwa and Shirakihara, 1994) making attachment of monogenean larvae more difficult as this is considered to be more successful in the littoral and benthic zones (Kearn, 1967). Moreover, there appeared to be geographical differences in the prevalence of *K. tanganicanus* on *S. tanganicae*, with higher values in the northern part (36%), compared to the central (0%) and southern parts of the lake (4.3%) (data from the dry season). This result could be correlated with the lower abundance of *S. tanganicae* in the southern part of the lake (Mannini *et al*., 1996) and therefore lower opportunities for parasites to infect this fish species (Bagge *et al.,* 2004). However, only little is known about the spatial dynamics of clupeid demographics (Mulimbwa and Shirakihara, 1994). Unfortunately, due to a lack of samples, seasonal variation in prevalence could not be tested in *L. miodon*. Interestingly, low infection intensity was observed in both monogenean species, ranging from one to 11 individuals. This result could be explained by a combination of the mostly pelagic lifestyle of clupeids preventing multiple infections, which are proposed to occur in the littoral zone (Rohde *et al.,* 1995) and the small size of the host species (Poulin, 2000).

Intra-specific morphological variability

The PCA revealed morphometric variation in haptoral sclerites in *K. tanganicanus* that was related to host species. Such intra-specific variability is commonly reported in monogeneans (Šimková *et al*., 2001a; Kaci-Chaouch *et al.,* 2008; Mladineo *et al*., 2013; Kmentová *et al*., 2016). Based on Fankoua *et al*., (2017), the shape and size of monogenean sclerotised structures could also be affected by the type of mounting medium. However, such an influence was minimized in our study by using GAP as well as Hoyer's medium for specimens from both host species. Remarkably, in this study, inter- and intra-specific morphological variation were of a similar magnitude (see Fig. 3). This situation is probably correlated with an adaptation to host habitat confirming results from previous studies showing greater morphometric variability of generalist species compared to specialists (Šimková *et al*., 2001a; Kaci-Chaouch *et al*., 2008). Since the MCO starts to develop once the haptor is fully developed (Kearn, 1968; Sanchez-Garcia *et al.,* 2015), all collected specimens were considered adults. Hence, the observed differences in haptoral measurements of specimens with developed MCO between the rainy and the dry season cannot be related to the stage of ontogenetic development but rather to external conditions such as temperature (Mo, 1991; Dávidová *et al*., 2005). Moreover, morphometric intra-specific

variability in the haptor was found to be influenced by host species size with significantly larger structures of *K. limnotrissae* and *K. tanganicanus* in bigger host specimens. However, this was not observed in *K. tanganicanus* collected from *L. miodon*, which confirms previous studies that questioned such correlations in monogeneans (Lakshmi Perera, 1992; Šimková *et al*., 2001a; Baker *et al*., 2005). Interestingly, a relation of some morphometric parameters to the geographic origin of both monogenean species was found supporting the potential use of monogeneans as tags for host species stock structure. However, seasonal differences in haptoral measurements of *K. tanganicanus* correspond with the geographical origin of samples and therefore we could not discern these two patterns based on our data set. A fixed pattern in parasite morphometric parameters could possibly indicate differentiation between northern and southern schools of both clupeid species and therefore prove the existence of school structure on a lake wide scale, which hitherto was not detected using fish genetics. However, morphometric intraspecific difference could also result from temporal isolation linked to host recruitment. Nevertheless, the temporal stability of this latitudinal morphometric differentiation has to be confirmed in studies on time series.

Surprisingly, a significantly different length of the accessory piece in *K. tanganicanus* related to host species was documented which could indicate a recent speciation process driven by an incipient reproductive barrier (Kritsky and Boeger, 2002). Although such a process could not be detected using ribosomal DNA markers, faster evolving regions e.g. from mitochondrial DNA or genome-scale data could clarify the situation (Bueno-Silva *et al.,* 2011). Moreover, the copulatory tube length of *K. tanganicanus* seems to be related to seasonality, with a difference between dry and rainy season, suggesting a link between the parasite's age/size and the size of the MCO. However, this pattern has to be tested through more detailed screening on a monthly basis. While no relation to the geographical origin of samples was detected, seasonal variation in copulatory organ development could be possibly correlated with the temperature dependent reproduction and survival of monogeneans (Buchmann, 1988; Šimková *et al*., 2001b; Tubbs *et al*., 2005) or the reproduction of host species (Šimková *et al*., 2005). However, water temperature in Lake Tanganyika is relatively stable with only 1 or 2˚C annual differences (Coulter and Spigel, 1991; Edmond *et al*., 1993). Moreover, while *S. tanganicae* shows a spawning peak prior to the onset of the rainy season, *L. miodon* seems to have multiple spawning periods throughout the year (Mulimbwa and Shirakihara, 1994; Mulimbwa *et al.,* 2014; Mulimbwa *et al.,* 2014). Therefore, we cannot conclude whether the larger MCO of monogeneans in the rainy season is connected with environmental factors or the spawning season of clupeids. Moreover, the lack of significant influence of any considered factor on the MCO of *K. limnotrissae* concurs with the multiple spawning periods of *L. miodon*.

Monogenean host-specificity in the lake's pelagic zone

While *Kapentagyrus limnotrissae* is host-specific to *Limnothrissa miodon*, *Kapentagyrus tanganicanus* infects both clupeid species in Lake Tanganyika. Although large phenotypic variation in *K. tanganicanus* related to host species was documented, the conspecificity of specimens was confirmed by molecular characterisation. Twenty-five monogenean species have been described from Lake Tanganyika, and most of them are classified as strict (infecting a single host species) or intermediate specialists (parasitizing on two or more congeneric host species). Thus far, *Cichlidogyrus casuarinus* is the only known intermediate generalist (following the terminology of Mendlová and Šimková, 2014) among Dactylogyridae from Lake Tanganyika, infecting pelagic bathybatine cichlids (Kmentová *et al*., 2016). Our findings suggest that *K. tanganicanus* is another intermediate generalist. This corroborates previous observations of lower host-specificity in monogeneans in the lake's pelagic zone, in contrast to the species-rich littoral habitat (Kmentová *et al*., 2016). The phenomenon of reduced host-specificity in the pelagic realm has been suggested to be correlated mainly with the lower host availability in this habitat (Klimpel *et al*., 2006, 2010; Schoelinck *et al*., 2012; Kmentová *et al*., 2016). However, schooling behaviour of clupeids forming large and, potentially, mixed-species groups (Plisnier *et al*., 2009; Van der Knaap *et al.,* 2014) could be one of the driving mechanisms of parasite host-switch or speciation (Poulin, 1992). This could have resulted in two species of *Kapentagyrus* infecting clupeids in Lake Tanganyika. Host species hybridisation might explain the more generalist life style of certain monogeneans due to an influence of host genetics on susceptibility to infection, host-specificity, and parasite speciation (Tinsley and Jackson, 1998; Vanhove *et al.,* 2011; Šimková *et al*., 2013). However, there are no reports of hybridisation among

Lake Tanganyika's clupeids (Wilson *et al*., 2008). Sharing of *K. tanganicanus* by both clupeid species, however, might be due to predator-prey transmission of parasites with direct lifecycles. This transmission mechanism was suggested by Strona (2015). Based on this scenario, the presence of *K. tanganicanus* on both clupeid species might be due to direct contact in host predator-prey interactions, as *L. miodon* is known to predate on *S. tanganicae*. This hypothesis is supported by previous studies showing strict host-specificity as an ancestral state in the genus *Dactylogyrus* (Šimková *et al*., 2006; Šimková and Morand, 2008), with a weak influence of inter-specific competition (Mouillot *et al*., 2005).

Phylogenetic position and origin of Kapentagyrus *in Lake Tanganyika*

Results from phylogenetic inference did not reveal a sister-taxon relationship between *Kapentagyrus* that infects the clupeids of Lake Tanganyika and *Cichlidogyrus*, indicating independent colonization of Lake Tanganyika. Interestingly, even though many clupeid species have been examined, no dactylogyrid monogenean has ever been reported from marine clupeids (Fig. 5). Although dactylogyrid species of *Parancyrocephaloides* Yamaguti, 1938 were described from *Clupea harengus* L., 1758 in Ramappa Lake in India (Kulkarni, 1969), the reported host species and locality are highly questionable as *Clupea harengus* does not occur in India or the Indian Ocean, see Laxmappa and Rao Bakshi (2016). Therefore, species of *Kapentagyrus* figure as the only dactylogyrid species ever reported from freshwater clupeids. Despite poor overall resolution, phylogenetic inference of dactylogyrids supported the separate position of *Kapentagyrus*. Based on this finding, it seems that freshwater sardines, after colonising African inland waters, lost their monogeneans typical for marine representatives and have been afterwards infected by a freshwater lineage. A similar process has been suggested for the monogeneans of cichlids in South America and Africa (Pariselle *et al*., 2011). However, the origin of this particular dactylogyrid lineage is uncertain as it was placed in a phylogenetically unresolved clade comprising both freshwater and marine representatives (see Fig. 4). A higher coverage in both the number of taxa and loci will be necessary to gain better insights in the biogeographic origins of the monogeneans infecting African freshwater clupeids.

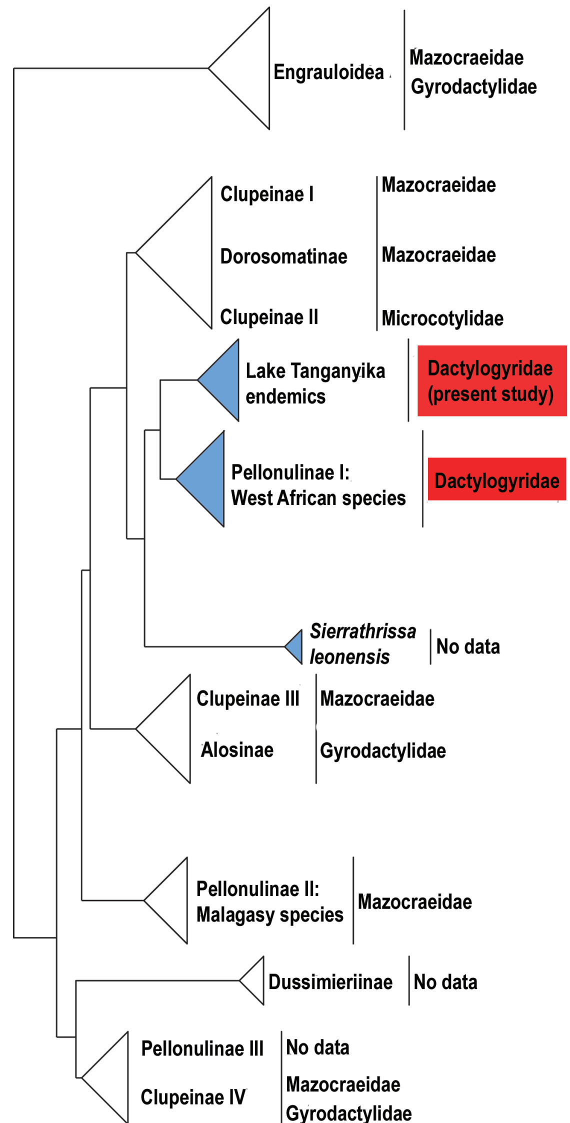


Figure 5. Schematic phylogenetic tree of Clupeidae adapted from Wilson, Teugels and Meyer (2008) indicating the position of continental African freshwater representatives (filled triangles) and the reported presence of monogenean families (dactylogyrids boxed).

Conclusions

This study provides a description of a new genus, *Kapentagyrus* supported by morphological and molecular data. Our results re-affirm that host specificity is lower in the pelagic zone of Lake Tanganyika, a conclusion also reached for parasites belonging to *Cichlidogyrus* (Pariselle *et al*., 2015; Kmentová *et al*., 2016). Phylogenetic patterns appear

to be inconclusive with regard to a colonisation of the originally marine clupeids by dactylogyrids in African freshwaters. However, we note that taxon coverage of other freshwater dactylogyrid species is still very poor and that sequence data for many more species are required to fully understand the obviously highly complex (co-)evolutionary pattern within this monogenean family. Interestingly, phenotypic variability in *K. tanganicanus* was identified related to host species identity with significant differences in haptoral hard parts. Moreover, significant differences in genital parts could possibly indicate the presence of an incipient reproductive barrier (Kritsky and Boeger, 2002). Moreover, the larger copulatory tubes found in *K. tanganicanus* collected in the rainy season could be related to the discontinuous reproduction of their hosts throughout the year. We reported significant intraspecific differences in morphometrics of the haptoral region between specimens collected from the northern, the central and the southern end of Lake Tanganyika. This pattern could indicate a geographic signature in monogenean infection caused by a lack of lake-wide migration in the host species. Further analyses focusing on parasite population genetic structure, phylogeography and demographic history linked with host data are required to reveal the history of this host-parasite system and its potential in recognizing fish population structure. Indeed, parasites, and especially monogeneans, because of their direct life cycle, potentially high substitution rates compared to their host and documented significant phenotypic changes related to geographic origin, might be used to track the history and structure of African clupeid species and fish populations. As clupeids are important fisheries targets in Lake Tanganyika, knowledge on their population structure, health and biology is fundamental to obtain a sustainable management of the species, from which all communities benefit.

Acknowledgements

The authors would like to thank: Ludmila Raisingerová, Chahrazed Rahmouni, Vercus Lumami Kapepula, Donatien Muzumani Risasi, Joseph Mbirize Ndalozibwa, Simon Kambale Mukeranya, Elias Bilali Mela and VLIR-UOS South Initiative ZEIN2015Z185 for field support. Walter Salzburger, Fabrizia Ronco and Adrian Indermaur are thanked for providing fish samples. We are grateful to Michiel Jorissen for useful discussions about this study. We also thank Tine Huyse, Jos Snoeks, Miguël Parrent (RMCA), Filip Volckaert (KU Leuven) and people in the parasitological group, Masaryk University, Brno and Research Centre of Hydrobiology, Uvira for their hospitality (mission statement number 031/MINRST/CRH-U/2016) and the RMCA for providing access to the collections. Research was supported by the Czech Science Foundation (GBP505/12/G112 – ECIP) as well as by a joint program between the Austrian agency for international mobility and cooperation in education, science and research and the Czech Ministry of Education, Youth and Sports (project number 8J18AT007). A research visit enabling access to the fish collections of RMCA was supported by SYNTHESYS (BE-TAF-5731). The referees are cordially thanked for their useful comments improving this study.

References

Bagge AM, Poulin R, Valtonen ET. 2004. Fish population size, and not density, as the determining factor of parasite infection: a case study. *Parasitology* 128: 305–313.

Baker TG, Viricel A, Meraziz L, de Buron I. 2005. Size variation of adult polyopisthocotylid *Metamicrocotyla macracantha* (Monogenea) in relation to host size. *Comparative Parasitology* 72: 179–182.

Blasco-Costa I, Míguez-Lozano R, Sarabeev V, Balbuena JA. 2012. Molecular phylogeny of species of *Ligophorus* (Monogenea: Dactylogyridae) and their affinities within the Dactylogyridae. *Parasitology International* 61: 619–627.

Brown KJ, Rüber L, Bills R, Day JJ. 2010. Mastacembelid eels support Lake Tanganyika as an evolutionary hotspot of diversification. *BMC Evolutionary Biology* 10: 188.

Bueno-Silva M, Boeger WA, Pie MR. 2011. Choice matters: Incipient speciation in *Gyrodactylus corydori* (Monogenoidea: Gyrodactylidae). *International Journal for Parasitology* 41: 657–667.

Buchmann K. 1988. Temperature-dependent reproduction and survival of *Pseudodactylogyrus bini* (Monogenea) on the European eel (*Anguilla anguilla*). *Parasitology Research* 75: 162–164.

Bychowsky BE, Nagibina LF. 1970. Contribution to the revision of the genus *Ancyrocephalus* Creplin, 1839 (Dactylogyrida, Ancyrocephalinae). *Parazitologiya* 3: 193–200.

Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics Application Note* 25: 1972–1973.

Chapman DW, van Well P. 1978. Growth and mortality of *Stolothrissa tanganicae*. *Transactions of the American Fisheries Society* 107: 26–35.

Coenen EJ, Hanek G, Kotilainen P. 1993. Shoreline classification of Lake Tanganyika based on the results of an aerial frame survey (29.09-03.10.1992). *FAO/FINNIDA Research for the Management of the Fisheries on Lake Tanganyika*: 11.

Cohen AS, Lezzar KE, Cole J, Dettman D, Ellis GS, Gonneea ME, Plisnier PD, Langenberg V, Blaauw M, Zilifi D. 2006. Late Holocene linkages between decade–century scale climate variability and productivity at Lake Tanganyika, Africa. *Journal of Paleolimnology* 36: 189–209.

Cohen AS, Soreghan MJ, Scholz CA. 1993. Estimating the age of formation of lakes: An example from Lake Tanganyika, East African Rift system. *Geology* 21: 511.

Coulter GW. 1970. Population changes within a group of fish species in Lake Tanganyika following their exploitation.

Journal of Fish Biology 2: 329–353.

Coulter GW. 1976. The biology of *Lates* species (Nile perch) in Lake Tanganyika, and the status of the pelagic fishery for *Lates* species and *Luciolates stappersii* (Blgr.). *Journal of Fish Biology* 9: 235–259.

Coulter GW. 1991a. Introduction. In: Coulter GW, ed. *Lake Tanganyika and its life*. London Oxford & New York: Natural History Museum Publications & Oxford University Press, 1–6.

Coulter GW. 1991b. Composition of the flora and fauna. In: Coulter GW, ed. *Lake Tanganyika and its life*. London Oxford & New York: Natural History Musem Publications & Oxford University Press, 200–274.

Coulter GW, Spigel RH. 1991. Hydrodynamics. In: Coulter GW, ed. *Lake Tanganyika and its life*. London Oxford & New York: Natural History Museum Publications & Oxford University Press, 49–75.

Criscione CD, Cooper B, Blouin MS. 2006. Parasite genotypes identify source populations of migratory fish more accurately than fish genotypes. *Ecology* 87: 823–828.

Danley PD, Husemann M, Ding B, Dipietro LM, Beverly EJ, Peppe DJ. 2012. The impact of the geologic history and paleoclimate on the diversification of East African cichlids. *International Journal of Evolutionary Biology*: 574851.

Dávidová M, Jarkovský J, Matějusová I, Gelnar M. 2005. Seasonal occurrence and metrical variability of *Gyrodactylus rhodei* Žitňan 1964 (Monogenea, Gyrodactylidae). *Parasitology Research* 95: 398–405.

Day JJ, Wilkinson M. 2006. On the origin of the *Synodontis* catfish species flock from Lake Tanganyika. *Biology Letters* 2: 548–552.

Dogiel VA, Bogolepova II. 1957. *Parasitic fauna of Lake Baikal fishes*. Trudy Baikalskoi Limnologicheskoi Stancii Akademiya Nauk SSR.

Eccles DH. 1992. Field guide to the freshwater fishes of Tanzania. *FAO Species Identification Sheets for Fishery Purposes*.

Edgar RC. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research* 32: 1792–1797.

Edmond JM, Stallard RF, Craig H, Craig V, Weiss RF, Coulter GW. 1993. Nutrient chemistry of the water column of Lake Tanganyika. *Limnology and Oceanography* 38: 725–738.

Ergens R, Lom J. 1970. *Causative agents of fish diseases*. Prague: Academia.

Erpenbeck D, Weier T, de Voogd NJ, Wörheide G, Sutcliffe P, Todd JA, Michel E. 2011. Insights into the evolution of freshwater sponges (Porifera: Demospongiae: Spongillina): Barcoding and phylogenetic data from Lake Tanganyika endemics indicate multiple invasions and unsettle existing taxonomy. *Molecular Phylogenetics and Evolution* 61: 231–236.

Eschmeyer WN, Fong JD. 2017. Catalog of Fishes. *Species by family/subfamily*.

Fankoua SO, Bitja Nyom AR, Bahanak DND, Bilong Bilong CF, Pariselle A. 2017. Influence of preservative and mounting media on the size and shape of monogenean sclerites. *Parasitology Research* 116: 2277–2281.

Filzmoser P, Gschwandtner M. 2017. mvoutlier: Multivariate outlier detection based on robust methods.: https://CRAN.R-project.org/package=mvoutlier.

Fryer G. 1991. Comparative aspects of adaptive radiation and speciation in Lake Baikal and the great rift lakes of Africa. *Hydrobiologia* 211: 137–146.

García-Varela M, Sereno-Uribe AL, Pinacho-Pinacho CD, Domínguez-Domínguez O, Pérez-Ponce de León G. 2016. Molecular and morphological characterization of *Austrodiplostomum ostrowskiae* Dronen, 2009 (Digenea: Diplostomatidae), a parasite of cormorants in the Americas. *Journal of Helminthology* 90: 174–185.

Gérard C, Hervé M, Réveillac E, Acou A. 2016. Spatial distribution and impact of the gill-parasitic *Mazocraes alosae* (Monogenea Polyopisthocotylea) on *Alosa alosa* and *A. fallax* (Actinopterygii, Clupeidae). *Hydrobiologia* 763: 371–379.

Glaubrecht M. 2008. Adaptive radiation of thalassoid gastropods in Lake Tanganyika, East Africa: morphology and systematization of a paludomid species flock in an ancient lake. *Zoosystematics and Evolution* 84: 71–122.

Gourène G, Teugels G. 1993. Position taxinomique de *Limnothrissa strappersii*, un clupéidé lacustre d'Afrique centrale. *Ichthyological Exploration of Freshwaters* 4: 367–374.

Grégoir AF, Hablützel PI, Vanhove MPM, Pariselle A, Bamps J, Volckaert FAM, Raeymaekers JAM. 2015. A link between host dispersal and parasite diversity in two sympatric cichlids of Lake Tanganyika. *Freshwater Biology* 60: 323–335.

Hablützel PI, Grégoir AF, Vanhove MPM, Volckaert FAM, Raeymaekers JAM. 2016. Weak link between dispersal and parasite community differentiation or immunogenetic divergence in two sympatric cichlid fishes. *Molecular Ecology* 25: 5451–5466.

Hablützel PI, Vanhove MPM, Deschepper P, Grégoir AF, Anna K. 2017. Parasite escape through trophic specialization in a species flock. *Journal of Evolutionary Biology* 30: 1437–1445.

Hablützel PI, Vanhove MPM, Deschepper P, Grégoir AF, Roose AK, Volckaert FAM, Raeymaekers JAM. 2017. Parasite escape through trophic specialization in a species flock. *Journal of Evolutionary Biology* 30: 1437-1445.

Hassouna N, Michot B, Bachellerie JP, Narbonne D. 1984. The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher eukaryotes. *Nucleic Acids Research* 12: 3563–3583.

Hauser L, Carvalho GR, Pitcher TJ. 1995. Morphological and genetic differentiation of the African clupeid *Limnothrissa miodon* 34 years after its introduction to Lake Kivu. *Journal of Fish Biology* 47: 127–144.

Hauser L, Carvalho GR, Pitcher TJ. 1998. Genetic population structure in the Lake Tanganyika sardine *Limnothrissa miodon*. *Journal of Fish Biology* 53: 413–429.

Hecky RE. 1991. The pelagic ecosystem. In: Coulter GW, ed. *Lake Tanganyika and its life*. London Oxford & New York: Natural History Musem & Oxford University Press, 91–110.

Hecky RE, Fee EJ, Kling HJ, Rudd JWM. 1981. Relationship between primary production and fish production in Lake Tanganyika. *Transactions of the American Fisheries Society* 110: 336–345.

Huyse T, Malmberg G. 2004. Molecular and morphological comparisons between *Gyrodactylus ostendicus* n. sp. (Monogenea: Gyrodactylidae) on *Pomatoschistus microps*

(Krøyer) and *G. harengi* Malmberg, 1957 on *Clupea harengus membras*. *Systematic Parasitology* 58: 105–113.

International Commission on Zoological Nomenclature. 2012. Amendment of articles 8, 9, 10, 21 and 78 of the International Code of Zoological Nomenclature to expand and refine methods of publication. *Zootaxa* 3450: 1–7.

Jombart T. 2008. Adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics* 24: 1403–1405.

Justine JL, Beveridge I, Boxshall GA, Bray RA, Miller TL, Moravec F, Trilles JP, Whittington ID. 2012. An annotated list of fish parasites (Isopoda, Copepoda, Monogenea, Digenea, Cestoda, Nematoda) collected from Snappers and Bream (Lutjanidae, Nemipteridae, Caesionidae) in New Caledonia confirms high parasite biodiversity on coral reef fish. *Aquatic Biosystems* 8: 22.

Kaci-Chaouch T, Verneau O, Desdevises Y. 2008. Host specificity is linked to intraspecific variability in the genus *Lamellodiscus* (Monogenea). *Parasitology* 135: 607–616.

Kearn GC. 1967. Experiments on host-finding and host-specificity in the monogenean skin parasite *Entobdella soleae*. *Parasitology* 57: 585–605.

Kearn GC. 1968. The development of the adhesive organs of some diplectanid, tetraonchid and dactylogyrid gill parasites (Monogenea). *Parasitology* 58: 149–163.

Klimpel S, Busch MW, Sutton T, Palm HW. 2010. Meso- and bathy-pelagic fish parasites at the Mid-Atlantic Ridge (MAR): Low host specificity and restricted parasite diversity. *Deep-Sea Research Part I: Oceanographic Research Papers* 57: 596–603.

Klimpel S, Palm HW, Busch MW, Kellermanns E, Rückert S. 2006. Fish parasites in the Arctic deep-sea: Poor diversity in pelagic fish species vs. heavy parasite load in a demersal fish. *Deep-Sea Research Part I: Oceanographic Research Papers* 53: 1167–1181.

Kmentová N, Gelnar M, Mendlová M, Van Steenberge M, Koblmüller S, Vanhove MPM. 2016. Reduced host-specificity in a parasite infecting non-littoral Lake Tanganyika cichlids evidenced by intraspecific morphological and genetic diversity. *Scientific Reports* 6: 39605.

Koblmüller S, Sefc KM, Sturmbauer C. 2008. The Lake Tanganyika cichlid species assemblage: Recent advances in molecular phylogenetics. *Hydrobiologia* 615: 5–20.

Koblmüller S, Sturmbauer C, Verheyen E, Meyer A, Salzburger W. 2006. Mitochondrial phylogeny and phylogeography of East African squeaker catfishes (Siluriformes: *Synodontis*). *BMC Evolutionary Biology* 6: 49.

Kritsky DC, Boeger WA. 2002. Neotropical Monogenoidea: 41. New and previously described species of Dactylogyridae (Platyhelminthes) from the gills of marine and freshwater perciform fishes (Teleostei) with proposal of a new genus and a hypothesis on phylogeny. *Zoosystema* 24: 7–40.

Kulkarni T. 1969. Studies on the monogenetic trematodes of fishes found in Hyderabad, Andhra Pradesh (India) - Part II. *Rivista di Parassitologia* 30: 263–282.

Kumar S, Stecher G, Tamura K, Gerken J, Pruesse E, Quast C, Schweer T, Peplies J, Ludwig W, Glockner F. 2016. MEGA7: Molecular evolutionary genetics analysis Version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874.

Kuris AM, Hechinger RF, Shaw JC, Whitney KL, Aguirre-Macedo L, Boch CA, Dobson AP, Dunham EJ, Fredensborg BL, Huspeni TC, Lorda J, Mababa L, Mancini FT, Mora AB, Pickering M, Talhouk NL, Torchin ME, Lafferty KD. 2008. Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature* 454: 515–518.

Lakshmi Perera KM. 1992. The effect of host size on large hamuli length of *Kuhnia scombri* (Monogenea: Polyopisthocotylea) from Eden, New South Wales, Australia. *International Journal for Parasitology* 22: 123–124.

Langenberg VT, Mwape LM, Tshibangu K, Tumba JM, Koelmans AA, Roijackers R, Salonen K, Sarvala J, Mölsä H. 2002. Comparison of thermal stratification, light attenuation, and chlorophyll- a dynamics between the ends of Lake Tanganyika. *Aquatic Ecosystem Health & Management* 5: 255–265.

Lavoué S, Konstantinidis P, Chen WJ. 2014. Progress in clupeiform systematics. In: Ganias K, ed. *Biology and ecology of sardines and anchovies*. CRC Press, 3–42.

Laxmappa B, Rao Bakshi R. 2016. A checklist of fishes of Telangana State, India. *International Journal of Fisheries and Aquatic Studies* 4: 35–42.

MacKenzie K. 1983. Parasites as biological tags in fish population studies. *Advances in Applied Biology* 7: 251–331.

Maddison WP, Maddison DR. 2017. Mesquite: a modular system for evolutionary analysis. Version 3.2 http://mesquiteproject.org.

Mannini P, Aro E, Katonda KI, Kassaka B, Mambona C, Milindi G, Paffen P, Verburg P. 1996. Pelagic fish stocks of Lake Tanganyika: biology and exploitation. FAO/FINNIDA research for the management of the fisheries of Lake Tanganyika. *GCP/RAF/271/FIN—TD/53 (En)*: 60.

Marijnissen SAE, Michel E, Daniels SR, Erpenbeck D, Menken SBJ, Schram FR. 2006. Molecular evidence for recent divergence of Lake Tanganyika endemic crabs (Decapoda: Platythelphusidae). *Molecular Phylogenetics and Evolution* 40: 628–634.

Marshall BE. 1993. Biology of the African clupeid *Limnothrissa miodon* with reference to its small size in artificial lakes. *Reviews in Fish Biology and Fisheries* 3: 17–38.

Meixner MJ, Lüter C, Eckert C, Itskovich V, Janussen D, von Rintelen T, Bohne AV, Meixner JM, Hess WR. 2007. Phylogenetic analysis of freshwater sponges provide evidence for endemism and radiation in ancient lakes. *Molecular Phylogenetics and Evolution* 45: 875–886.

Mendlová M, Šimková A. 2014. Evolution of host specificity in monogeneans parasitizing African cichlid fish. *Parasites & Vectors* 7: 69.

Mendoza-Garfias B, Pérez-Ponce de Léon G. 1998. Microcotílidos (Monogenea:Microcotylidae) parasitos de peces marinos de la Bahía de Chamela, Jalisco, México. *Anales del Instituto de Biología. Serie Zoología* 69: 139–153.

Messu Mandeng FD, Bilong Bilong CF, Pariselle A, Vanhove MPM, Bitja Nyom AR, Agnèse JF. 2015. A phylogeny of *Cichlidogyrus* spp. (Monogenea , Dactylogyridea) clarifies a host-switch between fish families and reveals an adaptive component to attachment organ morphology of this parasite genus. *Parasites & Vectors* 8: 582.

Michel E. 1995. Evolutionary diversification of rift lake gastropods: Morphology, anatomy, genetics and biogeography of *Lavigeria* (Mollusca: Thiaridae) in Lake Tanganyika. Unpublished thesis, The University of Arizona.

Milne I, Wright F, Rowe G, Marshall DF, Husmeier D, McGuire

G. 2004. TOPALi: software for automatic identification of recombinant sequences within DNA multiple alignments. *Bioinformatics* 20: 1806–1807.

Mladineo I, Šegvić-Bubić T, Stanić R, Desdevises Y. 2013. Morphological plasticity and phylogeny in a monogenean parasite transferring between wild and reared fish populations. *PLoS ONE* 8: e62011.

Mo TA. 1991. Variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic Salmon *Salmo salar* L. in laboratory experiments. *Systematic Parasitology* 20: 11–19.

Moore JES. 1897. The fresh-water fauna of Lake Tanganyika. *Nature* 56: 198–200.

Mouillot D, Šimková A, Morand S, Poulin R. 2005. Parasite species coexistence and limiting similarity: a multiscale look at phylogenetic, functional and reproductive distances. *Oecologia* 146: 269–278.

Mulimbwa N, Raeymaekers JAM, Sarvala J. 2014. Seasonal changes in the pelagic catch of two clupeid zooplanktivores in relation to the abundance of copepod zooplankton in the northern end of Lake Tanganyika. *Aquatic Ecosystem Health and Management* 17: 25–33.

Mulimbwa N, Sarvala J, Raeymaekers JAM. 2014. Reproductive activities of two zooplanktivorous clupeid fish in relation to the seasonal abundance of copepod prey in the northern end of Lake Tanganyika. *Belgian Journal of Zoology* 144: 77–92.

Mulimbwa N, Shirakihara K. 1994. Growth, recruitment and reproduction of sardines (*Stolothrissa tanganicae* and *Limnothrissa miodon*) in northwestern Lake Tanganyika. *Tropics* 4: 57–67.

Muschick M, Nosil P, Roesti M, Dittmann MT, Harmon L, Salzburger W. 2014. Testing the stages model in the adaptive radiation of cichlid fishes in East African Lake Tanganyika. *Proceedings of the Royal Society B: Biological Sciences* 281: 20140605.

Nelson JS. 2006. *Fishes of the world*. *4th ed*. New Jersey, USA: John Wiley & Sons.

Oliva ME, Gonzalez MT. 2004. Metazoan parasites of *Sebastes capensis* from two localities in northern Chile as tools for stock identification. *Journal of Fish Biology* 64: 170–175.

Paperna I. 1973. New species of Monogenea (Vermes) from African freshwater fish. A preliminary report. *Revue de Zoologie et de Botanique Africaines* 87: 505–518.

Paperna I. 1979. *Monogenea of inland water fish in Africa*. Royal Museum for Central Africa.

Pariselle A, Boeger WA, Snoeks J, Bilong Bilong CF, Morand S, Vanhove MPM. 2011. The monogenean parasite fauna of cichlids: a potential tool for host biogeography. *International Journal of Evolutionary Biology* 2011: 471480.

Pariselle A, Muterezi Bukinga F, Van Steenberge M, Vanhove MPM. 2015. Ancyrocephalidae (Monogenea) of Lake Tanganyika: IV: *Cichlidogyrus* parasitizing species of Bathybatini (Teleostei, Cichlidae): reduced host-specificity in the deep water realm? *Hydrobiologia* 748: 99–119.

Peart CR, Bills R, Wilkinson M, Day JJ. 2014. Nocturnal claroteine catfishes reveal dual colonisation but a single radiation in Lake Tanganyika. *Molecular Phylogenetics and Evolution* 73: 119–128.

Plisnier PD, Mgana H, Kimirei I, Chande A, Makasa L, Chimanga J, Zulu F, Cocquyt C, Horion S, Bergamino N, Naithani J, Deleersnijder E, André L, Descy JP, Cornet Y. 2009. Limnological variability and pelagic fish abundance (*Stolothrissa tanganicae* and *Lates stappersii*) in Lake Tanganyika. *Hydrobiologia* 625: 117–134.

Poll M, Gosse JP. 1995. *Genera des poissons d'eau douce de l'Afrique*. *Mémoire de la Classe des Sciences*. Académie royale de Belgique.

Poulin R. 1992. Determinants of host-specificity in parasites of freshwater fishes. *International Journal for Parasitology* 22: 753–758.

Poulin R. 2000. Variation in the intraspecific relationship between fish length and intensity of parasitic infection: biological and statistical causes. *Journal of Fish Biology* 56: 123–137.

Poulin R. 2007. *Evolutionary ecology of parasites*. *2nd ed*. (R Poulin, Ed.). Princeton, New Jersey: Princeton University Press.

Poulin R, Kamiya T. 2015. Parasites as biological tags of fish stocks: a meta-analysis of their discriminatory power. *Parasitology* 142: 145–155.

Poulin R, Morand S. 2004. *Parasite biodiversity*. Washington: Smithsonian Books.

Preston DL, Orlofske SA, Lambden JP, Johnson PTJ. 2013. Biomass and productivity of trematode parasites in pond ecosystems (D Tompkins, Ed.). *Journal of Animal Ecology* 82: 509–517.

Pugachev ON, Gerasev PI, Gussev A V., Ergens R, Khotenowsky I. 2009. *Guide to Monogenoidea of freshwater fish of Palaeartic and Amur regions* (P Galli, ON Pugachev, and D Kritsky, Eds.). Milan: Ledizione-LediPublishing.

R Core Team. 2013. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

R development core team. 2011. *R: A language and environment for statistical computing*. *R Foundation for Statistical Computing*. Vienna, Austria: the R Foundation for Statistical Computing.

Raeymaekers JAM, Hablützel PI, Grégoir AF, Bamps J, Roose AK, Vanhove MPM, Van Steenberge M, Pariselle A, Huyse T, Snoeks J, Volckaert FAM. 2013. Contrasting parasite communities among allopatric colour morphs of the Lake Tanganyika cichlid *Tropheus*. *BMC Evolutionary Biology* 13: 41.

Rambaut A, Suchard MA, Drummond AJ. 2014. Tracer v1.6, Available from http://beast.bio.ed.ac.uk/Tracer.

Rodríguez F, Oliver JL, Marín A, Medina JR. 1990. The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* 142: 485–501.

Rohde K. 1980. Diversity gradients of marine Monogenea in the Atlantic and Pacific Oceans. *Experientia* 36: 1368–1369.

Rohde K. 1988. Gill Monogenea of deep water and surface fish in southeastern Australia. *Hydrobiologia* 160: 271–283.

Rohde K, Hayward C, Heap M. 1995. Aspects of the ecology of metazoan ectoparasites of marine fishes. *International Journal for Parasitology* 25: 945–970.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.

Řehulková E, Mendlová M, Šimková A. 2013. Two new species of *Cichlidogyrus* (Monogenea: Dactylogyridae) parasitizing

the gills of African cichlid fishes (Perciformes) from Senegal: Morphometric and molecular characterization. *Parasitology Research* 112: 1399–1410.

Sanchez-Garcia N, Raga JA, Montero FE. 2015. Morphological and attachment changes of *Lamellodiscus theroni* (Monogenea: Diplectanidae) during its post-larval development on fish. *Folia Parasitologica* 62: 026.

Santos ME, Salzburger W. 2012. Evolution. How cichlids diversify. *Science (New York, N.Y.)* 338: 619–21.

Sefc K, Mattersdorfe K, Ziegelbecker A, Neuhüttler N, Steiner O, Goessler W, Koblmüller S. 2017. Shifting barriers and phenotypic diversification by hybridization. *Ecology Letters* 20: 651–662.

Schoelinck C, Cruaud C, Justine JL. 2012. Are all species of *Pseudorhabdosynochus* strictly host specific? - A molecular study. *Parasitology International* 61: 356–359.

Sigura A, Justine J-L. 2008. Monogeneans of the speckled blue grouper, *Epinephelus cyanopodus* (Perciformes, Serranidae), from off New Caledonia, with a description of four new species of *Pseudorhabdosynochus* and one new species of *Laticola* (Monogenea: Diplectanidae), and evidence of monogenean faunal changes according to the size of fish. *Zootaxa* 1695: 1–44.

Sinnappah ND, Lim LHS, Rohde K, Tinsley R, Combes C, Verneau O. 2001. A paedomorphic parasite associated with a neotenic amphibian host: phylogenetic evidence suggests a revised systematic position for sphyranuridae within anuran and turtle polystomatoineans. *Molecular Phylogenetics and Evolution* 18: 189–201.

Stamatakis A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.

Strona G. 2015. The underrated importance of predation in transmission ecology of direct lifecycle parasites. *Oikos* 124: 685–690.

Sturmbauer C, Baric S, Salzburger W, Rüber L, Verheyen E. 2001. Lake level fluctuations synchronize genetic divergences of cichlid fishes in African lakes. *Molecular Biology and Evolution* 18: 144–154.

Šimková A, Dávidová M, Papoušek I, Vetešník L. 2013. Does interspecies hybridization affect the host specificity of parasites in cyprinid fish? *Parasites & Vectors* 6: 95.

Šimková A, Desdevise Y, Gelnar M, Morand S. 2001a. Morphometric correlates of host specificity in *Dactylogyrus* species (Monogenea) parasites of European Cyprinid fish. *Parasitology* 123: 169–177.

Šimková A, Jarkovský J, Koubková B, Baruš V, Prokeš M. 2005. Associations between fish reproductive cycle and the dynamics of metazoan parasite infection. *Parasitology Research* 95: 65–72.

Šimková A, Morand S. 2008. Co-evolutionary patterns in congeneric monogeneans: a review of *Dactylogyrus* species and their cyprinid hosts. *Journal of Fish Biology* 73: 2210–2227.

Šimková A, Plaisance L, Matějusová I, Morand S, Verneau O. 2003. Phylogenetic relationships of the Dactylogyridae Bychowsky, 1933 (Monogenea: Dactylogyridea): The need for the systematic revision of the Ancyrocephalinae Bychowsky, 1937. *Systematic Parasitology* 54: 1–11.

Šimková A, Sasal P, Kadlec D, Gelnar M. 2001b. Water temperature influencing dactylogyrid species communities in roach, *Rutilus rutilus*, in the Czech Republic. *Journal of Helminthology* 75: 373–383.

Šimková A, Verneau O, Gelnar M, Morand S. 2006. Specificity and specialization of congeneric monogeneans parasitizing cyprinid fish. *Evolution* 60: 1023–1037.

Tinsley RC, Jackson JA. 1998. Correlation of parasite speciation and specificity with host evolutionary relationships. *International Journal for Parasitology* 28: 1573–1582.

Tubbs LA, Poortenaar CW, Sewell MA, Diggles BK. 2005. Effects of temperature on fecundity in vitro, egg hatching and reproductive development of *Benedenia seriolae* and *Zeuxapta seriolae* (Monogenea) parasitic on yellowtail kingfish *Seriola lalandi*. *International Journal for Parasitology* 35: 315–327.

Turner GF. 2007. Adaptive radiation of cichlid fish. *Current Biology* 17: 827–831.

Van der Knaap M, Katonda KI, De Graaf GJ. 2014. Lake Tanganyika fisheries frame survey analysis: Assessment of the options for management of the fisheries of Lake Tanganyika. *Aquatic Ecosystem Health & Management* 17: 4–13.

Vanhove MPM, Pariselle A, Van Steenberge M, Raeymaekers JAM, Hablützel PI, Gillardin C, Hellemans B, Breman FC, Koblmüller S, Sturmbauer C, Snoeks J, Volckaert FAM, Huyse T. 2015. Hidden biodiversity in an ancient lake: phylogenetic congruence between Lake Tanganyika tropheine cichlids and their monogenean flatworm parasites. *Scientific Reports* 5: 13669.

Vanhove MPM, Volckaert FAM, Pariselle A. 2011. Ancyrocephalidae (Monogenea) of Lake Tanganyika: I: Four new species of *Cichlidogyrus* from *Ophthalmotilapia ventralis* (Teleostei: Cichlidae), the first record of this parasite family in the basin. *Zoologia (Curitiba)* 28: 253–263.

Williams HH, MacKenzie K, McCarthy AM. 1992. Parasites as biological indicators of the population biology, migrations, diet, and phylogenetics of fish. *Reviews in Fish Biology and Fisheries* 2: 144–176.

Wilson AB, Teugels GG, Meyer A. 2008. Marine incursion: the freshwater herring of Lake Tanganyika are the product of a marine invasion into west Africa. *PLoS ONE* 3: e1979.

Ziętara MS, Lumme J. 2002. Speciation by host switch and adaptive radiation in a fish parasite genus *Gyrodactylus* (Monogenea, Gyrodactylidae). *Evolution* 56: 2445–2458.

Received: 8 December 2017
Revised and accepted: 14 May 2018
Published online: 12 September 2018
Editor: R. Sluys

Online supplementary information

S1. List of dactylogyrid species obtained from GenBank with their accession numbers for the rDNA region retrieved and their host species.

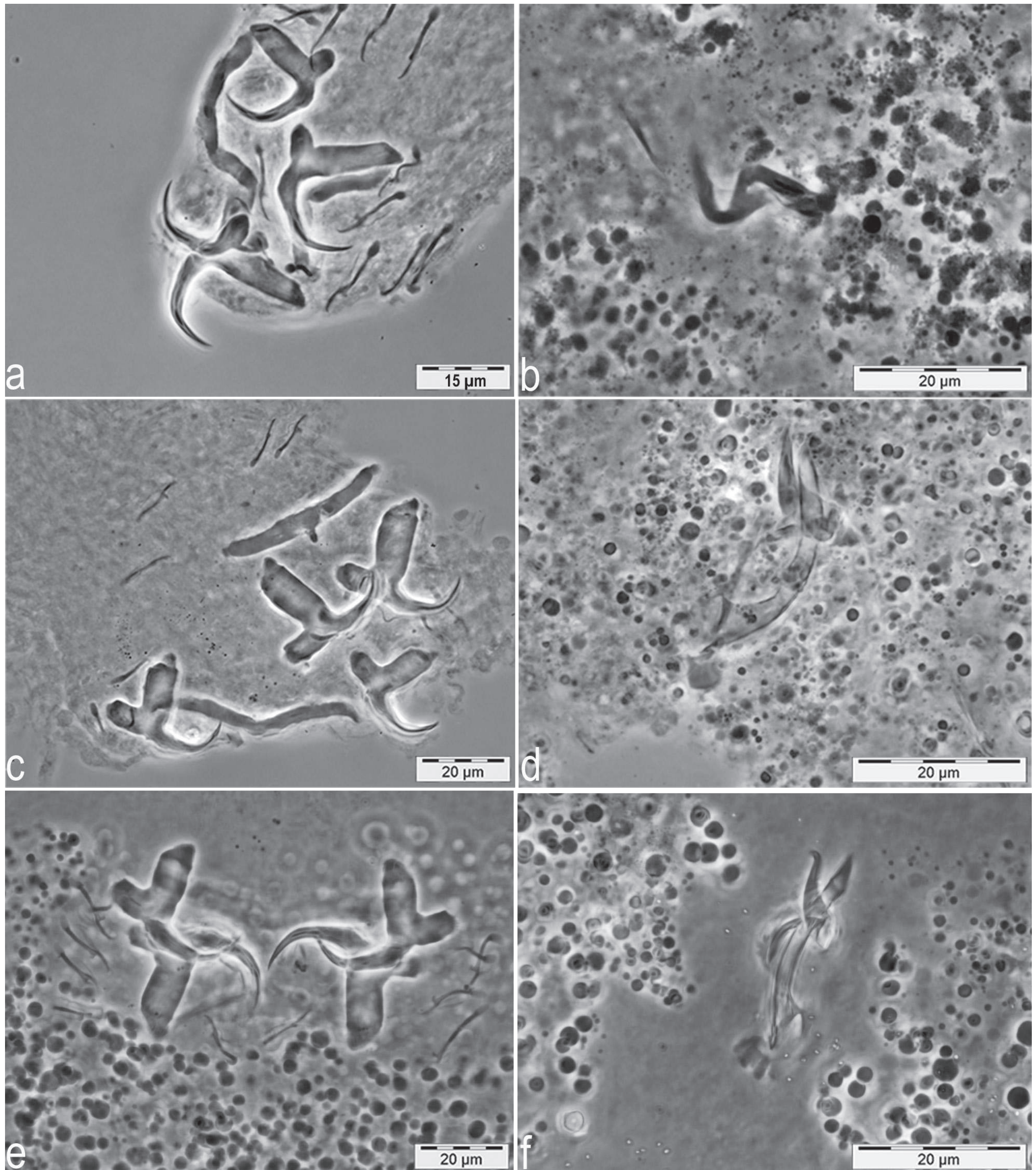


Figure 6. Haptoral and male genital sclerotised structures of monogenean species collected in this study (Hoyer's medium, phase-contrast photomicrographs). a) Opisthaptor of *Kapentagyrus limnotrissae* b) Male copulatory organ of *K. limnotrissae* c) Opisthaptor of *K. tanganicanus* from *L. miodon* d) Male copulatory organ of *K. tanganicanus* from *L. miodon* e) Opisthaptor of *K. tanganicanus* from *S. tanganicae* f) Male copulatory organ of *K. tanganicanus* from *S. tanganicae*.

Appendix

In this section, *Kapentagyrus* Kmentová, Gelnar and Vanhove, gen. nov. is described with *Ancyrocephalus limnotrissae* as type species for which re-description and new records are provided. Moreover, *Kapentagyrus tanganicanus* Kmentová, Gelnar and Vanhove, sp. nov. is described as new species displaying two different haptoral morphologies. The type series of the two species are deposited in the Royal Museum for Central Africa (RMCA); the Iziko South African Museum (SAMC), Cape Town, Republic of South Africa; the Finnish Museum of Natural History (MZH), Helsinki, Finland; the Muséum national d'Histoire naturelle (MNHN), Paris, France; and in the Natural History Museum (NHMUK), London, United Kingdom. Note that the authors of the new taxa are different from the authors of this paper; see article 50.1, recommendation 50A and 51E of the International Code of Zoological 3 Nomenclature (ICZN, 1999: Article 50.1, recommendation 50A and 51E).

Taxonomic account

Family: Dactylogyridae Yamaguti, 1963
Kapentagyrus Kmentová, Gelnar and Vanhove, **gen. nov.**
Type species: *Ancyrocephalus limnotrissae* Paperna, 1973 (original designation)

Other species. *Kapentagyrus tanganicanus*, *Kapentagyrus pellonulae* comb. nov. for *Ancyrocephalus pellonulae* Paperna, 1969

Type-host. *Limnothrissa miodon* (Boulenger, 1906) (Clupeidae).

Type-locality. Lake Tanganyika, Tanzania

Etymology. Since the species assigned to this new genus are known only from clupeids, the representatives of which are known as "kapenta" locally around part of Lake Tanganyika's shoreline, the first part of the genus name refers to a vernacular name of the host. The second part refers to the circular pattern of hooks or extensions and is frequently used in other monogenean genera. Gender: masculine.

Diagnosis. *Kapentagyrus* is a new genus of the family Dactylogyridae (Monogenea). Main diagnostic characters include the combination of (1) well-developed anchor roots together with (2) the presence of two V-shaped transversal haptoral bars without auricles.

Description. [Based on 106 specimens; Fig. 6, see measurements in Table 3.]. *Kapentagyrus*, and its type species *K. limnotrissae* are characterised by two pairs of anchors with well-incised roots and a regularly curved point. Ventral anchors slightly larger than dorsal anchors with more developed inner roots. Dorsal and ventral bar wide, V-shaped with constant width. Dorsal bar larger than ventral bar. Seven pairs of hooks, pairs 1 and 5 with same size, shorter than pairs 2, 3, 4, 6 and 7. Male copulatory organ formed by straight copulatory tube; accessory piece coiled once around the tube. No sclerotized vagina observed. Based on Paperna (1973, 1979), the internal anatomy of the type species comprises a single ovary, dextral vagina, one prostatic gland and seminal vesicle, post-ovarian testis, intestinal limbs that are not united, and a vas deferens which does not loop around the intestinal limbs. Examination of internal anatomy was not included in this study.

Discussion. The uniqueness of the proposed new genus lies in the regularly shaped anchors and bars with almost no difference in size or shape between ventral and dorsal side, the same size of the first and fifth pair of the marginal hooks and the S-shaped accessory piece twisted around the copulatory tube. The species, originally described as *Ancyrocephalus limnotrissae*, was chosen as type species because of the available genetic information and the original description containing internal soft parts (Paperna, 1979). Importantly, as mentioned in the original description of *Ancyrocephalus limnotrissae*, it differs from *Ancyrocephalus* sensu stricto Creplin, 1839 by the developed roots of both the dorsal and the ventral anchor, the longer anchor shafts, the different shape of the haptoral transversal bars, the non-triangular shape of the accessory piece of the MCO and by the presence of just one prostatic gland (Bychowsky and Nagibina, 1970; Paperna, 1979). A morphologically similar genus is *Cichlidogyrus* Paperna, 1960, which infects Levantine and African cichlids (including those in Lake Tanganyika), but which differs from *Kapentagyrus* by the presence of auricles in the dorsal bar and in often having a more asymmetrical dorsal anchor compared to the ventral one. Other monogenean genera known from African freshwaters with two pairs of anchors and similar dorsal and ventral bars are *Annulotrema* Paperna and Thurston, 1969 and *Afrocleidodiscus* Paperna, 1969. In both cases, there are differences either in the shape of the bars or in the more developed anchor roots of *Kapentagyrus*. Moreover, representatives of the two aforementioned genera were described from host species belonging to the fish families Alestidae and Distichodontidae, respectively. European parasites considered to belong to the Ancyrocephalidae sensu

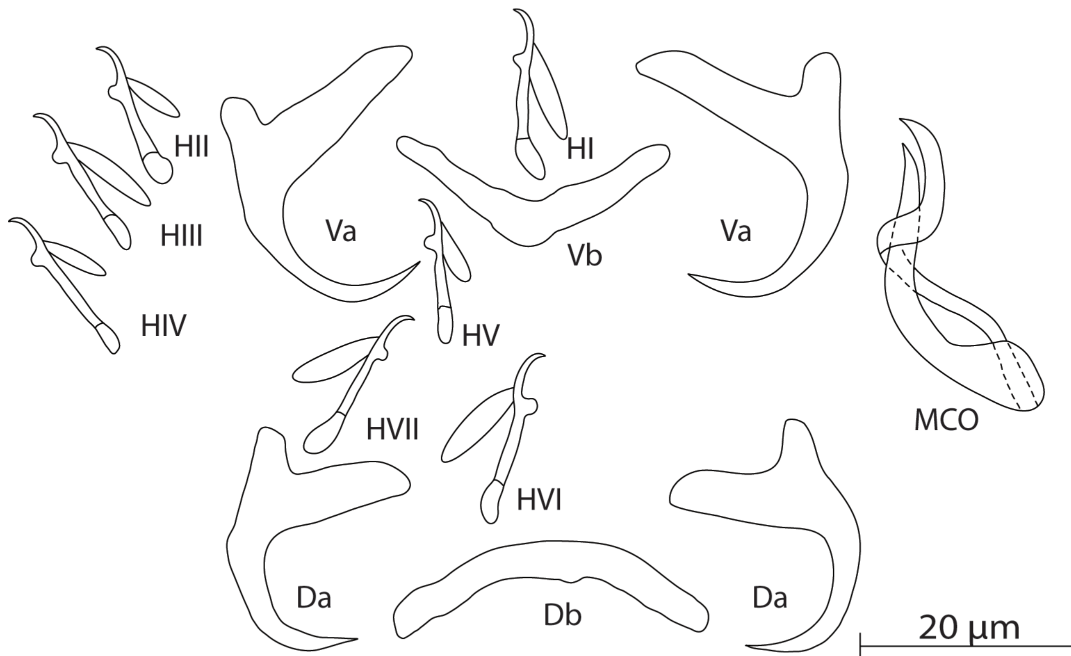


Figure 7. Sclerotized structures of *Kapentagyrus limnotrissae* collected from *Limnothrissa miodon*. Va-ventral anchors. Da-dorsal anchors. Db-dorsal bar. Vb-ventral bar. H-hooks (pairs I to V—ventral; pairs VI, VII—dorsal). MCO-male copulatory organ (ventral view)

lato include the genera *Haplocleidus* Mueller, 1936, *Urocleidus* Mueller, 1936 and *Actinocleidus* Mueller, 1934 but these all differ from *Kapentagyrus* in the shape of their haptoral bars and in the smaller-sized or near-undeveloped anchor roots. *Kapentagyrus* is morphologically similar to *Cleidodiscus* from North America but the latter genus differs in having near-undeveloped anchor roots and a relatively larger anchor shaft, compared to the anchors' base. Moreover, there are some marine dactylogyrid genera similar to *Ancyrocephalus*. Representatives of *Ligophorus* Euzet and Suriano, 1977 have well developed outer and inner anchor roots like *Kapentagyrus* but differ in the presence of auricles in the dorsal bar. Unlike *Kapentagyrus*, representatives of *Haliotrema* Johnston and Tiegs, 1922 and *Euryhaliotrema* Kritsky and Boeger, 2002 are characterised by an almost undeveloped outer root of the anchors compared to the inner one. A noticeable difference between *Kapentagyrus* and *Lethrinitrema* Lim and Justine, 2011 and *Bravohollisia* Bychowsky and Nagibina, 1970, respectively, is the wider base of the anchors' shaft in the latter two genera. Despite the observed similarities with other genera from different continents, the combination of (1) well-developed anchor roots together with (2) the presence of two V-shaped transversal haptoral bars without auricles is unique to the proposed monogenean genus *Kapentagyrus*. Based on the above-mentioned characteristics and its similarity to *K. limnotrissae*, *Ancyrocephalus pellonulae* described from the African freshwater clupeid *Pellonula leonensis* Boulenger, 1916 is here reassigned to *Kapentagyrus* as *Kapentagyrus pellonulae*.

Zoobank registration. To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN) (International Commission on Zoological Nomenclature, 2012), details of the species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:536737D1-44FE-4CF7-98B9-458AEDD6DC4D. The LSID for the new genus *Kapentagyrus* is urn:lsid:zoobank.org:act:D4D37CAB-F21C-46BB-B4AC-6A2D1BD8EE86.

Family: Dactylogyridae Yamaguti, 1963
Genus: *Kapentagyrus*
Kapentagyrus limnotrissae (Paperna, 1973) **comb. nov.**
Ancyrocephalus limnotrissae Paperna, 1979: plate XXVIII, figs.1-7
Figures: 6a, b, 7
Type-host. *Limnothrissa miodon* (Boulenger, 1906) (Clupeidae)

Type locality. Lake Tanganyika, Tanzania

Vouchers. MRAC MT. 38198-200,202 (6 specimens); MNHN HEL739-40 (4 specimens); NHMUK 2018.4.13.1 (4 specimens); SAMC-A089966 (6 specimens); MZH 10076-79 (4 specimens).

Additional localities. Bujumbura (3°23'S-29°22'E), Kalemie (5°56'S-29°12'E), Kigoma Bay (4°88'S-29°61'E), Kirango (7°37'S-30°59'E), Kivugwe (3°80'S-29°34'E), Lufubu Bay (8°38'S-30°47'E), Moba Bay (7°03'S-29°47'E), Mpulungu (8°46'S-31°07'E), Mvugo (4°29'S-29°57'E), Mvuna Island (7°26'S-30°32'E), near Ruzizi (2°50'S-29°02'E), Rumonge (3°97'S-29°43'E), Malagarasi River Delta (05°14'S-29°47'E)

Site of infection. Gills.

Infection parameters. 49 of 144 fish infected with 1 – 15 specimens. Based on the population samples that included at least over 20 host individuals, the average prevalence of *K. limnotrissae* was 35% with a mean infection intensity of 1.7.

Diagnosis. *Kapentagyrus limnotrissae* is the type species of the genus, infecting gills of *L. miodon*, characterized mainly by the proportion between the inner/outer root length of both ventral and dorsal anchors, which is around value 3.

Description. [Based on 106 specimens; Figs. 6 a and b; 7, see measurements in Table 2]. *Kapentagyrus limnotrissae* is characterised by a pair of dorsal and ventral anchors with more developed inner roots compared to the outer ones and a regularly curved point. Dorsal and ventral bars are V-shaped with similar branch lengths and constant width. Hooks: 7 pairs, pairs 2, 3, 4, 6 and 7 same size and slightly longer compared to pair 1 and 5. MCO formed by slightly curved copulatory tube narrowed at distal extremity and accessory piece coiled once around the tube. Sclerotized vagina not observed.

Discussion: Specimens of *K. limnotrissae* were identified based on comparison with the holotype material. Importantly, as mentioned in the original description, it differs from *Ancyrocephalus* sensu stricto Creplin, 1839 by the developed roots of both the dorsal and the ventral anchor, the longer anchor shafts, the different shape of the haptoral transversal bars and the non-triangular shape of the accessory

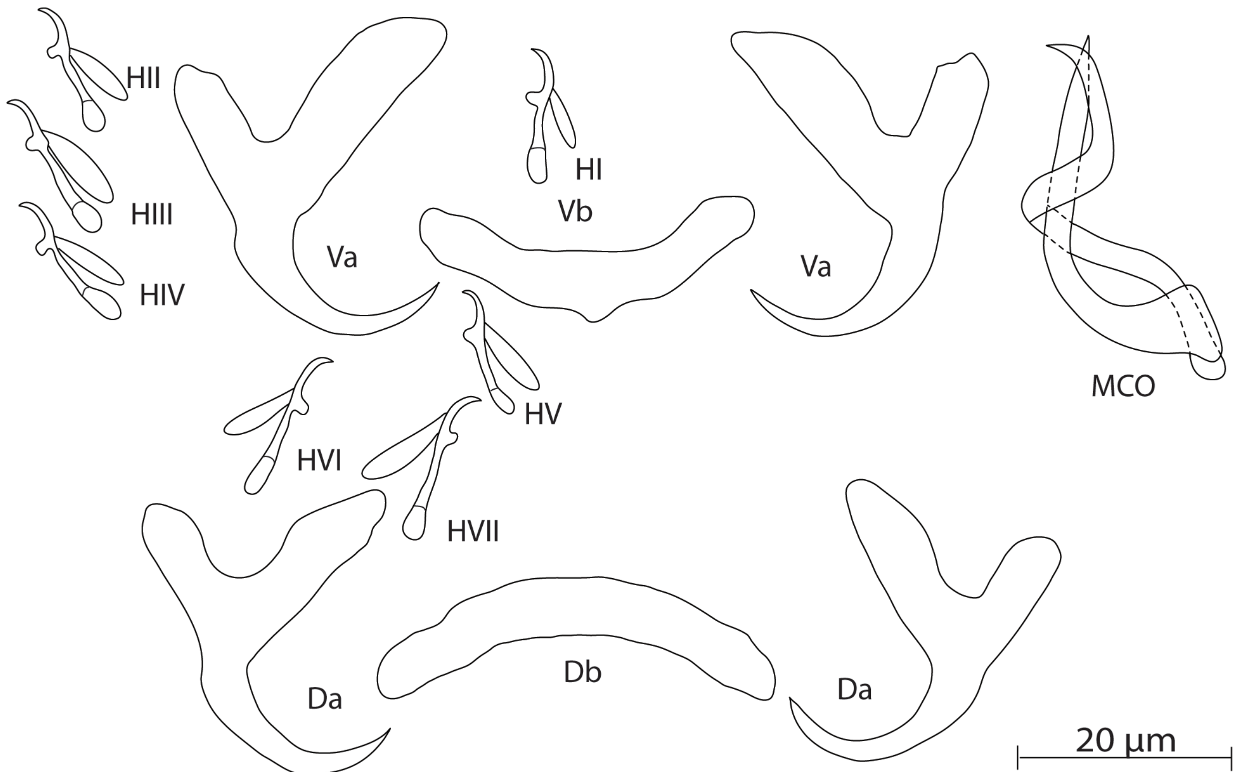


Figure 8. Sclerotized structures of *Kapentagyrus tanganicanus* collected from *Limnothrissa miodon*. Va-ventral anchors. Da-dorsal anchors. Db-dorsal bar. Vb-ventral bar. H-hooks (pairs I to V—ventral; pairs VI, VII—dorsal). MCO-male copulatory organ (ventral view)

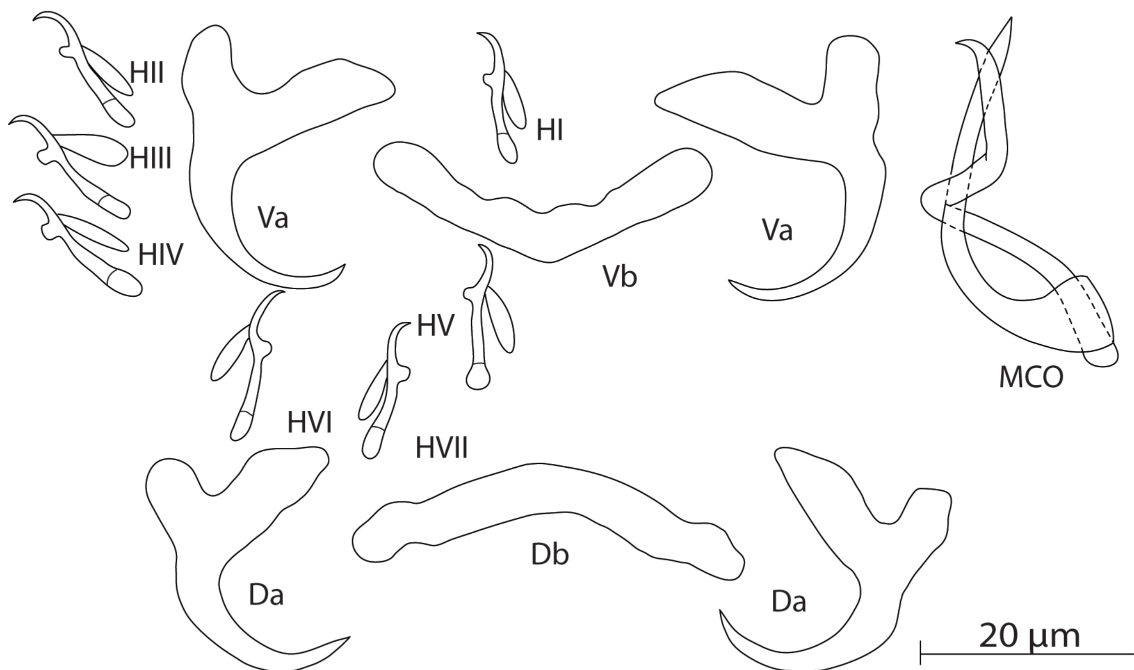


Figure 9. Sclerotized structures of *Kapentagyrus tanganicanus* collected from *Stolothrissa tanganicae*. Va-ventral anchors. Da-dorsal anchors. Db-dorsal bar. Vb-ventral bar. H-hooks (pairs I to V—ventral; pairs VI, VII—dorsal). MCO-male copulatory organ (ventral view)

piece of the MCO. Differential diagnosis with other congeners is provided in the description of *K. tanganicanus*.

Zoobank registration. To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN) (International Commission on Zoological Nomenclature, 2012), details of the species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:536737D1-44FE-4CF7-98B9-458AEDD6DC4D. The LSID for the new name *Kapentagyrus limnotrissae* is urn:lsid:zoobank.org:act:0B7EFDF3-9B45-42B5-94AB-B6ED07D397E5.

Family: Dactylogyridae Yamaguti, 1963
Genus: *Kapentagyrus*
Kapentagyrus tanganicanus Kmentová, Gelnar and Vanhove, **sp. nov.**
Figure: 6 c- f, 8, 9

Type host. *Stolothrissa tanganicae* Regan, 1917 (Clupeidae)

Additional host. *Limnothrissa miodon* (Boulenger, 1906) (Clupeidae)

Type locality. Uvira (3°22’ S 29°08’E)

Type material. Holotype: MRAC MT. 38201; Paratypes: MRAC MT. 38203-205 (5 specimens); MNHN HEL741-43 (4 specimens); NHMUK 2018.4.13.2-13.3 (6 specimens); SAMC-A089967-70 (11 specimens); MZH 10072-75 (4 specimens).

Additional localities. *S. tanganicae* - Bujumbura (3°23’S-29°22’E), Kalambo Lodge (8°59’S-31°18’E), Kalemie (5°56’S-29°12’E), Kigoma Bay (4°88’S-29°61’E), Mpulungu (8°46’S-31°07’E), Mvugo (4°29’S-29°57’E), Rumonge (3°97’S-29°43’E), Uvira (3°22’ S 29°08’E), Utinta Bay (7°11’S-30°52’E); *L. miodon* - Kasasa Bay (8°31’S-30°42’E), Kirango (7°37’S-30°59’E), Lufubu Bay (8°38’S-30°47’E), Luhanga (3°52’S-29°15’E), Mvugo (4°29’S-29°57’E), Mvuna Island (7°26’S-30°32’E), Rumonge (3°97’S-29°43’E)

Infection parameters: 46 of 241 specimens of *S. tanganicae* infected with 1 – 7 specimens. 27 of 144 specimens of *L. miodon* infected with 1 – 37 specimens. The average prevalence was 40% in *L. miodon* with a mean infection intensity of 3.5 and 18% in *S. tanganicae* with mean infection intensity 1.5. While higher prevalence values of *K. tanganicanus* were observed in *S. tanganicae* in the northern (21.5%) compared to the central (0%) and southern part of the

lake (4.2%), the mean infection intensity remained the same (4.5) in the northern and southern sub-basins.

Etymology. The species epithet is based on both the species epithet of the type host *Stolothrissa tanganicae* and the name of the ecosystem, Lake Tanganyika.

Diagnosis. *Kapentagyrus tanganicanus* is a monogenean species infecting gills of *L. miodon* and *S. tanganicae* in Lake Tanganyika, mainly characterized by the proportion between the inner/outer root length of both ventral and dorsal anchors, which is around value 2.

Description. [Based on 139 specimens; Figs. 6c-f; 8, 9, see measurements in Table 2.]. Dorsal and ventral anchors with different outer and inner root sizes and regularly curved points. Ventral anchors larger in total size with on average longer inner root compared to dorsal anchors. Hooks 7 pairs, pairs 2, 3, 4, 6 and 7 of same size and slightly longer compared to pairs 1 and 5. Dorsal and ventral bar wide, long, V-shaped with constant width. Dorsal bar with longer branches compared to ventral bar. Male copulatory organ formed by slightly curved copulatory tube and accessory piece coiled once around the tube. Sclerotized vagina not observed. Based on morphometric results showing a consistent pattern, phenotypic variation in *K. tanganicanus* from *L. miodon* and *S. tanganicae*, respectively, was observed (see Table 3).

Discussion. The most similar congener hitherto known is *K. limnotrissae*. These two species differ mainly by the more asymmetrical anchor roots in *K. limnotrissae*: the proportion inner/outer root length of both ventral and dorsal anchors is around 3, whereas this proportion is close to 2 in *K. tanganicanus*. Given that the size of the hooks is almost identical, the relative size of the anchors compared to the marginal hooks is greater in *K. tanganicanus*. Another monogenean species described from a freshwater clupeid host in Africa which was reassigned to *Kapentagyrus* is *K. pellonulae* infecting *Pellonula leonensis* in Lake Volta. Based on the original description by Paperna, 1969 and the holotype (MRAC MT. 35572) we see a high similarity to both species from Lake Tanganyika with differences mainly in the size of the MCO (the average size of the copulatory tube in *K. limnotrissae* and *K. tanganicanus* is 30.4±2.5 μm and 39.5±4.3 μm, respectively, compared to 25 μm in the original description of *K. pellonulae*) and the more similar length of inner and outer dorsal anchor roots in the case of *K. pellonulae*.

Zoobank registration. To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN) (International Commission on Zoological Nomenclature, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:536737D1-44FE-4CF7-98B9-458AEDD6DC4D. The LSID for the new name *Kapentagyrus tanganicanus* is urn:lsid:zoobank.org:act:1B59CB96-864B-4519-8F75-CF5F36782247.

Paper II

Kmentová N., Van Steenberge M., van den Audenaerde D.R., Nhiwatiwa T., Muterezi Bukinga F., Mulimbwa N'sibula T, Masilya Mulungula P., Gelnar M., Vanhove M. P. M. 2019. Co-introduction success of monogeneans infecting the fisheries target *Limnothrissa miodon* differs between two non-native areas: the potential of parasites as tag for introduction pathway. *Biological Invasions* 21(3): 757-773. DOI: https://doi.org/10.1007/s10530-018-1856-3 [Q1, IF (2018) = 2.897].

Publication is available at: https://link.springer.com/article/10.1007/s10530-018-1856-3

NK collected parasite specimens, obtained morphometric and genetic data, performed statistical analyses and wrote the manuscript. Overall contribution: c. 70%

Biol Invasions
https://doi.org/10.1007/s10530-018-1856-3

CrossMark

ORIGINAL PAPER

Co-introduction success of monogeneans infecting the fisheries target *Limnothrissa miodon* differs between two non-native areas: the potential of parasites as a tag for introduction pathway

Nikol Kmentová · Maarten Van Steenberge · Dirk F. E. Thys van den Audenaerde · Tamuka Nhiwatiwa · Fidel Muterezi Bukinga · Théophile Mulimbwa N'sibula · Pascal Masilya Mulungula · Milan Gelnar · Maarten P. M. Vanhove

Received: 16 January 2018 / Accepted: 9 October 2018
© Springer Nature Switzerland AG 2018

Abstract Fish have been widely translocated into non-native areas, commonly as fishery targets. Since fish figure as hosts of various parasite taxa, their introduction may pose often-underestimated threats to ecosystems. However, parasites can also serve to track host species' introduction routes when these would otherwise be unknown. To verify the potential of parasites in reconstructing invasion routes, we investigated two of the best-documented introductions: those of *Limnothrissa miodon* into lakes Kivu and Kariba. As a proof of concept, we investigate the possibility of using parasites to evaluate the effect of host size in the introduction pathway and to track the host origins of *L. miodon*. Combining historical collections and recent field samples, specimens of *L. miodon* from Lake Kivu and Lake Kariba were examined for monogenean flatworms. Intraspecific variation was investigated using morphometrics of the parasite's sclerotised structures. Three markers from the ribosomal DNA region were used for genetic parasite identification. In Lake Tanganyika, *L. miodon* is infected by two species of monogeneans, *Kapentagyrus limnotrissae* and *K. tanganicanus*. One of these species, *K. limnotrissae,* was found on *L. miodon* from Lake Kariba. In contrast, not a single monogenean individual was found in specimens from Lake Kivu. Morphometric results suggested that the origin of *K. limnotrissae* introduced into Lake Kariba may be

N. Kmentová (✉) · M. Gelnar · M. P. M. Vanhove
Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic
e-mail: kmentovan@mail.muni.cz

M. Van Steenberge · D. F. E. Thys van den Audenaerde
Biology Department, Royal Museum for Central Africa, Leuvensesteenweg 13, 3080 Tervuren, Belgium

M. Van Steenberge
Operational Directorate Taxonomy and Phylogeny, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1000 Brussels, Belgium

T. Nhiwatiwa
Lake Kariba Research Station, Department of Biological Sciences, University of Zimbabwe, 630 Churchill Avenue, Mt Pleasant, Harare, Zimbabwe

F. Muterezi Bukinga · T. Mulimbwa N'sibula · P. Masilya Mulungula
Département de Biologie, Centre de Recherche en Hydrobiologie, B.P. 73, Uvira, Democratic Republic of Congo

N. Kmentová · M. Van Steenberge · M. P. M. Vanhove
Laboratory of Biodiversity and Evolutionary Genomics, Department of Biology, University of Leuven, Ch. Deberiotstraat 32, 3000 Leuven, Belgium

M. P. M. Vanhove
Capacities for Biodiversity and Sustainable Development, Operational Directorate Natural Environment, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1000 Brussels, Belgium

Published online: 30 October 2018

the southern part of Lake Tanganyika, which corresponds to historical reports. Moreover, differences in the size of introduced fish, fry versus juveniles, were proposed as one of the factors influencing parasite occurrence in non-native areas. This supports the potential use of monogeneans as markers for host origin.

Keywords Lake Kivu · Lake Kariba · Comparative morphometrics · Intraspecific variability · Genetic characterisation

Introduction

Helminths are the most commonly detected parasite group co-introduced with non-native species, with fish as the most common alien hosts (Gozlan 2008; Lymbery et al. 2014). Parasite co-introduction and its possible impact on ecosystems is usually underestimated (Peeler et al. 2004; Lymbery et al. 2014). The success of parasite establishment in a non-native environment is affected by many factors such as the size of the founder host population (Anderson and May 1991; Sakai et al. 2001; Dlugosch and Parker 2008), the parasite's life cycle (direct vs. indirect) and environmental biotic and abiotic conditions (Taraschewski 2006; Lymbery et al. 2014). Moreover, the success of parasite co-introduction is influenced by host transportation, whereby factors such as salinity, the life stage of the introduced population or antiparasitic treatment might hamper parasite introduction (Mombaerts et al. 2014; Kvach et al. 2014). While the potential use of parasites as tags for host population, introduction pathway and historical distribution has been discussed for decades, there are only a small number of studies demonstrating this concept (Jiménez-García et al. 2001; Oliva and Gonzalez 2004; Huyse et al. 2015; Kmentová et al. 2018).

A taxonomically diverse range of fish has been anthropogenically introduced or translocated in Africa. Fish introductions out of their native range mainly occur with fishery target species like the Nile perch *Lates niloticus* (Linnaeus 1758) (Latidae), "tilapias" (*Oreochromis* spp., *Tilapia* spp.) (Cichlidae) and the clupeid *Limnothrissa miodon* (Boulenger, 1906) (Ogutu-Ohwayo and Hecky 1991). Other species acting as potential agents for disease control, such as the poeciliid *Gambusia affinis* (Baird & Girard, 1853), which feeds on the mosquito vectors of malaria, were translocated to non-native areas (Welcomme 1981).

Clupeids (Clupeiformes; Actinopterygii) form highly productive commercial stocks of worldwide importance (Naylor et al. 2000). Although they are primarily a marine family, more than half of the clupeid species can be found in brackish waters or freshwater. Some of them have adopted a continental lifestyle without any link to the marine realm. In African freshwaters, clupeids are represented by 27 species belonging to the Dorosomatinae (Lavoué et al. 2014). In this study, we focused on *Limnothrissa miodon*, a clupeid species endemic to Lake Tanganyika, and particularly on its non-native populations from lakes Kivu and Kariba.

Lake Tanganyika is the oldest and deepest of the African Great Lakes. It is famous for its explosive and adaptive evolution of many fish and other taxa (Salzburger et al. 2014). In contrast to the high species richness of fish in the littoral zone, the pelagic realm is mainly inhabited by two endemic clupeid species, *Limnothrissa miodon* (Boulenger, 1906) and *Stolothrissa tanganicae* Regan, 1917, both belonging to monotypic genera (Coulter 1991). Lake Tanganyika sprat (*S. tanganicae*) and sardines (*L. miodon*), together with their main predator, *Lates stappersii* (Boulenger, 1914), comprise up to 95% of commercial catches in the lake, with an estimated annual production in the range of 165,000 to 200,000 tons (Mölsä et al. 1999). Both clupeid species are short-lived and numerous. They show schooling behaviour, seasonal fluctuations in abundance, and form the main link between the planktonic and piscivorous trophic levels in the pelagic realm (Mulimbwa and Shirakihara 1994).

The Lake Tanganyika sardine, *L. miodon,* was introduced into several water bodies in Africa, including Lake Kivu (Spliethoff et al. 1983) and the

N. Kmentová · M. P. M. Vanhove
Centre for Environmental Sciences, Research Group Zoology: Biodiversity and Toxicology, Hasselt University, Agoralaan Gebouw D, 3590 Diepenbeek, Belgium

M. P. M. Vanhove
Zoology Unit, Finnish Museum of Natural History, University of Helsinki, P.O. Box 17, 00014 Helsinki, Finland

man-made reservoir Lake Kariba (Balon and Cache 1974). In addition, starting from Lake Kariba, this species further invaded the Cahora Bassa reservoir via the Zambezi River (Cross et al. 2011).

Lake Kivu is one of the Great African Lakes and is known for the vast amounts of carbon dioxide and methane in its anoxic waters, which cause unusual biochemical and limnological conditions. It has a species-poor fish fauna compared to the other Great Lakes (Beadle 1981) because of historical volcanic activity, periods of drought and the higher salinity and recent origin of the present-day lake (Snoeks et al. 1997). Lake Kariba is a man-made lake that was constructed by damming the Zambezi River in 1958.

Limnothrissa miodon was introduced to both non-native areas to fill in the empty pelagic niche. However, initially, *S. tanganicae* was planned to be introduced into Lake Kivu as a fishery target (Collart 1960; Dumont 1986). The introduction of both clupeid species, rather than just *Stolothrissa tanganicae*, was unintentional. The success of *L. miodon* is probably due to its greater habitat and diet plasticity (Mulimbwa and Shirakihara 1994).

As the introduction of *S. tanganicae* to Lake Kivu in 1959 was unsuccessful, *L. miodon* was targeted for introduction into Lake Kariba in 1967. Transport of adult sardines was not recommended because of their fragile skin that becomes severely damaged upon contact and becomes susceptible to infection. Therefore, fry occurring in shallow water at night were chosen for transport because they were easier to handle. The fry were scooped with large containers to avoid high fry density, and a tranquilizer was added. As the species migrates to deeper water during the day, the containers were transported by plane at night and emptied into Lake Kivu and Lake Kariba (Collart 1960; Bell-Cross and Bell-Cross 1971). Based on available reports, the origin of the population currently inhabiting Lake Kivu was the northern end of Lake Tanganyika near Bujumbura in Kabezi (Collart 1960). Fish for Lake Kariba, however, were caught near Mpulungu and Kasaba Bay at the south-western coast of Lake Tanganyika (Bell-Cross and Bell-Cross 1971). In contrast to Lake Kivu, where only fry were introduced, some somewhat larger specimens were also present in the transports to Lake Kariba (Bell-Cross and Bell-Cross 1971).

In Lake Tanganyika, *L. miodon* is infected with two species of dactylogyrid monogeneans, *Kapentagyrus limnotrissae* (Paperna 1973) and *K. tanganicanus* Kmentová, Gelnar & Vanhove, 2018 (Paperna 1973; Kmentová et al. 2018). Monogeneans (Platyhelminthes) are parasitic flatworms with a direct life cycle (they infect a single host species). They occur worldwide and are mainly ectoparasites on the gills, skin and fins of fish (Pugachev et al. 2009). Parasites with a direct life cycle have an increased chance of establishment after translocation compared to parasites where more than one host is involved in the life cycle (Bauer 1991). Importantly, monogeneans and other parasites are considered to be potential tags for the characterisation of host stock structure (Oliva and Gonzalez 2004; Criscione et al. 2006). Monogeneans have already been used to reconstruct their host's historical distribution (Lumme et al. 2016) but also their introduction route (Huyse et al. 2015). Although co-introductions can be viewed as natural experiments to test the potential of parasites in detecting their host's origin, very few introductions are sufficiently documented to allow testing this. The introductions of *L. miodon*, however, do provide us with such a case, as the procedures were described in detail for Lake Kivu (Collart 1960) and Lake Kariba (Bell-Cross and Bell-Cross 1971). Based on historical reports, solely sardine fry was introduced to Lake Kivu (Collart 1960), while some somewhat larger sardine specimens are thought to have been potentially introduced to Lake Kariba (Bell-Cross and Bell-Cross 1971). Infection of dactylogyrid monogeneans on fry was reported as a result of high fish population densities and stress in farmed/artificial conditions (Paperna 1963; Thoney and Hargis 1991; Jalali and Barzegar 2005) and the gills of fry populations in natural environments are usually less affected or not affected (Pugachev et al. 2009). Therefore, we hypothesize a higher co-introduction success of monogenean parasites in the latter case, as sardine fry are not known to be infected by monogeneans (11 specimens from Uvira (northern basin of Lake Tanganyika) and 9 specimens from Bujumbura (MRAC MT. 43554-64) of *L. miodon* below 5.0 cm standard length were not infected by monogeneans, suggesting that sardine fry are not infected by monogeneans: unpublished results).

This study is designed as a natural experiment in two different ways: 1) Since some larger specimens of *L. miodon* were transported to Lake Kariba whereas only fry was introduced to Lake Kivu, we can test the effect of host life stage on the co-introduction of

monogeneans. In this study, the potential presence of monogenean species in their non-native range was verified by using a combination of morphological and molecular identification. 2) Additionally, intraspecific geographical variation in morphology was reported in dactylogyrids, including in species of *Kapentagyrus* Kmentová, Gelnar & Vanhove 2018 which infect *L. miodon* in Lake Tanganyika (Kmentová et al. 2018). We investigated whether the morphology of introduced parasites might indicate the geographic origin of the host population which was used in the introduction. To date, few parasitological surveys have been conducted in Lake Kivu (Baer and Fain 1958; Vercammen-Grandjean 1960) and only a small portion of host species have been investigated in Lake Kariba (Douëllou 1993; Barson et al. 2010). Although a previous study reported on the presence of a species of *Kapentagyrus* on *L. miodon* in Lake Kariba (Douëllou 1991), only one species was known from *L. miodon* at that time (*Kapentagyrus limnotrissae* (Paperna, 1973)) with the second species, *K. tanganicanus* discovered at a later date. As such, the presence of the latter species remained to be checked.

Materials and methods

Sampling

Individuals of *Limnothrissa miodon* from two non-native areas, Lake Kivu and Lake Kariba, were examined (Fig. 1). Fish specimens from the Rwandese side of Lake Kivu originated from the ichthyology collection of the Royal Museum for Central Africa (RMCA) (Tervuren, Belgium). Fresh specimens were obtained by scientists from the Unité d'Enseignement et de Recherche en Hydrobiologie Appliquée (UERHA) of the department of Biology of the Institut Supérieur Pédagogique (ISP) of Bukavu located at the Congolese side of the lake (see Table 1). Fish from Lake Kariba were caught by gillnets during several months in 2016 and the beginning of 2017 in Sanyati East Basin (see Table 1). In total, gills and fins of 251 fish specimens were examined following the standard protocol of Ergens and Lom (1970). Monogeneans were mounted on a slide with a drop of water, which was later replaced by Hoyer's medium, and covered with a cover slip that was fixed with nail polish. With the exception of museum specimens, at least two monogenean individuals from each infected fish were cut in two, followed by the transfer of the anterior body part into an Eppendorf tube (1.5 ml) containing

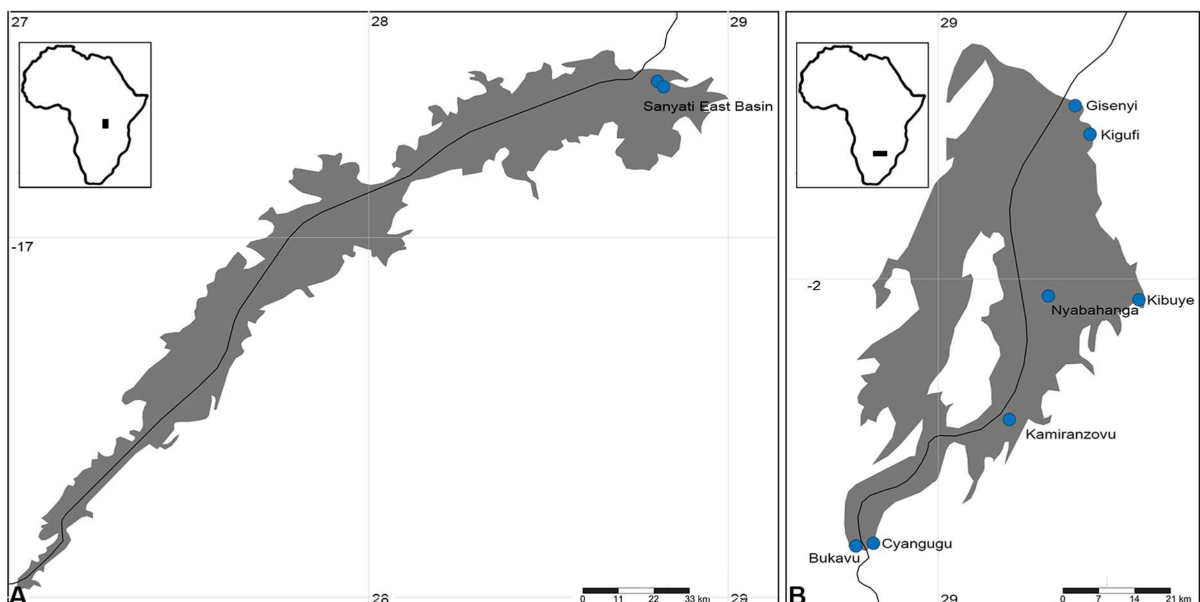


Fig. 1 Geographical positions of sampling localities in **a** Lake Kariba and **b** Lake Kivu. Map created using SimpleMappr software v7.0.0. (available at http://www.simplemappr.net. Accessed July 25, 2017)

Table 1 An overview of host specimens examined for monogeneans parasites with localities and infection parameters

Locality (geographic coordinates)	Date of sampling	Number of fish specimens (accession number in RMCA)	Number of monogenean individuals	Prevalence (%)	Infection intensity/one gill chamber (range)	Abundance/ one gill chamber (range)
Lake Kariba, Sanyati East Basin (16°59′S–28°82′E)	14.04.2016	19 (HU 49-67)	0	0	0	0
Lake Kariba, Sanyati East Basin (16°59′S–28°82′E)	05.05.2016	23 (HU 81–104)	0	0	0	0
Lake Kariba, Sanyati East Basin (16°59′S–28°82′E)	07.06.2016	23 (HU 105–121)	0	0	0	0
Lake Kariba, Sanyati East Basin (16°59′S–28°82′E)	05.07.2016	19 (HU 68–80)	0	0	0	0
Lake Kariba, Sanyati East Basin (16°59′S–28°82′E)	05.01.2017	22 (HU 151–170)	32	50	1.5 (1–10)	0.8 (0–10)
Lake Kariba, Sanyati East Basin (16°60′S–28°87′E)	06.12.2017	9 (HU 171–179)	26	55.5	1.5 (2–10)	0.8 (0–10)
Lake Kivu, Kigufi (1°75′S–29°28′E)	08.08.1979	12 (MRAC 79031.0010–11, 28, 29,30)	0	0	0	0
Lake Kivu, Gissenyi (1°70′S–29°25′E)	15.08.1979	2 (MRAC 79031.0071,72)	0	0	0	0
Lake Kivu, Kibuye (2°06′S–29°34′E)	17.08.1979	21(MRAC 79031.0086–88, 111, 112)	0	0	0	0
Lake Kivu, Cyangugu (2°47′S–28°90′E)	04.09.1979	13 (MRAC 79031.0197-200)	0	0	0	0
Lake Kivu, Kamiranzovu (2°25′S–29°13′E)	07.09.1979	34 (MRAC 79031.0284-88)	0	0	0	0
Lake Kivu, Kamiranzovu (2°25′S–29°13′E)	26.12.1979	2 (MRAC P80029.1164,65)	0	0	0	0
Lake Kivu, Nyabahanga (2°04′S–29°22′E)	27.08.1981	10 (MRAC 81055.8524, 54–57, 90228,29)	0	0	0	0
Lake Kivu, near Bukavu (2°29′S–28°51′E)	11.08.2016	42 (MRAC P. 2016.20)	0	0	0	0

99% ethanol. In addition, 80 specimens of *L. miodon* fry under 3.1 cm of standard length, originating from Chituta Bay, the southern basin of Lake Tanganyika, were examined (8°43′S, 31°09′E). Parasite identification and measurements were carried out using an Olympus BX51 microscope. Specimens were compared with type material of *K. limnotrissae* and *K. tanganicanus*, respectively, deposited in the RMCA (MRAC MT.35572 and MT.38201). Fish tissue samples from Lake Kariba were deposited in the collection of the research group Zoology: Biodiversity and Toxicology of Hasselt University under accession number HU hostvouchers HU 49–179. Fish tissue samples from Lake Kivu were deposited in the ichthyology collection of the RMCA under collection number MRAC P. 2016.20 and parasite voucher specimens are available in the invertebrate collection of the RMCA (MRAC MT. 38237-8 and 38450-60).

Morphometrics

Since monogenean taxonomy is mainly based on the parasites' sclerotized structures, 25 different variables of the hard parts of the haptor and male copulatory organ (MCO) were measured for species identification (see Table 2). Measurements were taken using an Olympus BX51 microscope with incorporated phase contrast and the Olympus Stream Motion software at a magnification × 1000 (objective × 100 immersion, ocular × 10). Terminology was based on Řehulková et al. (2013). To check for intraspecific phenotypic diversity (in haptor morphology), measurements were analysed using multivariate statistical techniques in the R (R development core team 2013) adegenet package (Jombart 2008), where a principal component analysis (PCA) was conducted on a covariance matrix with 19 measured and standardised variables. Outliers were identified and removed using Mahalanobis distances in the mvoutlier package (Filzmoser and Gschwandtner 2017). Morphometric data generated in this study were compared with previously published data on *Kapentagyrus limnotrissae* from Lake Tanganyika (Kmentová et al. 2018) which stemmed from specimens from all three subbasins (Danley et al. 2012). Since significant intraspecific variation of *K. limnotrissae* among subbasins was documented (Kmentová et al. 2018), comparisons for the different subbasins were made separately. The assumption of normality was tested by Shapiro–Wilk's W test implemented in the stats package (R development core team 2013). Morphological differences between monogeneans from the native and introduced range were also tested using multiple one-way MANOVA in the package stats as a set of independent tests, with Pillai's test of significance and Bonferroni's correction (α value of 0.05/number of variables). To test the significance of intraspecific differences in haptor and MCO structures, Mann–Whitney U tests were performed in STATISTICA 12. The assumption of homogeneous variance within sample groups was verified by Levene's test.

Molecular characterisation

Total genomic DNA was extracted following Zavodna et al. (2008): ethanol evaporation took place in a vacuum centrifuge and the tissue was homogenized in 200 µl of extraction buffer (100 mM Tris–HCl, 10 mM EDTA, 100 mM NaCl, 1% SDS, 0.06 mg Proteinase K, 1.5 mM dithiothreitol) and incubated at 56 °C overnight. After incubation, proteins were precipitated using 10 M ammonium acetate (1/3 of the lysate volume). The lysate was then vortexed, centrifuged at the highest speed (13,800 rpm) and the supernatant containing DNA was precipitated using a double volume of ice-cold 100% ethanol. Following centrifugation, the DNA pellet was washed using 70% ethanol. Finally, the DNA pellet was air-dried and dissolved in 60 µl of sterile Millipore water. To confirm parasite species identification genetically, we used three nuclear fragments: from the small and large ribosomal subunit gene (18 and 28 rDNA) and internal transcribed spacer 1 (ITS-1). Partial 18S rDNA together with ITS-1 were amplified using the S1 (5′-ATTCCGATAACGAACGAGACT-3′) (Sinnappah et al. 2001) and Lig5.8R (5′-GATACTCGAGCC GAGTGATCC-3′) (Blasco-Costa et al. 2012) primers. Each reaction mix contained 1.5 units of *Taq* Polymerase, 1X buffer containing 0.1 mg/ml BSA, 1.5 mM $MgCl_2$, 200 mM dNTPs, 0.8 mM of each primer and 3 µl of isolated DNA (concentration was not measured) in a total reaction volume of 30 µl under the following conditions: 2 min at 95 °C, 39 cycles of 1 min at 95 °C, 1 min at 55 °C and 1 min and 30 s at 72 °C, and finally 10 min at 72 °C. Primers C1 (5′-ACCCGCTGAATTTAAGCAT-3′) and D2 (5′-TGGTCCGTGTTTCAAGAC-3′) (Hassouna et al. 1984) were used for amplification of the partial

Table 2 Comparison of measurements performed on haptoral and genital hardparts of *Kapentagyrus limnotrissae* (Paperna, 1973) studied in Kmentová et al. (2018) and recorded in this study (a—mean value ± standard deviation, b—range)

Parameters (μm)	*K. limnotrissae* (Kmentová et al. 2018)	*K. limnotrissae* (present study)
Total length	458.1 ± 128.5 (n = 21); (286.2–748.3)	420.2 (n = 10); (352.4–556.9)
Total width	143.9 ± 26.2 (n = 20); (97.8–220.3)	114.1 (n = 10); (77.9–152.5)
Ventral anchor		
Total length	26.2 ± 1.5 (n = 99); (22.3–31.8)	25.9 ± 2.53 (n = 30); (19.9–30.5)
Length to notch	18.8 ± 1.6 (n = 98); (15.5–28.3)	18.9 ± 1.83 (n = 30); (14.3–22.8)
Inner root length	15.6 ± 1.4 (n = 99); (11.4–18.6)	15 ± 1.95 (n = 29); (18–29)
Outer root length	5.0 ± 0.8 (n = 94); (3.6–7.9)	5.27 ± 0.85 (n = 24); (4–7.4)
Point length	8.1 ± 1.1 (n = 90); (5.3–11.0)	7.58 ± 1.2 (n = 21); (5.8–10.2)
Dorsal anchor		
Total length	21.48 ± 1.55 (n = 88); (18.7–26.1)	21.7 ± 1.31 (n = 15); (19.7–23.5)
Length to notch	16.5 ± 1.3 (n = 87); (13.8–20.5)	16.9 ± 1.39 (n = 15); (14.4–19.4)
Inner root length	11.2 ± 1.3 (n = 87); (7.8–14.6)	12.1 ± 1.53 (n = 14); (9.7–14.4)
Outer root length	4.5 ± 1.0 (n = 84); (2.5–8.0)	4.7 ± 0.74 (n = 14); (3.2–6)
Point length	7.8 ± 1.1 (n = 84); (5.1–10.9)	7.5 ± 1.34 (n = 15); (5.1–10.6)
Ventral bar		
Branch length	16.7 ± 3.0 (n = 84); (12.5–33.3)	21 ± 2.63 (n = 30); (15.8–27.4)
Thickness at midlength	4.4 ± 0.7 (n = 86); (3.0–7.0)	6.3 ± 0.98 (n = 30); (4.7–8.7)
Dorsal bar		
Branch length	17.4 ± 3 (n = 69); (12.0–27.1)	22.9 ± 2.2 (n = 22); (17.7–26.1)
Branch maximum width	4.2 ± 0.7 (n = 75); (2.9–6.3)	5.95 ± 0.89 (n = 22); (4–7.8)
Hooks		
Pair I	14.4 ± 1.4 (n = 67); (11.4–17.9)	13.9 ± 1.36 (n = 18); (12.2–6.3)
Pair II	15.4 ± 1.3 (n = 63); (12.1–18.2)	15.7 ± 1.24 (n = 22); (13.2–18)
Pair III	15.9 ± 1.2 (n = 68); (13.3–19.3)	16.4 ± 1.36 (n = 23); (14–18.9)
Pair IV	16.2 ± 1.1 (n = 59); (13.0–19.3)	17.2 ± 1.36 (n = 17); (15–20)
Pair V	14.2 ± 1.6 (n = 35); (9.3–17)	11.1 ± 2.65 (n = 5); (7–13.3)
Pair VI	16.3 ± 1.1 (n = 34); (13.0–18.8)	16.6 ± 1.3 (n = 17); (14.2–19.2)
Pair VII	16.7 ± 1.4 (n = 24); (14.3–20.8)	17.2 ± 0.93 (n = 8); (16.3–19)
Pair I. II. III. IV. VI. VII average size	15.5 ± 1.5 (n = 350); (9.3–20.8)	16 ± 1.4 (n105 =); (12.2–20)
Copulatory tube curved length	30.4 ± 2.5 (n = 75); (25.1–38.3)	31 ± 2.3 (n = 21); (25.5–35.2)
Accessory piece curved length	36.1 ± 3.6 (n = 69); (28.0–47.1)	34 ± 4 (n = 20); (29.2–45.8)

28S rDNA gene. Each PCR reaction contained 1.5 unit of *Taq* Polymerase, 1X buffer containing 0.1 mg/ml BSA, 1.5 mM $MgCl_2$, 200 mM dNTPs, 0.5 mM of each primer and 50 ng of genomic DNA in a total reaction volume of 30 μl under the following conditions: 2 min at 94 °C, 39 cycles of 20 s at 94 °C, 30 s at 58 °C and 1 min and 30 s at 72 °C, and finally 10 min at 72 °C. The PCR products were visualized using horizontal gel electrophoresis using a GoldView stained agarose gel (1%) followed by enzymatic cleaning of the positive samples using 1 μl of ExoSAP-IT reagent and 2,5 μl of PCR product under the following conditions: 15 min at 37 °C and 15 min at 80 °C. Identical primers as in the amplification reactions were used for sequencing with a Big Dye Chemistry Cycle Sequencing Kit 3.1, following the manufacturer's recommendations. Fragments were cleaned using the BigDye XTerminator® Purification Kit and visualized on an ABI3130 capillary sequencer. Sequences were visually inspected and corrected using

MEGA v7 (Kumar et al. 2016) and aligned using MUSCLE (Edgar 2004) under default distance measures as implemented in MEGA v7. Previously published sequences of *Kapentagyrus limnotrissae* (GenBank accession numbers MH071808 and MH071782) were added to the dataset. Sequences obtained in the present study were deposited in the NCBI GenBank under the accession numbers MH620705 and MH623076.

Results

Morphological and molecular characterisation

Based on 136 fish individuals, no monogenean parasites were recorded from Lake Kivu. In total, 58 monogenean individuals were collected from 115 individuals of *L. miodon* from Lake Kariba (Table 1). Morphological identification combined with genetic characterisation revealed the presence of only one parasite species: *Kapentagyrus limnotrissae*. The observed prevalence in Lake Kariba ranged from 0 to 55.5%. An average infection intensity of 1.5 individuals was documented in both positive samplings in Lake Kariba. No monogenean parasites were found in 80 specimens of sardine fry.

The amplified fragments of 18S, ITS-1 and 28S rDNA from 9 individuals were 451, 328 and 650 base pairs long, respectively. No intraspecific differences either among individuals collected from Lake Kariba and Lake Tanganyika or between the lakes were found.

Taxonomic account

New record

Family: Dactylogyridae Yamaguti, 1963

Genus: *Kapentagyrus* Kmentová, Gelnar & Vanhove, 2018

Species: *K. limnotrissae*

Type-host*:* *Limnothrissa miodon* (Boulenger, 1906) (Clupeidae)

Type-locality: Lake Tanganyika, Tanzania

Vouchers: MRAC MT. 38237-8 and 38450-60.

Additional locality: Sanyati East Basin, Lake Kariba (16°59′S–28°82′E; 16°60′S–28°87′E)

Site of infection: Gills.

Infection parameters: 16 of 115 *L. miodon* infected with 1 – 10 specimens (Table 1).

Species identification was based on morphology (Fig. 2) and morphometrics (Table 2) of sclerotized structures. The presence of two pairs of anchors with well-incised roots and a regularly curved point, slightly larger ventral compared to dorsal anchors with more developed inner roots and V-shaped bars with similar branch lengths and constant width, enabled identification to the genus level. The proportion of the inner/outer root length of both ventral and dorsal anchors of around 3, combined with a straight copulatory tube and a coiled accessory piece, correspond to the original description of *K. limnotrissae* and the redescription provided by Kmentová et al. (2018).

Morphometrics

Intraspecific phenotypic variability was analysed by PCA using 19 haptoral variables (the length of the

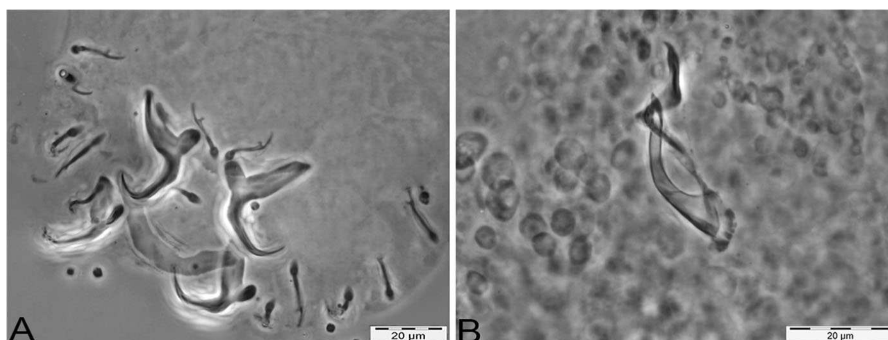


Fig. 2 Sclerotised haptoral and male genital structures of *Kapentagyrus limnotrissae* from Lake Kariba (Hoyer's medium, phase-contrast photomicrographs). **a** Opisthaptor, **b** male copulatory organ

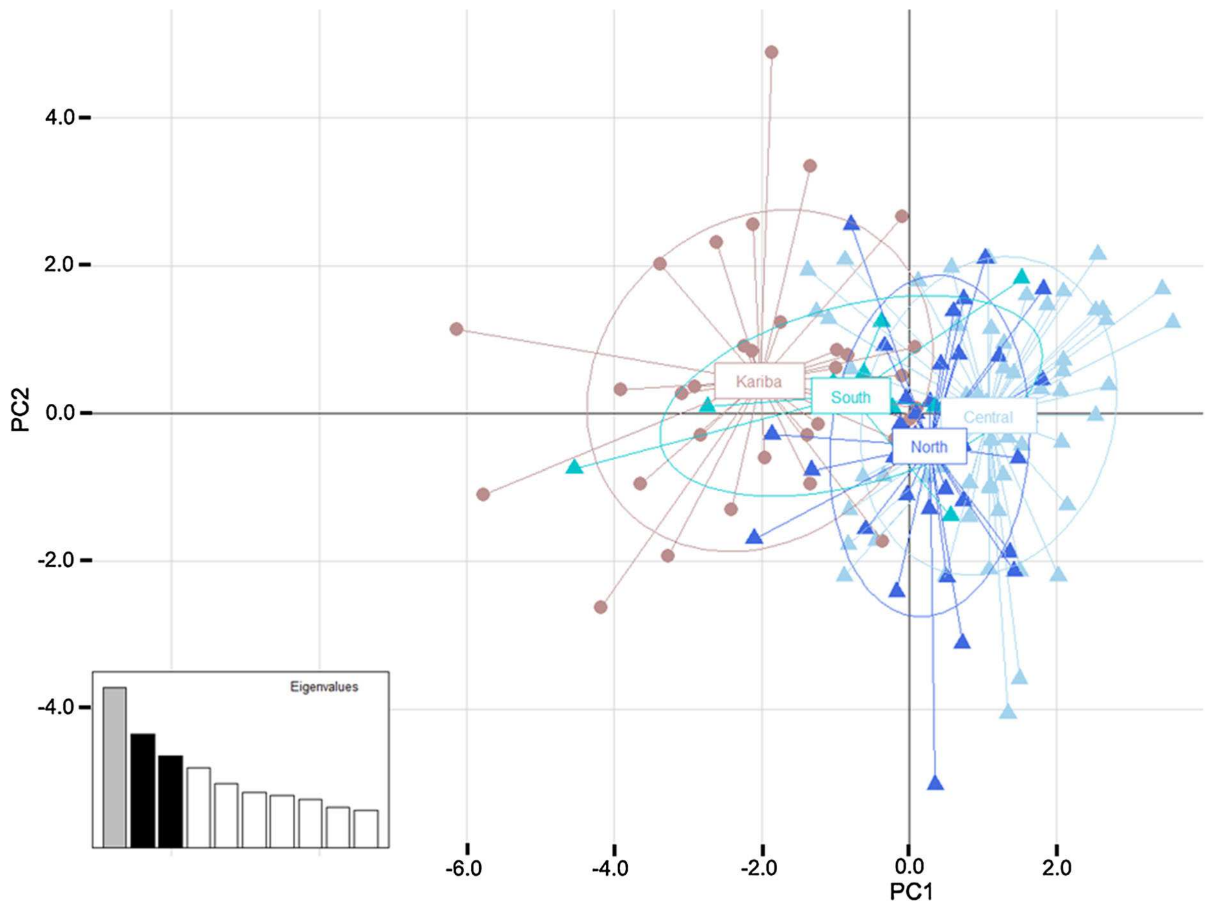


Fig. 3 A biplot of PCA (first two axes) based on measurements of haptoral sclerotized structures of *K. limnotrissae* from Lake Tanganyika and Lake Kariba. Symbols denote the lake origin of specimens (dot—Lake Kariba, triangle—Lake Tanganyika), colour is used to specify the subbasins of Lake Tanganyika

sixth and seventh pair of hooks were omitted given the small number of replicates) of 127 individuals of *K. limnotrissae*, 95 of which were from Lake Tanganyika and stem from a previous study (Kmentová et al. 2018) (Fig. 3). The first PCA axis, which explained 16.6% of the variation, failed to clearly separate specimens originating from Lake Kariba and Lake Tanganyika. The five variables with the highest contribution were the branch length and thickness of both bars and the outer root length of the ventral anchor. Other PCs did not show a clearer separation. Morphometric results were then compared with the samples from Lake Tanganyika divided in groups based on their subbasin origin. Multiple one-way MANOVA, after applying strict Bonferroni's correction, revealed that specimens from the southern basin turned out to be more similar to the population from Lake Kariba compared to the other two subbasins. In contrast to the significant difference in length of the dorsal and ventral bar branches and of the fourth pair of marginal hooks between the central and the northern subbasin of Lake Tanganyika and Lake Kariba, respectively, no difference in these parameters was reported between specimens from Lake Kariba and the southern subbasin of Lake Tanganyika (see Table 3).

Copulatory organ variables from a total of 88 individuals of *K. limnotrissae* originating from Lake Kariba (21) and Lake Tanganyika stem from a previous study (Kmentová et al. 2018) (67) were compared. Mann–Whitney U tests showed no difference in MCO structures between *K. limnotrissae* from Lake Kariba and Lake Tanganyika (copulatory tube $- Z_{1,86} = -1.10$; $p > 0.05$; accessory piece $- Z_{1,79} = -1.89$; $p > 0.05$) (Fig. 4).

Table 3 Results of one-way MANOVA tests performed on haptor measurements of *Kapentagyrus limnotrissae* (Paperna, 1973) from Lake Tanganyika (LT) and Lake Kariba, respectively

Lake Kariba (Sanyati East Basin)

Parameter	Subbasin (LT)	Df, residuals	F-value	p value	Significant in α = 0.003 (Bonferroni's correction)
	North	1, 66	4.1046	3.782e−05, < 0.001	Yes
	Central	1, 90	11.252	1.028e−14, < 0.001	Yes
	South	1, 39	1.1863	0.3502, > 0.05	No
Dorsal bar					
Branch length	North	1, 66	20.037	3.078e−05, < 0.001	Yes
	Central	1, 90	83.312	1.868e−14, < 0.001	Yes
	South	1, 39	0.063	0.8034, > 0.05	No
Branch maximum width	North	1, 66	28.379	1.293e−06, < 0.001	Yes
	Central	1, 90	61.136	9.611e−12, < 0.001	Yes
	South	1, 39	6.580	0.0142, < 0.05	No
Ventral bar					
Branch length	North	1, 66	14.534	0.00030, < 0.001	Yes
	Central	1, 90	49.579	3.666e−10, < 0.001	Yes
	South	1, 39	6.228	0.01692, < 0.05	No
Thickness at midlength	North	1, 66	42.694	1.082e−08, < 0.001	Yes
	Central	1, 90	103.17	2.2e−16, < 0.001	Yes
	South	1, 39	7.654	0,00816, < 0.01	No
Hooks					
Pair IV	North	1, 66	8.119	0.00183, < 0.01	Yes
	Central	1, 90	6.163	0.00149, < 0.01	Yes
	South	1, 39	1.075	0.3062, > 0.05	No
Pair V	North	1, 66	5.955	0.01736, < 0.05	No
	Central	1, 90	6.058	0.01575, < 0.05	No
	South	1, 39	4.668	0.03696, < 0.05	No
Inner root length of dorsal anchor	North	1, 66	2.229	0.1402, > 0.05	No
	Central	1, 90	4.383	0.0391, < 0.05	No
	South	1, 39	0	0.9961, > 0.05	No
Ventral anchor					
Outer root length	North	1, 66	6.023	0.01573, < 0.05	No
	Central	1, 90	4.201	0.0433, < 0.05	No
	South	1, 39	0.324	0.5725, > 0.05	No
Point length	North	1, 66	3.823	0.05478, > 0.05	No
	Central	1, 90	4.824	0.03064, < 0.05	No
	South	1, 39	0.147	0.7037, > 0.05	No

Only significantly different variables between the lakes (subbasins) are listed

Discussion

Co-introduction of the monogenean *K. limnotrissae* from Lake Tanganyika with *L. miodon* to Lake Kariba was documented by combining morphological and genetic results. On the other hand, *L. miodon* was seen to be free of monogenean infection in Lake Kivu, where this sardine was also introduced. Intraspecific

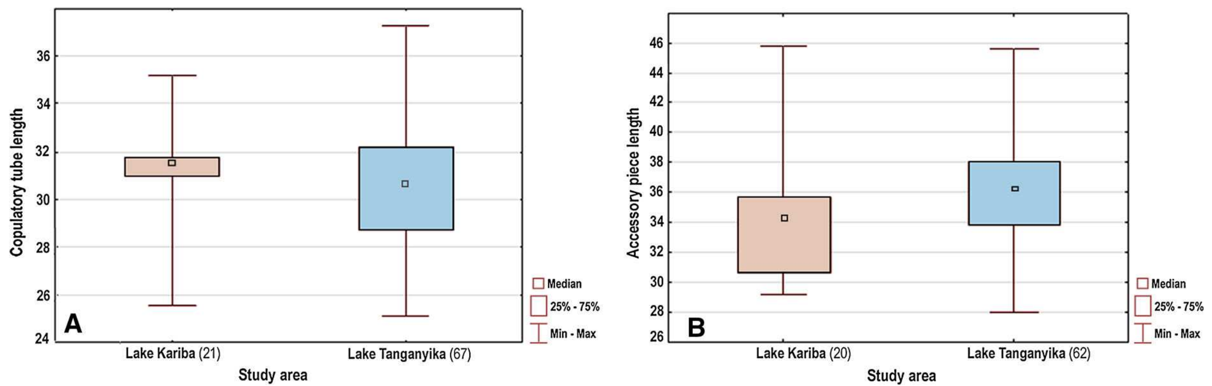


Fig. 4 Box-plot graph with male copulatory organ structures of *K. limnotrissae* defined by study area: **a** copulatory tube length; **b** accessory piece length. The number of specimens is indicated in brackets

diversity of *K. limnotrissae* was analysed to evaluate morphological differences between native and introduced populations. This has potential for the identification of host origins. The effect of host life stage on parasite co-introduction is discussed. Co-introduction of *K. tanganicanus* was not detected.

Kapentagyrus limnotrissae in Lake Kariba

The higher observed abundance (1.5 vs. 0.6 individuals/gill chamber) and prevalence [56 vs. 35% and 70% reported by Douëllou (1991)] of *K. limnotrissae* in Lake Kariba compared to its native Lake Tanganyika is in contrast to previously studied monogenean introductions finding the opposite pattern (Ondračková et al. 2010; Sheath et al. 2015; Gabagambi and Skorping 2017; Sarabeev et al. 2017). Interestingly, the relatively faster growth but smaller size of *L. miodon* was reported in Lake Kariba compared to natural lakes (Lake Tanganyika and Lake Kivu), probably as a result of unstable conditions and high predation pressure (Marshall 1987, 1993). Therefore, we can suggest that a higher parasite prevalence of *K. limnotrissae* in Lake Kariba could be caused by different environmental conditions such as predation pressure or host life history (Dunn 2009; Gabagambi and Skorping 2017; Sarabeev et al. 2017). However, the observed differences in prevalence could also be the consequence of different abiotic factors in Lake Kariba compared to Lake Tanganyika, such as temperature or water chemical composition (Coche 1974; Edmond et al. 1993), as these factors are known to influence monogenean population dynamics (Buchmann 1988; Šimková et al. 2001; Marchiori et al. 2015).

Seasonal differences in the prevalence and abundance of *K. limnotrissae* were documented in Lake Kariba (see Table 1). The pattern seems to follow changes in water temperature, which reaches its maximum of 30 °C in January and its minimum of 17 °C in July (Balon and Cache 1974). This absence of monogeneans in the colder period of the year corresponds to previous studies on dactylogyrids in temporal climates (Šimková et al. 2001; Marchiori et al. 2015). As the hatching of monogenean eggs is temperature-dependent (Whittington and Kearn 2011), the lack of seasonal temperature differences in Lake Tanganyika explains the year-round abundance of *K. limnotrissae* in the latter lake. However, differences in *K. limnotrissae* prevalence between native and non-native localities as well as within Lake Kariba need to be further tested over several years to reveal the general pattern of the parasites' population dynamics (Hudson et al. 2002).

Although *L. miodon* does not seem to have been infected by monogeneans native to Lake Kariba, Douëllou (1991) mentioned the presence of eight endoparasite species infecting this sardine in Lake Kariba which have not yet been reported in the population from Lake Tanganyika (Kmentová et al. 2018). This result indicates parasite spill-back of native fauna to the introduced *L. miodon* and is explained by the generally lower host specificity in fish of endoparasites' larval stages compared to monogeneans (Cribb et al. 2001; Jensen and Bullard 2010). Finally, it would be interesting to investigate the effect of the combined stressors, predators and

increased parasite loads on the introduced sardines, as interactions between parasitism and predation are known to exist (Hudson et al. 1992; Rohlenová et al. 2011).

Another question regarding the infection of *K. limnotrissae* in Lake Kariba is why only one of the species of *Kapentagyrus* infecting *L. miodon* (Kmentová et al. 2018) was co-introduced. There are two possible scenarios: either *K. tanganicanus* was not co-introduced, as it possibly might not have been present in the source population of *L. miodon*, or it may not have survived the environmental conditions in Lake Kariba. However, based on present knowledge and available data, we cannot determine which of these two possibilities are the cause.

Co-introduced flatworm parasites have been observed to cause population decline (Tanum 1983; Johnsen and Jensen 1988; Britton et al. 2011) and extinction of native fish fauna (Zholdasova 1997). However, considering the generally high host-specificity of dactylogyrid monogeneans and the fact that *K. limnotrissae* is strictly host-specific in Lake Tanganyika, the potential for spill-over to native fish seems low. Co-introduction of monogenean species without any known impact on the native fauna has been documented (Truter et al. 2017). However, monitoring the potential presence of non-native monogenean species on the local fish fauna in Lake Kariba is recommended as parasite spill-over does not always occur in a predictable manner (Jiménez-García et al. 2001).

Testing the possibility of inferring host origin using the morphometrics of *K. limnotrissae*

To check for possible differences between native and introduced parasite populations, measurements of the parasites' sclerotized structures were analysed. Knowledge of stock structure and the degree of mixing among populations is crucial for the management of *L. miodon* not only in Lake Kariba but also in other areas of its distribution. Parasites are considered potential biological tags revealing host structure (Poulin and Kamiya 2015). PCA showed only a slight differentiation between the specimens from Lake Kariba and Lake Tanganyika as well as among Lake Tanganyika's subbasins. Morphometric variables were tested using MANOVA and sub-tests were discussed, as possibly one or a few variables, rather than the full set of haptoral variables, could indicate the host's subbasin fidelity. As rather continuous morphometric variability in *K. limnotrissae* found in PCA was reported, tests on haptor morphometrics indicated a greater level of similarity of the specimens from Lake Kariba with the individuals from the southern compared to the northern and central subbasin of Lake Tanganyika. This result corresponds with the documented origin of the introduced *L. miodon*, namely Mpulungu and Kasaba Bay, both located in the southern part of Lake Tanganyika. The low percentage of explained variation in the PCA indicates that there is either continuous phenotypic plasticity among the specimens from different subbasins/lakes, or that the potential geographic segregation of parasite populations does not affect all haptoral variables to the same extent (Vignon et al. 2011). While significant differences in haptor morphometrics were revealed by sub-tests of MANOVA, no significant differences in MCO characteristics between Lake Tanganyika and Lake Kariba were observed. Phenotypic variation without a genetic basis has already been observed in various parasite taxa (Stunkard 1957; Pérez Ponce de Leon 1995; Mariniello et al. 2004; Steinauer et al. 2007; Ondračková et al. 2012; Kmentová et al. 2016; Truter et al. 2017). Even if the intraspecific variability was not mirrored in the three rDNA gene portions amplified in this study, highly variable markers with a faster rate of molecular evolution, such as mitochondrial genes, may identify potential divergence as a consequence of founder effects, adaptation or geographical isolation (Steinauer et al. 2007; Dlugosch and Parker 2008).

Release from monogenean infection in Lake Kivu

In contrast to Lake Kariba, the introduced population of *L. miodon* in Lake Kivu consisted only of small fry. Since monogenean infection has been reported to depend on the size of fish fry (Bagge and Valtonen 1999), introducing only fry will have decreased the possibility of monogenean co-introduction with *L. miodon*. Therefore, the absence of monogenean parasites of *L. miodon* in Lake Kivu can be explained by the host's life stage. Moreover, no monogenean parasite was found in 80 specimens of sardine fry examined as a part of this study. This hypothesis is also supported by the fact that there is no report documenting any type of antiparasitic treatment

before or after the respective translocations of *L. miodon* (Collart 1960; Bell-Cross and Bell-Cross 1971). In previous studies, characterisation of the monogenean parasite fauna matched the suggested method of transport of invasive goby species, with differences between arrival with ballast water and active dispersal (Mombaerts et al. 2014; Huyse et al. 2015). However, different biochemical and limnological conditions (Degens et al. 1973; Schmid and Wüest 2012) together with a different surface temperature compared to Lake Tanganyika (26 and 24 °C) (Katsev et al. 2014) may have also precluded the establishment of sardine-infecting monogeneans in Lake Kivu. Moreover, founder effects related to the small population size of introduced parasites could have influenced the parasites' ability to adapt (Gavrilets and Hastings 1996). However, distinguishing between the effect on the establishment success of a particular parasite of the translocation procedure or of different environmental conditions is impossible without an experimental study. The enemy release hypothesis suggests that a lack of parasite infections can increase the invasion success of alien species (Colautti et al. 2004). According to Guillard et al. (2012) *L. miodon* is well established in Lake Kivu, showing the same schooling behaviour, seasonal fluctuations and cannibalistic behaviour as in Lake Tanganyika (de Iongh et al. 1983; Spliethoff et al. 1983; Hauser et al. 1995). The species impacts the community composition of zooplankton (de Iongh et al. 1995). The invasion success of the sardine is probably also correlated with the absence of planktivorous competitors and predators (Snoeks 2000).

Conclusion

The main question of our study was whether we could use parasites to investigate host introductions. While parasite co-introduction with the fishery target *L. miodon* to Lake Kariba was documented, a release of monogenean infection into Lake Kivu is suggested. Two possible scenarios to explain the current situation in Lake Kivu were proposed: monogenean parasites not having been translocated, as the founder population consisted only of fry, would support previous studies highlighting introduction conditions as crucial for parasite survival. Therefore, the absence of monogenean parasites in some introduced areas could be the result of circumstances surrounding host translocation and host life stage. This should be considered as a parameter when considering fish introductions. The other possibility is that the parasites were unsuccessfully established because of differences in biotic and abiotic conditions in their native area compared to Lake Kivu. Experimental studies are needed to discern between these two scenarios. In contrast, the increased prevalence of *K. limnotrissae* in Lake Kariba compared to Lake Tanganyika was reported. This pattern was suggested to be due to different environmental conditions such as different predation pressure, differences in host life history or in abiotic conditions, as these factors are known to influence monogenean population dynamics (Buchmann 1988; Šimková et al. 2001; Marchiori et al. 2015). Despite only slight phenotypic differences in morphology of *K. limnotrissae* between populations from its native range and from Lake Kariba, our results revealed a greater similarity to the specimens from the southern part of Lake Tanganyika using morphometric results. This finding corresponds with historical reports about the introduction events. Therefore, the potential of *K. limnotrissae* as a tag for its host's origins was supported and should be further scrutinised by detailed genetic characterisation, including fast evolving markers.

Acknowledgements The authors would like to thank Tine Huyse, Jos Snoeks, Miguël Parrent (RMCA), Filip Volckaert (KU Leuven) and others in the parasitological group, Masaryk University, Brno and the Research Centre of Hydrobiology, Uvira for their hospitality, and the RMCA for providing access to the collections. Walter Salzburger from the University of Basel is acknowledged for providing us with samples. We are grateful to Federico Calboli from the University of Leuven for useful discussion about the statistical methodology. Special thanks go to Maxwell Barson from the University of Zimbabwe for providing literature and delivering samples. John Suire is acknowledged for proofreading of the manuscript.

Funding This study was supported by ECIP (European Centre of Ichthyoparasitology); project No. P505/12/G112. Work in the collections of RMCA (Royal Museum for Central Africa) was financed by the SYNTHESYS Project (http://www.synthesys.info/) (BE-TAF-5731) which is financed by European Community Research Infrastructure Action under the FP7 Integrating Activities Programme.

References

Anderson RM, May RM (1991) Infectious diseases of humans : dynamics and control. Oxford University Press, Oxford

Baer JG, Fain A (1958) *Bothriocephalus* (*Clestobothrium*) *kivuensis* n. sp., cestode parasite of a barbel of Lake Kivu. Ann la Soc R Zool Belgique 88:287–302

Bagge AM, Valtonen ET (1999) Development of monogenean communities on the gills of roach fry (*Rutilus rutilus*). Parasitology 118:479–487

Balon EK, Cache AG (1974) Lake Kariba: a manmade tropical ecosystem in Central Africa. Dr. W. Junk b.v., Publishers, Hague

Barson M, Přikrylová I, Vanhove MPM, Huyse T (2010) Parasite hybridization in African *Macrogyrodactylus* spp. (Monogenea, Platyhelminthes) signals historical host distribution. Parasitology 137:1585–1595. https://doi.org/10.1017/S0031182010000302

Bauer ON (1991) Spread of parasites and diseases of aquatic organisms by acclimatization: a short review. J Fish Biol 39:679–686. https://doi.org/10.1111/j.1095-8649.1991.tb04398.x

Beadle LC (1981) Volcanic-barrier lakes: Lake Kivu. The inland waters of tropical Africa. 2nd edn. Longman House, London, New York

Bell-Cross G, Bell-Cross B (1971) Introduction of *Limnothrissa miodon* and *Limnocaridina tanganicae* from Lake Tanganyika to Lake Kariba. Fish Res Bull Zambia 5:207–214

Blasco-Costa I, Míguez-Lozano R, Sarabeev V, Balbuena JA (2012) Molecular phylogeny of species of *Ligophorus* (Monogenea: Dactylogyridae) and their affinities within the Dactylogyridae. Parasitol Int 61:619–627. https://doi.org/10.1016/j.parint.2012.06.004

Britton JR, Pegg J, Williams CF, Clayton D, Copp G, Giraud T (2011) Pathological and ecological host consequences of infection by an introduced fish parasite. PLoS ONE 6:e26365. https://doi.org/10.1371/journal.pone.0026365

Buchmann K (1988) Temperature-dependent reproduction and survival of *Pseudodactylogyrus bini* (Monogenea) on the European eel (*Anguilla anguilla*). Parasitol Res 75:162–164. https://doi.org/10.1007/BF00932717

Coche AG (1974) Limnological study of a tropical reservoir. In: Balon EK, Cache AG (eds) Lake Kariba: a man-made tropical ecosystem in central Africa. Dr. W. Junk b.v., Publishers, Hague, p 246

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success explained by the enemy release hypothesis? Ecol Lett 7:721–733. https://doi.org/10.1111/j.1461-0248.2004.00616.x

Collart A (1960) L'introduction du *Stolothrissa tanganicae* (Ndagala) au lac Kivu. Bull Agric du Congo Belge 51:975–985

Coulter GW (1991) Pelagic fish. In: Coulter GW (ed) Lake Tanganyika and its life. Natural History Musem & Oxford University Press, London & Oxford, pp 111–150

Cribb TH, Bray RA, Littlewood DTJ (2001) The nature and evolution of the association among digeneans, molluscs and fishes. Int J Parasitol 31:997–1011. https://doi.org/10.1016/S0020-7519(01)00204-1

Criscione CD, Cooper B, Blouin MS (2006) Parasite genotypes identify source populations of migratory fish more accurately than fish genotypes. Ecology 87:823–828

Cross K, Niskanen L, Dragisic C, Barrera L, Canter Weikel M (2011) Development and policy plans for the future. In: Darwall WR, Smith K, Allen D et al (eds) The diversity of life in African freshwaters: Underwater, under threat. An analysis of the status and distribution of freshwater species throughout mainland Africa. IUCN, Cambridge, pp 290–305

Danley PD, Husemann M, Ding B, Dipietro LM, Beverly EJ, Peppe DJ (2012) The impact of the geologic history and paleoclimate on the diversification of East african cichlids. Int J Evol Biol. https://doi.org/10.1155/2012/574851

de Iongh HH, Spliethoff PC, Frank VG (1983) Feeding habits of the clupeid *Limnothrissa miodon* (Boulenger), in Lake Kivu. Hydrobiologia 102:113–122. https://doi.org/10.1007/BF00006074

de Iongh HH, Spliethoff PC, Roest F (1995) The impact of an introduction of sardine into Lake Kivu. In: Pitcher TJ, Hart PJB (eds) The impact of species changes in African Lakes. Springer, Dordrecht, pp 277–297

Degens ET, von Herzen RP, Wong H-K, Deuser WG, Jannasch HW (1973) Lake Kivu: structure, chemistry and biology of an East African rift lake. Geol Rundsch 62:245–277. https://doi.org/10.1007/BF01826830

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449. https://doi.org/10.1111/j.1365-294X.2007.03538.x

Douëllou L (1991) Survey of fish parasites in Lake Kariba. Highlights Res Program Univ Zimb 3:49–50

Douëllou L (1993) Monogeneans of the genus *Cichlidogyrus* Paperna, 1960 (Dactylogyridae: Ancyrocephalinae) from cichlid fishes of Lake Kariba (Zimbabwe) with descriptions of five new species. Syst Parasitol 25:159–186. https://doi.org/10.1007/BF00007007

Dumont HJ (1986) The Tanganyika sardine in Lake Kivu: another ecodisaster for Africa? Environ Conserv 13:143. https://doi.org/10.1017/S0376892900036742

Dunn AM (2009) Parasites and biological invasions. In: Rollinson D, Hay S (eds) Natural history of host-parasite interactions. Academic Press, pp 161–184

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797. https://doi.org/10.1093/nar/gkh340

Edmond JM, Stallard RF, Craig H, Craig V, Weiss RF, Coulter GW (1993) Nutrient chemistry of the water column of Lake Tanganyika. Limnol Oceanogr 38:725–738. https://doi.org/10.4319/lo.1993.38.4.0725

Ergens R, Lom J (1970) Causative agents of fish diseases. Academia, Prague

Filzmoser P, Gschwandtner M (2017) mvoutlier: multivariate outlier detection based on robust methods. https://cran.r-project.org/package=mvoutlier. Accessed 29 Oct 2017

Gabagambi NP, Skorping A (2017) Spatial and temporal distribution of *Ligula intestinalis* (Cestoda: Diphyllobothriidea) in usipa (*Engraulicypris sardella*) (Pisces: Cyprinidae) in Lake Nyasa. J Helminthol. https://doi.org/10.1017/s0022149x17000724

Gavrilets S, Hastings A (1996) Founder effect speciation: a theoretical reassessment. Am Nat 147:466–491. https://doi.org/10.1086/285861

Gozlan RE (2008) Introduction of non-native freshwater fish: is it all bad? Fish Fish 9:106–115. https://doi.org/10.1111/j.1467-2979.2007.00267.x

Guillard J, Darchambeau F, Mulungula PM, Descy J-P (2012) Is the fishery of the introduced Tanganyika sardine (*Limnothrissa miodon*) in Lake Kivu (East Africa) sustainable? J Great Lakes Res 38:524–533. https://doi.org/10.1016/j.jglr.2012.05.004

Hassouna N, Michot B, Bachellerie J-P, Narbonne D (1984) The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher eukaryotes. Nucleic Acids Res 12:3563–3583

Hauser L, Carvalho GR, Pitcher TJ (1995) Morphological and genetic differentiation of the African clupeid *Limnothrissa miodon* 34 years after its introduction to Lake Kivu. J Fish Biol 47:127–144. https://doi.org/10.1111/j.1095-8649.1995.tb06049.x

Hudson PJ, Dobson AP, Newborn D (1992) Do parasites make prey vulnerable to predation? Red grouse and parasites. J Anim Ecol 61:681. https://doi.org/10.2307/5623

Hudson PJ, Dobson AP, Arneberg P, Begon ME, Cattadori IM, Greenman JV, Heesterbeek JAP, Hudson PJ, Newborn D, Pugliese A, Rizzoli A, Rosa' R, Rosso F, Wilson K (2002) Parasites and host population dynamics. In: Hudson PJ, Rizzoli A, Grenfell BT, Heesterbeek JAP, Dobson AP (eds) The ecology of wildlife diseases. Oxford University Press, Oxford, p 197

Huyse T, Vanhove MPM, Mombaerts M, Volckaert FAM, Verreycken H (2015) Parasite introduction with an invasive goby in Belgium: double trouble? Parasitol Res 114:2789–2793. https://doi.org/10.1007/s00436-015-4544-6

Jalali B, Barzegar M (2005) Dactylogyrids (Dactylogyridae: Monogenea) on Common Carp (*Cyprinus carpio* L.) in Freshwaters of Iran and description of the pathogenicity of *D. sahuensis*. J Agric Sci 7:9–16

Jensen K, Bullard SA (2010) Characterization of a diversity of tetraphyllidean and rhinebothriidean cestode larval types, with comments on host associations and life-cycles. Int J Parasitol 40:889–910. https://doi.org/10.1016/J.IJPARA.2009.11.015

Jiménez-García MI, Vidal-Martínez VM, López-Jiménez S (2001) Monogeneans in introduced and native cichlids in México: evidence for transfer. J Parasitol 87:907–909. https://doi.org/10.1645/0022-3395(2001)087%5b0907:MIIANC%5d2.0.CO;2

Johnsen BO, Jensen AJ (1988) Introduction and establishment of *Gyrodactylus salaris* Malmberg, 1957, on Atlantic salmon, *Salmo salar* L., fry and parr in the River Vefsna, northern Norway. J Fish Dis 11:35–45. https://doi.org/10.1111/j.1365-2761.1988.tb00521.x

Jombart T (2008) Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Katsev S, Aaberg AA, Crowe SA, Hecky RE (2014) Recent warming of lake Kivu. PLoS ONE 9:e109084. https://doi.org/10.1371/journal.pone.0109084

Kmentová N, Gelnar M, Mendlová M, Van Steenberge M, Koblmüller S, Vanhove MPM (2016) Reduced host-specificity in a parasite infecting non-littoral Lake Tanganyika cichlids evidenced by intraspecific morphological and genetic diversity. Sci Rep 6:39605. https://doi.org/10.1038/srep39605

Kmentová N, Van Steenberge M, Raeymaekers JAR, Koblmüller S, Hablützel PI, Muterezi Bukinga F, Mulimbwa N'sibula T, Masilya Mulungula P, Nzigidahera B, Ntakimazi G, Gelnar M, Vanhove MPM (2018) Monogenean parasites of sardines in Lake Tanganyika: diversity, origin and intra-specific variability. Contrib Zool 87(2):105–132

Kumar S, Stecher G, Tamura K, Gerken J, Pruesse E, Quast C, Schweer T, Peplies J, Ludwig W, Glockner F (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054

Kvach Y, Kornyychuk Y, Mierzejewska K, Rubtsova N, Yurakhno V, Grabowska J, Ovcharenko M (2014) Parasitization of invasive gobiids in the eastern part of the Central trans-European corridor of invasion of Ponto-Caspian hydrobionts. Parasitol Res 113:1605–1624. https://doi.org/10.1007/s00436-014-3791-2

Lavoué S, Konstantinidis P, Chen W-J (2014) Progress in clupeiform systematics. In: Ganias K (ed) Biology and ecology of sardines and anchovies. CRC Press, Boca Raton, pp 3–42

Lumme J, Mäkinen H, Ermolenko AV, Gregg JL, Ziętara MS (2016) Displaced phylogeographic signals from *Gyrodactylus arcuatus*, a parasite of the three-spined stickleback *Gasterosteus aculeatus*, suggest freshwater glacial refugia in Europe. Int J Parasitol 46:545–554. https://doi.org/10.1016/J.IJPARA.2016.03.008

Lymbery AJ, Morine M, Kanani HG, Beatty SJ, Morgan DL (2014) Co-invaders: the effects of alien parasites on native hosts. Int J Parasitol Parasites Wildl 3:171–177. https://doi.org/10.1016/j.ijppaw.2014.04.002

Marchiori NC, Gonçalves ELT, Tancredo KR, Pereira-Junior J, Garcia JRE, Martins ML, Marchiori NC, Gonçalves ELT, Tancredo KR, Pereira-Junior J, Garcia JRE, Martins ML (2015) Effect of water temperature and salinity in oviposition, hatching success and infestation of *Aphanoblastella mastigatus* (Monogenea, Dactylogyridae) on Rhamdia quelen. Braz J Biol 75:245–252. https://doi.org/10.1590/1519-6984.14014

Mariniello L, Ortis M, D'Amelio S, Petrarca V (2004) Morphometric variability between and within species of Ligophorus Euzet & Suriano, 1977 (Monogenea: Ancyrocephalidae) in the Mediterranean Sea. Syst Parasitol 57:183–190. https://doi.org/10.1023/B:SYPA.0000019080.43784.06

Marshall BE (1987) Growth and mortality of the introduced Lake Tanganyika clupeid, *Limnothrissa miodon*, in Lake Kariba. J Fish Biol 31:603–615. https://doi.org/10.1111/j.1095-8649.1987.tb05265.x

Marshall BE (1993) Biology of the African clupeid *Limnothrissa miodon* with reference to its small size in artificial lakes. Rev Fish Biol Fish 3:17–38. https://doi.org/10.1007/BF00043296

Mölsä H, Reynolds JE, Coenen EJ, Lindqvist OV (1999) Fisheries research towards resource management on Lake

Tanganyika. Hydrobiologia 407:1–24. https://doi.org/10.1023/A:1003712708969
Mombaerts M, Verreycken H, Volckaert FAM, Huyse T (2014) The invasive round goby *Neogobius melanostomus* and tubenose goby *Proterorhinus semilunaris*: two introduction routes into Belgium Running title: invasive round and tubenose gobies in Belgium. Aquat Invasions 9:305–314
Mulimbwa N, Shirakihara K (1994) Growth, recruitment and reproduction of sardines (*Stolothrissa tanganicae* and *Limnothrissa miodon*) in northwestern Lake Tanganyika. Tropics 4:57–67. https://doi.org/10.3759/tropics.4.57
Naylor RL, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MCM, Clay J, Folke C, Lubchenco J, Mooney H, Troell M (2000) Effect of aquaculture on world fish supplies. Nature 405:1017–1024. https://doi.org/10.1038/35016500
Ogutu-Ohwayo R, Hecky RE (1991) Fish ntroductions in Africa and some of their implications. Can J Fish Aquat Sci 48:8–12
Oliva ME, Gonzalez MT (2004) Metazoan parasites of *Sebastes capensis* from two localities in northern Chile as tools for stock identification. J Fish Biol 64:170–175. https://doi.org/10.1111/j.1095-8649.2004.00295.x
Ondračková M, Francová K, Dávidová M, Polačik M, Jurajda P (2010) Condition status and parasite infection of *Neogobius kessleri* and *N. melanostomus* (Gobiidae) in their native and non-native area of distribution of the Danube River. Ecol Res 25:857–866. https://doi.org/10.1007/s11284-010-0716-0
Ondračková M, Matějusová I, Grabowska J (2012) Introduction of *Gyrodactylus perccotti* (Monogenea) into Europe on its invasive fish host, Amur sleeper (*Perccottus glenii*, Dybowski 1877). Helminthologia 49:21–26. https://doi.org/10.2478/s11687-012-0004-3
Paperna I (1963) Dynamics of *Dactylogyrus vastator* Nybelin (Monogenea) populations on the gills of carp fry in fish ponds. Bamidgeh 15:31–50
Paperna I (1973) New species of Monogenea (Vermes) from African freshwater fish. A preliminary report. Rev Zool Bot Afr 87:505–518
Peeler EJ, Gardiner R, Thrush MA (2004) Qualitative risk assessment of routes of transmission of the exotic fish parasite *Gyrodactylus salaris* between river catchments in England and Wales. Prev Vet Med 64:175–189. https://doi.org/10.1016/j.prevetmed.2004.05.005
Pérez Ponce de Leon G (1995) Host-induced morphological variability in adult *Posthodiplostomum minimum* (Digenea: Neodiplostomidae). J Parasitol. 81:818. https://doi.org/10.2307/3283989
Poulin R, Kamiya T (2015) Parasites as biological tags of fish stocks: a meta-analysis of their discriminatory power. Parasitology 142:145–155. https://doi.org/10.1017/S0031182013001534
Pugachev ON, Gerasev PI, Gussev AV, Ergens R, Khotenowsky I (2009) Guide to Monogenoidea of freshwater fish of Palaeartic and Amur regions. Ledizione-LediPublishing, Milan
R development core team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
Řehulková E, Mendlová M, Šimková A (2013) Two new species of *Cichlidogyrus* (Monogenea: Dactylogyridae) parasitizing the gills of African cichlid fishes (Perciformes) from Senegal: Morphometric and molecular characterization. Parasitol Res 112:1399–1410. https://doi.org/10.1007/s00436-013-3291-9
Rohlenová K, Morand S, Hyršl P, Tolarová S, Flajšhans M, Šimková A (2011) Are fish immune systems really affected by parasites? An immunoecological study of common carp (*Cyprinus carpio*). Parasit Vectors 4:120. https://doi.org/10.1186/1756-3305-4-120
Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305–332. https://doi.org/10.1146/annurev.ecolsys.32.081501.114037
Salzburger W, Van Bocxlaer B, Cohen AS (2014) Ecology and evolution of the African Great Lakes and their faunas. Annu Rev Ecol Evol Syst 45:519–545. https://doi.org/10.1146/annurev-ecolsys-120213-091804
Sarabeev V, Balbuena JA, Morand S (2017) Testing the enemy release hypothesis: abundance and distribution patterns of helminth communities in grey mullets (Teleostei: Mugilidae) reveal the success of invasive species. Int J Parasitol 47:687–696. https://doi.org/10.1016/j.ijpara.2017.05.006
Schmid M, Wüest A (2012) Stratification, mixing and transport processes in Lake Kivu. In: Descy J-P, Darchambeau F, Schmid M (eds) Lake Kivu: limnology and biogeochemistry of a tropical great lake. Springer, Dordrecht, pp 13–29
Sheath DJ, Williams CF, Reading AJ, Britton JR (2015) Parasites of non-native freshwater fishes introduced into England and Wales suggest enemy release and parasite acquisition. Biol Invasions 17:2235–2246. https://doi.org/10.1007/s10530-015-0857-8
Šimková A, Sasal P, Kadlec D, Gelnar M (2001) Water temperature influencing dactylogyrid species communities in roach, *Rutilus rutilus*, in the Czech Republic. J Helminthol 75:373–383. https://doi.org/10.1017/S0022149X01000580
Sinnappah ND, Lim L-HS, Rohde K, Tinsley R, Combes C, Verneau O (2001) A paedomorphic parasite associated with a neotenic amphibian host: phylogenetic evidence suggests a revised systematic position for sphyranuridae within anuran and turtle polystomatoineans. Mol Phylogenet Evol 18:189–201. https://doi.org/10.1006/mpev.2000.0877
Snoeks J (2000) Ancient lakes: biodiversity, ecology and evolution. Adv Ecol Res 31:17–38. https://doi.org/10.1016/S0065-2504(00)31005-4
Snoeks J, De Vos L, Thys van den Audenaerde DFE (1997) The ichthyogeography of Lake Kivu. S Afr J Sci 93:579–584
Spliethoff PC, Longh HH, Frank VG (1983) Success of the introduction of the fresh water clupeid *Limnothrissa miodon* (Boulenger) in Lake Kivu. Aquac Res 14:17–31. https://doi.org/10.1111/j.1365-2109.1983.tb00050.x
Steinauer ML, Nickol BB, Ortí G (2007) Cryptic speciation and patterns of phenotypic variation of a highly variable acanthocephalan parasite. Mol Ecol 16:4097–4109. https://doi.org/10.1111/j.1365-294X.2007.03462.x
Stunkard HW (1957) Intraspecific variation in parasitic flatworms. Syst Zool 6:7. https://doi.org/10.2307/2411703

Tanum K (1983) Studier av taksonomi og vertsforhold hos *Gyrodactylus*-arteT pa laksefisk av slektene Salmo og Salvelinus i Norge. Hovedfagsoppgave i spesiell Zool parasittolog Thesis, University of Oslo, p 99

Taraschewski H (2006) Hosts and parasites as aliens. J Helminthol 80:99–128. https://doi.org/10.1079/JOH2006364

Thoney DA, Hargis WJ (1991) Monogenea (platyhelminthes) as hazards for fish in confinement. Annu Rev Fish Dis 1:133–153. https://doi.org/10.1016/0959-8030(91)90027-H

Truter M, Přikrylová I, Weyl OLF, Smit NJ (2017) Co-introduction of ancyrocephalid monogeneans on their invasive host, the largemouth bass, *Micropterus salmoides* (Lacepéde, 1802) in South Africa. Int J Parasitol Parasites Wildl. https://doi.org/10.1016/j.ijppaw.2017.06.002

Vercammen-Grandjean PH (1960) Les Trematodes du lac Kivu Sud (Vermes). Royal Museum for Central Africa, Tervuren

Vignon M, Pariselle A, Vanhove MPM (2011) Modularity in attachment organs of African *Cichlidogyrus* (Platyhelminthes: Monogenea: Ancyrocephalidae) reflects phylogeny rather than host specificity or geographic distribution. Biol J Linn Soc 102:694–706. https://doi.org/10.1111/j.1095-8312.2010.01607.x

Welcomme RL (1981) Register of international transfers of inland fish species. FAO Fish Tech Pap 213:120

Whittington ID, Kearn GC (2011) Hatching Strategies in Monogenean (Platyhelminth) Parasites that Facilitate Host Infection. Integr Comp Biol 51:91–99. https://doi.org/10.1093/icb/icr003

Zavodna M, Sandland GJ, Minchella DJ (2008) Effects of intermediate host genetic background on parasite transmission dynamics: a case study using *Schistosoma mansoni*. Exp Parasitol 120:57–61. https://doi.org/10.1016/j.exppara.2008.04.021

Zholdasova I (1997) Sturgeons and the Aral Sea ecological catastrophe. Environ Biol Fishes 48:373–380. https://doi.org/10.1023/A:1007329401494

Paper III

Kmentová N., Koblmüller S., Van Steenberge M., Artois T., De Keyzer E., Muterezi Bukinga F., Mulimbwa N'sibula T, Masilya Mulungula P., Gelnar M., Vanhove M. P. M. Population structure and demographic history of *Kapentagyrus* spp. (Monogenea: Dactylogyridae) infecting sardine species (Teleostei: Clupeidae) in Lake Tanganyika: an indication of near-panmictic lake-wide occurrence. Submitted in *International Journal for Parasitology* (August 2019).

NK contributed to the material collection, obtained morphometric, geomorphometric and molecular data, performed statistical analyses, contributed to data interpretation and wrote the manuscript. Overall contribution: c. 70%

1 **Population structure and demographic history of *Kapentagyrus* spp.**
2 **(Monogenea: Dactylogyridae) infecting sardine species (Teleostei: Clupeidae) in**
3 **Lake Tanganyika: an indication of near-panmictic lake-wide occurrence**

4 **Nikol Kmentová[1,2,3*], Stephan Koblmüller[4], Maarten Van Steenberge[3,4,5,6], Joost A.M.**
5 **Raeymaekers[3,7,8], Tom Artois[2], Els L.R. De Keyzer[3,9], Fidel Muterezi Bukinga[10], Théophile**
6 **Mulimbwa N'sibula[10], Pascal Masilya Mulungula[10], Gaspard Ntakimazi[11], Milan Gelnar[1],**
7 **Maarten P.M. Vanhove[1,2,3,12]**

8 [1] Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611
9 37 Brno, Czech Republic
10 [2] Hasselt University, Centre for Environmental Sciences, Research Group Zoology: Biodiversity
11 & Toxicology, Agoralaan Gebouw D, B-3590 Diepenbeek, Belgium
12 [3] Laboratory of Biodiversity and Evolutionary Genomics, Department of Biology, University of
13 Leuven, Ch. Deberiotstraat 32, B-3000 Leuven, Belgium
14 [4] Institute of Biology, University of Graz, Universitätsplatz 2, A-8010 Graz, Austria
15 [5] Operational Directorate Taxonomy and Phylogeny, Royal Belgian Institute of Natural Sciences,
16 Vautierstraat 29, B-1000 Brussels, Belgium
17 [6] Biology Department, Royal Museum for Central Africa, Leuvensesteenweg 13, 3080, Tervuren,
18 Belgium
19 [7] Centre for Biodiversity Dynamics, Department of Biology, Norwegian University of Science and
20 Technology, N-7491 Trondheim, Norway
21 [8] Faculty of Biosciences and Aquaculture, Nord University, N-8049 Bodø, Norway
22 [9] Capacities for Biodiversity and Sustainable Development (CEBioS), Operational Directorate
23 Natural Environment, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000,
24 Brussels, Belgium

25 [10]Centre de Recherche en Hydrobiologie, Département de Biologie, B.P. 73 Uvira, Democratic
26 Republic of Congo
27 [11] Département de Biologie, Université du Burundi, Campus Mutanga, B.P. 2700, Bujumbura,
28 Burundi
29 [12] Zoology Unit, Finnish Museum of Natural History, University of Helsinki, P.O.Box 17, Helsinki
30 FI-00014, Finland
31 *corresponding author: kmentovan@mail.muni.cz, Laboratory of Parasitology, Department of
32 Botany and Zoology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
33 Note: Supplementary data associated with this article
34
35
36
37
38
39
40
41
42
43
44
45
46

47

48

49 **Abstract**

50 Lake Tanganyika is the oldest and deepest of the African Great Lakes and harbours one of the
51 most diverse fish assemblages on earth. Its two sardines, *Limnothrissa miodon* and *Stolothrissa*
52 *tanganicae*, constitute a major part of total fisheries catches, making them indispensable for
53 local food provision. As parasites were proposed as indicators of stock structure in highly
54 mobile pelagic hosts, we examined the monogeneans infecting these clupeids (*Kapentagyrus*,
55 Dactylogyridae) to explore the parasites' potential as tags for their hosts' population structure
56 and patterns of demographic history.

57 To assess parasite population structure and its link with their hosts' origin, samples originated
58 from several localities including all three subbasins of the lake. Intraspecific morphological
59 variation of the sclerotised structures of 380 monogeneans was analysed in detail using
60 morphometrics and geomorphometrics, respectively. Genetic population structure was
61 assessed in 246 individuals based on a 415 bp fragment of the mitochondrial COI gene.

62 Despite the lack of clear geographical morphological differentiation preventing identification of
63 unique phenotypes, some of the haptoral parameters showed significant differences related to
64 the sampling site origin. The lack of purely geographical population structure in parasites was
65 further supported by a high proportion of shared haplotypes and a pattern of seemingly
66 unrestricted gene flow between populations. However, significant genetic differentiation
67 between some of the analysed populations is suggested to possibly reflect temporal
68 differentiation as well as incipient speciation related to host species identity of *K. tanganicanus.*
69 Moreover, hybridisation between species of *Kapentagyrus* was found, representing the first

70 case of hybridisation in dactylogyrid monogeneans. Notably, both parasite species underwent
71 recent demographic expansion that might be linked to paleohydrological events. Overall, the
72 near-panmictic population proposed for both species of *Kapentagyrus* corresponds with the
73 weak north-south diversification reported in one of the host species studied in detail so far.

74 **Keywords:** *Kapentagyrus limnotrissae, Kapentagyrus tanganicanus*, Near-panmictic population,
75 Phenotypic plasticity, Fisheries targets

76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93

94

95

96 **1. Introduction**

97 In the African Great Lakes, the pelagic realm generally harbours a lower species diversity than
98 the littoral habitat. This is most likely caused by the lack of potential barriers to gene flow
99 (Kirchberger et al., 2012; Shaw et al., 2000). Lake Tanganyika's pelagic zone is dominated by
100 two clupeid species (*Limnothrissa miodon* (Boulenger, 1906) and *Stolothrissa tanganicae* Regan,
101 1917) and their latid predators. The two sardines combined make up 65% (in mass) of total
102 catches in Lake Tanganyika, making them indispensable for the local fisheries and food security
103 in the countries bordering the lake (Mölsä et al., 1999). Clupeids also have an important
104 function in the lake's food chain, as they form a link between the planktonic and the
105 piscivorous level (Coulter, 1991) and are the main food source for at least 16 piscivorous fish
106 species (Brichard, 1978). Lake Tanganyika sardines are parasitized by two species of
107 *Kapentagyrus* (Kmentová, Gelnar & Vanhove, 2018) (Monogenea, Dactylogyridae),
108 *Kapentagyrus limnotrissae* (Paperna, 1973) and *Kapentagyrus tanganicanus* (Kmentová, Gelnar
109 & Vanhove, 2018). Both parasite species have a lake-wide distribution throughout the year
110 (Kmentová et al., 2018). While *K. limnotrissae* is host specific to *L. miodon*,
111 *K. tanganicanus* has a more generalist lifestyle and infects both *L. miodon* and *S. tanganicae*. In
112 this species, two distinct morphotypes related to sardine species identity have been observed
113 (see graphical abstract).

114 Just like marine clupeids, sardines in Lake Tanganyika are short-lived species with mostly a one
115 year lifespan (max. 3 years), characterised by reproduction in inshore spawning grounds and a
116 schooling behaviour that follows the nocturnal vertical migration of the plankton (Mandima,
117 1999; Mulimbwa and Shirakihara, 1994). In the unique freshwater pelagic ecosystem of Lake

118 Tanganyika, the migration of the sardine species is poorly understood, but suggested to be
119 linked to seasonal changes in plankton distribution (Kurki et al., 1999; Plisnier et al., 2009).
120 Generally, the delineation of pelagic fish stocks, so crucial for fisheries management, is
121 challenging (Emmett et al., 2005). Traditional methods to track the movement of fish
122 populations, such as GPS or colour labelling, require handling of fishes and are no option for
123 sardine*s* because of the fragility of their skin and their susceptibility to changes in pressure
124 (James et al., 1988). More recently, lake-wide genome screening of *Stolothrissa tanganicae*
125 using SNPs did not identify a clear population structure in this sardine species, suggesting a
126 near-panmictic population (De Keyzer et al., 2019). However, differences in chemical
127 composition of otoliths in both species (Sako et al., 2005) and a significant pattern of isolation
128 by distance along a north-south gradient in *S. tanganicae* (De Keyzer et al., 2019) indicate
129 limitations in long-distance migration. Recently, a combination of host- and parasite genetics
130 has been proposed as an integrative approach to reconstruct host population structure
131 (Catalano et al., 2014) or stock structure over small geographical and temporal scales (Baldwin
132 et al., 2012). Monogenean parasites are ideal targets for such research for several reasons.
133 Foremost, the direct life cycle and often high host specificity of monogeneans prevents their life
134 history from being influenced by any than the targeted host taxon (Catalano et al., 2014).
135 Secondly, due to their short generation time, monogeneans may accumulate genetic changes
136 more rapidly than their hosts (Poulin, 2007). Thirdly, their faster mutation rate in comparison to
137 that of their hosts may reflect historical events that are too recent to be inferred from host
138 genetics (Nieberding and Olivieri, 2007): parasites as a “magnifying glass”. However, only few
139 studies used monogeneans in such a multidisciplinary approach to date. Pettersen et al. (2015)
140 used a portion of the cytochrome c oxidase of *Gyrodactylus thymalli* Žitňan, 1960 combined
141 with dehydrogenase subunit 5 to indirectly infer barriers in gene flow of grayling (*Thymallus*

142 *thymallus* L.) Monogenean genetics was also used to track the historical distribution of clariid
143 catfishes in Africa (Barson et al., 2010) as well as to reconstruct introduction pathways in
144 *Perccottus glenii* Dybowski, 1877 (Ondračková et al., 2012), and was proposed as potential tool
145 for cichlid biogeography (Pariselle et al., 2011).

146 Several steps need to be considered before using parasites as tags for host population
147 structure, including parasite species identification, the availability of more than one genetic
148 marker to verify the existence of cryptic species, and the presence and temporal stability of the
149 selected parasite species across the host's geographic range (Mattiucci et al., 2004; Vilas et al.,
150 2005). All above-mentioned criteria are fulfilled in the system studied here. Since the
151 morphology of their sclerotised structures was shown to vary along a north-south gradient
152 (Kmentová et al., 2018), species of *Kapentagyrus* are proposed as candidates to help unravel
153 clupeid population structure in Lake Tanganyika. Moreover, several periods of draught in the
154 past led to low lake levels and at times even separation in up to four paleolakes corresponding
155 with the current subbasins (Danley et al., 2012). Such events repeatedly caused periods of
156 population separation via migration barriers followed by periods of secondary admixis across
157 the north-south gradient. These left their signature in the genetics of various taxa (Sturmbauer
158 et al., 2001) and influenced their current population structure (Nevado et al., 2013; Sefc et al.,
159 2017; Sturmbauer et al., 2017), or their demographic history, even in the barrier-free pelagic
160 realm (Koblmüller et al., 2019). As such a lake level fluctuation in the past was shown to have
161 influenced demographic history of cichlid fishes and their respected monogenean species in a
162 similar way (Kmentová et al., 2016; Koblmüller et al., 2019), we assume the demographic
163 history of *Kapentagyrus* spp. to be connected with past population trajectories of sardine hosts.
164 We aim to test two species of *Kapentagyrus* as potential markers for 1) sardine stock structure,
165 as we expect more population structure in parasites compared with their hosts because of the

166 magnifying glass effect (Nieberding and Olivieri, 2007); and 2) we will investigate the recent

167 demographic history of *Kapentagyrus* spp. as we believe it to be connected with lake's

168 hydrological history. We ask whether an integrative approach, using comprehensive

169 intraspecific morphometric, geomorphometric and genetic data will shed light on the spatial

170 and spatio-temporal stock structure, and history of clupeid species in Lake Tanganyika. We

171 further ask whether we would see similar patterns in host-specific versus more generalist

172 species of *Kapentagyrus*.

173 **2. Material and methods**

174 2.1. Sampling design

175 In total, 380 monogenean individuals collected out of 497 host specimens were morphologically

176 analysed in this study combining fresh samples listed in Kmentová et al. (2018) and specimens

177 collected during the field work in 2018 (see Table 1). As clupeids are highly mobile pelagic fish

178 (De Keyzer et al., 2019; Marshall, 1993; Mulimbwa and Mannini, 1993), monogeneans were

179 collected from ethanol-preserved fish samples collected along the lake's shoreline within two

180 days in April 2018 (off Bujumbura, Kalemie, Mpulungu and Uvira). Our sampling design

181 therefore enabled us to analyse the spatial population structure of these monogenean

182 populations without the potential effect of school migration. Additionally, to compare spatio-

183 temporal patterns in the parasites' morphology, fresh specimens collected within two days in

184 August 2016 (off Kalemie and Uvira) and within two weeks in Mpulungu 2018 were included in

185 this study. To increase the resolution of population genetic analyses, samples from the above-

186 mentioned sampling events were combined with fresh specimens from Baraka in 2017,

187 Mpulungu in 2016, Mvugo in 2016 and Mvuna Island in 2015. In total, 246 individuals of

188 *Kapentagyrus* spp. were genetically characterised (see Table 1). Fish specimens were either

189 bought at fish markets in the above-mentioned cities or caught with gills nets during

190 experimental fishing. Fishes were identified to species level *in situ*. Voucher specimens of both
191 targeted clupeid species are part of the ichthyology collection of the Royal Museum for Central
192 Africa in Tervuren (RMCA 2016.20). Since the host specimens from some localities were fixed in
193 ethanol prior to dissection, two different fixation methods were used for the parasites. While
194 monogenean individuals collected from fresh fish specimens were placed on a slide in a drop of
195 glycerine ammonium picrate solution in 1:1 ratio (GAP), ethanol-preserved samples were
196 cleaned of host tissue in a drop of water followed by adding Hoyer's solution, in both cases
197 followed by fixation under a cover slip. All collected monogenean species were identified as
198 either *K. limnotrissae* or *K. tanganicanus*. Infection parameters are listed in Table 1.

199 2.2. Morphometrics and geomorphometrics

200 Morphological variation on a lake wide geographical scale was inferred via both morphometric
201 and geomorphometric approaches. Haptoral and male copulatory hardparts of the two species
202 of *Kapentagyrus* were measured and photographed using an Olympus BX51 microscope with
203 incorporated phase contrast at a magnification of 1000x (objective x100 immersion, ocular x10)
204 with MicroImage v3.1. In total, we obtained 23 different morphometric parameters following
205 the terminology of Řehulková et al. (2013).

206 Geomorphometric data were obtained by digitising the shape of dorsal and ventral anchor,
207 respectively. For this we used tps Dig v2.30 (Rohlf, 2006) from the thin-plate spline (TPS)
208 packages (Rohlf, 2006). We chose the anchors for geomorphometric analyses as their shape
209 was successfully used in intraspecific studies on members of *Ligophorus* Euzet & Suriano, 1977
210 (Monogenea, Dactylogyridae) (Rodríguez-González et al., 2015). The shape of other
211 monogeneans' sclerotised structures, such as the shape of bars and marginal hooks, was shown
212 highly related to the method of sample preparation (Vignon et al., 2011). Eight fixed landmarks
213 were selected on each of the anchors. Additionally, semi-landmarks were placed in equal

214 intervals on each anchor, resulting in 98 of them in the case of *K. limnotrissae* and 102 in *K.*

215 *tanganicanus* (see Fig S1).

216 2.3. DNA extraction and genetic characterisation

217 Monogeneans were stored in 99% ethanol prior to DNA isolation. Subsequently, ethanol was

218 evaporated using a vacuum centrifuge and lysis buffer poured onto the specimens. Whole

219 genomic DNA was extracted using either Qiagen DNeasy Blood & Tissue Kit or Nucleospin

220 Tissue Genomic DNA kit following the manufacturer's instructions. The extracted DNA was

221 eluted in a volume of 50 µl.

222 Part of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene was amplified using a

223 nested PCR reaction in view of the low content of genomic DNA extracted from in most cases

224 1/3 of the worm. The first PCR reaction was performed with ASmit1 (5'-

225 TTTTTTGGGCATCCTGAGGTTTAT-3') (Littlewood et al., 1997) and Schisto3 (5'-

226 TAATGCATMGGAAAAAAACA-3') (Lockyer et al., 2003) primers in 24 µl of PCR mix (one unit of

227 *Taq* Polymerase, 1X buffer containing 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.8 mM of each primer) for

228 a total reaction volume of 25 µl. It was carried out under the following conditions: initial

229 denaturation at 95°C for 5 min, then 40 cycles of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C,

230 and final elongation for 7 min at 72°C. The nested PCR with ASmit1 and ASmit2 (5'-

231 TAAAGAAAGAACATAATGAAAATG-3') (Littlewood et al., 1997) primers followed the same

232 protocol as the first one with 1:100 dilution of template DNA. The final PCR products were

233 enzymatically purified using 1 µl of ExoSAP-IT reagent and 2.5 µl of PCR product under the

234 following conditions: 15 min at 37 °C and 15 min at 80 °C. The same primers as in the

235 amplification reactions were used for sequencing with a Big Dye Chemistry Cycle Sequencing Kit

236 v3.1. DNA sequences were visually inspected and corrected using MEGA v7 (Kumar et al., 2016)

237 and aligned using MUSCLE (Edgar, 2004) under default distance measures as implemented in

238 MEGA. COI sequences are deposited in NCBI GenBank under the accession numbers
239 MK598125-323. Corresponding nuclear data of the same isolates are available on GenBank
240 under the accession numbers MK521656-MK521659 (28S rDNA portion of *K. limnotrissae*
241 individuals identified as hybrids), MK521661-MK521664 (18S and ITS-1 rDNA portion of *K.*
242 *limnotrissae* individuals identified as hybrids) and MK522517-520 (28S, 18S and ITS-1 region of
243 *K. tanganicanus* collected from *Stolothrissa tanganicae*).
244
245 2.4. Data analysis
246
247 2.4.1. Morphological differentiation
248 To avoid any possible influence of ethanol fixation on the size and shape of sclerotised
249 structures, the samples were subdivided into six different sample sets according to parasite
250 species, host species and the fixative used. These sample sets were always analysed separately.
251 Samples in all analyses and sample sets were grouped based on the sampling site origin to
252 check for possible geographical structure in both species of *Kapentagyrus.* As preliminary
253 analyses indicated a significant influence of host size on morphological characters, individuals
254 from Kalemie 2018 were analysed as two groups using 12 cm of host standard length (SL) as a
255 cut off value (referred to as Kalemie 2018 Big and Kalemie 2018 Small, respectively). As the
256 same pattern was discovered with the fresh samples from Mpulungu, a threshold in SL of host
257 specimens was set to 10 cm (referred to as Mpulungu 2018 Big and Mpulungu 2018 Small,
258 respectively).

259 To evaluate intraspecific and intrahost variation, a principal component analysis (PCA) of
260 haptoral morphometric parameters was performed in the R package adegenet (Jombart, 2008).
261 Missing data were replaced by the average value for each morphological character. To increase

262 the resolution of the resulting pattern, morphological characters with more than 50% missing
263 data were excluded prior to the analysis. Overall significance of the particular morphological
264 characters was tested via a linear model approach followed by F-statistics and a generalised
265 linear model approach followed by Chi Square statistics, respectively, with the factors sampling
266 site origin and host size (and interaction), conducted in R package stats (R Core Team, 2013).
267 Significance between the groups was assessed post-hoc by ANOVA and Kruskall-Wallis tests,
268 respectively, with Bonferroni correction. Only morphological parameters without significant
269 influence of host size as well as reciprocal interaction between the sampling site origin and host
270 size were considered as informative for geographical structure related to sampling site origin of
271 the specimens. Sampling sites with insufficient number of specimens (<10) were excluded from
272 the analyses.

273 Configurations of fixed landmarks were superimposed using Generalized Full Procrustes
274 Analyses (Cox and Cox, 1989; Zelditch et al., 2012), under the Least Squares criterion to
275 minimize bending energy with respect to a mean reference form. Canonical variate analyses
276 (Klingenberg and Monteiro, 2005) and PCA using fixed landmarks only were performed in
277 MorphoJ v2.0 (Klingenberg, 2011). A permutation test with 10,000 iterations was used to
278 statistically validate pairwise differences between the pre-defined groups. Additionally, the
279 overall shape of both anchors, captured using fixed landmarks and semi-landmarks, was
280 analysed using tps Relw v1.49. A Relative Warp Analysis (RWA) (Rohlf, 1993) was performed
281 with the Procrustes coordinates. In order to give all landmarks equal weight, the scaling option
282 was set to $\alpha = 0$. Sampling sites with insufficient number of specimens (<6 as in the case of
283 dorsal anchor of *K. limnotrissae* from Uvira 2016) were excluded from the analyses.

284 Relationships between the individual scores inferred with PCA and RWA analyses, respectively,
285 and the host size were checked via linear regression analyses. This was done for each of the six

286 sample sets and within the respective groups. Further, t and F-statistics were calculated in the R
287 package stats (R Core Team, 2013). Correlation between the host size and each of the tested
288 morphological or shape character within each sample set and group was tested via Pearson's
289 and Kendall's correlation coefficient, respectively. Additionally, t and Z statistics, respectively,
290 were calculated in the R package ggpubr (Kassambara, 2018). All sample sets were visually
291 inspected for outliers, which were excluded from the analyses. Normality of the data was
292 checked by Shapiro-Wilks test in R package onewaytests (Dag et al., 2018). The homogeneity of
293 variance among groups within each datasets was assessed by Levene's tests in the R package
294 car (Fox and Weisberg, 2011). Biplots of PC and RW scores were visualised with the packages
295 ggplot2 (Wickham, 2009) and factoextra (Kassambara and Mundt, 2017).

296 2.4.2. Genetic structure

297 Genetic diversity of both monogenean species infecting sardines in Lake Tanganyika was
298 studied based on 415 bp of the COI gene. Since the population genetic structure of *S.*
299 *tanganicae* was already examined (De Keyzer et al., 2019), diversity indices in COI gene were
300 calculated using for this host species as well (Genbank accession numbers MH290064-159).
301 Moreover, unpublished data were used to obtain genetic diversity indices for the COI region of
302 *L. miodon*.

303 The number of haplotypes and polymorphic sites, haplotype diversity and nucleotide diversity
304 were calculated using Arlequin v3.5 (Excoffier and Lischer, 2010). The genealogy of COI
305 haplotypes was inferred by means of a Median Joining network in PopART v1.71 (Leigh and
306 Bryant, 2015).

307 Differentiation among pre-defined populations was estimated by Fst in Arlequin (Excoffier and
308 Lischer, 2010): for *K. tanganicanus* collected from *L. miodon* and *S. tanganicae*, respectively, in

309 Uvira 2016, as well as for five populations ex *L. miodon* with at least 17 individuals available.
310 Analysis of molecular variance (AMOVA) was used to test the significance of population
311 structure by the F-statistics of *K. tanganicanus* at the level of subbasins within Lake Tanganyika.
312 Unfortunately, for *K. limnotrissae*, sample size was generally too small to allow for any
313 meaningful population genetic analyses.

314 2.4.3. Demographic history

315 To test for signals of past population expansion mismatch distributions as well as two different
316 neutrality test statistics, Tajima's D (Tajima, 1989) and Fu's FS (Fu, 1997), were calculated in
317 Arlequin. The sum of square deviations (SSD) and raggedness index (rg) were used to assess the
318 fit of the observed mismatch distributions to the expectations based on growth parameter
319 estimates. Past population size trajectories were further investigated employing a Bayesian
320 coalescent approach - Bayesian skyline plot (Drummond et al., 2005) - as implemented in BEAST
321 v1.8.2. (Suchard et al., 2018). The substitution rate was set to 10% per million years, which is
322 lower that the rates previously used for other monogenean taxa (Meinilä et al., 2004), and
323 should take into account the comparatively longer generation time of *Kapentagyrus*. Two
324 independent MCMC runs of 300 million generations each at a sampling frequency of 30,000
325 were conducted, with a burn-in of the first 10% of sampled generations. The number of
326 grouped intervals was set to 5. Verification of effective sample sizes (ESS > 200 for all
327 parameters), tracing MCMC runs and visualisation of past population size changes were done in
328 Tracer v1.6 (Rambaut et al., 2018).

329 **3. Results**

330 3.1 Morphological variation

331 Since Kmentová et al. (2018) documented clear morphological differences in sclerotised
332 structures between two species of *Kapentagyrus* as well as between the host-dependent
333 morphotypes of *K. tanganicanus*, the three parasite/host species combinations were analysed
334 separately (see graphical abstract). Additionally, given the effect of preservation on sclerotised
335 structures, separate analyses were done for each of the two preservation methods. These were
336 treated as spatial (ethanol-preserved) and spatio-temporal (fresh) sample sets. As such, six
337 different sample sets were analysed creating three different datasets each for each sample set:
338 a) morphometrics of haptoral structures, and geomorphometrics of b) dorsal and c) ventral
339 anchors.

340 3.1.1 *Kapentagyrus limnotrissae* ex *Limnothrissa miodon*

341 Two sample sets, both containing specimens of *K. limnotrissae* ex *L. miodon* from three
342 different groups, were analysed separately. PCA did not reveal any unequivocal geographical
343 separation, based on haptoral morphometric parameters in *K. limnotrissae*, along any of the PC
344 axes, in any of the two datasets (Fig. 2A&B). However, differentiation was visible between the
345 specimens from Mpulungu 2018 and Uvira 2016 along the first as well as the second axis (Fig.
346 2B). In all but three of the six comparisons tested, at least one the morphological parameters
347 was found to differ significantly. The length of the outer root of the ventral anchor was the only
348 morphological character that was significantly related to the sampling site in both datasets (see
349 Fig. 1 and Table S1).

350 In *K. limnotrissae*, a clearer differentiation between Uvira and Bujumbura 2018 was visible in
351 the PCA biplot of fixed landmarks of the dorsal than of the ventral anchor (Fig. 2C and Fig. S2A).
352 This differentiation was further reflected in the result of CVA (Table S2). The shape of the
353 ventral anchor was significantly different between the specimens from Uvira and Kalemie 2016,
354 and between Kalemie 2016 and Mpulungu 2018 (detailed results presented in Table S1). The

355 results of RWA (including sliding landmarks) followed the pattern obtained via PCA but did not
356 provide higher resolution (Fig. S2).

357 No effect of host size on the position of specimens in neither of the presented biplots was
358 detected (not shown). Overall intraspecific morphological variation of *K. limnotrissae* related to
359 the sampling site origin is summarised in Fig. 1B. An overview of measurements from haptoral
360 as well as from the male copulatory organ region is listed in Table S3.

361 3.1.2 *Kapentagyrus tanganicanus* ex *Limnothrissa miodon*

362 In these sample sets, specimens of two host sized categories from Kalemie 2018 and Mpulungu
363 2018 were analysed separately (see M&M). In total, two sample sets, both containing
364 specimens of *K. tanganicanus* ex *L. miodon* from five and four different groups, respectively,
365 were analysed. A gradient was visible along the first PC axis of haptoral morphometric
366 parameters. Here, specimens from Mpulungu 2018 were intermediate between those from
367 Kalemie 2018 and Uvira 2018 (Fig 2A). This separation was further reflected in the number of
368 significantly different characters related to sampling site along the lake's coastline (see Table
369 S1). A separation was visible along the first PC axis of haptoral morphometric parameters of
370 specimens collected in Mpulungu 2018 from those from Kalemie 2016 and Uvira 2016 (Fig. 2B).
371 Only two morphological parameters differed significantly between Mpulungu 2018 and Uvira
372 2016 (see Table S1). A single one differed significantly between the two host size categories
373 from Kalemie 2018 and Mpulungu 2018.

374 In *K. tanganicanus* ex *L. miodon*, the PCAs of the shape of both anchors, based on a fixed
375 landmark geomorphometric approach, reflected the gradient visible in the biplot of the
376 haptoral morphometric approach in the both spatial and the spatio-temporal sample set (Fig.
377 3C-F). This differentiation was further supported by the results of CVA. Here, a significant

378 difference was seen in either both or in at least one of the anchors in comparisons between
379 each of the studied group. The sole exception was the comparison between Mpulungu 2018
380 and Uvira 2018, where no difference found (detailed results presented in Table S2). Moreover,
381 the shape of the ventral and of both anchors was found to be significantly different between
382 the two host size categories from Kalemie 2018 and Mpulungu 2018, respectively. The results
383 of RWA (including sliding landmarks) followed the pattern obtained via PCA but did not provide
384 higher resolution (Fig. S3).

385 A significant effect of host size was detected only on the individual PC scores for the dorsal
386 anchor of the spatio-temporal sample dataset ($F_{1,64}$=7.0753, P=0.010). The overall intraspecific
387 morphological variation in *K. tanganicanus* ex *L. miodon* related to the sampling site origin is
388 summarised in Fig. 1C. An overview of measurements from the haptoral as well as the male
389 copulatory organ region are given in Table S4.

390 3.1.3 *Kapentagyrus tanganicanus* ex *Stolothrissa tanganicae*

391 In total, two sample sets, both containing specimens of *K. tanganicanus* ex *S. tanganicae* from
392 four and two different groups, respectively, were analysed. Clear differentiation was visible in
393 the second PC axis of haptoral morphometric parameters between the specimens from Kalemie
394 2018 and Uvira 2018 (Fig. 4A). A single morphological character, the size of the first pair of
395 marginal hooks, was identified as being significantly related to the sampling site. Additionally, a
396 separation in the spatio-temporal dataset was reported along the first together with the second
397 PC axis between specimens from Mpulungu 2018 and Uvira 2016 (Fig. 4B). Similarly to the
398 previous sample set, only one morphological character, the length to the notch of the dorsal
399 anchor, was identified as being significantly related to group origin. This variable was not
400 correlated with the host size differences between the specimens.

401 In *K. tanganicanus* ex *S. tanganicae,* the position of specimens along the first axis of the PCA of
402 the anchors' shape based on a fixed landmark geomorphometric approach mirrored the
403 pattern seen in haptoral morphometric characters in both sample sets (Fig. 4C-F). In the spatial
404 sample set, the shape of ventral anchor was reported as significantly related to the sampling
405 site between specimens from Kalemie 2018 and Uvira 2018. In the spatio-temporal sample set,
406 the shape of botch anchors was significantly different between Mpulungu 2018 and Uvira 2016
407 (detailed resulted presented in Table S2). The results of RWA (including sliding landmarks)
408 followed the pattern obtained via PCA but did not provide higher resolution (Fig. S4).

409 No effect of the host size on the position of specimens in neither of the presented biplots was
410 detected. Overall intraspecific morphological variation of *K. tanganicanus* ex *S. tanganicae*
411 related to sampling site is summarised in Fig. 1D. An overview of measurements from the
412 haptoral as well as from the male copulatory organ region is listed in Table S5.

413 In total, a sole morphological character, namely length to notch of dorsal anchor, was reported
414 as significantly related to group in all six datasets. Two additional characters, branch length of
415 ventral bar and a total length of ventral anchor, were found significantly group dependent in
416 specimens of *Kapentagyrus* spp. collected from *L. miodon*. Finally, the length of the first pair of
417 marginal hooks was found as a common character significantly related to the group of *K.*
418 *tanganicanus* collected from different host species (see 3.1.2). For further details see Table S1.

419 3.2 Population genetics

420 All population genetic analyses were based on a 415 bp fragment of COI. The number of
421 haplotypes found per species was 19 for *K. limnotrissae* and 60 for *K. tanganicanus*,
422 respectively. There was no evident clustering of *K. tanganicanus* according to host species and
423 therefore no indication of potential host-related cryptic diversity or incipient speciation (see

424 Fig. 5A). Similar levels of nucleotide and haplotype diversity were observed between the two
425 parasite species and one of the host species: *S. tanganicae*. However, the other host species, *L.*
426 *miodon*, had higher genetic diversity than both species of *Kapentagyrus*. Lower genetic diversity
427 was calculated also for *K. tanganicanus* when only individuals collected from *S. tanganicae*
428 were included (Table 2). Haplotype networks indicated neither geographic, nor school-related
429 structure in either species. All networks showed a star-like topology with a single dominant
430 haplotype (see Fig. 5B-D). Satellite haplotypes were mostly separated by a single mutation from
431 the central haplotypes. Fst values significantly different from zero were reported between
432 individuals of *K. tanganicanus* from different host species (Fst= 0.06677; P= 0.01119). Fst values
433 significantly different from zero were also recorded in *K. tanganicanus* ex *L. miodon* among
434 several sampling localities (see enclosed table in Fig. 5). Results of AMOVA calculated for *K.*
435 *tanganicanus* ex *L. miodon* at the level of subbasins showed most of the variation to be present
436 within populations (96.85%) in comparison to 1.67% among populations within subbasins and
437 1.47% among subbasins.

438 3.3 Demographic history

439 Signatures of population expansion were detected in both monogenean species. The unimodal
440 mismatch distribution was well supported by a non-significant SSD and rg, indicating recent
441 population expansion in both *Kapentagyrus* species (see Fig. 6A, B). Recent population growth
442 in all species was further supported by significantly negative values of Fu's FS (-20.97992,
443 P<0.001) in *K. limnotrissae* and -27.89273 (P<0.001) in *K. tanganicanus* and Tajima's D (-
444 2.47932, P<0.001) in *K. limnotrissae* and -2.40750 (P< 0.001) in *K. tanganicanus*. Mismatch
445 analyses dated the onset of population expansion to 11.8 KYA in *K. limnotrissae* (95% CI: 6.5 –
446 16.8 KYA) and to 17.6 KYA in *K. tanganicanus* (95% CI: 3.3 – 30.1 KYA).

447 Using Bayesian Skyline Plot analysis of *K. tanganicanus*, the past trajectory of population size
448 was reconstructed back to more than 15 KYA, with the start of population growth estimated
449 around 12 KYA (see Fig. 6D) and the time to the most recent common ancestor (TMRCA)
450 around 70.9 KYA (95% HDP: 15.6 – 143.1 KYA). Due to the insufficient number of
451 haplotypes/individuals, BSP could not track past changes of effective population size back to
452 more than 7 KYA in the case of *K. limnotrissae* (see Fig. 6C). No sign of population growth was
453 observed and the TMRCA was estimated at 14.4 KYA (95% HDP: 6.5 – 24.1 KYA).

454 Based on the comparison of rDNA markers published in Kmentová et al. (2018) and the
455 obtained COI sequences of the same specimens, nuclear–mitochondrial discordance was
456 documented for four individuals collected from *L. miodon* (out of 62 individuals for which rDNA
457 and COI mtDNA regions were sequenced) (see Fig. 5A). For two of these four cases,
458 morphological vouchers are available (specimens on slides deposited under xxx-xxx in RMCA).
459 Their morphology and haplotype of 28S and/or 18S and ITS-1 rDNA (MK521656-59 and
460 MK521661-64) nuclear markers are indicative of *K. limnotrissae* and the COI mitochondrial
461 haplotype of *K. tanganicanus.*

462 **Discussion**

463 In our study, geographic population structure and demographic history of monogenean species
464 infecting sardine hosts in Lake Tanganyika were investigated in an attempt to indirectly
465 investigate the stock structure and demographic history of these fisheries targets.
466 Morphological comparison of the parasites' sclerotised structures did not show clear patterns
467 of differentiation according to sampling site. Although none of the approaches used identified a
468 morphological character unambiguously specific to geographic origin, significant differences
469 between some of them indicate so far unpatterned differentiation related to environmental
470 and/or host-morphology related differences. Moreover, the genetic part of the study suggests a

471 near-panmictic lake-wide population with only temporal differentiation. Finally, both species of
472 *Kapentagyrus* showed a similar pattern of recent population expansion.

473 *Monogeneans as tags for sardine geographical population structure: different host, different*
474 *story*

475 The pelagic environment, promoting dispersal, and large effective population sizes limit genetic
476 drift and differentiation in fishes (Gonzalez and Zardoya, 2007; Kinsey et al., 1994; Koblmüller
477 et al., 2019; Martínez et al., 2006; Waples, 1987). On the other hand, patchiness in
478 phytoplankton occurrence as it was proposed in Lake Tanganyika (Phiri and Shirakihara, 1999;
479 Plisnier et al., 2009; van Zwieten et al., 2002), may promote isolation by distance of sardines via
480 restricted migration (Zardoya et al., 2004). Although the migration pattern of clupeids in the
481 lake has not been resolved yet, some isolation by distance along a north-south gradient was
482 detected, suggesting limits to lake-wide migration in *S. tanganicae* (De Keyzer et al., 2019). This
483 was also seen in the chemical composition of otoliths in both species (Sako et al., 2005). Based
484 on our comprehensive study, in some cases, morphometrics of monogenean haptoral as well as
485 male copulatory organs structures showed significant intraspecific shape variation with respect
486 to sampling site. However, none of the approaches used identified a morphological character
487 unambiguously specific to locality of origin for neither of the sample sets. Interestingly, even
488 though the shape of both anchors mirrored the pattern seen in overall haptoral morphology,
489 the detailed morphology of neither of these structures did provide higher resolution to figure
490 as a clear tag for the geographic origin of a monogenean individual.

491 Interestingly, differences in the number of morphometric characters related to sampling site
492 differed between the sample sets. While a maximum of two characters was informative in the
493 case of *K. limnotrissae*, a species specific to *L. miodon*, this number was markedly higher in *K.*
494 *tanganicanus* collected from the same host species. As host-size dependent intensity of

495 infection between *Kapentagyrus* spp. collected from *L. miodon* was observed (sympatric
496 occurrence of both species was documented only in 49 out of 355 infected specimens), this
497 discrepancy might be explained by divergence in migration capacity between schools as
498 clupeids are known to form size-dependent units (Misund, 1993). Moreover, the number of
499 morphological characters with significantly different lengths between the groups of *K.*
500 *tanganicanus* ex *S. tanganicae* was lower compared to the individuals collected from *L. miodon*.
501 Such a pattern might be related to the difference in ecology between the host species.
502 However, this result could be also influenced by the small number of specimens collected from
503 *S. tanganicae* and the fact that some of the characters from the ventral anchor might have
504 been devaluated due to the correlation with host size, which could not have been
505 differentiated.

506 Significantly different morphological characters were not repeatedly found between the
507 sampling sites in 2016 or between the specimens from the same localities in 2018. This
508 suggests dependency of phenotypic differentiation on actual environmental conditions rather
509 than fidelity to a certain geographic location in *Kapentagyrus* spp. and consequently of their
510 sardine host species. Nevertheless, non-heritable morphological variation, dependent on
511 geographical origin, can be explained only by variation in environmental and/or host
512 morphology related differences affecting parasite's morphology. These can cause geographical
513 patterns via restricted hosts' and consequently parasites' migration or via similar
514 environmental conditions in geographically-isolated localities. This could explain why
515 geographically-isolated specimens of *K. limnotrissae* from Mpulungu clustered together with
516 those from Kalemie and not with Uvira (Fig. 3A).

517 In previous studies, temperature (Brazenor et al., 2018; Dávidová et al., 2005; Ergens and
518 Gelnar, 1985; Mo, 1993), pollutants (Beaumont, 1997) or other environmental factors (Cable

519 and Harris, 2002; Olstad et al., 2009) were shown to affect the morphology of monogeneans.
520 The observed phenotypic plasticity of *Kapentagyrus* spp. with respect to geographical origin
521 could hence be explained by environmental factors directly influencing the parasites'
522 morphology or indirectly via morphology of hosts. Interestingly, spatio-temporal variation
523 visible in sampling groups containing fresh specimens collected in Mpulungu 2018 supported
524 the hypothesis of environmentally-dependent variation, specific to actual locality and time. In
525 Lake Tanganyika, geographical and seasonal variation in thermal stratification, the level of
526 oxygen (Hecky et al., 1978; Langenberg et al., 2002), pH (Plisnier et al., 1999), chemical (Degens
527 et al., 1971) and phytoplankton composition (Descy et al., 2005) or algae succession (Agawin et
528 al., 2000) were reported. They are driven mostly by wind conditions (Hecky et al., 1978;
529 Langenberg et al., 2003). Indeed, season, geography and host body size were already reported
530 in a previous study to significantly influence several haptoral parameters (Kmentová et al.,
531 2018). However, no experimental data for representatives of *Kapentagyrus* are currently
532 available and therefore no unequivocal explanation can be provided.

533 Given the egg hatching phase in dactylogyrid life history, we assume that monogenean
534 infection happens in spawning grounds as reproduction is one of the only times for both
535 species to spend in the littoral zone along the lake's coastlines (Mulimbwa and Shirakihara,
536 1994). Such a synchronisation would therefore mean a direct effect on parasites' population
537 structure supporting the magnifying glass theory. As no clear geographical structure was visible
538 in the haplotype network of neither of the three monogenean/host species combination, we
539 suggest geographical panmixia with temporal effects in both species of monogeneans infecting
540 Lake Tanganyika's clupeids. The COI-based median joining networks of both monogenean
541 species exhibited a similar core-satellite topology with similar levels of variation in haplotype
542 and gene diversity. Nevertheless, and despite shared COI haplotypes, significant genetic

543 divergence among some of the pre-defined populations of *K. tanganicanus* from *L. miodon* was
544 detected based on Fst. The observed temporal genetic structure without clear geographical
545 pattern could be explained by random genetic drift across generations related to overfishing of
546 declining sardine stocks in the lake (Mölsä et al., 1999). Moreover, AMOVA revealed that the
547 majority of the variation was found within populations rather than among them. Therefore, the
548 reported genetic divergence of *K. tanganicanus* among pre-defined geographical populations
549 can be influenced by stochasticity related to small sample size and short fragment length rather
550 than persisting gene flow barriers. Notably, genetic diversity of *K. tanganicanus* in COI is
551 comparable to that of the host: *S. tanganicae*. However, a higher maximum divergence
552 between haplotypes was observed. When only individuals collected from this host species were
553 included, this value was lower. Nucleotide diversity in *L. miodon* is much higher than in both
554 species of *Kapentagyrus* (see Table 2). Generally, the genetic structure of parasites is heavily
555 influenced by the dispersal ability of their host (Miura et al., 2006). The overall geographical
556 panmixia seen in *Kapentagyrus* spp. is assumed to be maintained by random variation in
557 parental contribution to the next generation as reproduction is suggested to be synchronised
558 with the sardines' breeding behaviour. Alternatively, monogenean reproduction in the pelagic
559 habitat connected with plankton larval dispersal could cause differences in local genetic
560 diversity of parasites, known as fluctuating genetic patchiness (Hellberg et al., 2002). Neither in
561 *S. tanganicae* (De Keyzer et al., 2019), nor in *K. tanganicanus*, migration barriers were
562 observed. However, genome-wide population data showed signs of isolation by distance (De
563 Keyzer et al., 2019). As the genetic results of both monogenean species infecting *L. miodon*
564 show no clear and consistent geographical population structure, near-panmictic population of
565 this host species is suggested. This corresponds with the general biology of clupeids, with
566 assumed lake-wide migration patterns (Hauser et al., 1998; Mulimbwa and Shirakihara, 1994)

567 that would restrict geographical population differentiation in parasites, too. In general, studies
568 conducted on marine sardines do not show strong school structure neither using genetic
569 markers (García-Rodríguez et al., 2011; Gonzalez and Zardoya, 2007; Kinsey et al., 1994) nor fish
570 tags (Clark, 1945).

571 Our data did not show a magnifying glass effect suggested for directly-transmitted parasites. As
572 the magnifying glass effect depends on the shorter generation time in parasites than in hosts,
573 multiple spawning of sardines each year, together with their short life span may erase the
574 expected effect of faster molecular evolution in parasites. Alternatively, even counting on the
575 existence of a shorter generation time in *Kapentagyrus* spp. compared to their hosts, the
576 existence of common breeding sites of sardines would most likely erase such a pattern in the
577 next spawning season. Even though the existence of reproductive barriers between individuals
578 collected from different sardine schools is not supported, there is an indication of restricted
579 migration of fish reflected in both the morphological as well as the genetic part of the study.
580 Expanding our limited knowledge of host and parasite population dynamics is needed to
581 identify the cause of the apparent lack of population structure. In order to further evaluate the
582 magnifying potential of *Kapentagyrus* spp., genome-wide markers should be applied and
583 compared with similar data on the host species.

584 The core-satellite structure of the haplotype networks and the lower haplotype as well as
585 nucleotide diversity in both studied species of *Kapentagyrus* also indicates more recent
586 diversification in comparison to *Cichlidogyrus casuarinus* Pariselle, Muterezi Bukinga &
587 Vanhove, 2015, a monogenean species infecting bathybatine benthopelagic cichlids in Lake
588 Tanganyika (Kmentová et al., 2016; Pariselle et al., 2015). This could be connected with limited
589 allopatric divergence linked to the overall higher dispersal capacity and population densities of
590 clupeids in the lake compared to pelagic cichlid species (Coulter, 1991; Koblmüller et al., 2019).

591 Interestingly, morphological differentiation of *K. tanganicanus* influenced by host species
592 detected in a previous study (Kmentová et al., 2018) was supported by genetic differentiation
593 of the specimens sampled off Uvira in 2016. Unlike in the case of *C. casuarinus*, our results
594 indicate genetic differentiation of *K. tanganicanus* with respect to the sardine host species
595 probably after a recent host switch. However, given the uniformity in three nuclear gene
596 fragments, the low Fst value and the many shared COI haplotypes of *K. tanganicanus* collected
597 from different host species, we hypothesize speciation is hampered by hosts forming mixed
598 schools or might have started just recently. This should be further verified by genetic
599 characterisation of more individuals combined with genome wide data. The results fit the
600 scenario of relatively low rate of intraspecific divergence in barrier-free pelagic compared to
601 littoral fish species (Koblmüller et al., 2019, 2015).

602 *Nuclear–mitochondrial discordance*

603 We found indications for potential mitochondrial introgression of *K. tanganicanus* into *K.*
604 *limnotrissae* as the COI haplotype of *K. tanganicanus* was present in four specimens identified
605 as *K. limnotrissae* (based on nuclear rDNA regions, and for two of them, confirmed
606 morphologically). As all four individuals were homozygous at all three nuclear loci studied and
607 identical to other individuals of *K. limnotrissae*, they are definitely not F1 hybrids of
608 *Kapentagyrus* spp. Given the broader host range of *K. tanganicanus* compared to its congener,
609 this introgression could result from a recent host switch and a demographic expansion of *K.*
610 *tanganicanus* (Barson et al., 2010; Rieseberg et al., 2007; Seixas et al., 2018). However, our type
611 of data and the low number cases of mito-nuclear discordance does not allow clear
612 differentiation among incomplete lineage sorting, historical introgression, or contemporary
613 hybridisation. Nevertheless, as no intermediate nuclear haplotype was captured, the presence
614 of a mitochondrial genome of one species in the nuclear environment of another suggests

615 mitochondrial introgression followed by backcrossing into the paternal species and eventual
616 dilution and loss of alleles inherited from the maternal species rather than ongoing
617 hybridisation events (Okamoto et al., 2010). A historical hybridisation event would support the
618 above-mentioned scenario of a recent host switch of *K. tanganicanus* followed by temporal
619 differentiation of infection as sympatric occurrence of both species was documented only in 49
620 out of 355 examined specimens of *L. miodon*. Moreover, the apparent morphological similarity
621 in the MCO of the two parasite species contradicts the incomplete lineage sorting often linked
622 to monogenean intrahost speciation (Jarkovský et al., 2004). Although hybridisation has been
623 reported in gyrodactylid monogeneans (Barson et al., 2010; Schelkle et al., 2012), this is the
624 first case in dactylogyrid monogeneans. The rarity of such events in monogeneans is probably
625 related to the lack of similar studies on other monogenean species, as hybridisation is believed
626 to be one of the drivers of evolution (Franssen et al., 2015; Hedrick, 2013; Huyse et al., 2013;
627 King et al., 2015; Schelkle et al., 2012), and to impact the host range of parasites (Henrich et al.,
628 2013; Huyse et al., 2009).

629 *Demographic history*

630 Recent population expansion was suggested for both species of *Kapentagyrus.* The time of
631 population growth reflected in the demographic history reconstruction for *K. tanganicanus*
632 corresponded with global climate changes and subsequent lake level rise. Indeed, 10 KYA is the
633 estimated end of the last Little Ice Age, which corresponded with the end of a dry period in East
634 Africa (McGlue et al., 2008). Other studies discussed the possible influence of sea level changes
635 and climate oscillations in monogenean demographic history (Wang et al., 2016; Yan et al.,
636 2016). We suggest that expansion and population growth of *Kapentagyrus* spp. are connected
637 with rising lake levels and such a pattern is expected to be present in the sardine host species.
638 The influence of such events on population size in lake's pelagic zone was already reported for

639 bathybatine cichlids (Koblmüller et al., 2019) and their monogenean parasite *C. casuarinus*
640 (Kmentová et al., 2016). The onset of population expansion and the time to the most recent
641 common ancestor was estimated for both species of *Kapentagyrus* to have been more sudden
642 and recent than for *C. casuarinus* (using the same substitution rate). One possible explanation
643 for this difference could be the different life-style and biomass of the hosts, host range of the
644 parasites and difference in substitution rates.

645 In conclusion, no clear geographical structure related to sampling site of neither *Kapentagyrus*
646 spp. was found, suggesting ongoing gene flow throughout Lake Tanganyika. The morphology of
647 neither of the two species did hold as a suitable marker for host populations within the lake
648 with only so far rather temporal than geographical differentiation detected. Therefore, our
649 results correspond with the pattern of a lake-wide near-panmictic population of *S. tanganicae*
650 based on COI and RADseq data. Nevertheless, significant morphological diversification
651 documented for some of the tested characters is assumed to be caused by similar
652 environmental conditions in geographically isolated localities, potentially combined with
653 restricted host migration. Moreover, as significant genetic differentiation was found between
654 some of the analysed populations, genomic data over serial temporal sampling for
655 *Kapentagyrus* spp. are needed to increase resolution and track host migration. Despite
656 uniformity in ribosomal gene portions, indications for incipient speciation in *K. tanganicanus*
657 according to host species were found using a mitochondrial marker. No magnifying glass effect
658 in the mitochondrial gene portion of *Kapentagyrus* spp. in comparison to their sardine hosts
659 was detected. Furthermore, mitonuclear discordance suggests past hybridisation between the
660 two species of *Kapentagyrus*, which is the first documented case of hybridisation in
661 dactylogorid monogeneans. Furthermore, our findings provide additional support for the
662 impact of drastic lake level changes also on organisms inhabiting the lake’s pelagic zone.

663 **Acknowledgments**

664 The authors would like to thank to Ludmila Raisingerová, Chahrazed Rahmouni, Holger
665 Zimmermann and Aneesh P. H. Bose who helped to collect host samples. Michiel Jorissen is
666 gratefully acknowledged for fruitful discussion about monogenean morphological variability
667 and help in the lab. Special thanks go to Filip Volckaert (KU Leuven) and people in the
668 parasitological group at Masaryk University, Brno, Research Group Zoology at Hasselt
669 University, Research Centre of Hydrobiology in Uvira and Fisheries Research Unit in Mpulungu
670 for their hospitality.

671 **Funding**

672 Research has been supported by the Czech Science Foundation (P505/12/G112 (ECIP) and
673 GA19-13573S), EMBRC Belgium - FWO project GOH3817N as well as by a joint program
674 between the Austrian agency for international mobility and cooperation in education, science
675 and research and the Ministry of Education, Youth and Sports (project number 8J18AT007), the
676 Austrian Agency for International Cooperation in Education and Research (OEAD; project
677 number CZ 08/2018; to SK). MPMV was co-financed by institutional funding of the Finnish
678 Museum of Natural History and the Belgian Directorate-General for Development Cooperation
679 and Humanitarian Aid (CEBioS program). ELRDK was supported by the Belgian Development
680 Cooperation through VLIR-UOS (VLADOC scholarship NDOC2016PR006 to ELRDK and South
681 Initiative project CD2018SIN218A101). Fieldwork was carried out with the approval of the
682 competent local authorities under mission statement 031/MINRST/CRH-U/2016 and the
683 permission of the Fisheries Department of Zambia and under a study permit issued by the
684 government of Zambia (SP 008732).

685

686 **References**

687 Agawin, N.S.R., Duarte, C.M., Agustí, S., 2000. Nutrient and temperature control of the
688 contribution of picoplankton to phytoplankton biomass and production. Limnol. Oceanogr.
689 45, 591–600. https://doi.org/10.4319/lo.2000.45.3.0591

690 Baldwin, R.E., Banks, M.A., Jacobson, K.C., 2012. Integrating fish and parasite data as a holistic
691 solution for identifying the elusive stock structure of Pacific sardines (*Sardinops sagax*).
692 Rev. Fish Biol. Fish. 22, 137–156. https://doi.org/10.1007/s11160-011-9227-5

693 Barson, M., Přikrylová, I., Vanhove, M.P.M., Huyse, T., 2010. Parasite hybridization in African
694 *Macrogyrodactylus* spp. (Monogenea, Platyhelminthes) signals historical host distribution.
695 Parasitology 137, 1585–1595. https://doi.org/10.1017/S0031182010000302

696 Beaumont, M., 1997. Book review: Sublethal and chronic effects of pollutants on freshwater
697 fish, edited by R. Müller and R. Lloyd. Regul. Rivers Res. Manag. 13, 95–96.
698 https://doi.org/10.1002/(SICI)1099-1646(199701)13:1<95::AID-RRR428>3.0.CO;2-V

699 Brazenor, A.K., Saunders, R.J., Miller, T.L., Hutson, K.S., 2018. Morphological variation in the
700 cosmopolitan fish parasite *Neobenedenia girellae* (Capsalidae: Monogenea). Int. J.
701 Parasitol. 48, 125–134. https://doi.org/10.1016/J.IJPARA.2017.07.009

702 Brichard, P., 1978. Fishes of Lake Tanganyika.

703 Cable, J., Harris, P.D., 2002. Gyrodactylid developmental biology: historical review, current
704 status and future trends. Int. J. Parasitol. 32, 255–80.
705 https://doi.org/https://doi.org/10.1016/S0020-7519(01)00330-7

706 Catalano, S.R., Whittington, I.D., Donnellan, S.C., Gillanders, B.M., 2014. Parasites as biological
707 tags to assess host population structure: Guidelines, recent genetic advances and

708 comments on a holistic approach. Int. J. Parasitol. Parasites Wildl. 3, 220–6.
709 https://doi.org/10.1016/j.ijppaw.2013.11.001

710 Clark, F.N., 1945. Results of tagging experiments in California on Sardine (*Sardinops caerulea*).
711 Fish. Bull. 61, 93.

712 Coulter, G.W., 1991. Pelagic Fish, in: Coulter, G.W. (Ed.), Lake Tanganyika and Its Life. Natural
713 History Musem & Oxford University Press, London Oxford & New York, pp. 111–150.

714 Cox, C.F., Cox, M.A.A., 1989. Procrustes analysis, in: Cox, C.F., Cox, M.A.A. (Eds.),
715 Multidimentional Scaling. Second Edition. Chapman & Hall, London, pp. 123–139.
716 https://doi.org/10.1137/1028043

717 Dag, O., Dolgun, A., Konar, N.M., 2018. onewaytests: an R package for one-way tests in
718 independent groups designs. R J. 10, 175–199.

719 Danley, P.D., Husemann, M., Ding, B., Dipietro, L.M., Beverly, E.J., Peppe, D.J., 2012. The impact
720 of the geologic history and paleoclimate on the diversification of East African cichlids. Int.
721 J. Evol. Biol. 574851. https://doi.org/10.1155/2012/574851

722 Dávidová, M., Jarkovský, J., Matějusová, I., Gelnar, M., 2005. Seasonal occurrence and metrical
723 variability of *Gyrodactylus rhodei* Žitňan 1964 (Monogenea, Gyrodactylidae). Parasitol. Res.
724 95, 398–405. https://doi.org/10.1007/s00436-005-1311-0

725 De Keyzer, E.L.R., De Corte, Z., Van Steenberge, M., Raeymaekers, J.A.M., Calboli, F.C.F.,
726 Kmentová, N., N'Sibula Mulimbwa, T., Virgilio, M., Vangestel, C., Mulungula, P.M.,
727 Volckaert, F.A.M., Vanhove, M.P.M., 2019. First genomic study on Lake Tanganyika sprat
728 *Stolothrissa tanganicae*: a lack of population structure calls for integrated management of
729 this important fisheries target species. BMC Evol. Biol. 19, 6.

730 https://doi.org/10.1186/s12862-018-1325-8

731 Degens, E.T., Von Herzen, R.P., Wong, H.-K., 1971. Lake Tanganyika: Water chemistry,
732 sediments, geological structure. Naturwissenschaften 58, 229–241.
733 https://doi.org/10.1007/BF00602986

734 Descy, J.P., Hardy, M.A., Sténuite, S., Pirlot, S., Leporcq, B., Kimirei, I., Sekadende, B., Mwaitega,
735 S.R., Sinyenza, D., 2005. Phytoplankton pigments and community composition in Lake
736 Tanganyika. Freshw. Biol. 50, 668–684. https://doi.org/10.1111/j.1365-2427.2005.01358.x

737 Drummond, A.J., Rambaut, A., Shapiro, B., Pybus, O.G., 2005. Bayesian coalescent inference of
738 past population dynamics from molecular sequences. Mol. Biol. Evol. 22, 1185–1192.
739 https://doi.org/10.1093/molbev/msi103

740 Edgar, R.C., 2004. MUSCLE: Multiple sequence alignment with high accuracy and high
741 throughput. Nucleic Acids Res. 32, 1792–1797. https://doi.org/10.1093/nar/gkh340

742 Emmett, R.L., Brodeur, R.D., Miller, T.D., Pool, S.S., Krutzikowsky, G.K., Bentley, P.J., Mccrae, J.,
743 2005. Pacific sardine (*Sardinops sagax*) abundance, distribution, and ecological
744 relationships in the Pacific Northwest. Calif. Coop. Ocean. Fish. Investig. Rep. 46, 122–143.

745 Ergens, R., Gelnar, M., 1985. Experimental verification of the effect of temperature on the size
746 of the hard parts of haptor of *Gyrodactylus katharineri* Malberg 1964. Folia Parasitol.
747 (Praha). 32, 377–380.

748 Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform
749 population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10, 564–567.
750 https://doi.org/10.1111/j.1755-0998.2010.02847.x

751 Fox, J., Weisberg, S., 2011. An {R} companion to applied regression, Second. ed. Sage, Thousand

752 Oaks {CA}.

753 Franssen, F., Bilska-Zając, E., Deksne, G., Sprong, H., Pozio, E., Rosenthal, B., Rozycki, M., van
754 der Giessen, J., 2015. Genetic evidence of interspecies introgression of mitochondrial
755 genomes between *Trichinella spiralis* and *Trichinella britovi* under natural conditions.
756 Infect. Genet. Evol. 36, 323–332. https://doi.org/10.1016/J.MEEGID.2015.10.005

757 Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking
758 and background selection. Genetics 147, 915–25.

759 García-Rodríguez, F.J., García-Gasca, S.A., Cruz-Agüero, J.D. La, Cota-Gómez, V.M., 2011. A
760 study of the population structure of the Pacific sardine *Sardinops sagax* (Jenyns, 1842) in
761 Mexico based on morphometric and genetic analyses. Fish. Res. 107, 169–176.
762 https://doi.org/10.1016/J.FISHRES.2010.11.002

763 Gonzalez, E.G., Zardoya, R., 2007. Relative role of life-history traits and historical factors in
764 shaping genetic population structure of sardines (*Sardina pilchardus*). BMC Evol. Biol. 7,
765 197. https://doi.org/10.1186/1471-2148-7-197

766 Hauser, L., Carvalho, G.R., Pitcher, T.J., 1998. Genetic population structure in the Lake
767 Tanganyika sardine *Limnothrissa miodon*. J. Fish Biol. 53, 413–429.
768 https://doi.org/10.1111/j.1095-8649.1998.tb01040.x

769 Hecky, R.E., Fee, E.J., Kling, H., Rudd, J.W.M., 1978. Studies on the planktonic ecology of Lake
770 Tanganyika. Winnipeg, Man.:Fisheries and Marine Service.

771 Hedrick, P.W., 2013. Adaptive introgression in animals: examples and comparison to new
772 mutation and standing variation as sources of adaptive variation. Mol. Ecol. 22, 4606–
773 4618. https://doi.org/10.1111/mec.12415

774 Hellberg, M.E., Burton, R.S., Neigel, J.E., Palumbi, S.R., 2002. Genetic assessment of connectivity
775 among marine populations. Bull. Mar. Sci. 70, 273–290.

776 Henrich, T., Benesh, D.P., Kalbe, M., 2013. Hybridization between two cestode species and its
777 consequences for intermediate host range. Parasit. Vectors 6, 33.
778 https://doi.org/10.1186/1756-3305-6-33

779 Huyse, T., Van den Broeck, F., Hellemans, B., Volckaert, F.A.M., Polman, K., 2013. Hybridisation
780 between the two major African schistosome species of humans. Int. J. Parasitol. 43, 687–
781 689. https://doi.org/10.1016/J.IJPARA.2013.04.001

782 Huyse, T., Webster, B.L., Geldof, S., Stothard, J.R., Diaw, O.T., Polman, K., Rollinson, D., 2009.
783 Bidirectional introgressive hybridization between a cattle and human schistosome species.
784 PLoS Pathog. 5, e1000571. https://doi.org/10.1371/journal.ppat.1000571

785 James, A.G., Hutchings, L., Brownell, C.L., Horstman, D.A., 1988. Methods of capture and
786 transfer to the laboratory of wild pelagic fish. South African J. Mar. Sci. 6, 17–21.
787 https://doi.org/10.2989/025776188784480519

788 Jarkovský, J., Morand, S., Šimková, A., Gelnar, M., 2004. Reproductive barriers between
789 congeneric monogenean parasites (*Dactylogyrus*: Monogenea): Attachment apparatus
790 morphology or copulatory organ incompatibility? Parasitol. Res. 92, 95–105.
791 https://doi.org/10.1007/s00436-003-0993-4

792 Jombart, T., 2008. Adegenet: A R package for the multivariate analysis of genetic markers.
793 Bioinformatics 24, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

794 Kassambara, A., 2018. ggpubr: “ggplot2” based publication ready plots.

795 Kassambara, A., Mundt, F., 2017. factoextra: extract and visualize the results of multivariate

796 data analyses.

797 King, K.C., Stelkens, R.B., Webster, J.P., Smith, D.F., Brockhurst, M.A., 2015. Hybridization in
798 parasites: consequences for adaptive evolution, pathogenesis, and public health in a
799 changing w. PLOS Pathog. 11, e1005098. https://doi.org/10.1371/journal.ppat.1005098

800 Kinsey, S.T., Orsoy, T., Bert, T.M., Mahmoudi, B., 1994. Population structure of the Spanish
801 sardine *Sardinella aurita*: natural morphological variation in a genetically homogeneous
802 population. Mar. Biol. 118, 309–317. https://doi.org/10.1007/BF00349798

803 Kirchberger, P.C., Sefc, K.M., Sturmbauer, C., Koblmüller, S., 2012. Evolutionary history of Lake
804 Tanganyika's predatory deepwater cichlids. Int. J. Evol. Biol. 2012, 716209,
805 10.1155/2012/716209. https://doi.org/10.1155/2012/716209

806 Klingenberg, C.P., 2011. MORPHOJ: an integrated software package for geometric
807 morphometrics. Mol. Ecol. Resour. 11, 353–357. https://doi.org/10.1111/j.1755-
808 0998.2010.02924.x

809 Klingenberg, C.P., Monteiro, L.R., 2005. Distances and directions in multidimensional shape
810 spaces: implications for morphometric applications. Syst. Biol. 54, 678–688.
811 https://doi.org/10.1080/10635150590947258

812 Kmentová, N., Gelnar, M., Mendlová, M., Van Steenberge, M., Koblmüller, S., Vanhove, M.P.M.,
813 2016. Reduced host-specificity in a parasite infecting non-littoral Lake Tanganyika cichlids
814 evidenced by intraspecific morphological and genetic diversity. Sci. Rep. 6, 39605.
815 https://doi.org/10.1038/srep39605

816 Kmentová, N., Van Steenberge, M., Raeymaekers, J.A.R., Koblmüller, S., Hablützel, P.I., Muterezi
817 Bukinga, F., Mulimbwa N'sibula, T., Masilya Mulungula, P., Nzigidahera, B., Ntakimazi, G.,

818 Gelnar, M., Vanhove, M.P.M., 2018. Monogenean parasites of sardines in Lake Tanganyika:
819 diversity, origin and intra-specific variability. Contrib. to Zool. 87, 105–132.

820 Koblmüller, S., Odhiambo, E.A., Sinyinza, D., Sturmbauer, C., Sefc, K.M., 2015. Big fish, little
821 divergence: phylogeography of Lake Tanganyika's giant cichlid, *Boulengerochromis*
822 *microlepis*. Hydrobiologia 748, 29–38. https://doi.org/10.1007/s10750-014-1863-z

823 Koblmüller, S., Zangl, L., Börger, C., Daill, D., Vanhove, M.P.M., Sturmbauer, C., Sefc, K.M., 2019.
824 Only true pelagics mix: comparative phylogeography of deepwater bathybatine cichlids
825 from Lake Tanganyika. Hydrobiologia 832, 93–103. https://doi.org/10.1007/s10750-018-
826 3752-3

827 Kumar, S., Stecher, G., Tamura, K., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J.,
828 Ludwig, W., Glockner, F., 2016. MEGA7: Molecular evolutionary genetics analysis Version
829 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.
830 https://doi.org/10.1093/molbev/msw054

831 Kurki, H., Vuorinen, I., Bosma, E., Bwebwa, D., 1999. Spatial and temporal changes in copepod
832 zooplankton communities of Lake Tanganyika, in: From Limnology to Fisheries: Lake
833 Tanganyika and Other Large Lakes. Springer Netherlands, Dordrecht, pp. 105–114.
834 https://doi.org/10.1007/978-94-017-1622-2_10

835 Langenberg, V.T., Mwape, L.M., Tshibangu, K., Tumba, J.-M., Koelmans, A.A., Roijackers, R.,
836 Salonen, K., Sarvala, J., Mölsä, H., 2002. Comparison of thermal stratification, light
837 attenuation, and chlorophyll- a dynamics between the ends of Lake Tanganyika. Aquat.
838 Ecosyst. Health Manag. 5, 255–265. https://doi.org/10.1080/14634980290031956

839 Langenberg, V.T., Sarvala, J., Roijackers, R., 2003. Effect of wind induced water movements on
840 nutrients, chlorophyll- a , and primary production in Lake Tanganyika. Aquat. Ecosyst.

841 Health Manag. 6, 279–288. https://doi.org/10.1080/14634980301488

842 Leigh, J.W., Bryant, D., 2015. PopART : full-feature software for haplotype network
843 construction. Methods Ecol. Evol. 6, 1110–1116. https://doi.org/10.1111/2041-
844 210X.12410

845 Littlewood, D.T.J., Rohde, K., Clough, K.A., 1997. Parasite speciation within or between host
846 species? - Phylogenetic evidence from site-specific polystome monogeneans. Int. J.
847 Parasitol. 27, 1289–1297. https://doi.org/10.1016/S0020-7519(97)00086-6

848 Lockyer, A.E., Olson, P.D., Ostergaard, P., Rollinson, D., Johnston, D.A., Attwood, S.W.,
849 Southgate, V.R., Horak, P., Snyder, S.D., Le, T.H., Agatsuma, T., McManus, D.P., Carmichael,
850 A.C., Naem, S., Littlewood, D.T.J., 2003. The phylogeny of the Schistosomatidae based on
851 three genes with emphasis on the interrelationships of *Schistosoma* Weinland, 1858.
852 Parasitology 126, 203–24. https://doi.org/https://doi.org/10.1017/S0031182002002792

853 Mandima, J.J., 1999. The food and feeding behaviour of *Limnothrissa miodon* (Boulenger, 1906)
854 in Lake Kariba, Zimbabwe, in: From Limnology to Fisheries: Lake Tanganyika and Other
855 Large Lakes. Springer Netherlands, Dordrecht, pp. 175–182. https://doi.org/10.1007/978-
856 94-017-1622-2_16

857 Marshall, B.E., 1993. Biology of the African clupeid *Limnothrissa miodon* with reference to its
858 small size in artificial lakes. Rev. Fish Biol. Fish. 3, 17–38.
859 https://doi.org/10.1007/BF00043296

860 Martínez, P., González, E.G., Castilho, R., Zardoya, R., 2006. Genetic diversity and historical
861 demography of Atlantic bigeye tuna (*Thunnus obesus*). Mol. Phylogenet. Evol. 39, 404–416.
862 https://doi.org/10.1016/j.ympev.2005.07.022

863 Mattiucci, S., Abaunza, P., Ramadori, L., Nascetti, G., 2004. Genetic identification of *Anisakis*
864 larvae in European hake from Atlantic and Mediterranean waters for stock recognition. J.
865 Fish Biol. 65, 495–510. https://doi.org/10.1111/j.0022-1112.2004.00465.x

866 McGlue, M.M., Lezzar, K.E., Cohen, A.S., Russell, J.M., Tiercelin, J.-J., Felton, A.A., Mbede, E.,
867 Nkotagu, H.H., 2008. Seismic records of late Pleistocene aridity in Lake Tanganyika, tropical
868 East Africa. J. Paleolimnol. 40, 635–653. https://doi.org/10.1007/s10933-007-9187-x

869 Meinilä, M., Kuusela, J., Ziętara, M.S., Lumme, J., 2004. Initial steps of speciation by geographic
870 isolation and host switch in salmonid pathogen *Gyrodactylus salaris* (Monogenea:
871 Gyrodactylidae). Int. J. Parasitol. 34, 515–526.
872 https://doi.org/10.1016/j.ijpara.2003.12.002

873 Misund, O., 1993. Dynamics of moving masses: variability in packing density, shape, and size
874 among herring, sprat, and saithe schools. ICES J. Mar. Sci. 50, 145–160.
875 https://doi.org/10.1006/jmsc.1993.1016

876 Miura, O., Torchin, M.E., Kuris, A.M., Hechinger, R.F., Chiba, S., 2006. Introduced cryptic species
877 of parasites exhibit different invasion pathways. Proc. Natl. Acad. Sci. U. S. A. 103, 19818–
878 23. https://doi.org/10.1073/pnas.0609603103

879 Mo, A., 1993. Seasonal variation of haptoral hard parts of *Gyrodactylus derjavini* Mikailov 1975
880 (Monogenea: Gyrodactylidae) on brown trout *Salmo trutta* L. parr and Atlantic salmon
881 *Salmo salar* L. parr in the River Sandviksela, N. Syst. Parasitol. 26, 225–231.

882 Mölsä, H., Reynolds, J.E., Coenen, E.J., Lindqvist, O.V., 1999. Fisheries research towards
883 resource management on Lake Tanganyika. Hydrobiologia 407, 1–24.
884 https://doi.org/10.1023/A:1003712708969

885 Mulimbwa, N., Mannini, P., 1993. Demographic characteristics of *Stolothrissa tanganicae*,
886 *Limnothrissa miodon* and *Lates stappersii* in the Northwestern (Zairean) waters of Lake
887 Tanganyika. CIFA Occas. Pap.

888 Mulimbwa, N., Shirakihara, K., 1994. Growth, recruitment and reproduction of sardines
889 (*Stolothrissa tanganicae* and *Limnothrissa miodon*) in northwestern Lake Tanganyika.
890 Tropics 4, 57–67. https://doi.org/10.3759/tropics.4.57

891 Nevado, B., Mautner, S., Sturmbauer, C., Verheyen, E., 2013. Water-level fluctuations and
892 metapopulation dynamics as drivers of genetic diversity in populations of three
893 Tanganyikan cichlid fish species. Mol. Ecol. 22, 3933–3948.
894 https://doi.org/10.1111/mec.12374

895 Nieberding, C.M., Olivieri, I., 2007. Parasites: proxies for host genealogy and ecology? Trends
896 Ecol. Evol. 22, 156–165. https://doi.org/10.1016/j.tree.2006.11.012

897 Okamoto, M., Nakao, M., Blair, D., Anantaphruti, M.T., Waikagul, J., Ito, A., 2010. Evidence of
898 hybridization between *Taenia saginata* and *Taenia asiatica*. Parasitol. Int. 59, 70–74.
899 https://doi.org/10.1016/j.parint.2009.10.007

900 Olstad, K., Bachmann, L., Bakke, T. a, 2009. Phenotypic plasticity of taxonomic and diagnostic
901 structures in gyrodactylosis-causing flatworms (Monogenea, Platyhelminthes).
902 Parasitology 136, 1305–1315. https://doi.org/10.1017/S0031182009990680

903 Ondračková, M., Matějusová, I., Grabowska, J., 2012. Introduction of *Gyrodactylus perccotti*
904 (Monogenea) into Europe on its invasive fish host, Amur sleeper (*Perccottus glenii*,
905 Dybowski 1877). Helminthologia 49, 21–26. https://doi.org/10.2478/s11687-012-0004-3

906 Pariselle, A., Boeger, W.A., Snoeks, J., Bilong Bilong, C.F., Morand, S., Vanhove, M.P.M., 2011.

907 The monogenean parasite fauna of cichlids: a potential tool for host biogeography. Int. J.
908 Evol. Biol. 2011, 471480, 10.4061/2011/471480. https://doi.org/10.4061/2011/471480

909 Pariselle, A., Muterezi Bukinga, F., Van Steenberge, M., Vanhove, M.P.M., 2015.
910 Ancyrocephalidae (Monogenea) of Lake Tanganyika: IV: *Cichlidogyrus* parasitizing species
911 of Bathybatini (Teleostei, Cichlidae): reduced host-specificity in the deepwater realm?
912 Hydrobiologia 748, 99–119. https://doi.org/10.1007/s10750-014-1975-5

913 Pettersen, R.A., Mo, T.A., Hansen, H., Vøllestad, L.A., 2015. Genetic population structure of
914 *Gyrodactylus thymalli* (Monogenea) in a large Norwegian river system. Parasitology 142,
915 1693–1702. https://doi.org/10.1017/S003118201500133X

916 Phiri, H., Shirakihara, K., 1999. Distribution and seasonal movement of pelagic fish in southern
917 Lake Tanganyika. Fish. Res. 41, 63–71. https://doi.org/10.1016/S0165-7836(99)00008-9

918 Plisnier, P.-D., Chitamwebwa, D., Mwape, L., Tshibangu, K., Langenberg, V., Coenen, E., 1999.
919 Limnological annual cycle inferred from physical-chemical fluctuations at three stations of
920 Lake Tanganyika, in: Lindqvist, O.V., Mölsä, H., Salonen, K., Sarvala, J. (Eds.), From
921 Limnology to Fisheries: Lake Tanganyika and Other Large Lakes. Springer Netherlands,
922 Dordrecht, pp. 45–58. https://doi.org/10.1007/978-94-017-1622-2_4

923 Plisnier, P.D., Mgana, H., Kimirei, I., Chande, A., Makasa, L., Chimanga, J., Zulu, F., Cocquyt, C.,
924 Horion, S., Bergamino, N., Naithani, J., Deleersnijder, E., André, L., Descy, J.P., Cornet, Y.,
925 2009. Limnological variability and pelagic fish abundance (*Stolothrissa tanganicae* and
926 *Lates stappersii*) in Lake Tanganyika. Hydrobiologia 625, 117–134.
927 https://doi.org/10.1007/s10750-009-9701-4

928 Poulin, R., 2007. Evolutionary ecology of parasites. 2nd ed. Princeton University Press,
929 Princeton, New Jersey.

930 R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for
931 Statistical Computing, Vienna, Austria.

932 Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior summarization
933 in Bayesian phylogenetics using Tracer 1.7. Syst. Biol 67, 2.
934 https://doi.org/10.1093/sysbio/syy032

935 Řehulková, E., Mendlová, M., Šimková, A., 2013. Two new species of *Cichlidogyrus*
936 (Monogenea: Dactylogyridae) parasitizing the gills of African cichlid fishes (Perciformes)
937 from Senegal: Morphometric and molecular characterization. Parasitol. Res. 112, 1399–
938 1410. https://doi.org/10.1007/s00436-013-3291-9

939 Rieseberg, L.H., Kim, S.-C., Randell, R.A., Whitney, K.D., Gross, B.L., Lexer, C., Clay, K., 2007.
940 Hybridization and the colonization of novel habitats by annual sunflowers. Genetica 129,
941 149–165. https://doi.org/10.1007/s10709-006-9011-y

942 Rodríguez-González, A., Míguez-Lozano, R., Llopis-Belenguer, C., Balbuena, J.A., 2015.
943 Phenotypic plasticity in haptoral structures of *Ligophorus cephali* (Monogenea:
944 Dactylogyridae) on the flathead mullet (*Mugil cephalus*): a geometric morphometric
945 approach. Int. J. Parasitol. 45, 295–303. https://doi.org/10.1016/j.ijpara.2015.01.005

946 Rohlf, F., 2006. Tpsdig, digitize landmarks and outlines. Version 2.10. Stony Brook, NY: State
947 University.

948 Rohlf, F.J., 1993. Relative warp analysis and an example of its application to mosquito wings, in:
949 Marcus, L., Bello, E., Garcia-Valdecasas, A. (Eds.), Contributions to Morphometrics. Stony
950 Brook, NY: State University, New York, pp. 132–158.

951 Sako, A., O'Reilly, C.M., Hannigan, R., Bickford, N., Johnson, R.L., 2005. Variations in otolith

952 elemental compositions of two clupeid species, *Stolothrissa tanganicae* and *Limnothrissa*
953 *miodon* in Lake Tanganyika. Geochemistry Explor. Environ. Anal. 5, 91–97.
954 https://doi.org/10.1144/1467-7873/03-039

955 Schelkle, B., Faria, P.J., Johnson, M.B., van Oosterhout, C., Cable, J., 2012. Mixed infections and
956 hybridisation in monogenean parasites. PLoS One 7, e39506.
957 https://doi.org/10.1371/journal.pone.0039506

958 Sefc, K., Mattersdorfe, K., Ziegelbecker, A., Neuhüttler, N., Steiner, O., Goessler, W., Koblmüller,
959 S., 2017. Shifting barriers and phenotypic diversification by hybridization. Ecol. Lett. 20,
960 651–662.

961 Seixas, F.A., Boursot, P., Melo-Ferreira, J., 2018. The genomic impact of historical hybridization
962 with massive mitochondrial DNA introgression. Genome Biol. 19, 91.
963 https://doi.org/10.1186/s13059-018-1471-8

964 Shaw, P.W., Turner, G.F., Idid, M.R., Robinson, R.L., Carvalho, G.R., 2000. Genetic population
965 structure indicates sympatric speciation of Lake Malawi pelagic cichlids. Proc. R. Soc. B
966 Biol. Sci. 267, 2273–2280. https://doi.org/10.1098/rspb.2000.1279

967 Sturmbauer, C., Baric, S., Salzburger, W., Rüber, L., Verheyen, E., 2001. Lake level fluctuations
968 synchronize genetic divergences of cichlid fishes in African lakes. Mol. Biol. Evol 18, 144–
969 154.

970 Sturmbauer, C., Börger, C., Van Steenberge, M., Koblmüller, S., 2017. A separate lowstand lake
971 at the northern edge of Lake Tanganyika? Evidence from phylogeographic patterns in the
972 cichlid genus *Tropheus*. Hydrobiologia 791, 51–68. https://doi.org/10.1007/s10750-016-
973 2939-8

974 Suchard, M.A., Lemey, P., Baele, G., Ayres, D.L., Drummond, A.J., Rambaut, A., 2018. Bayesian
975 phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evol. 4.
976 https://doi.org/10.1093/ve/vey016

977 Tajima, F., 1989. Statistical methods to test for nucleotide mutation hypothesis by DNA
978 polymorphism. Genetics 123, 585–595. https://doi.org/PMC1203831

979 van Zwieten, P.A.M., Roest, F.C., Machiels, M.A.M., van Densen, W.L.T., 2002. Effects of inter-
980 annual variability, seasonality and persistence on the perception of long-term trends in
981 catch rates of the industrial pelagic purse-seine fishery of northern Lake Tanganyika
982 (Burundi). Fish. Res. 54, 329–348. https://doi.org/10.1016/S0165-7836(01)00267-3

983 Vignon, M., Pariselle, A., Vanhove, M.P.M., 2011. Modularity in attachment organs of African
984 *Cichlidogyrus* (Platyhelminthes: Monogenea: Ancyrocephalidae) reflects phylogeny rather
985 than host specificity or geographic distribution. Biol. J. Linn. Soc. 102, 694–706.
986 https://doi.org/10.1111/j.1095-8312.2010.01607.x

987 Vilas, R., Criscione, C.D., Blouin, M.S., 2005. A comparison between mitochondrial DNA and the
988 ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth
989 parasites. Parasitology 131, 839. https://doi.org/10.1017/S0031182005008437

990 Wang, M., Yan, S., Brown, C.L., Shaharom-harrison, F., Shi, S., Yang, T., 2016. Phylogeography of
991 *Tetrancistrum nebulosi* (Monogenea, Dactylogyridae) on the host of mottled spinefoot
992 (*Siganus fuscescens*) in the South China Sea, inferred from mitochondrial COI and ND2
993 genes. Mitochondrial DNA 27, 3865–3875.
994 https://doi.org/10.3109/19401736.2014.971240

995 Waples, R.S., 1987. A multispecies approach to the analysis of gen flow in marine shore fishes.
996 Evolution (N. Y). 41, 385. https://doi.org/10.2307/2409146

997 Wickham, H., 2009. Ggplot2 : elegant graphics for data analysis. Springer.

998 Yan, S., Wang, M., Yang, C.-P., Zhi, T.-T., Brown, C.L., Yang, T.-B., 2016. Comparative
999 phylogeography of two monogenean species (Mazocraeidae) on the host of chub
1000 mackerel, *Scomber japonicus*, along the coast of China. Parasitology 143, 594–605.
1001 https://doi.org/10.1017/S0031182016000160

1002 Zardoya, R., Castilho, R., Grande, C., Favre-Krey, L., Caetano, S., Marcato, S., Krey, G., Patarnello,
1003 T., 2004. Differential population structuring of two closely related fish species, the
1004 mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*), in the
1005 Mediterranean Sea. Mol. Ecol. 13, 1785–1798. https://doi.org/10.1111/j.1365-
1006 294X.2004.02198.x

1007 Zelditch, M., Swiderski, D.L., Sheets, H.D., 2012. Geometric morphometrics for biologists : a
1008 primer. Elsevier, London.

1009 Table 1: Number of fish specimens of (a) *Limnothrissa miodon* and (b) *Stolothrissa tanganicae* examined for monogenean parasites along with

1010 locality, basin and infection parameters. Values for *Kapentagyrus limnotrissae* and *Kapentagyrus tanganicanus* are shown before and after the slash,

1011 respectively).

Locality (geographic coordinates. year)	Locality – basins (Danley et al. 2012)	Number of fish specimens	Number of monogenean individuals	Prevalence (%)	Infection intensity	Abundance (range)	Number of COI haplotypes (Genbank a.n)	Number of microscopic slides (a.n. in RMCA)
				(a) *Limnothrissa miodon*				
Baraka (4°05'S 29°06'E; 29.7.2017)	The northern basin	24	10/63	16.7/41.7	2.5/5.4	0.4 (0-4)/2.1 (0-15)	-/26 (MK598222-47)	XXX/XXX
Bujumbura (3°23'S-29°22'E; 12.4.2018)	The northern basin	30	108/4	83/10	4.3/1.3	3.6 (0-17)/ 0.1 (0-2)	-/-	XXX/XXX
Kalemie (5°56'S-29°12'E; 11.8.2016)	The central basin	10	55/5	80/33	6.9/1.7	5.5 (0-15)/0.5(0-2)	23 (MK598078-100)/21 (MK598145-65)	XXX/XXX
Kalemie (12.4.2018)	The central basin	20	24/204	25/70	4.8/14.6	1.2 (0-11)/ 10.2 (0-37)	4 (MK598125-28)/8 (MK598137-44)	XXX/XXX
Mpulungu (8°46'S-31°07'E; 19.8.2016)	The southern basin	2	1/3	50/50	1/3	0.5(0-1)/1.5(0-3)	1 (MK598114)/3 (MK598248-50)	XXX/XXX
Mpulungu (7.4. – 21.4.2018)	The southern basin	81	60/452	28/63	2.6/9	0.7 (0-8)/ 5.6 (0-42)	6 (MK598115-20)/18 (MK598251-68)	XXX/XXX
Mvugo (4°18’S-29°34’E. 4.8.2016)	The northern basin	6	9/25	50/100	3/4.2	1.5 (0-3)/4.2 (1-10)	2 (MK598112-13)/17 (MK598205-21)	XXX/XXX
Mvuna Island (7°26’S-	The southern basin	6	11/5	50/50	3.7/1.7	1.8 (0-8)/0.8 (0-3)	5 (MK598101-5) /2 (MK598177-8)	XXX/XXX

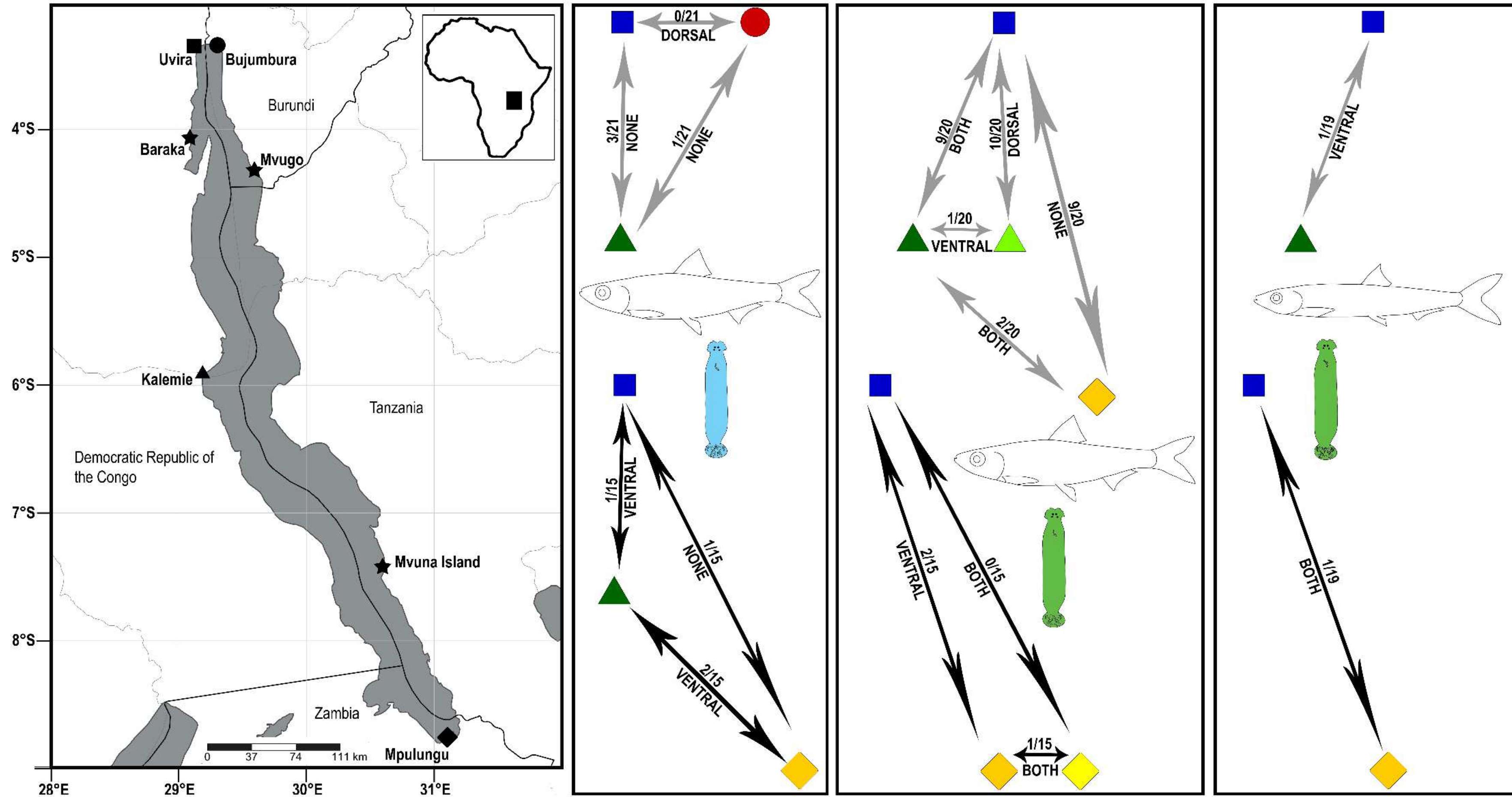
30°36′E 1.4.2015)								
Uvira (3°22′ S 29°08′E. 10.8. – 20.8.2016)	The northern basin	41	12/28	35/40	1.7/3.5	0.6 (0-3)/1.4(0-9)	6 (MK598106-11)/37 (MK598166-76, 79-204)	XXX/XXX
Uvira (12.4.2018)	The northern basin	30	43/70	53/76.7	2.7/3.0	1.4 (0-7)/ 2.3 (0-8)	4 (MK598121-24)/8 (MK598129-36)	XXX/XXX
(b) *Stolothrissa tanganicae*								
Bujumbura (12.4.2018)	The northern basin	30	5	16.7	1	0.17 (0-1)	-	XXX/XXX
Kalemie (11.8.2016)	The central basin	33	0	0	0	0	-	XXX/XXX
Kalemie (12.4.2018)	The central basin	30	44	66.7	2.2	1.5 (0-5)	7 (MK598317-23)	XXX/XXX
Mpulungu (19.8.2016)	The southern basin	18	2	11.1	1	0.11 (0-1)	3 (MK598269, 81-82)	XXX/XXX
Mpulungu (7.4. – 21.4.2018)	The southern basin	84	107	52.4	2.4	1.3 (0-13)	11 (MK598270-80)	XXX/XXX
Uvira (10.8. – 20.8.2016)	The northern basin	27	31	44	2.6	1.1 (0-6)	29 (MK598283-311)	XXX/XXX
Uvira (12.4.2018)	The northern basin	25	12	28	1.7	0.5 (0-3)	5 (MK598312-16)	XXX/XXX

Table 2: Genetic diversity indices of species of *Kapentagyrus*, and their hosts *Stolothrissa tanganicae* (De Keyzer et al., 2019) and *Limnothrissa miodon* (De Keyzer et al., unpublished) inferred from the COI mtDNA region.

Species	N	H	S	Hd	π	Max. divergence (%)
K. limnotrissae	51	19	18	0.6329±0.0800	0.002241±0.001742	1.2
K. tanganicanus	195	60	68	0.7341±0.0348	0.003800±0.002515	3.1
K. tanganicanus* ex *L. miodon	140	53	64	0.8066±0.0339	0.004499±0.002869	3.1
K. tanganicanus* ex *S. tanganicae	55	14	17	0.4983±0.0838	0.001991±0.001604	1.4
S. tanganicae	96	46	48	0.8583±0.0337	0.003543±0.002174	1.2
L. miodon	69	38	45	0.9250±0.0236	0.008909±0.004799	3.2

N sample size, H number of haplotypes, S number of polymorphic sites, Hd haplotype diversity, π nucleotide diversity.

1015 **Figure captions**



1016

1017 **Fig. 1: Sampling localities in Lake Tanganyika with an overview of significant results of morphometric (above arrow) and geomorphometrics**

1018 **(below arrow) between the specimens of *Kapentagyrus* spp. from the respected sampling sites.** A) *K. limnotrissae*, B) *K. tanganicanus* ex *L.*

1019 *miodon* and C) *K. tanganicanus* ex *S. tanganicae*. Results are presented as number of significantly different records out of all analysed

1020 morphometric parameters between the respected groups. In the case of geomorphometrics, difference in either ventral, dorsal, both or none of

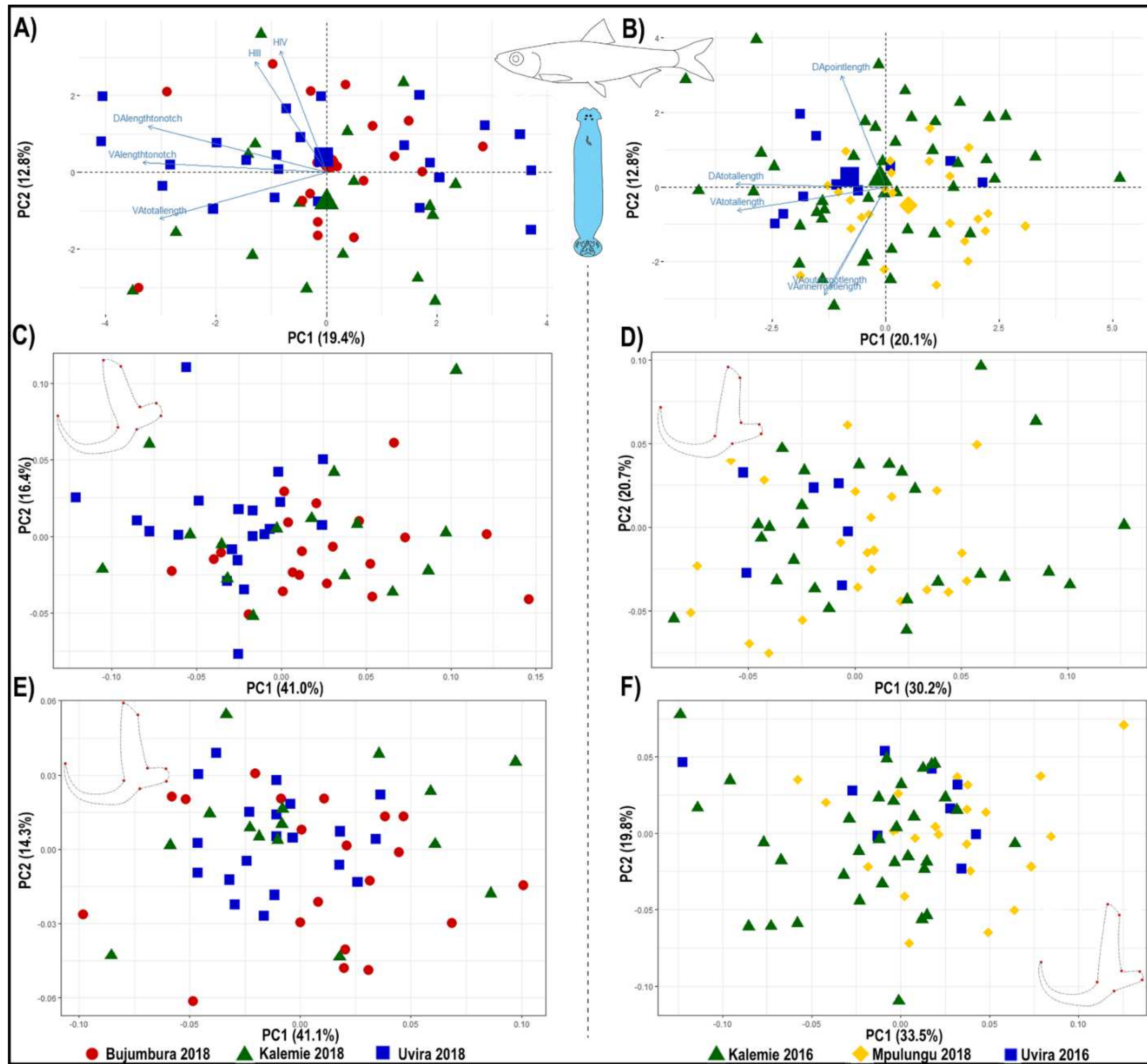
1021 the anchors is mentioned. Shapes of signs correspond with the sampling site origin of specimens in respective analyses. Colours of arrows refer to

1022 the separation based on fixative medium (grey – ethanol preserved specimens, black – fresh specimens). Map created using SimpleMappr

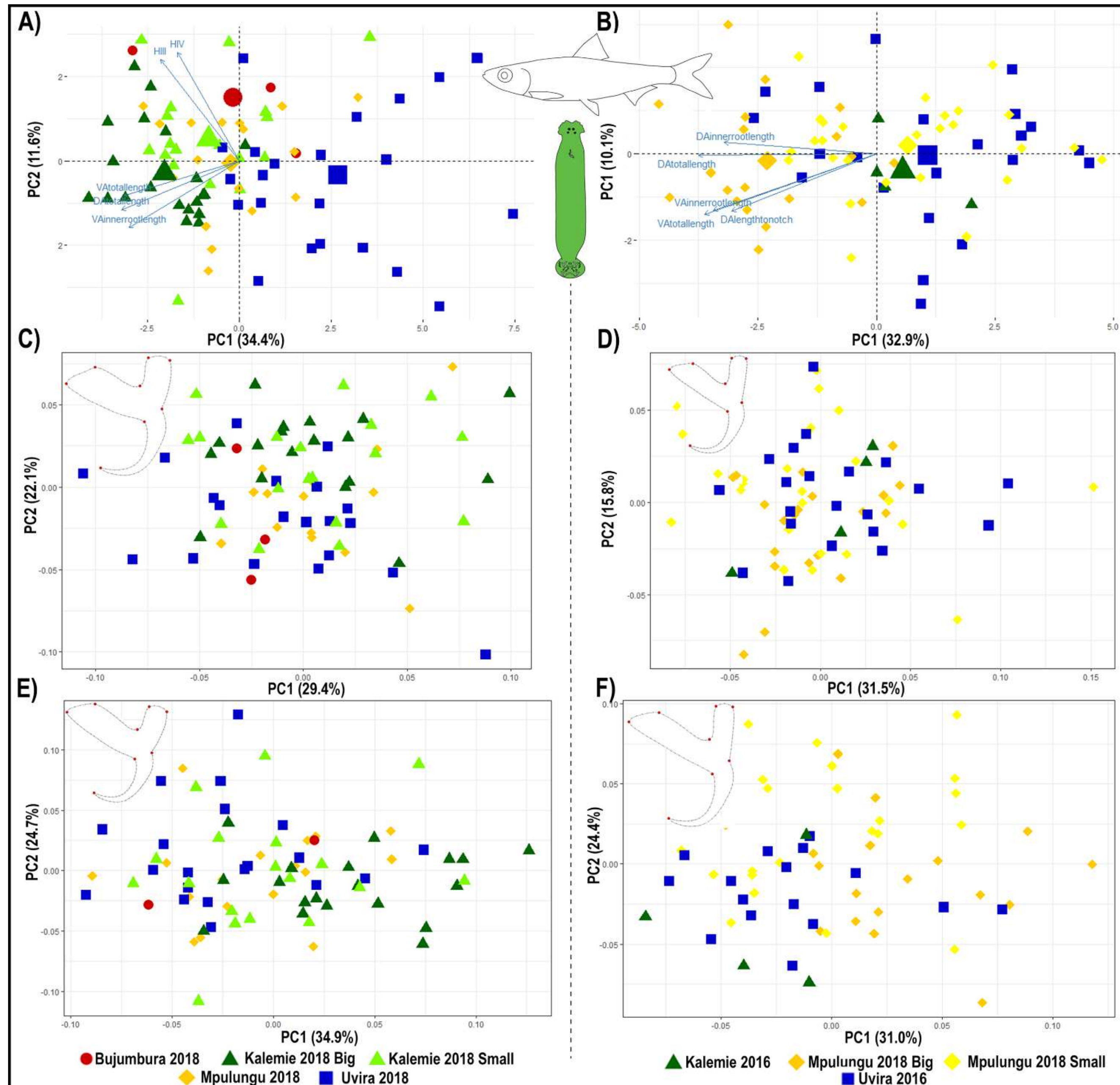
1023 software v7.0.0. (available at http://www.simplemappr.net. Accessed January 20, 2019).

1024

1025



1026 Fig. 2: **Biplots showing the variation in haptoral structures of *K. limnotrissae*. Only the first two axes are shown.** A) PCA of haptoral
1027 measurements with five best contributing variables indicates by arrows, ethanol-preserved specimens (fifth and seventh pair of marginal hooks
1028 excluded due to data missingness, the average position for each group is indicated by larger size of the symbol); B) PCA of haptoral measurements
1029 with five best contributing variables indicates by arrows, fresh specimens (second to seventh pair of marginal hooks excluded due to data
1030 missingness the average position for each group is indicated by larger size of the symbol); C) PCA based on Procrustes distances of eight fixed
1031 landmarks describing the shape of the monogeneans' dorsal anchor, ethanol preserved specimens; D) PCA based on Procrustes distances of eight
1032 fixed landmarks describing the shape of the monogeneans' dorsal anchor, fresh specimens; E) PCA based on Procrustes distances of eight fixed
1033 landmarks describing the shape of the monogeneans' ventral anchor, ethanol preserved specimens; F) PCA based on Procrustes distances of eight
1034 fixed landmarks describing the shape of the monogeneans' ventral anchor, ethanol preserved specimens. Consensus anchor's shape for the
1035 respected analysis is shown.
1036


A)
B)
C)
D)
E)
F)
PC1 (34.4%)
PC2 (11.6%)
PC1 (32.9%)
PC1 (10.1%)
PC1 (29.4%)
PC2 (22.1%)
PC1 (31.5%)
PC2 (15.8%)
PC1 (34.9%)
PC2 (24.7%)
PC1 (31.0%)
PC2 (24.4%)
HIII
HIV
VAtotallength
DAtotallength
VAinnerrootlength
DAinnerrootlength
DAtotallength
VAinnerrootlength
VAtotallength
DAlengthtonotch
Bujumbura 2018
Kalemie 2018 Big
Kalemie 2018 Small
Mpulungu 2018
Uvira 2018
Kalemie 2016
Mpulungu 2018 Big
Mpulungu 2018 Small
Uvira 2016

1038 **Fig. 3: Biplots showing the variation in haptoral structures of *K. tanganicanus* collected from *L. miodon* in this study. Only the first two axes are**

1039 **shown.** A) PCA of haptoral measurements with five best contributing variables indicates by arrows, ethanol-preserved specimens (fifth to seventh

1040 pair of marginal hooks excluded due to data missingness, the average position for each group, is indicated by larger size of the symbol); B) PCA of

1041 haptoral measurements with five best contributing variables indicates by arrows, fresh specimens (second to seventh pair of marginal hooks

1042 excluded due to data missingness, the average position for group is indicated by larger size of the symbol); C) PCA based on Procrustes distances

1043 of eight fixed landmarks describing the shape of the monogeneans' dorsal anchor, ethanol preserved specimens; D) PCA based on Procrustes

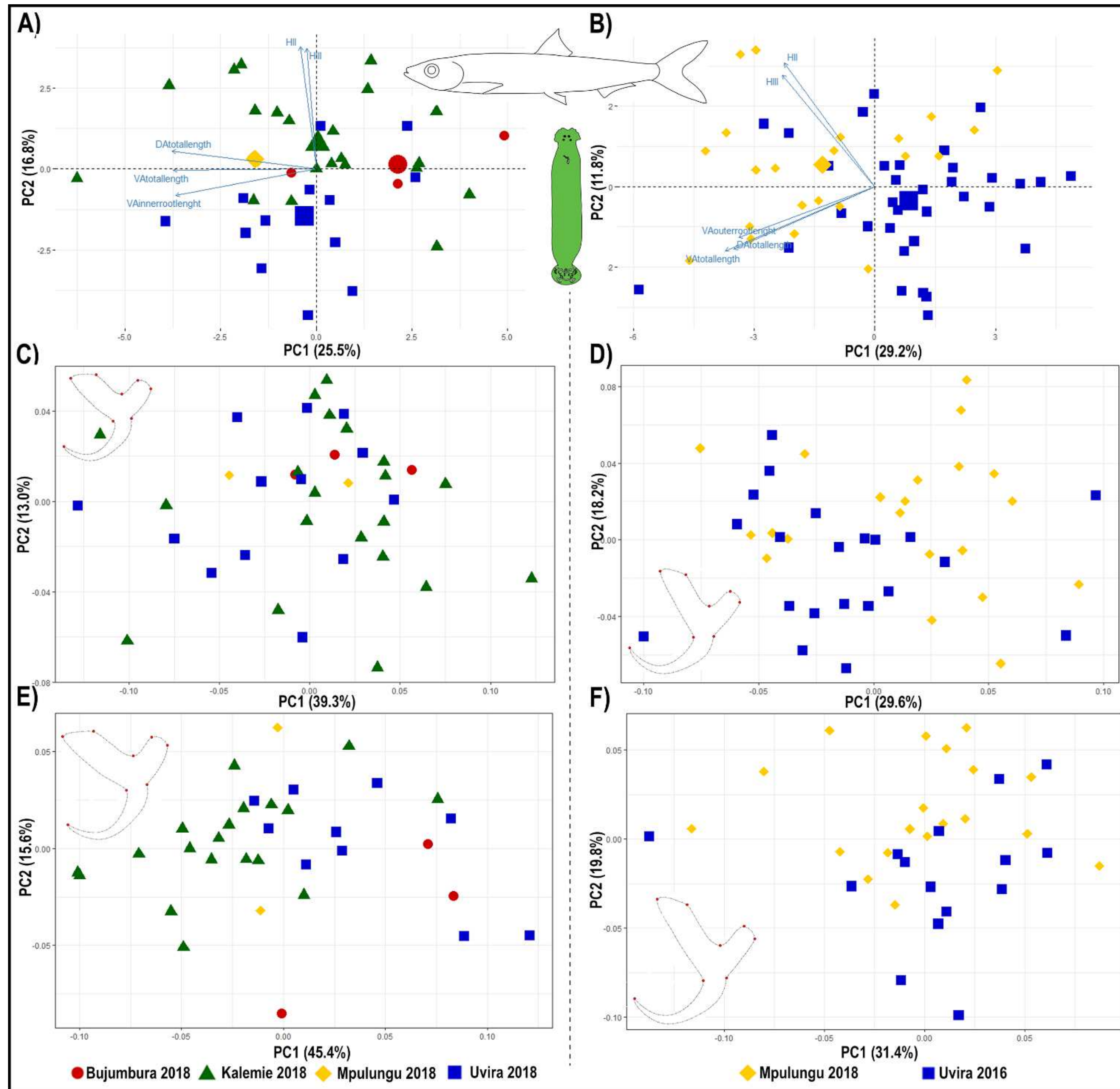
1044 distances of eight fixed landmarks describing the shape of the monogeneans' dorsal anchor, fresh specimens; E) PCA based on Procrustes

1045 distances of eight fixed landmarks describing the shape of the monogeneans' ventral anchor, ethanol preserved specimens; F) PCA based on

1046 Procrustes distances of eight fixed landmarks describing the shape of the monogeneans' ventral anchor, ethanol preserved specimens. Consensus

1047 anchor's shape for the respected analysis is shown.

1048


A)
B)
C)
D)
E)
F)
PC1 (25.5%)
PC2 (16.8%)
PC1 (29.2%)
PC2 (11.8%)
PC1 (39.3%)
PC2 (13.0%)
PC1 (29.6%)
PC2 (18.2%)
PC1 (45.4%)
PC2 (15.6%)
PC1 (31.4%)
PC2 (19.8%)
Bujumbura 2018
Kalemie 2018
Mpulungu 2018
Uvira 2018
Mpulungu 2018
Uvira 2016

1050 **Fig. 4: Biplots showing the variation in haptoral structures of *K. tanganicanus* collected from *S. tanganicae* in this study. Only the first two axes**

1051 **are shown.** A) PCA of haptoral measurements with five best contributing variables indicates by arrows, ethanol-preserved specimens (fifth and

1052 seventh pair of marginal hooks excluded due to data missingness, the average position for each group is indicated by larger size of the symbol); B)

1053 PCA of haptoral measurements with five best contributing variables indicates by arrows, fresh specimens (sixth and seventh pair of marginal

1054 hooks excluded due to data missingness, the average position for each group is indicated by larger size of the symbol); C) PCA based on Procrustes

1055 distances of eight fixed landmarks describing the shape of the monogeneans' dorsal anchor, ethanol preserved specimens; D) PCA based on

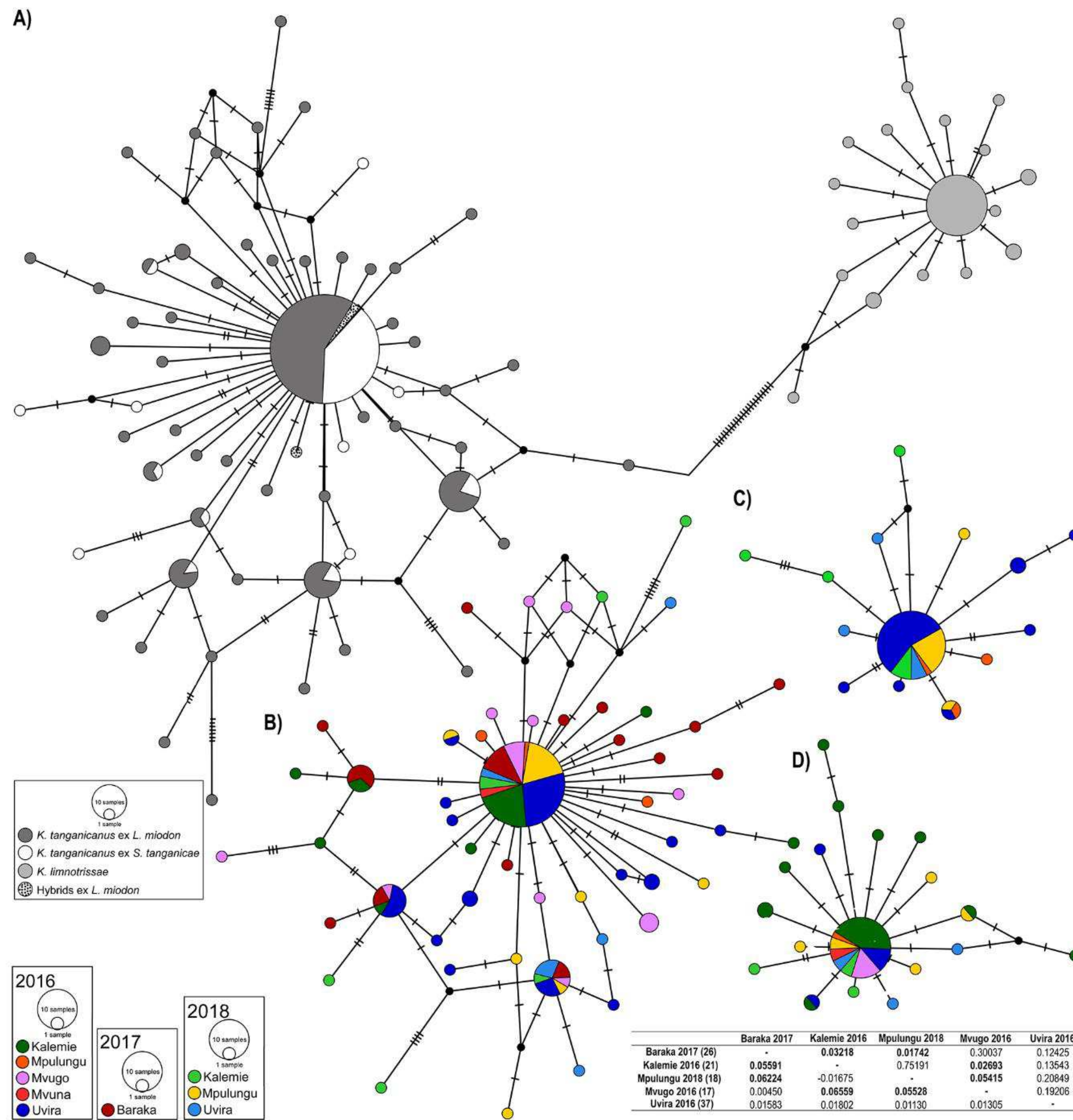
1056 Procrustes distances of eight fixed landmarks describing the shape of the monogeneans' dorsal anchor, fresh specimens; E) PCA based on

1057 Procrustes distances of eight fixed landmarks describing the shape of the monogeneans' ventral anchor, ethanol preserved specimens; F) PCA

1058 based on Procrustes distances of eight fixed landmarks describing the shape of the monogeneans' ventral anchor, ethanol preserved specimens.

1059 Consensus anchor's shape for the respected analysis is shown.

1060



	Baraka 2017	Kalemie 2016	Mpulungu 2018	Mvugo 2016	Uvira 2016
Baraka 2017 (26)	-	**0.03218**	**0.01742**	0.30037	0.12425
Kalemie 2016 (21)	**0.05591**	-	0.75191	**0.02693**	0.13543
Mpulungu 2018 (18)	**0.06224**	-0.01675	-	**0.05415**	0.20849
Mvugo 2016 (17)	0.00450	**0.06559**	**0.05528**	-	0.19206
Uvira 2016 (37)	0.01583	0.01802	0.01130	0.01305	-

1061

1062 **Fig. 5: Genetic population structure of *Kapentagyrus* spp. based on COI sequences.** Median joining haplotype network of A) *K. tanganicanus* and

1063 *K. limnotrissae* with hybrid individuals; B) *K. tanganicanus* ex *L. miodon*; C) *K. tanganicanus* ex *S. tanganicae*; D) *K. limnotrissae*. The circles

1064 represent different haplotypes with their size proportional to the number of individuals represented. Haplotypes are connected with lines,

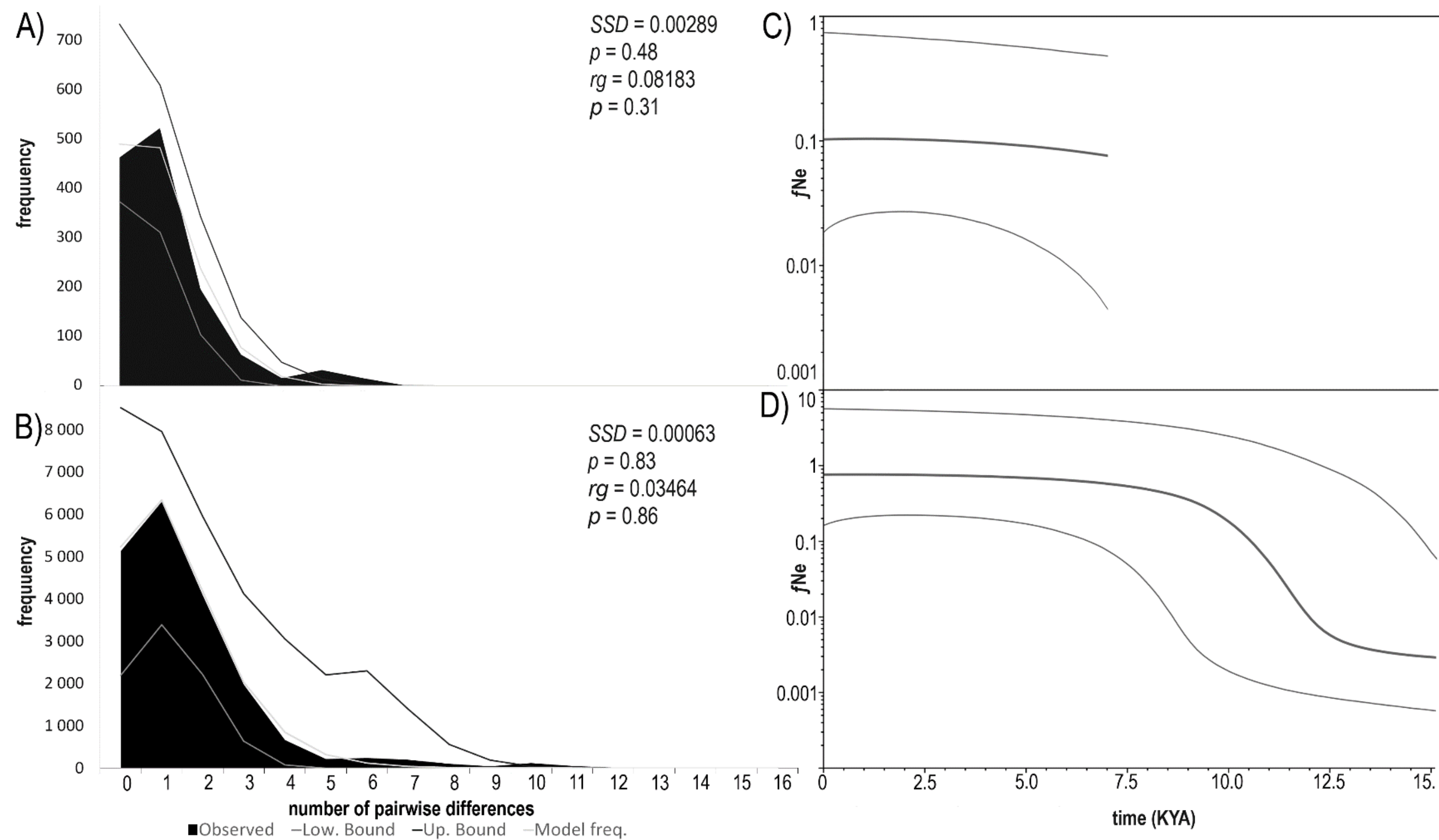
1065 indicating the number of mutations. Small black circles indicate hypothetical haplotypes, predicted by the model. Colours represent sampling

1066 events and host species, respectively, as mentioned in the legends. Genetic differentiation among geographically pre-defined subpopulations of *K.*

1067 *tanganicanus* ex *L. miodon* listed in the enclosed table. Pairwise FST estimates below diagonal and respective p-values above diagonal. Significant

1068 results with α=0.05 are marked in bold. Number of monogenean individuals in brackets.

1069



1070

1071 Fig. 6: **Demographic history of *Kapentagyrus* spp.** Mismatch distribution of A) *K. limnotrissae*; B) *K. tanganicanus*. The black bars show the

1072 observed frequency of pairwise differences. The grey lines refer to the expected distribution based on parameter estimates (plus 95% confidence

1073 limits) under a model of population growth. The sum of squared differences (SSD) and raggedness index (rg) and their respective p-values are

1074 given to describe the fit of the observed distribution to the expectations based on growth parameter estimates, as well as τ, the modal value of

1075 the mismatch distribution. Bayesian Skyline plot (BSP) of C) *K. limnotrissae* based on 415 base pairs of COI sequences; D) *K. tanganicanus* based on

1076 415 base pairs of COI sequences. BSPs show the effective populations size through time, assuming a substitution rate of 10% per site per million

1077 years in *Kapentagyrus* spp. The thick line represents the median values; the thin lines denote 95% highest posterior density (HPD) intervals. The y-

1078 axis represents the population size parameter (product of female effective population size, ƒNe, and mutation rate, μ).

1079

1080

1081

1082

1083

1084

1085

1086

1087

1088

1089

1090

1091

1092

1093

Supplementary tables

Table S1: Results of mixed linear model approach (lm or glm with F and Chi Square statistics, respectively) analysing the effect of sampling site origin in spatial as well as spatio-temporal sample sets corrected for the host size on morphometric parameters measured from *Kapentagyrus limnotrissae* and *K. tanganicanus* (haptoral morphologies from *L. miodon* and *S. tanganicae*, respectively). Only significant parameters of post-hoc testing between the respected sampling sites after Bonferroni correction are listed. Abbreviation of sampling sites with the year of collection and host size categories (as set mentioned methodological part for *K. tanganicanus* ex *L. miodon*): B – Bujumbura, K – Kalemie, M – Mpulungu, U - Uvira.

	K. limnotrissae	***K. tanganicanus* ex *L. miodon***	***K. tanganicanus* ex *S. tanganicae***
Dorsal anchor			
Total length	-	$X^2_{3,77}$=16.055 (P=0.001) K 2018 - U 2018 (P<0.001) K 2018 Small - U 2018 (P=0.005) M 2018 - U 2018 (P=0.052)	-
Length to notch	$X^2_{2,59}$=7.657 (P=0.005) B 2018 - K 2018 (P=0.008)	$X^2_{3,77}$= 13.808 (P=0.003) K 2018 Big - U 2018 (P<0.001) K 2018 Small - U 2018 (P=0.123) M 2018 - U 2018 (P=0.006)	$F_{1,54}$=5.141 (P=0.027) M 2018 - U 2016 (P=0.013)
Length of inner root	-	$F_{3,72}$= 7.071 (P=0.002) M 2018 Big - M 2018 Small (P<0.001) M 2018 Big- U 2016 (P<0.001)	-
Length of outer root	-	$X^2_{3,71}$= 6.794 (P=0.033) M 2018 Big - U 2016 (P=0.013)	-
Point length	$F_{2,58}$=2.020 (P=0.057) K 2018 - U 2018 (P=0.053)	-	-
Dorsal bar			
Maximum width	-	$F_{3,73}$=4.653 (P=0.005) K 2018 Big - U 2018 (P<0.001) K 2018 Small - U 2018 (P<0.001) M 2018 - U 2018 (P=0.043)	-
Ventral bar			

Branch length	$F_{2,77}$=4.694 (P=0.012) (no significant difference between respected sampling sites)	$X^2_{3,77}$=24.662 (P<0.001) K 2018 Big - U 2018 (P<0.001) K 2018 Small - U 2018 (P=0.004) M 2018 - U 2018 (P=0.012)	-
Maximum width	-	$X^2_{3,76}$=13.102 (P=0.004) K 2018 Big - U 2018 (P<0.001) K 2018 Small - U 2018 (P=0.004) M 2018 - U 2018 (P=0.012)	-
Marginal hooks			
HI	-	$X^2_{3,71}$=19.581 (P<0.001) K 2018 Big - U 2018 (P=0.002) K 2018 Small - U 2018 (P=0.001) M 2018 - U 2018 (P=0.011)	$X^2_{1,28}$=7.904 (P=0.005) K 2018 - U 2018 (P=0.005)
HII	-	$F_{3,69}$=5.816 (P=0.001) K 2018 Big - U 2018 (P=0.037) K 2018 Small - U 2018 (P=0.001) M 2018 - U 2018 (P=0.003)	-
HIV	-	$F_{3,53}$=3.435 (P=0.02) K 2018 Small - U 2018 (P=0.005) M 2018 - U 2018 (P=0.220)	-
HVI	-	$F_{3,49}$=4.057 (P=0.012) K 2018 Small - U 2018 (P=0.009) M 2018 - U 2018 (P=0.018)	-
Ventral anchor			
Total length	$\chi^2_{2,79}$=8.571 (P=0.014) M 2018 - K 2016 (P=0.009)	$X^2_{3,77}$=11.473 (P=0.009) K 2018 Big – K Small 2018 (P=0.001) K 2018 Big – M 2018 (P<0.001) K 2018 Big - U 2018 (P<0.001)	-
Length to notch	-	$F_{3,77}$=5.456 (P=0.002) K 2018 Big - M 2018 (P=0.002) K 2018 Big - U 2018 (P<0.001) K 2018 Small - U 2018 (P=0.004)	-
Length of outer root	$F_{2,79}$=3.898 (P=0.025) M 2018 - K 2016 (P=0.002) $F_{2,59}$=5.876 (P=0.005) K 2018 - U 2018 (P=0.019)	-	-
MCO			

Accessory piece	$F_{2,59}$=5.876 (P=0.005) K 2018 - U 2018 (P=0.019)	-	Not tested

1101

1102 Table S2: Procrustes distances based on a canonical variate analysis. P-values (in brackets) in bold mark significance at the level of α=0.05).

1103 Results for the dorsal anchor and ventral anchor are listed below/above the diagonal, respectively.

Kapentagyrus limnotrissae				
Locality (N_{ind})	Bujumbura 2018 (20)	Kalemie 2018 (15)	Uvira 2018 (20)	
Bujumbura 2018 (20)	-	0.0255 (0.3264)	0.0278 (0.0709)	
Kalemie 2018 (16)	0.0315 (0.2717)	-	0.0249 (0.2404)	
Uvira 2018 (20)	**0.0607 (0.0001)**	0.0417 (0.0660)	-	
	Kalemie 2016 (33)	Mpulungu 2018 (23)	Uvira 2016 (9)	
Kalemie 2016 (27)	-	**0.0548 (0.0001)**	**0.0522 (0.0111)**	
Mpulungu 2018 (23)	0.0334 (0.1023)	-	0.0461 (0.0510)	
Uvira 2016 (6)	0.0510 (0.1274)	0.0417 (0.2492)	-	
Kapentagyrus tanganicanus* ex *Limnothrissa miodon				
Locality (N_{ind})	Kalemie 2018 Big (18)	Kalemie 18 Small (18)	Mpulungu 2018 (17)	Uvira 2018 (20)
Kalemie 2018 Big (19)	-	**0.0467 (0.0076)**	**0.0524 (0.0018)**	**0.0702 (<.0001)**
Kalemie 18 Small (18)	0.0219 (0.5782)	-	0.0251 (0.4782)	0.0372 (0.0676)
Mpulungu 2018 (14)	**0.0417 (0.0147)**	0.0391 **(0.0197)**	-	0.0319 (0.1361)
Uvira 2018 (19)	**0.0437 (0.0018)**	0.0435 **(0.0026)**	0.0333 (0.0555)	-
	Mpulungu 2018 Big (19)	Mpulungu 2018 Small (24)	Uvira 2016 (16)	
Mpulungu 2018 Big (18)	-	0.0440 **(0.0076)**	**0.0504 (0.0022)**	
Mpulungu 2018 Small (25)	0.0361 **(0.0241)**	-	**0.0517 (0.0006)**	
Uvira 2016 (21)	0.0319 (0.0665)	0.0349 **(0.0226)**	-	
Kapentagyrus tanganicanus* ex *Stolothrissa tanganicae				
Locality (N_{ind})	Kalemie 2018 (19)	Uvira 2018 (10)		
Kalemie 2018 (20)	-	3.154 **(<.0001)**		
Uvira 2018 (13)	0.037 (0.156)	-		
	Mpulungu 2018 (19)	Uvira 2016 (15)		
Mpulungu 2018 (21)	-	0.046 **(0.013)**		
Uvira 2016 (21)	**0.046 (0.003)**	-		

1104

1105 Table S3: Comparison of measurements performed on *Kapentagyrus limnotrissae* haptoral and genital hard parts described. Standard deviation
1106 is shown only for cases with more 10 and more individuals (a – mean value±standard deviation, b – range).

	Ethanol preserved specimens			Fresh specimens		
	Bujumbura 2018	Kalemie 2018	Uvira 2018	Kalemie 2016	Mpulungu 2018	Uvira 2016
Dorsal anchor						
Total length	20.7±1.6[a] (18.8 - 24.3)[b]; n=20	20.3±1.7 (17.6 - 25.1); n=18	19.7±1.0 (17.3 - 22.0); n=21	21.4±1.4 (18.7 - 24.6); n=43	21.0±1.1 (18.8 - 22.9); n=24	22.2 (19.3 - 24.8); n=9
Length to notch	17±0.6 (16.1 - 18.2); n=20	16±0.9 (14.6 - 17.6); n=18	16.5±1.0 (14.0 - 17.7); n=21	16.4±1.1 (14.0 - 18.9); n=43	16.3±0.9 (14.6 - 18.5); n=24	16.9 (15.8 - 18.9); n=9
Inner root length	11.7±1.0 (9.7 - 13.3); n=20	11.8±0.6 (10.8 - 13.1); n=18	11.9±0.8 (10.2 - 13.2); n=20	11.3±1.2 (9.0 - 13.7); n=42	11.6±1.1 (9.9 - 15.6); n=24	11.4 (9.7 - 13.7); n=9
Outer root length	4.5±0.5 (3.5 - 5.4); n=20	4.8±0.7 (3.5 - 5.9); n=17	4.9±0.6 (3.9 - 6.4); n=21	4.4±0.9 (3.1 - 7.7); n=41	4.9±0.7 (3.4 - 6.1); n=24	4.7 (3.8 - 5.7); n=9
Point length	7.6±0.7 (6.1 - 8.8); n=20	7.3±0.4 (6.4 - 8.1); n=17	7.8±0.6 (6.9 - 9.2); n=21	7.6±1.1 (5.2 - 10.0); n=42	7.5±1.1 (5.1 - 9.7); n=23	8.3 (7.5 - 10.3); n=9
Dorsal bar						
Branch length	22.0±1.8 (18.4 - 25.6); n=23	22.6±3.0 (16.9 - 28.7); n=15	21.2±1.6 (17.8 - 23.7); n=22	16.3±1.7 (12.0 - 21.5); n=39	16.3±1.1 (14.5 - 18.0); n=23	16.4 (15.6 - 17.5); n=9
Branch maximum width	5.4±0.7 (3.8 - 6.9); n=23	5.3±0.8 (3.7 - 6.8); n=15	5.5±0.5 (4.5 - 6.4); n=21	4.1±0.5 (3.0 - 5.6); n=40	4.3±0.5 (3.0 - 5.1); n=22	4.2±0.2 (3.9 - 4.4); n=8
Ventral anchor						
Total length	25.2±1.6 (20.6 - 27.8); n=22	25.2±1.3 (21.4 - 26.8); n=18	24.7±1.7 (19.0 - 27.0); n=23	26.2±1.8 (20.3 - 31.8); n=43	25.3±1.0 (23.3 - 27.1); n=26	26.3±1.5 (23.2 - 28.1); n=10
Length to notch	18.9±0.9 (17.1 20.4); n=22	18.5±0.9 (16.8 - 20.0); n=18	18.9±1.3 (15.6 - 20.7); n=23	18.7±1.2 (15.5 - 22.2); n=43	18.4±1.1 (15.7 - 20.0); n=26	18.5±1.0 (16.5 - 19.9); n=10
Inner root length	16.2±0.9 (14.3 - 17.4); n=22	16.1±0.8 (14.6 - 17.7); n=17	16.4±0.9 (14.7 - 17.7); n=23	15.8±1.4 (13.2 - 18.6); n=44	15.9±0.7 (14.6 - 17.1); n=26	15.3±1.2 (13.4 - 17.3); n=10
Outer root length	5.2±0.5 (4.4 - 6.3); n=21	5.3±0.4 (4.5 - 6.1); n=16	5.4±0.5 (4.5 - 6.3); n=23	4.8±0.7 (3.7 - 6.5); n=44	5.4±0.5 (4.2 - 6.3); n=26	5.3 (4.8 - 6.2); n=9
Point length	8.1±0.6 (7.0 - 9.5); n=22	7.8±0.5 (6.8 - 8.7); n=18	8.2±0.7 (6.8 - 9.7); n=23	8.1±1.0 (6.0 - 10.4); n=39	7.7±0.7 (6.2 - 9.1); n=26	8.7 (7.3 - 10.6); n=9
Ventral bar						
Branch length	20.4±1.6 (17.9 - 23.7); n=22	19.8±2.0 (17.7 - 26.4); n=18	19.2±1.6 (16.2 - 22.8); n=21	15.3±1.1 (12.5 - 18.0); n=43	14.7±1.0 (12.2 - 17.0); n=25	15.7 (14.4 - 16.6); n=9
Branch maximum width	5.5±0.7 (4.2 - 7.2); n=21	5.3±0.6 (4.1 - 6.6); n=18	5.4±0.7 (4.2 - 6.5); n=21	4.3±0.6 (3.0 - 5.6); n=43	4.0±0.6 (2.9 - 5.5); n=24	4.4 (4.0 - 4.7); n=9

Hooks						
Pair I	15.3±1.1 (13.1 - 17.0); n=18	14.7±1.0 (12.8 - 16.5); n=17	15.3±0.8 (13.9 - 17.2); n=17	14.8±1.1 (12.0 - 16.7); n=29	15.0±0.9 (13.1 - 16.3); n=15	14.8±0.7 (13.7 - 15.7); n=10
Pair II	16.1±1.5 (13.3 - 19.4); n=21	16.1±1.2 (13.8 - 18.2); n=15	16.4±0.9 (15.0 - 18.3); n=20	15.4±1.0 (13.6 - 17.1); n=25	15.7±0.9 (14.0 - 17.2); n=11	15.3±1.3 (13.3 - 18.2); n=10
Pair III	16.5±1.1 (14.6 - 19.1); n=22	16.1±1.3 (13.4 - 18.5); n=17	16.4±1.1 (14.5 - 18.2); n=21	15.8±1.2 (14.3 - 19.3); n=24	15.8±1.3 (13.6 - 17.2); n=11	16.3±0.9 (15.3 - 17.5); n=10
Pair IV	17.±1.2 (14.5 - 19.6); n=20	16.7±1.2 (14.6 - 18.3); n=15	16.7±1.2 (15.0 - 18.3); n=14	16.0±0.9 (14.2 - 18.0); n=23	16.4±1.3 (14.0 - 18.4); n=10	16.2±0.9 (14.3 - 17.5); n=10
Pair V	16.1±1.0 (14.5 - 17.8); n=21	15.2 (13.0 - 17.4); n=9	15.6±0.8 (14.1 - 16.6); n=14	14.6±1.2 (12.8 - 17.0); n=19	14.8 (13.2 - 15.9); n=7	14.8 (12.9 - 15.8); n=5
Pair VI	16.8±1.1 (14.4 - 19.2); n=20	16.6±1.2 (14.9 - 18.6); n=14	16.7±1.1 (14.0 - 18.4); n=15	16.1±0.8 (14.6 - 17.1); n=15	16.7 (15.0 - 18.7); n=8	15.4 (13.0 - 16.9); n=7
Pair VII	16.3±0.8 (14.7 - 18.0); n=12	16.4 (15.1 - 18.1); n=9	16.6±0.9 (15.0 - 17.8); n=12	16.2±1.3 (14.3 - 17.9); n=11	16.6 (15.3 - 18.1); n=7	16.2 (15.2 - 17.4); n=6
Male copulatory organ						
Copulatory tube curved length	28.3±1.3 (26.4 - 31.5); n=19	29.5±2.5 (24.2 - 35.3); n=16	28.4±2.2 (23.0 - 32.2); n=24	30.1±2.0 (26.2 - 33.8); n=36	32.2 (28.6 - 34.9); n=3	30.0 (27.5 - 32.5); n=7
Accessory piece curved length	33.6±2.6 (27.9 - 39.1); n=20	34.2±3.2 (30.3 - 40.2); n=16	31.6±2.6 (26.7 - 36.4); n=23	34.4±3.1 (28.0 - 41.4); n=35	33.3 (30.1 - 38.3); n=3	34.4 (32.2 - 45.6); n=7

1107

1108

1109

1110

1111

1112

1113

1114

1115 Table S4: Comparison of measurements performed on *Kapentagyrus tanganicanus* ex *Limnothrissa miodon* haptoral and genital hard parts
1116 described. Standard deviation is shown only for cases with more 10 and more individuals (a – mean value±standard deviation, b – range).

	Ethanol preserved specimens					**Fresh specimens**			
	Bujumbura 2018	Kalemie 2018 Big	Kalemie 2018 Small	Mpulungu 2018	Uvira 2018	Kalemie 2016	Mpulungu 2018 Big	Mpulungu 2018 Small	Uvira 2016
Dorsal anchor									
Total length	27.2±1.6 (22.9 - 30.1); n=18	28.7±1.4 (26.3 - 31.4); n=21	27.0±1.8 (23.2 - 30.0); n=18	24.6±2.5 (20.0 - 28.0); n=23	27.3 (26.9 - 27.9); n=24	28.5±1.8 (24.3 - 32.2); n=4	29.7±1.4 (27.2 - 32.2); n=19	27.5±1.6 (24.3 - 31.3); n=26	27.1 (25.1 - 28.8); n=24
Length to notch	19.9 (18.5 - 21.6); n=5	21.3±1.1 (18.4 - 23.9); n=21	20.6±1.0 (18.6 - 22.6); n=18	21.5±2.2 (19.0 - 28.7); n=15	19.4±1.4 (16.9 - 21.8); n=23	19.9 (19.5 - 20.5); n=4	20.9±1.1 (19.3 - 22.8); n=19	20.0±1.6 (16.8 - 24.0); n=26	20.0±1.2 (17.9 - 21.9); n=23
Inner root length	14.8 (14.1 - 15.5); n=5	17.1±1.3 (13.5 - 19.1); n=21	16.5±1.5 (13.5 - 18.7); n=18	16.1±1.3 (13.2 - 17.7); n=15	15.3±1.7 (12.1 - 18.1); n=23	14.9 (13.9 - 16.4); n=4	17.0±1.1 (14.4 - 19.0); n=19	15.3±1.2 (12.5 - 17.1); n=26	14.7±1.5 (11.1 - 16.5); n=23
Outer root length	8.2 (7.2 - 8.7); n=5	8.3±0.8 (7.0 - 10.3); n=21	8.7±1.0 (6.6 - 10.7); n=17	8.6±0.8 (6.9 - 10.0); n=15	8.3±1.1 (5.2 - 10.3); n=23	8.8 (6.9 - 9.8); n=4	9.3±1.1 (7.8 - 12.9); n=19	8.7±1.2 (5.8 - 11.5); n=25	8.3±1.0 (6.8 - 10.2); n=23
Point length	8.9 (8.1 - 9.7); n=5	8.3±0.7 (7.1 - 9.5); n=21	8.2±0.7 (7.0 - 9.5); n=17	8.1±0.7 (7.0 - 9.3); n=15	7.8±0.7 (6.5 - 8.9); n=23	8.0 (7.5 - 8.7); n=4	8.6±1.1 (6.8 - 10.8); n=19	8.1±0.9 (6.1 - 10.0); n=25	8.5±1.3 (5.7 - 11.4); n=23
Dorsal bar									
Branch length	29.1 (25.9 - 33.3); n=5	29.8±2.5 (24.9 - 34.6); n=21	27.8±2.4 (22.5 - 32.3); n=17	28.6±2.1 (25.4 - 33.8); n=14	27.1±2.6 (22.9 - 34.7); n=23	21.3 (20.1 - 22.3); n=4	22.5±1.9 (18.7 - 25.6); n=19	20.9±1.6 (18.7 - 23.5); n=22	20.9±1.4 (18.4 - 23.6); n=22
Branch maximum width	7.3 (6.0 - 8.6); n=5	8.4±0.6 (7.4 - 9.7); n=20	8.4±1.2 (6.4 - 10.2); n=17	7.9±1.5 (5.4 - 10.3); n=14	6.9±1.0 (5.2 - 8.6); n=23	6.2 (5.7 - 7.1); n=4	6.4±0.8 (4.8 - 7.6); n=19	6.0±0.9 (4.0 - 8.8); n=22	6.0±1.0 (4.0 - 8.7); n=22
Ventral anchor									
Total length	30.4 (29.3 - 31.5); n=5	34.3±1.8 (30.7 - 38.6); n=20	31.2±2.2 (24.9 - 34.8); n=18	30.8±2.6 (25.3 - 33.4); n=17	28.8±3.0 (24.9 - 33.9); n=23	32.1 (31.0 - 33.4); n=4	34.8±2.0 (30.1 - 38.0); n=19	31.6±2.4 (27.3 - 35.6); n=25	31.8±2.3 (27.8 - 36.7); n=23
Length to notch	22.3 (20.9 - 23.6); n=5	23.2±0.7 (21.7 - 24.3); n=20	22.2±1.3 (19.6 - 24.5); n=18	21.8±0.8 (20.1 - 23.1); n=17	20.9±1.6 (17.4 - 23.6); n=23	21.6 (20.6 - 22.8); n=4	22.2±0.9 (20.5 - 23.9); n=19	21.2±1.1 (18.0 - 22.8); n=25	21.1±1.7 (16.5 - 24.3); n=23

Inner root length	20.4 (19.5 - 21.3); n=5	21.8±1.1 (19.7 - 24.0); n=21	21.1±1.6 (17.0 - 23.4); n=18	20.3±1.5 (16.8 - 23.1); n=17	19.1±2.2 (13.3 - 22.0); n=23	19.5 (19.0 - 19.9); n=4	21.3±1.8 (16.1 - 23.1); n=19	18.9±1.6 (15.4 - 21.3); n=25	18.6±1.8 (14.9 - 21.3); n=23
Outer root length	8.5 (8.0 - 8.9); n=5	9.4±1.3 (6.9 - 11.8); n=21	9.0±1.2 (6.3 - 10.9); n=18	9.5±1.1 (7.9 - 11.4); n=16	9.1±1.0 (7.2 - 10.5); n=22	9.2 (7.7 - 10.7); n=4	10.4±1.1 (8.2 - 12.8); n=19	9.2±1.1 (6.9 - 11.2); n=25	9.1±1.5 (6.3 - 11.4); n=23
Point length	9.1 (8.8 - 9.4); n=5	8.6±0.8 (7.1 - 10.4); n=18	8.4±1.1 (6.8 - 10.3); n=17	8.8±1.2 (7.0 - 12.0); n=17	8.4±1.0 (6.2 - 10.7); n=23	9.1 (8.2 - 10.1); n=4	8.6±1.0 (6.2 - 10.1); n=19	8.7±1.0 (6.9 - 10.7); n=24	9.0±1.1 (7.4 - 11.7); n=23
Ventral bar									
Branch length	22.3 (19.3 - 24.6); n=5	25.9±2.2 (21.4 - 29.4); n=21	25.3±2.0 (20.9 - 30.5); n=18	24.0±2.0 (21.4 - 28.5); n=17	21.9±2.3 (17.6 - 26.5); n=22	18.2 (16.8 - 19.1); n=3	19.6±1.2 (17.6 - 22.2); n=18	19.4±2.0 (16.0 - 23.4); n=24	18.9±3.5 (14.5 - 32.7); n=23
Branch maximum width	8.3 (7.3 - 9.4); n=5	8.7±1.1 (6.8 - 11.3); n=20	8.0±1.3 (4.1 - 9.5); n=18	8.1±1.1 (6.3 - 10.4); n=16	6.7±1.1 (5.4 - 9.2); n=22	6.2 (4.5 - 7.7); n=3	6.8±0.8 (4.8 - 7.9); n=17	6.2±0.8 (4.9 - 7.6); n=24	6.1±1.0 (4.4 - 8.2); n=23
Hooks									
Pair I	15.1 (14.0 - 16.0); n=5	15.1±0.8 (14.1 - 17.6); n=19	15.2±1.2 (13.0 - 18.9); n=18	14.9±0.8 (13.8 - 16.9); n=14	13.9±0.9 (12.5 - 16.0); n=21	13.2 (13.1 - 13.2); n=4	13.4±0.8 (12.3 - 14.7); n=13	14.1±0.6 (13.1 - 15.2); n=15	13.4±1.2 (10.0 - 14.7); n=16
Pair II	15.9 (14.8 - 17.0); n=5	15.4±1.1 (12.9 - 17.7); n=19	16.0±0.8 (15.2 - 18.2); n=18	15.8±0.9 (14.3 - 17.3); n=13	14.5±1.2 (12.0 - 16.5); n=19	13.3 (13.2 - 13.3); n=4	14.2±0.7 (12.5 - 14.8); n=10	13.7 (12.9 - 15.1); n=6	14.7±1.3 (13.1 - 17.8); n=15
Pair III	16.7 (15.9 - 17.4); n=5	15.8±1.0 (14.4 - 17.7); n=20	16.1±1.2 (12.9 - 18.9); n=17	15.7±1.0 (13.9 - 17.1); n=15	14.4±1.6 (11.3 - 16.5); n=18	14.8 (14.7 - 14.9); n=4	14.4 (12.4 - 16.2); n=8	14.3±0.8 (12.8 - 15..3); n=10	14.7±0.9 (13.0 - 15.9); n=16
Pair IV	17.8 (17.6 - 17.9); n=5	15.8±1.0 (13.4 - 17.1); n=10	16.2±0.7 (15.4 - 17.6); n=16	15.7±1.0 (14.0 - 17.3); n=13	14.7±1.4 (12.5 - 17.0); n=15	15.2 (14.3 - 16.0); n=4	15.0 (14.2 - 15.4); n=5	14.5±0.7 (13.1 - 15.7); n=10	15.1±1.0 (12.5 - 16.7); n=16
Pair V	15.6 (16.4 - 17.4); n=5	14.3±1.3´6 (11.6 - 17.6); n=15	15.3±1.0 (12.9 - 17.3); n=17	15.3±0.8 (14.3 - 16.4); n=7	15.2 (13.4 - 17.3); n=9	13.4 (13.3 - 13.4); n=2	14.3 (13.7 - 15.1); n=5	14.2±1.2 (11.4 - 15.6); n=10	15.2±1.2 (13.6 - 17.5); n=13
Pair VI	15.5 (15.2 - 15.8); n=5	16.0±1.0 (14.8 - 17.7); n=10	16.1±0.9 (14.0 - 17.9); n=18	16.2±0.6 (154 - 17.5); n=10	15.2±0.5 (14.3 - 16.0); n=12	14.8 (14.7 - 14.8); n=3	14.9 (14.3 - 15.5); n=3	14.6 (14.0 - 15.6); n=8	14.9±0.7 (13.9 - 15.9); n=14
Pair VII	15.6 (16.4 - 17.4); n=5	16.2 (13.6 - 19.6); n=8	16.4±0.8 (15.0 - 17.8); n=16	15.3±0.8 (14.3 - 16.4); n=7	15.2 (13.4 - 17.3); n=9	13.4 (13.3 - 13.4); n=2	14.7 (13.5 - 15.4); n=4	14.4 (13.3 - 15.6); n=8	15.2±1.2 (13.6 - 17.5); n=13

Male copulatory organ									
Copulatory tube curved length	35.7 (34.2 - 37.2); n=5	38.6± (32.3 - 44.0); n=22	39.1±2.6 (34.9 - 44.5); n=11	38.0±2.2 (34.1 - 42.0); n=15	37.7±3.5 (30.0 - 44.5); n=44	39.8 (34.4 - 43.7); n=4	-	-	40.9±3.3 (32.8 - 43.6); n=11
Accessory piece curved length	44.3 (42.1 - 46.5); n=5	48.5± (37.1 - 58.8); n=22	44.7±3.8 (40.9 - 49.6); n=10	48.0±4.0 (40.0 - 53.2); n=14	46.9±2.5 (36.5 - 56.6); n=41	52.8 (52.5 - 53.0); n=4	-	-	54.9±3.2 (47.9 - 60.0); n=11

1117

1118

1119

1120

1121

1122

1123

1124

1125

1126

1127

1128

1129

1130

1131

1132 Table S5: Comparison of measurements performed on *Kapentagyrus tanganicanus* ex *Stolothrissa tanganicae* haptoral and genital hard parts
1133 described. Standard deviation is shown only for cases with more 10 and more individuals (a – mean value±standard deviation, b – range).

	Ethanol preserved specimens			Fresh specimens		
	Bujumbura 2018	Kalemie 2018	Mpulungu 2018	Uvira 2018	Mpulungu 2018	Uvira 2016
Dorsal anchor						
Total length	21,1[a] (19,1 - 21,8)[b]; n=3	21,7±1,9 (18,1 - 25,2); n=19	22,6 (21,9 - 23,1); n=5	21,8±1,1 (20,0 - 23,7); n=13	21,8± (18,8 - 26,1); n=22	21,8±1,7 (17,5 - 25,9); n=33
Length to notch	18,1 (16,6 - 18,7); n=3	18,5±1,5 (15,8 - 21,7); n=19	19,4 (18,5 - 20,4); n=5	18,6±1,3 (15,2 - 20,7); n=13	17,8±1,0 (17,1 - 20,5); n=22	17,8±1,0 (15,3 - 19,8); n=34
Inner root length	12,2 (9,5 - 12,4); n=3	12,4±1,3 (9,8 - 14,4); n=19	12,9 (11,9 - 13,6); n=5	12,5±0,9 (11,3 - 14,1); n=13	11,4±1,7 (9,1 - 16,3); n=22	10,9±1,3 (7,8 - 14,8); n=33
Outer root length	6,6 (5,6 - 6,9); n=3	6,7±0,8 (5,3 - 8,7); n=19	5,9 (5,3 - 7,4); n=5	6,6±0,8 (6,0 - 8,6); n=13	6,1±0,8 (4,8 - 7,7); n=22	5,9±0,9 (4,2 - 7,8); n=32
Point length	7,5 (7,0 - 7,9); n=3	7,1±0,8 (5,7 - 8,4); n=19	7,1 (6,8 - 7,7); n=5	7,3±0,7 (6,3 - 9,5); n=13	7,9±0,9 (5,2 - 8,7); n=22	7,7±0,9 (5,9 - 9,6); n=32
Dorsal bar						
Branch length	25,3 (24,2 - 30,1); n=3	25,5±2,9 (21,5 - 31,6); n=18	25,2 (19,7 - 30,4); n=4	25,6±2,5 (20,2 - 29,3); n=13	20,7±2,0 (16,9 - 25,0); n=22	19,6±1,5 (14,9 - 23,0); n=32
Branch maximum width	6,1 (4,3 - 5,8); n=3	6,4±1,0 (4,4 - 8,3); n=18	6,6 (6,1 - 8,0); n=4	6,3±0,5 (5,5 - 7,5); n=12	4,7±0,5 (4,3 - 6,0); n=20	4,6±0,6 (3,7 - 6,5); n=32
Ventral anchor						
Total length	24,8±3,4 (20,9 - 27,1); n=3	25,1±1,7 (22,1 - 28,4); n=20	25,6 (22,7 - 29,9); n=5	24,9±1,7 (21,5 - 27,1); n=12	24,6±2,1 (22,2 - 29,8); n=20	24,8±2,3 (21,3 - 32,8); n=32
Length to notch	19,3±1,6 (17,8 - 20,9); n=3	19,7±1,4 (16,8 - 21,7); n=20	19,1 (18,0 - 20,8); n=5	19,7±1,0 (19,1 - 21,8); n=11	19,0±1,4 (15,9 - 21,1); n=20	19,5±1,4 (16,2 - 22,2); n=33
Inner root length	15,6±1,9 (13,7 - 17,3); n=3	15,0±1,8 (11,3 - 18,6); n=20	16,1 (14,0 - 17,1); n=5	15,4±1,3 (13,6 - 18,6); n=12	13,9±2,0 (9,2 - 17,6); n=20	13,3±2,0 (9,0 - 18,2); n=32
Outer root length	7,0±0,2 (6,5 - 6,9); n=3	6,9±0,6 (6,1 - 8,5); n=20	6,7 (4,6 - 8,3); n=5	7,0±0,7 (6,3 - 9,1); n=12	6,8±1,0 (5,2 - 9,1); n=20	6,8±1,1 (4,3 - 9,8); n=33
Point length	8,0±0,8 (6,7 - 8,3); n=3	7,8±0,9 (6,4 - 9,6); n=20	8,6 (8,0 - 9,1); n=5	8,0±0,6 (7,4 - 9,6); n=12	8,6±1,2 (5,0 - 10,3); n=17	7,8±1,3 (5,0 - 10,5); n=31
Ventral bar						
Branch length	24,5±1,5 (22,1 - 25,1); n=3	25,0±3,5 (20,3 - 31,7); n=15	20,0 (18,8 - 22,5); n=3	23,9±2,3 (20,4 - 27,0); n=12	18,5±2,2 (13,6 - 23,5); n=19	18,1±2,4 (14,2 - 23,6); n=30
Branch maximum width	6,5±0,6 (6,0 - 7,1); n=3	6,6±1,2 (4,6 - 8,8); n=15	6,6 (6,2 - 6,8); n=3	6,5±0,9 (4,0 - 7,8); n=12	4,5±0,5 (4,5 - 6,2); n=16	4,4±1,0 (3,0 - 6,7); n=31

Hooks						
Pair I	13,7±0,3 (13,6 - 14,1); n=3	13,8±0,9 (12,4 - 15,7); n=17	14,0 (13,2 - 14,8); n=5	13,6±0,9 (11,7 - 14,3); n=12	12,9±1,0 (11,4 - 14,5); n=14	12,8±1,0 (9,7 - 14,3); n=27
Pair II	14,7±0,9 (13,5 - 14,8); n=3	14,8±0,7 (13,5 - 15,7); n=17	13,5 (12,9 - 14,2); n=4	14,2±1,0 (12,6 - 15,6); n=13	13,5±0,9 (12,2 - 15,3); n=17	13,3±1,1 (10,8 - 15,4); n=23
Pair III	14,6±0,4 (14,5 - 15,3); n=3	14,9±0,9 (12,8 - 16,5); n=16	14,9 (14,6 - 15,2); n=3	14,6±0,9 (12,5 - 15,7); n=13	15,9±0,6 (12,8 - 15,1); n=17	13,2±0,7 (12,0 - 14,4); n=25
Pair IV	14,7±0,1 (14,6 - 14,8); n=3	15,3±1,3 (13,0 - 17,8); n=17	14,9 (14,6 - 15,2); n=3	14,9±1,6 (12,0 - 16,8); n=9	13,7±0,9 (12,4 - 15,5); n=15	13,5±1,0 (11,3 - 15,0); n=21
Pair V	14,4±1,3 (12,9 - 14,8); n=3	14,3±0,9 (13,0 - 15,4); n=9	14,2 (13,8 - 14,6); n=3	13,7±1,0 (11,2 - 14,5); n=8	13,1±0,9 (11,5 - 14,6); n=12	12,8±0,8 (11,4 - 14,1); n=18
Pair VI	14,8±0,8 (13,9 - 15,1); n=3	15,0±0,7 (13,9 - 16,5); n=14	15,8 (15,4 - 16,1); n=3	14,7±0,6 (13,3 - 15,0); n=11	14,1±0,7 (13,0 - 15,3); n=14	14,1±0,8 (12,0 - 15,4); n=15
Pair VII	15,3±0,2 (14,0 - 14,3); n=3	15,8±0,7 (14,8 - 16,8); n=8	15,7 (14,3 - 16,8); n=4	14,8±0,9 (12,8 - 15,7); n=12	13,8±0,9 (12,0 - 15,0); n=12	13,6±1,0 (11,0 - 15,5); n=15
Male copulatory organ						
Copulatory tube curved length	40,3 (40,0 - 40,5); n=3	38,0±3,3 (32,4 - 46,0); n=15	39,4 (36,2 - 43,2); n=2	39,1 (33,0 - 42,8); n=6	40,0±2,6 (36,6 - 42,7); n=6	40,6 (38,3 - 44,0); n=7
Accessory piece curved length	43,0 (40,5 - 45,5); n=3	47,0±3,7 (40,0 - 55,1); n=15	48,0 (45,2 - 49,3); n=2	42,9 (32,6 - 54,2); n=6	47,6±5,0 (42,3 - 55,5); n=6	48,6 (44,4 - 56,0); n=7

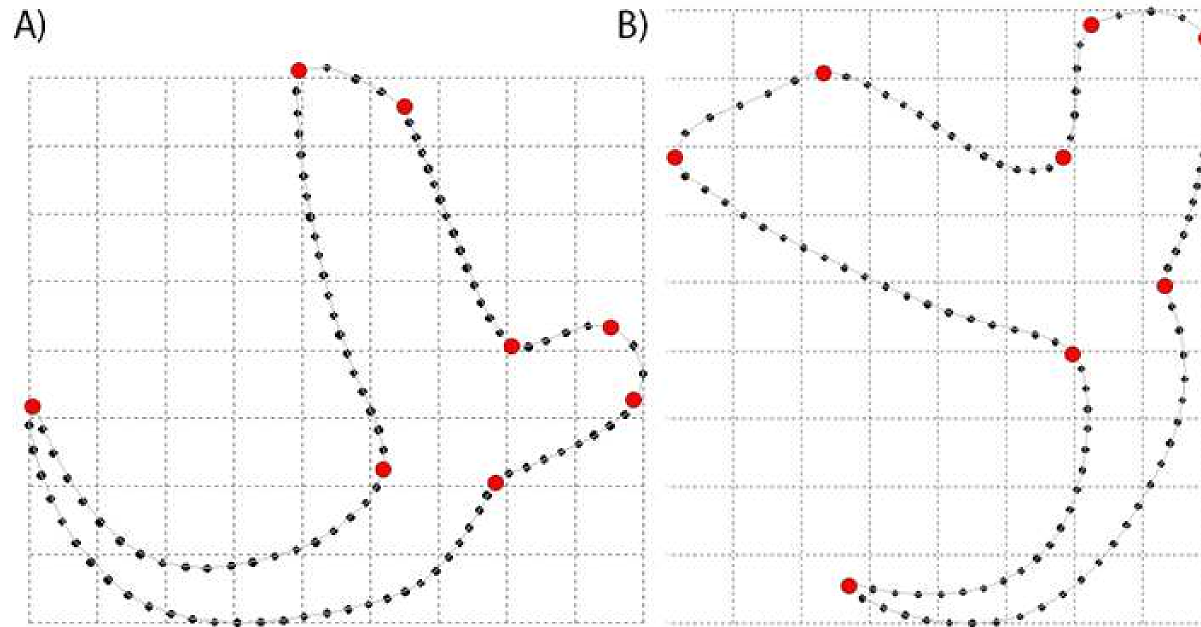
1134

1135

1136

1137

1138 **Supplementary figures**



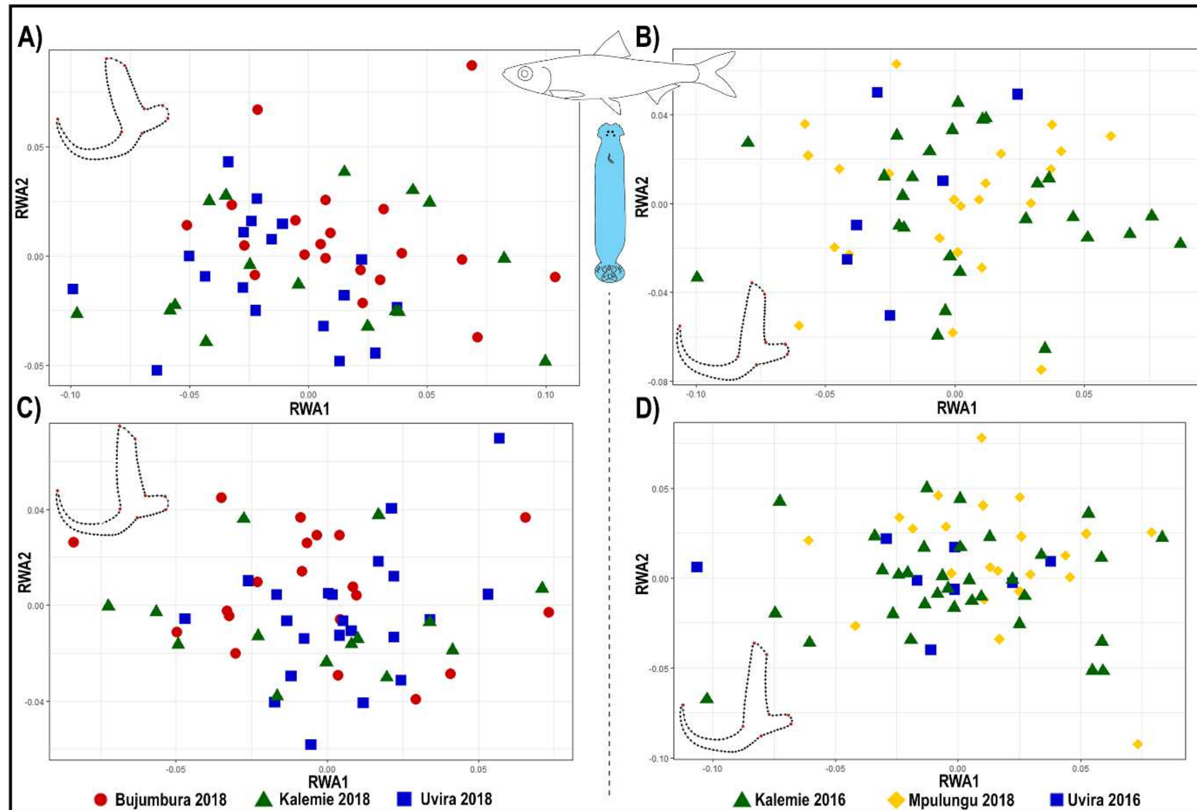
1139

1140 Fig. S1: Position of fixed landmarks (big points) as well as semi-landmarks (small points) in dorsal anchor of A) *K. limnotrissae*; B) *K. tanganicanus*.

1141 Number and position of landmarks according to species of *Kapentagyrus* was respected in ventral anchor.

1142

1143



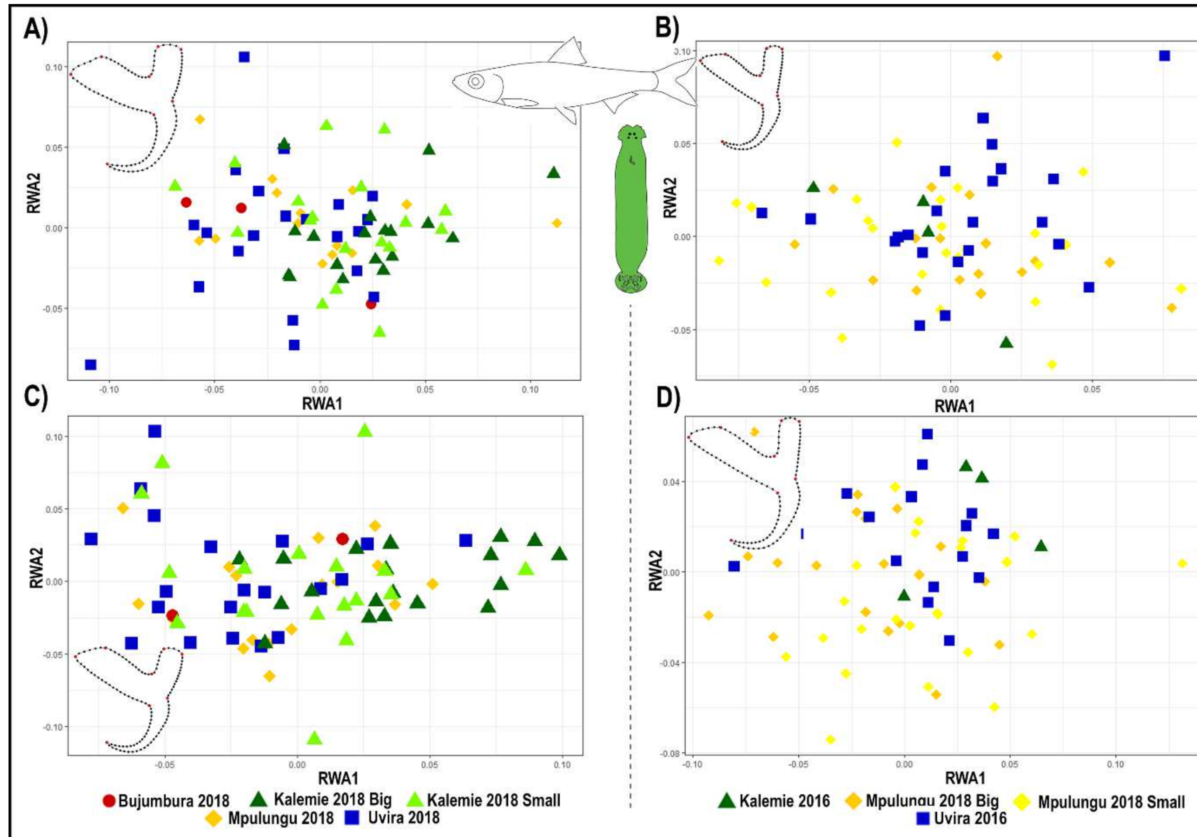
1144 Fig. S2: Biplots showing the shape variation in haptoral structures of *K. limnotrissae* in this study. Only the first two axes are shown. A) RWA of dorsal

1145 anchor using semi-landmark sliding approach, fresh specimens; B) RWA of dorsal anchor using semi-landmark sliding approach, fresh specimens; C)

1146 RWA of ventral anchor using semi-landmark sliding approach, fresh specimens; D) RWA of ventral anchor using semi-landmark sliding approach,

1147 fresh specimens. Symbols denote sampling site origin with the year of sampling. Consensus anchor's shape for the respected analysis is shown.

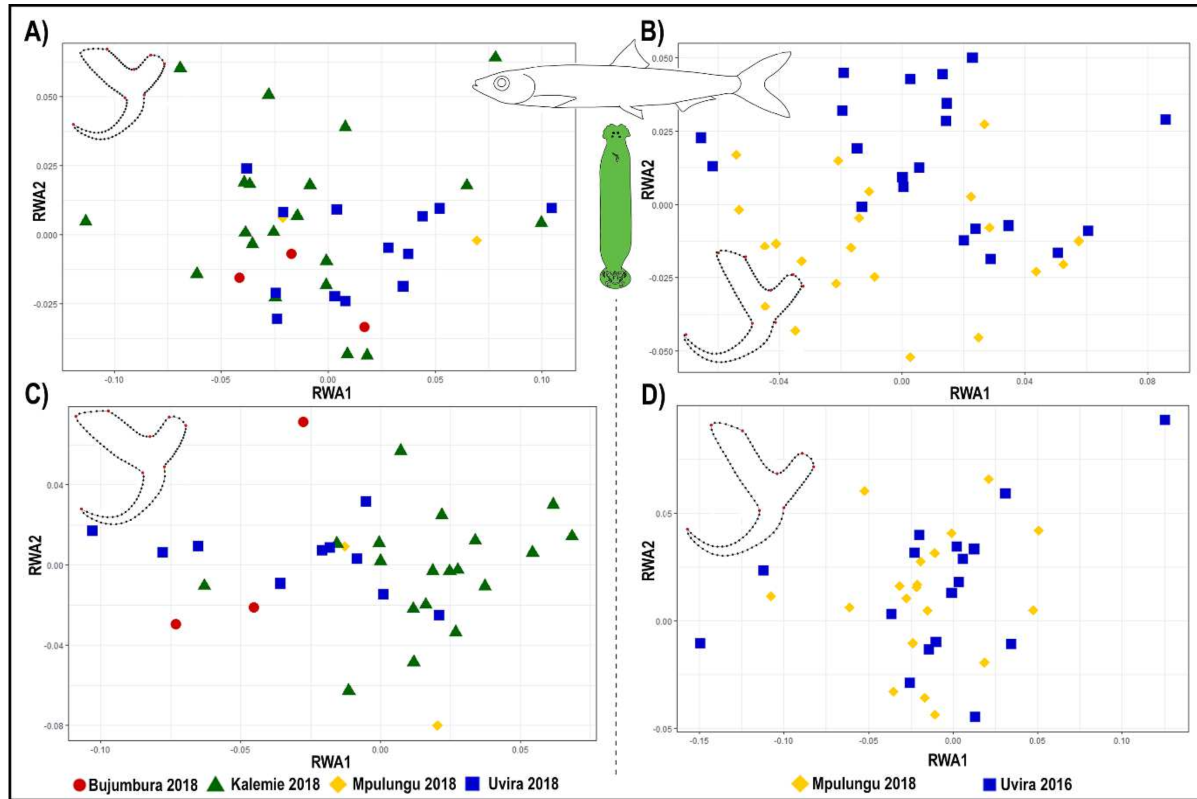
1148



1149

1150 Fig. S3: Biplots showing the shape variation in haptoral structures of *K. tanganicanus* ex *L. miodon* in this study. Only the first two axes are shown. A)
1151 RWA of dorsal anchor using semi-landmark sliding approach, fresh specimens; B) RWA of dorsal anchor using semi-landmark sliding approach, fresh
1152 specimens; C) RWA of ventral anchor using semi-landmark sliding approach, fresh specimens; D) RWA of ventral anchor using semi-landmark sliding
1153 approach, fresh specimens. Symbols denote sampling site origin with the year of sampling. Consensus anchor's shape for the respected analysis is
1154 shown.

1155



1156

1157 Fig. S4: Biplots showing the shape variation in haptoral structures of *K. tanganicanus* ex *S. tanganicae* in this study. Only the first two axes are shown.
1158 A) RWA of dorsal anchor using semi-landmark sliding approach, fresh specimens; B) RWA of dorsal anchor using semi-landmark sliding approach,
1159 fresh specimens; C) RWA of ventral anchor using semi-landmark sliding approach, fresh specimens; D) RWA of ventral anchor using semi-landmark
1160 sliding approach, fresh specimens. Symbols denote sampling site origin with the year of sampling. Consensus anchor's shape for the respected
1161 analysis is shown.

1162

1163

Paper IV

Kmentová N., Koblmüller S., Van Steenberge M., Artois T., Muterezi Bukinga F., Mulimbwa N'sibula T, Masilya Mulungula P., Muzumani Risasi D., Gelnar M., Vanhove M. P. M. Failure to diverge in African Great Lakes: the case of *Dolicirroplectanum lacustre* comb. nov. (Monogenea, Diplectanidae) infecting latid hosts. Accepted in *Journal of Great Lakes Research* [Q1, IF (2018) = 2.175].

NK contributed to material collection, obtained morphometric and molecular data, performed statistical and phylogenetic analyses, contributed to the interpretation of results and wrote the manuscript. Overall contribution: c. 70%.

1 # Failure to diverge in African Great Lakes: the case of
2 # *Dolicirroplectanum lacustre* comb. nov. (Monogenea,
3 # Diplectanidae) infecting latid hosts

4 **Nikol Kmentová[1,2,3*], Stephan Koblmüller[4], Maarten Van Steenberge[3,5,6], Tom Artois[2],**
5 **Fidel Muterezi Bukinga[7], Théophile Mulimbwa N'sibula[7], Donatien Muzumani Risasi[7],**
6 **Pascal Masilya Mulungula[7], Milan Gelnar[1], Maarten P.M. Vanhove[1,2,3,8]**

7 [1] Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2,
8 611 37 Brno, Czech Republic

9 [2] Hasselt University, Centre for Environmental Sciences, Research Group Zoology:
10 Biodiversity & Toxicology, Agoralaan Gebouw D, B-3590 Diepenbeek, Belgium

11 [3] Laboratory of Biodiversity and Evolutionary Genomics, Department of Biology, University
12 of Leuven, Ch. Deberiotstraat 32, B-3000 Leuven, Belgium

13 [4] Institute of Biology, University of Graz, Universitätsplatz 2, A-8010 Graz, Austria

14 [5] Operational Directorate Taxonomy and Phylogeny, Royal Belgian Institute of Natural
15 Sciences, Vautierstraat 29, B-1000 Brussels, Belgium

16 [6] Biology Department, Royal Museum for Central Africa, Leuvensesteenweg 13, 3080,
17 Tervuren, Belgium

18 [7] Centre de Recherche en Hydrobiologie, Département de Biologie, B.P. 73 Uvira,
19 Democratic Republic of Congo

20 [8] Finnish Museum of Natural History, Zoology Unit, University of Helsinki, Finland

21 * corresponding author: kmentovan@mail.muni.cz, +420 723 004 419

22

23

24

25

26 **Abstract**

27 Speciation of fish in the African Great Lakes has been widely studied. Surprisingly, extensive
28 speciation in parasites was only recently discovered in these biodiversity hotspots, notably in
29 monogeneans (Platyhelminthes) from Lake Tanganyika. *Diplectanum* is a monogenean genus
30 of which only a single species is known from the Great Lakes: *Diplectanum lacustre*
31 (Diplectanidae) living on latid perches of Lake Albert. Despite their primary marine origin,
32 latids have diversified in African freshwaters including several Great Lakes. In better-studied
33 marine diplectanid species, incongruence between morphological and genetic differentiation
34 was documented. As freshwater systems provide more opportunities for speciation than the
35 marine realm, we ask whether diplectanids of *Lates* spp. of the Great Lakes underwent similar
36 diversification as their hosts.

37 Fresh and museum specimens of five African latid species (*Lates angustifrons, L. mariae, L.*
38 *microlepis, L. niloticus, L. stappersii*) were examined for the presence of monogenean gill
39 parasites. Monogeneans were characterised morphologically via morphometrics of sclerotised
40 structures and genetically using nuclear ribosomal and mitochondrial markers.

41 Continuous morphological variation was documented in these parasites. In addition, the
42 genetic distance, based on the COI region, between parasites of geographically isolated host
43 species did not reach the level typically associated with distinct diplectanid species.
44 Therefore, a single species of a newly described genus, *Dolicirroplectanum lacustre* gen. nov.
45 comb. nov. is suggested to infect latid species in the examined basins. We discuss this
46 parasite's failure to diverge in the light of the congruence between the rate of molecular
47 evolution in COI and host historical distribution.

48

49 **Keywords:** parasitic flatworm - *Lates* – DNA barcoding - evolutionary history - Nile perches

50

51

52

53

54

55

56 **Introduction**

57 African Great Lakes are known for their species rich flocks of cichlid fish, that are well
58 established models in evolutionary biology (Salzburger, 2018). Remarkably, Lake
59 Tanganyika is characterised by extraordinary diversity and high degrees of endemism not
60 only of cichlids but also other fish families (Salzburger et al., 2014) as well as invertebrate
61 taxa (Coulter, 1991) including parasitic flatworms (Pariselle et al., 2015). Monogeneans
62 (Platyhelminthes) are mainly parasites of fish. They display a high level of host specificity
63 believed to be connected with their direct life cycle (a single host needed) combined with the
64 adaptive evolution of monogenean hardparts responsible for attachment (the haptor in the
65 posterior part of the body) and reproduction (Poulin, 2002).

66 Parasite speciation mirroring host diversification was reported for monogeneans infecting
67 tropheine cichlids in Lake Tanganyika (Vanhove et al., 2015). However, the history of
68 monogenean interactions with their hosts does not always feature only co-speciation events.
69 For example, in *Dactylogyrus* Diesing, 1850 infecting cyprinid fishes, diversification can be
70 mainly explained by intrahost speciation (Šimková et al., 2004). In the case of *Cichlidogyrus*
71 *casuarinus* Pariselle, Muterezi Bukinga & Vanhove, 2015, a monogenean infecting deepwater
72 cichlids in Lake Tanganyika, no specificity or preference was detected towards its various
73 well-diverged host species (Kmentová et al., 2016), a process called "failure to diverge"
74 (Brooks, 1979).

75 Among the parasitic flatworms known to infect lates perches (Latidae) are diplectanids
76 (Diplectanidae), a monogenean family with more than 250 species described worldwide,
77 mainly from marine perciform fishes (Domingues and Boeger, 2008). The genus *Lates* L.,
78 1758 consists of 11 species, seven of which inhabit African freshwaters, with the rest to be
79 found in marine, brackish and freshwater habitats in the Indo-Pacific region (Otero, 2004).
80 While seven diplectanid species from three different genera were documented from *Lates*

81 *calcarifer* (Bloch, 1790) from the Indo-Pacific region (Tingbao et al., 2006), only one species
82 was described from African *Lates* spp. so far: *Diplectanum lacustre* Thurston & Paperna,
83 1969 infecting *Lates niloticus* L. from Lake Volta and the Victoria Nile near Lake Albert
84 (Paperna and Thurston, 1969), and from near Cairo in Egypt (Ergens, 1981). The native range
85 of *L. niloticus* includes most major river basins and Great Lakes in the Nilo-Sudanic region
86 and large parts of the Congo basin (Paugy et al., 2003). Importantly, *L. niloticus* was
87 introduced to Lake Victoria for fisheries with a dramatic impact on the local environment
88 (Ogutu-Ohwayo, 1995). In Lake Tanganyika, four endemic latid fishes, *Lates angustifrons*
89 Boulenger, 1906, *Lates mariae* Steindachner, 1909, *Lates microlepis* Boulenger, 1898 and
90 *Lates stappersii* (Boulenger, 1914), with different habitat preferences, are present (Poll,
91 1953).
92 Small interspecific morphological differences and high levels of phenotypic plasticity render
93 the species status of some diplectanids questionable (Poisot et al., 2011; Schoelinck et al.,
94 2012; Wu et al., 2005) with unclear phylogenetic relationships within and between some of
95 the genera (Villar-Torres et al., 2019). In the 21st century, species delineation is often based
96 on a combination of morphological and molecular data (Schlick-Steiner et al., 2010).
97 Integrative techniques revealed problems in various taxonomic groups, especially in soft-
98 bodied organisms or lineages with heteromorphic life stages such as parasitic flatworms
99 (Georgieva et al., 2013; Rahmouni et al., 2017). Species identification using specific
100 molecular tags derived from the cytochrome *c* oxidase subunit 1 gene (COI) in the
101 mitochondrial DNA, known as DNA barcoding, was successfully implemented in many
102 taxonomic groups such as fishes (Hubert et al., 2008), mammals (Francis et al., 2010) and
103 lepidopteran insects (Hebert et al., 2003). However, this approach proved problematic in
104 many other taxa (DeSalle et al., 2005; Will and Rubinoff, 2004) including monogenean
105 flatworms (Vanhove et al., 2013). So far, little correlation between host specificity and

106 taxonomic diversification was found in diplectanid monogeneans (Desdevises et al., 2001;
107 Villar-Torres et al., 2019). However, the latter studies were conducted in a marine system
108 with no real geographic barrier between host species that even form mixed schools. As
109 freshwater systems provide more opportunities for speciation than the marine realm, and
110 given the age of Lakes Albert and Tanganyika, which are situated in different basins, these
111 lakes are a perfect study system to investigate diplectanid evolution under allopatry. We
112 hypothesize that diplectanid monogeneans infecting latids belong to different species in Lake
113 Albert and Lake Tanganyika. If so, is there a congruence between the level of morphological
114 and molecular diversification in diplectanid parasites infecting African of *Lates* spp.? Within
115 Lake Tanganyika, we ask whether diplectanids of *Lates* spp. underwent similar diversification
116 as their hosts, or whether they rather failed to diverge like the above-mentioned *C.*
117 *casuarinus*, a monogenean infecting bathybatine cichlids. These are, like latids, non-littoral
118 fishes, and this lack of parasite specificity is considered an adaptation of low host availability
119 outside of the littoral zone (Kmentová et al., 2016).
120
121 **Material & Methods**
122 **Sampling**
123 Fish samples of five latid species (*Lates angustifrons, L. mariae, L. microlepis, L. niloticus, L.*
124 *stappersii*) were examined in this study. Samples included specimens of all *Lates* species
125 from the ichthyology collection of the Royal Museum for Central Africa (RMCA) (Tervuren,
126 Belgium) and fresh specimens from recent field expeditions (2010, 2016, 2017 and 2018). At
127 Lake Albert, fresh specimens of *L. niloticus* were obtained from local fishermen (Nzunzu,
128 Uganda). For Lake Tanganyika, the four endemic latid species (*Lates angustifrons, L. mariae,*
129 *L. microlepis* and *L. stappersii*) were either caught with gill nets from the experimental
130 fishing unit of the Centre de Recherche en Hydrobiologia-Uvira (CRH) (Uvira, Democratic

131 Republic of the Congo) or obtained from local fish markets (see Table 1). To provide a
132 broader geographical range for morphological comparison, fish specimens of *L. niloticus*
133 from seven additional localities throughout the host's range were examined. In total, gills (one
134 side in the case of museum specimens) of 158 fish specimens from 20 localities in African
135 freshwaters (see Table 1) were examined following the standard protocol of Ergens & Lom
136 (Ergens and Lom, 1970). In the field, fresh monogenean specimens were either mounted on
137 slides using a solution of glycerine ammonium picrate (GAP) or using Hoyer's medium in the
138 case of ethanol-fixed specimens from Lake Albert and specimens retrieved from the museum
139 collection. Some of the individuals were cut in three parts with the anterior and posterior parts
140 mounted on slides for morphological characterisation and the rest preserved in 99% ethanol
141 for genetic analyses. To characterize internal anatomy, some specimens were stained using
142 the Carmine method described by Justine (2005) without the initial step of putting a live
143 parasite under a cover slip. Parasite identification and description were carried out using an
144 Olympus BX51 microscope equipped with a drawing tube and OLYMPUS KL 1500 LED
145 illumination. Specimens were compared with the holotype (MRAC MT.35572) and voucher
146 material (MRAC MT.35573) of *D. lacustre*. Drawings were edited with a graphics tablet
147 compatible with Adobe Illustrator CS6 16.0.0 and Adobe Photoshop CS6 13.0. Fish tissue
148 samples were deposited in the ichthyology collection of the RMCA under collection number
149 2016.20.P for Lake Tanganyika and 2016.036.P for Lake Albert. Parasite voucher specimens
150 are available from the invertebrate collection of the RMCA, the Iziko South African Museum
151 (SAMC), Cape Town, Republic of South Africa; the Muséum national d'Histoire naturelle
152 (MNHN), Paris, France; the Natural History Museum (NHMUK), London, United Kingdom;
153 and the Finnish Museum of Natural History (MZH), Helsinki, Finland.
154
155

156 **Morphometrics**

157 Measurements of sclerotised structures were taken at a magnification of 1000× (objective ×
158 100 immersion, ocular × 10) using an Olympus BX51 microscope with incorporated phase
159 contrast and the software Digital Image Analysis v4. In total, 29 parameters of the hardparts
160 of the haptoral and copulatory organs were measured for morphometric characterisation and a
161 detailed redescription (see Fig. 1). Terminology was based on Justine & Henry (2010). To
162 investigate the level of morphological differentiation (haptor morphology), raw measurements
163 were analysed by multivariate statistical techniques in R (R Core Team, 2013). Principal
164 component analyses (PCAs) were conducted with scaled variables on 17 morphological
165 characters of the haptor using the package adegenet (Jombart, 2008). Results of the PCA were
166 visualised with the packages ggplot2 (Wickham, 2009) and factoextra (Kassambara and
167 Mundt, 2017). To visualise the variance in the total size of the ventral anchor, a density plot
168 using uncorrected measurements was drawn using ggplot2 and factoextra. A Kruskall-Wallis
169 test of multiple comparison with Bonferroni's post-hoc correction via Dunn's test,
170 implemented in the package FSA (Ogle et al., 2019), respectively, was conducted to test the
171 relation of the host species and the catch locality to copulatory organ measurements,
172 respectively. The assumption of normality was tested by Shapiro-Wilk's W tests implemented
173 in stats. The assumption of homogeneous variance within sample groups was tested by
174 Levene's test in the R package lawstat (Gastwirth et al., 2017).

175 **Molecular characterisation**

176 Morphological characterisation was combined with genetic characterisation using tissue
177 samples of the central part of some of the parasite individuals collected from fresh fish
178 specimens from Lake Tanganyika and Lake Albert, as described above. No fresh material was
179 available from other locations. To genetically verify parasite species delineation, we used
180 three different nuclear sequence fragments, from the small and large ribosomal subunit gene

181 (18 and 28 rDNA) and the first internal transcribed spacer region (ITS-1). To assess
182 intraspecific genetic diversity, part of the mitochondrial COI gene was used. Whole genomic
183 DNA was extracted using the Qiagen Blood and Tissue Isolation Kit following the
184 manufacturer's instructions with some modifications (samples in ATL buffer (180 µl) with
185 protein kinase (20 µl) were kept in 1.5 ml Eppendorf tubes overnight at room temperature).
186 The DNA extract was concentrated to a volume of 80 µl in 1.5 ml Eppendorf tubes using a
187 vacuum centrifuge and stored at a temperature of -20 °C. Partial 18S rDNA and ITS-1 were
188 amplified using the S1 (5´-ATTCCGATAACGAACGAGACT-3´) (Sinnappah et al., 2001)
189 and Lig5.R (5´-GATACTCGAGCCGAGTGATCC-3´) (Blasco-Costa et al., 2012) primers.
190 Each reaction mix contained 1.5 unit of *Taq* polymerase, 1X buffer containing 0.1 mg/ml
191 bovine serum albumin (BSA), 1.5 mM $MgCl_2$, 200 mM dNTPs, 0.8 mM of each primer and 3
192 µl of isolated DNA (concentration was not measured) in a total reaction volume of 30 µl
193 under the following conditions: 2 min at 95 ºC, 39 cycles of 1 min at 95 ºC, 1 min at 55 ºC
194 and 1 min and 30 s at 72 ºC, and finally 10 min at 72 ºC. Primers C1 (5´-
195 ACCCGCTGAATTTAAGCAT-3´) and D2 (5´-TGGTCCGTGTTTCAAGAC-3´) (Hassouna
196 et al., 1984) were used for amplification of the partial 28S rDNA gene. Each PCR reaction
197 contained 1.5 unit of *Taq* polymerase, 1X buffer containing 0.1 mg/ml BSA, 1.5 mM $MgCl_2$,
198 200 mM dNTPs, 0.5 mM of each primer and 5 µl of isolated DNA (concentration was not
199 measured) in a total reaction volume of 30 µl under the following conditions: 2 min at 94 ºC,
200 39 cycles of 20 seconds at 94 ºC, 30 seconds at 58 ºC and 1 min and 30 s at 72 ºC, and finally
201 10 min at 72 ºC. Part of the mitochondrial COI gene was amplified using ASmit1 (5'-
202 TTTTTTGGGCATCCTGAGGTTTAT-3') combined with Schisto3 (5'-
203 TAATGCATMGGAAAAAAACA-3'), and with ASmit2 (5'-
204 TAAAGAAAGAACATAATGAAAATG-3') in case of nested PCR (Littlewood et al., 1997).
205 For both primer combinations, the amplification reaction contained 24 µl of PCR mix (one

206 unit of *Taq* polymerase, 1X buffer containing 2 mM $MgCl_2$, 0.1 mg/ml BSA, 0.2 mM dNTPs,
207 0.8 mM of each primer) with 1 µl of isolated DNA (concentration was not measured) in a
208 total reaction volume of 25 µl and was performed under the following conditions: initial
209 denaturation at 95°C for 5 min and then 40 cycles of 1 min at 94°C, 1 min at 50°C and 1 min
210 at 72°C, and final elongation for 7 min at 72°C. Amplification success was checked by
211 agarose gel electrophoresis and for positive samples, 2.5 µg of PCR product was
212 enzymatically cleaned up using 1 µl of ExoSAP-IT reagent under the following conditions: 15
213 min at 37 ºC and 15 min at 80 ºC. After cycle sequencing of purified PCR products using
214 BigDye v3.1, following the manufacturer's recommendations, fragments were cleaned up
215 using the BigDye XTerminator® Purification Kit and visualized on an ABI3130 capillary
216 sequencer. Electropherograms were visually inspected, corrected and sequences were aligned
217 using MUSCLE (Edgar, 2004) under default settings as implemented in MEGA v7 (Kumar et
218 al., 2016), together with selected previously published sequences of representatives of
219 Diplectanidae (see Table S1). The newly obtained haplotype sequences were deposited in
220 NCBI GenBank under the accession numbers MK937579-MK937581 (28S rDNA),
221 MK937574-MK937576 (18S+ITS-1 rDNA) and MK908145- MK908196 (COI mtDNA).
222
223 **Genetic distances and phylogeny**
224 The consistency of all alignments was checked and corrected under the "automated 1" option
225 in trimAL v1.2, which uses a heuristic search to find the best method for trimming the
226 alignment (Capella-Gutiérrez et al., 2009). As there is a lack of available ITS sequences of
227 diplectanid species, phylogenetic analyses were based on two regions: 18S and 28S rDNA.
228 These two regions were analysed separately because of the lack of species for which both
229 regions are available. Topali v2.5 (Milne et al., 2004) was used to identify the best fitting
230 model of molecular evolution based on the Bayesian information criterion (28S rDNA: GTR

231 + Γ, gamma shape parameter of 0.461; 18S rDNA: K2P + Γ, gamma shape parameter of
232 0.130). For each gene, pairwise distances were calculated using both the most appropriate
233 evolutionary model and, to compare with previous studies, uncorrected pairwise distances.
234 The number of haplotypes and polymorphic sites, haplotype diversity and nucleotide diversity
235 were calculated using ARLEQUIN v3.5 (Excoffier and Lischer, 2010). Phylogenetic analyses
236 were carried out using maximum likelihood (ML) and Bayesian inference (BI) in RAxML v8
237 (Stamatakis, 2014) and MrBayes v3.2.0 (Ronquist et al., 2012), respectively. A ML tree was
238 inferred using RAxML's standard tree search algorithm and bootstrap support was calculated
239 using the option with an automated number of replicates to obtain stable support values under
240 the frequency stopping criterion (Stamatakis, 2014). Bayesian inference was based on two
241 independent runs (100,000,000 generations, sampled every 1,000th generation following a
242 burn-in of 10%). Parameter convergence and run stationarity were assessed in Tracer v1.6
243 (http://beast.bio.ed.ac.uk). As Dactylogyridae and Diplectanidae were shown to be sister taxa
244 (Šimková et al., 2003), *Dactylogyrus extensus* (Mueller and Van Cleave, 1932) (sequence
245 from: Šimková, Matějusová, & Cunningham, 2006) together with *Cichlidogyrus*
246 *attenboroughi* Kmentová, Gelnar & Vanhove, 2016 (sequence from: Kmentová et al. (2018)
247 in the case of 28S rDNA region were selected as outgroup for phylogenetic inference.
248 Phylogenetic trees were edited in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/)
249 and Adobe Photoshop CS6. Phylogenetic relationships among COI haplotypes were inferred
250 by means of a Median Joining network (Bandelt et al., 1999) in PopART 1.7104 (Leigh and
251 Bryant, 2015).
252
253 **Results**
254 A single diplectanid species, morphologically identified as *Diplectanum lacustre* was
255 recorded from three of the four species of *Lates* from Lake Tanganyika (*L. angustifrons, L.*

256 *mariae, L. microlepis*) and from *L. niloticus* from Lakes Albert, Kossou, Nasser and Victoria,
257 from the Taja River in Sierra Leone and from the Bahr-Sara, mouth of the Mandoul River in
258 Tchad. In total, 473 parasite specimens were collected (for more details see Table 1 and Fig.
259 2). Based on morphological characterisation and phylogenetic reconstruction (see Figs. 7&8),
260 a new genus *Dolicirroplectanum* gen. nov. is described with *Dolicirroplectanum lacustre*
261 comb. nov. as the type species. The internal anatomy is characterised, including the
262 sclerotised vagina, prostatic reservoir and seminal vesicle, which were absent in the original
263 description of *D. lacustre* comb. nov. Measurements of the parasite's internal organs and
264 sclerotised haptoral and copulatory structures are presented in Table 2.

265 **Taxonomy and species redescription**

266 ***Dolicirroplectanum* gen. nov. Kmentová, Gelnar & Vanhove (Fig. 3 - 5)**

267 **Family:** Diplectanidae Monticelli, 1903

268 **Genus:** *Dolicirroplectanum* gen. nov.

269 **Type species:** *Dolicirroplectanum lacustre* (Thurston & Paperna, 1969)

270 **Type host:** *Lates niloticus* L. (Latidae)

271 **Type locality:** Lake Volta, Ghana; Lake Albert, Uganda

272 **Site:** Gills

273 **Additional hosts:** *L. angustifrons, L. mariae, L. microlepis*

274 **Other species:** *Dolicirroplectanum penangi* comb. nov. for *Diplectanum penangi* Liang &
275 Leong, 1991 (original designation)

276 **Material examined:** type material: MRAC MT. 35572, vouchers: MNHN HEL744-47 (4
277 specimens), USNPC 180-A 3-7; MRAC. MT. 38206-10, 38913-39058 (243 specimens), MZH

278 10067-71 (6 specimens), SAMC-A089971-72 (6 specimens), NHMUK 2018.4.13.4-13.7 (8
279 specimens)

280 **Zoobank registration:** To comply with the regulations set out in article 8.5 of the amended
281 2012 version of the International Code of Zoological Nomenclature (ICZN) (International
282 Commission on Zoological Nomenclature, 2012), details of the genus have been submitted to
283 ZooBank. The Life Science Identifier (LSID) of the article is
284 urn:lsid:zoobank.org:pub:209675D6-2EBE-4E37-84CB-DA59994F7B2. The LSID for the
285 new genus *Dolicirroplectanum* is urn:lsid:zoobank.org:act:89BFF3C5-271B-4482-98E0-
286 AC667AA6611D.

287 **Etymology:** The genus name derives from Latin and refers to the barrel shape of the male
288 copulatory organ, noticeably wider than in other diplectanid genera.

289 **Diagnosis:** Tegument smooth. Genital pore opening posterior to male copulatory organ (MCO).
290 Genital atrium sclerotised. MCO wide, robust, composed of two nested tubes. Prostatic
291 reservoir simple. Seminal vesicle sinistral. Accessory copulatory organ absent. Squamodiscs
292 ventral, dorsal; rows of bone-shaped rodlets with open rings. Superficial root of ventral anchor
293 reduced. Parasites of perciform fishes (*Lates* spp.). Vagina sclerotised or muscular.

294 **Description:**

295 Multiple pairs of head organs, two pairs of eye-spots. No tegument scales were observed.
296 *Dolicirroplectanum* gen. nov. is characterised by two pairs of dorsal and ventral anchors with
297 a regularly curved shaft point, a large and wide ventral bar and two dorsal bars. Dorsal anchors
298 smaller than ventral ones and without developed outer root. 14 marginal hooklets of similar size
299 and relatively small compared to other haptoral structures. Two squamodiscs, ventral and
300 dorsal, formed by concentric open rows of bone-shaped rodlets of similar width in all rows.

301 Intestinal bifurcation follows pharynx, oesophagus absent. Caeca simple, terminate blindly.
302 Testis spherical, intercaecal. Vas deferens emerges from anterior part of testis, enlarges into
303 seminal vesicle. Seminal vesicle single in the middle region of body, transforms into elongated
304 duct connected with sclerotized part of copulatory organ. Prostatic reservoir simple. Slightly
305 sclerotized MCO composed of two straight tubes, one inside the other, almost as wide as long.
306 Ovary intercaecal, pre-testicular, encircles right caecum. Oviduct passes medially to oötype,
307 surrounded by Mehlis' gland, oötype short, enters into uterus. Uterus sinistral. Vaginal atrium
308 sclerotised or muscular.
309 **Discussion:** Species of *Dolicirroplectanum* gen. nov. can be distinguished by the combination
310 of: 1) presence of a robust barrel-shaped MCO formed by two narrow nested tubes, almost as
311 wide as long, 2) absence of an accessory piece, 3) squamodiscs composed of bone-shaped
312 rodlets forming open rings, 4) superficial roots of ventral anchor reduced, 5) a simple prostatic
313 reservoir not separated into zones, 6) seminal vesicle as an expansion of vas deferens, 7) ovary
314 intercaecal, pre-testicular, encircles right caecum and 8) a lack of tegumental scales. The status
315 of *Dolicirroplectanum* gen. nov. is supported by its placement outside of the clade including
316 *Diplectanum aequans* (Wagener, 1857), the type species of *Diplectanum* (Figs. 7&8).
317 Particularly, *Dolicirroplectanum* gen. nov. differs from other diplectanids including *D. aequans*
318 by the short but wide sclerotised part of the MCO. In contrast to *D. aequans*, a simple prostatic
319 reservoir is present. Conversely, a prostatic reservoir separated into three zones is one of the
320 specific characters for *Diplectanum* sensu stricto mentioned in Domingues & Boeger (2009).
321 *Diplectanum penangi* has all the diagnostic features attributed to *Dolicirroplectanum* gen. nov.
322 The position within the genus was supported by its position in a phylogenetic reconstruction,
323 clustering with *Dolicirroplectanum lacustre* comb. nov. (Figs. 6&7). The holotype of *D.*
324 *penangi* comb. nov. could not be verified as the specimen was not provided by Lee Kong Chian
325 Natural History Museum in Singapore and as the digital pictures we received were taken at

326 insufficient magnification/resolution. Therefore, voucher material deposited in the National
327 Museum of Natural History in Washington and the National Museum of Natural History in
328 Paris was checked instead, and the two nested copulatory tubes and simple prostatic reservoir
329 were found to be present in *D. penangi* comb. nov. together with other characteristics mentioned
330 in its original description (Fig. 5).

331 **Redescription**

332 **Family:** Diplectanidae Monticelli, 1903

333 **Genus:** *Dolicirroplectanum*

334 *Dolicirroplectanum lacustre* comb. nov. (Thurston & Paperna, 1969)

335 **Synonyms:** *Diplectanum lacustre*

336 **Zoobank registration:** To comply with the regulations set out in article 8.5 of the amended
337 2012 version of the International Code of Zoological Nomenclature (ICZN) (International
338 Commission on Zoological Nomenclature, 2012), details of the species have been submitted to
339 ZooBank. The Life Science Identifier (LSID) of the article is
340 urn:lsid:zoobank.org:pub:209675D6-2EBE-4E37-84CB-DA59994F7B2. The LSID for the
341 new name *Dolicirroplectanum lacustre* is urn:lsid:zoobank.org:act:423241C3-777D-4F86-
342 A70F-02B4D74F9E66.

343 **Figures:** 3, 44

344 **Material examined:** holotype: MRAC MT. 35572, paratype: MRAC MT. 35573

345 **Vouchers:** MRAC. MT. 38206-10, 38913-39058 (243 specimens), MZH 10067-71 (6
346 specimens), MNHN HEL744-47 (4 specimens), SAMC-A089971-72 (6 specimens), NHMUK
347 2018.4.13.4-13.7 (8 specimens)

348 **Type host:** *Lates niloticus* L. (Latidae)

349 **Type locality:** Lake Volta, Ghana; Lake Albert, Uganda

350 **Site:** Gills

351 **Additional hosts:** *L. angustifrons, L. mariae, L. microlepis*

352 **Additional localities:** Bahr-Sara, Tchad (08°56'N-17°58'E); Kisumu, Lake Victoria (00°06'S-
353 34°45'E), Lake Kossou, Egypt (07°10'N-05°20'E), Lake Nasser, Egypt (24°05'N-33°00'E),
354 Luxor market, Egypt (25°42'N 32°38'E), Njala, riv. Taja, Sierra Leone (08°06'N-12°04'E), Lake
355 Albert – Nyawiega (01°28'N-30°56'E); Nzunzu (1°19'N, 30°72'E); Lake Tanganyika – Crock
356 Island (8°42'S-31°07'E), Katukula (8°35'S-31°10'E), Mpulungu (8°46'S-31°07'E); Rumonge
357 (3°97'S-29°43'E); Sumbu Bay (8°31'S-30°29'E); Bujumbura (3°23'S-29°22'E); Ilagala (5°12'S-
358 29°50'E); Kilomoni (4°20′S, 29°09′E); Mulembwe (6°07′S, 29°16′E); Nyanza (4°20'S-
359 29°35'E); Edith Bay (6°30'S-29°55'E); Uvira (3°22′ S 29°08′E)

360 Infection parameters: 4 of 8 *Lates angustifrons* infected with 1 – 15 specimens, 15 of 23 *L.*
361 *mariae* infected with 1 – 18 specimens, 21 of 31 *L. microlepis* infected with 1 – 53 specimens.
362 1 of 1 *L. niloticus* from Bahr-Sara infected with 2 specimens, 2 of 3 *L. niloticus* from Kisumu
363 (Lake Victoria) infected with 1-7 specimens, 4 of 5 *L. niloticus* from Lake Kossou infected with
364 5-9 specimens, 1 of 5 *L. niloticus* from Lake Nasser infected with 1 specimen, 1 of 3 *L. niloticus*
365 from Nyawiega (Lake Albert) infected with 2 specimens, 5 of 11 *L. niloticus* from Nzuzu (Lake
366 Albert) infected with 2-10 specimens, 1 of 1 *L. niloticus* from Nzuzu (Lake Albert) infected
367 with 2 specimens.

368 **Diagnosis:** *Dolicirroplectanum lacustre* comb. nov. is a monogenean infecting gills of
369 freshwater African latid species distinguished from its congener by the width of the outer root
370 of the ventral anchor. The copulatory tube is oriented anteriorly.

371 **Description:** Tegument thin, smooth. Three pairs of head organs, two pairs of eye-spots, the
372 posterior ones larger and closer together. Two squamodiscs, ventral squamodisc larger than

373 dorsal, both consist of 9-12 concentric rows of bone-shaped rodlets, the two distal rows of
374 which are composed of only rudimentary rodlets. Two pairs of anchors, rudiment of inner root
375 and wide base of outer root in dorsal anchor. Marginal hooklets (14) of similar size. Ventral bar
376 tapering towards extremities with terminal auricles. Dorsal bar broadening towards the centre
377 of the haptor area. Testis post-ovarial, thin vas deferens along the dextral intestinal caecum.
378 Single seminal vesicle in the middle of the body. Simple prostatic reservoir. MCO robust and
379 formed by two nested tubes. Copulatory tube oriented anteriorly. Ovary looping around the left
380 intestinal caecum towards the oviduct, surrounded by Mehlis' glands located near oötype.
381 Uterus simple tube towards vagina. Vagina is formed by a complex of sclerotized structures
382 consisting of an elongated primary canal followed by a secondary tube opening into an anterior
383 duct; duct continues into distal sclerotized part ending in blade-shaped structure. Orientation of
384 sclerotized vagina with blade-shaped end always anterior. Sclerotised vagina can be absent.
385 Vitellaria dense, located around outer wall of intestinal caeca.

386 **Discussion:** *Dolicirroplectanum lacustre* comb. nov. resembles its congener
387 *Dolicirroplectanum penangi* comb. nov. infecting *Lates calcarifer* in Asia. The type species of
388 the genus can be easily distinguished from *D. penangi* comb. nov. by the comparative
389 morphology of the anchors, especially the thinner outer root in *D. penangi* comb. nov. (see Fig.
390 5). Contrary to *D. lacustre* comb. nov., a sclerotised vagina was not observed in *D. penangi*
391 comb. nov. (Liang and Leong, 1991). Our findings are based only on a combination of the
392 original description of *D. penangi* comb. nov. and voucher material deposited in the National
393 Museum of Natural History in Washington and the National Museum of Natural History in
394 Paris as the Lee Kong Chian Natural History Museum in Singapore refused to provide the
395 holotype material.

396 **Morphometric variation**

397 Morphological variation was visualized based on a PCA performed on 17 standardised
398 haptoral morphometric parameters from 148 individuals. The first PC explained 48.4 % of the
399 variation in the data, the second one 10.1 %. Results show a high level of variability in
400 specimens of *D. lacustre* comb. nov. infecting *L. niloticus* and a continuous size gradient not
401 related to the locality of origin with an intermediate position of specimens from Lake Kossou
402 and Lake Victoria along the first axis. Moreover, individuals collected in the Taja River seem
403 to be separated from the others. Interestingly, two morphotypes were retrieved from different
404 fish specimens in Lake Albert (Lake Albert1 and Lake Albert2). Therefore, the morphology
405 of *D. lacustre* comb. nov. does not seem to be influenced by neither geographical nor host
406 species origin (Fig. 8A). Moreover, two specimens from Lake Albert (belonging to Lake
407 Albert2) were, based on the haptoral sclerotised structures and MCO, more similar to those
408 collected outside the lake (see Table 2). The position of specimens in the scatterplot was
409 mainly influenced by the size of dorsal anchors, maximum width of the dorsal bar and length
410 of both squamodiscs. However, almost all parameters were correlated with the first axis.
411 Other PCs did not show a clearer separation. The length of the dorsal anchor was shown to be
412 related to the combination of host species and geographic origin, in a gradient, with two
413 morphotypes recognised in Lake Albert (Lake Albert1 and Lake Albert2), as visualised in a
414 density plot (Fig. 8B).

415 MCO parameters from 91 individuals of *D. lacustre* comb. nov. were compared. Significantly
416 wider and longer copulatory organs were observed in specimens collected from *L. niloticus*
417 (n=31), than in those collected from *L. microlepis* (n=38) (Bonferroni's post-hoc correction,
418 MCO length $Z_{2,87}$=-6.48, P<0.001, MCO width $Z_{2,89}$=-6.74, P<0.001) and *L. mariae* (n=22)
419 (Bonferroni's post-hoc correction, MCO length $Z_{2,87}$=-4.98, P<0.001, MCO width $Z_{2,89}$=-4.25,
420 P<0.001). The influence of geographical origin was tested only for samples from these three
421 host species from Lake Albert and Lake Tanganyika as there was an insufficient number of

422 high-quality specimens from other localities and *L. angustifrons*, respectively. In both
423 parameters of the MCO, a significantly larger size was observed in specimens from Lake
424 Albert, morphotype Lake Albert1 (Bonferroni's post-hoc correction, MCO length – $Z_{1,89}$ =
425 6.61, $P < 0.001$, MCO width – $Z_{1,87}$ = 6.41, $P < 0.001$).

426 Apart from these size differences, the variable presence of a sclerotised vagina was
427 documented (see Table 2), also including data from two previous records of the species
428 (Ergens, 1981; Thurston and Paperna, 1969).

429 **Genetic characterisation and phylogeography**

430 Uncorrected p-distances between *D. lacustre* comb. nov. collected from Lake Tanganyika and
431 Lake Albert, respectively, varied among the amplified regions from 0.5% in 18S rDNA (441
432 base pairs (bp)), 1.1% in 28S rDNA (810 bp) to 9% in ITS-1 rDNA (478 bp) and 9.0 – 10.2%
433 in COI mtDNA (412 bp). In previous studies, the ability to align ITS-1 sequences was used as
434 a criterion for diplectanid species delineation (Poisot et al., 2011; Wu et al., 2007). No
435 intralacustrine variability in rDNA regions was detected. Sequences of the ITS-1 region of all
436 populations of *D. lacustre* comb. nov. in our study were alignable and included 19 indels. For
437 comparison with the threshold of 14.5% difference in the COI region to distinguish intra- and
438 interspecific diversity proposed for diplectanids by Vanhove et al. (2013), genetic distances
439 were also calculated using the K2P model (Kimura, 1980), under which they amounted to 9.6
440 – 10.7%. Intralacustrine variation in COI was higher in Lake Albert than in Lake Tanganyika
441 (Table 3). The haplotype network showed two distinct haplogroups, corresponding to the two
442 lakes (Fig. 9). Identical COI haplotypes were shared among individuals of *D. lacustre* comb.
443 nov. collected from *L. mariae* originating from the central subbasin (Mulembwe) and *L.*
444 *microlepis* collected from the northern and southern subbasins of Lake Tanganyika (Uvira and
445 Mpulungu).

446 **Phylogeny**

447 Phylogenetic inference at the family level (Diplectanidae) was based on two separate
448 alignments of the 28S and 18S nuclear rDNA with 33 and 15 taxa, respectively (Table S1).
449 The alignment of 28S rDNA and 18S rDNA totalled 803 and 482 bp, respectively.

450 Phylogenetic analyses of 28S rDNA placed the haplotypes of *Dolicirroplectanum lacustre*
451 comb. nov. in a monophyletic clade sister to *Dolicirroplectanum penangi* comb. nov.
452 collected from *Lates calcarifer* in Asia (Fig. 6). The tree obtained from the 18S rDNA
453 fragment placed *D. lacustre* comb. nov. in a poorly resolved clade with species of
454 *Pseudorhabdosynochus* Yamaguti, 1958 and *Echinoplectanum* Justine and Euzet (2006) (Fig.
455 7). ML and BI produced the same topologies. In both phylogenetic trees, the previous notion
456 of *Diplectanum* appeared polyphyletic with the type species, *Diplectanum aequans*, placed
457 outside the clade including species of *Dolicirroplectanum* gen. nov., hence supporting the
458 erection of a new genus.

459 **Discussion**

460 The main aim of this study was to examine the level of diversification in diplectanid parasites
461 infecting latid hosts in two of the African Great Lakes, Lakes Albert and Tanganyika.
462 Moreover, museum specimens from throughout the host's range were added to provide a
463 broader geographical range for morphological comparison. Morphological and molecular
464 characterisation identified a single species in both lakes, reassigned to *Dolicirroplectanum*
465 gen. nov. Despite the persistent geographic separation between Lakes Albert and Tanganyika
466 for 9 MYA (Cohen et al., 1993), and the speciation of the hosts, their respective populations
467 of *D. lacustre* comb. nov. have not reached the level of morphological and genetic
468 differentiation typically associated with distinct species. Hence, we conclude that this is an
469 example of a lineage that failed to speciate.

470 **Diplectanid species infecting latid fishes in Africa – molecular and morphological**
471 **perspectives**

472 The monophyly of *Diplectanum* was already rejected in previous studies (Chotnipat et al.,
473 2015; Villar-Torres et al., 2019) with *Dolicirroplectanum lacustre* comb. nov. being classified
474 outside of *Diplectanum* sensu stricto (Chotnipat et al., 2015; Domingues and Boeger, 2008).
475 The phylogenetic reconstructions based on ribosomal regions place *D. lacustre* comb. nov. in
476 a separate lineage together with *D. penangi* comb. nov. infecting an Asian latid species, *L.*
477 *calcarifer*, but outside the clade that includes *D. aequans*, the type species of *Diplectanum.*
478 This, combined with a detailed morphological characterisation, leads us to propose the new
479 genus *Dolicirroplectanum* gen. nov., now including *D. lacustre* comb. nov. and *D. penangi*
480 comb. nov. Overall, the phylogenetic position of other diplectanid genera corresponds with
481 the study by Villar-Torres et al. (2019). The genetic distance between *D. lacustre* comb. nov.
482 from Lake Tanganyika and Lake Albert, and *D. penangi* comb. nov., is 7.9% based on the
483 28S rDNA fragment. This is comparable to the situation in *Laticola latesi* (Tripathi, 1957)
484 and *L. paralatesi* (Nagibina, 1976), which infect *L. calcarifer* in Hainan province, China
485 (Tingbao et al., 2006). However, these diplectanid species occur sympatrically, infecting a
486 single host species, whereas there is no contact between *L. calcarifer* and the species of *Lates*
487 from Lakes Albert and Tanganyika.

488 Interestingly, copulatory tube width and length differ between most of the parasite individuals
489 collected from *L. niloticus* from Lake Albert and three of Lake Tanganyika's species, *L.*
490 *angustifrons*, *L. microlepis* and *L. mariae,* respectively. Differences in the MCO may be the
491 basis for species delineation in diplectanids (e.g. in *Echinoplectanum*: Sigura & Justine,
492 2008). However, the mean values of individuals of *L. niloticus* from other localities (Lake
493 Kossou, Lake Victoria) do not differ from Lake Tanganyika's specimens. Moreover, two
494 specimens from Lake Albert (belonging to Lake Albert2) are, based on the haptoral

495 sclerotised structures and the MCO, more similar to those collected outside this lake (see
496 Table 2). A complex pattern of morphological variation emerging from the other populations
497 of *D. lacustre* comb. nov., with a lot of overlapping features between host species and
498 localities (Fig. 8 and Table 2), does not suggest the existence of different species. The
499 intermediate position of some specimens, particularly from Lake Albert (referred to as Lake
500 Albert2), prevents a clear correlation between parasite morphotype, host species identity
501 and/or geographic origin. Future genetic characterisation of such morphotypes is needed to
502 address diversification of *D. lacustre* comb. nov. in detail. Internal anatomy is documented
503 only in fresh specimens from Lake Albert and Lake Tanganyika, with high levels of
504 intralacustrine variation and without structural differences in organisation between these
505 lakes.

506 Moreover, the impossibility to align ITS-1 rDNA sequences is generally considered as an
507 indicator for diplectanid species delineation (Wu et al., 2005a; Poisot et al., 2011). Since
508 haplotypes from the two populations of *D. lacustre* comb. nov. in our study are alignable, the
509 hypothesis of a single species is supported. Also, as the model-corrected genetic distance of
510 10.7% over the COI fragment does not reach the “best-compromise threshold” (Meier et al.,
511 2006) for barcoding of 14.5% proposed by Vanhove et al. (2013) for diplectanids infecting
512 Indo-Pacific groupers, we consider all specimens in our study as conspecific and belonging to
513 *D. lacustre* comb. nov. Its records therefore increased from four to ten areas (see Table 1 and
514 taxonomic part of the result section).

515 Based on differences in the size of haptoral structures and the split ends of the internal tube of
516 the MCO (see Table 2 and Fig. 4), specimens from Taja River might be considered as
517 belonging to a different species. This could be explained by the long separation between the
518 Upper Guinean and Nil ichthyofaunal provinces (Roberts, 1975). However, more samples and

519 molecular data from Taja River are needed to confirm the identity of the species of
520 *Dolicirroplectanum* infecting *L. niloticus* in the Upper Guinean province.

521 **Host range of the diplectanid monogeneans infecting lates perches in Lake Tanganyika**

522 Based on our results, the host species list of *D. lacustre* comb. nov. was extended with three
523 of the four latid species from Lake Tanganyika. No monogeneans were found on *L. stappersii*.
524 A potential reason could be its different life style compared to other latid species in the lake.
525 In contrast to its congeners, *L. stappersii* is truly pelagic throughout its life, usually not
526 moving into inshore waters (Mannini et al., 1999; Mulimbwa and Mannini, 1993). Short-lived
527 and slow swimming monogenean larvae (oncomiracidia) are assumed to infect fish hosts in
528 littoral habitats, typically synchronised with their hosts' period of reproduction (Whittington
529 et al., 1999). Therefore, there is less chance for parasite infection in *L. stappersii* compared to
530 other latid species (see Rohde, 1980; Rohde et al., 1995). Moreover, there is no sign of
531 species diversification in Lake Tanganyika as we found haplotypes shared between latid hosts
532 and between subbasins.

533 The African Great Lakes are highly biodiverse areas with a remarkable species richness and a
534 high level of endemism (Salzburger et al., 2014). Parasite diversification linked with host
535 speciation was recently discovered in monogeneans belonging to *Cichlidogyrus* Paperna,
536 1960 infecting littoral cichlids of Lake Tanganyika (Vanhove et al., 2015). Interestingly, also
537 in Lake Tanganyika's pelagic zone, the same pattern as in *D. lacustre* comb. nov., apparently
538 without host preference or host-related speciation processes, was observed in *Cichlidogyrus*
539 *casuarinus*, a parasite of bathybatine cichlids (Kmentová et al., 2016). However, the
540 haplotype and nucleotide diversity in the COI region are remarkably lower in *D. lacustre*
541 comb. nov. (0.517 and 0.001 compared to 0.987 and 0.0205, respectively). Host species
542 hybridisation might explain the more generalist life style of certain monogeneans due to an
543 influence of host genetics on the susceptibility to infection, host specificity, and parasite

544 speciation (Šimková et al., 2013; Vanhove et al., 2011). However, there are no reports of
545 hybridisation among latid species (Otero, 2004). Moreover, a lack of host-related speciation in
546 diplectanids was observed in *Pseudorhabdosynochus cyanopodus* Sigura & Justine, 2008
547 infecting two deep-sea grouper species in New Caledonia (Schoelinck et al., 2012) with a
548 maximum intraspecific distance of 1.2% in the COI region compared to 0.7% in *D. lacustre*
549 comb. nov. in Lake Tanganyika. Our results therefore correspond with previous studies in
550 marine and freshwater habitats where decreased host specificity in pelagic ecosystems was
551 proposed to increase the chance of finding a host if host species exhibit low population
552 densities (Kmentová et al., 2016; Rohde, 1980; Schoelinck et al., 2012).

553 Despite the generally high degree of endemism of macrofauna in Lake Tanganyika (Coulter,
554 1991; Salzburger et al., 2014; Snoeks, 2000), this might not be reflected in all
555 microinvertebrate taxa. *Dolicirroplectanum lacustre* comb. nov. and some other monogenean
556 species are found to naturally occur both within and outside Lake Tanganyika. *Gyrodactylus*
557 *sturmbaueri* Vanhove, Snoeks, Volckaert & Huyse, 2011 was described from *Simochromis*
558 *diagramma* (Günther, 1894), but also parasitizes on *Pseudocrenilabrus philander* (Weber,
559 1897) in Lake Kariba and the River Nwanedi (Zahradníčková et al., 2016). *Cichlidogyrus*
560 *mbirizei* Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle 2012, *C. halli* (Price and
561 Kirk 1967) and *Scutogyrus gravivaginus* (Paperna and Thurston 1969) are known from an
562 endemic tilapia, *Oreochromis tanganicae* (Günther, 1894), but were also reported from other
563 species of *Oreochromis* Günther, 1889 and other cichlids in Africa (Douëllou, 1993; Pariselle
564 and Euzet, 2009) as well as cage-cultured tilapia species in Asia (Lim et al., 2016; Mohd
565 Agos et al., 2016). This highlights the ability of some monogenean species to survive in a
566 wide range of environments and host species (Huyse et al., 2006).

567

568 **Rate of molecular evolution of *Dolicirroplectanum lacustre* comb. nov. and its**
569 **implications**

570 For want of paleontological data, substitution rates in parasitic flatworms are typically
571 estimated using host fossils or calibrated with paleogeographical events and assuming that
572 parasite speciation follows that of the hosts (Meinilä et al., 2004). With a mean distance of
573 10% between the Lake Tanganyika and Lake Albert populations, the substitution rate in our
574 412 bp COI mtDNA region, using the end of rifting in the eastern African Rift Valley 9 MYA
575 (Cohen et al., 1993) for the age of the most recent common ancestor of their hosts, is
576 estimated at 0.5%/MY.

577 Often, molecular evolution of parasites is considered and was proven to be faster than within
578 the homologous loci of their hosts (Hafner et al., 1994; Huyse et al., 2005). Surprisingly, *D.*
579 *lacustre* comb. nov. appears to have a slower rate of molecular evolution in its mitochondrial
580 DNA than most fish taxa (1-4% in cytochrome *b*) (Bermingham et al., 1997; He and Chen,
581 2007; Muss et al., 2001). However, preliminary molecular data of African latids show even
582 less difference over the COI region (4.5-6% uncorrected p-distance between Lake Tanganyika
583 and Lake Albert, own unpublished data) than their monogenean parasite. Therefore, the
584 widespread hypothesis of a faster evolutionary rate of parasites compared to hosts, based on
585 their shorter generation time (Cable and Harris, 2002; Thomas et al., 2010) does hold in the
586 case of *D. lacustre* comb. nov. The link between the rate of morphological and molecular
587 evolution varies among different taxa (Omland, 1997) with studies showing either rate
588 decoupling (Poisot et al., 2011) or rate correlation. However, it seems that in the case of *D.*
589 *lacustre* comb. nov., there is a correlation between a slow rate of molecular evolution in the
590 COI gene, which is a structural coding marker known to be under balancing selection (Wu et
591 al., 1997), and failure to diverge seen in the lack of speciation.

592 There are three possible scenarios explaining the observed situation in *D. lacustre* comb. nov.
593 First, the rate of evolution of latids and their parasites is slower in comparison to other fish
594 (Bermingham et al., 1997; Muss et al., 2001) and other monogenean taxa. Indeed, the

595 mutation rate of *D. lacustre* comb. nov. seems to be much lower than the 13.7 – 20.3% per
596 million years estimated for *Gyrodactylus* von Nordmann, 1832 by Meinilä et al. (2004). This
597 can be explained by their different life history, as diplectanids lack the asexual reproduction
598 that has also led to a high species richness promoted by host switches and peripatric
599 speciation processes in *Gyrodactylus* (Boeger et al., 2003). The failure to diverge of *D.*
600 *lacustre* comb. nov. in Lake Tanganyika then corresponds with the hypothesis suggesting that
601 a lower rate of molecular evolution resulted in low diversification.

602 Secondly, the invasion of the studied latid lineage could be more recent than the lakes'
603 formation, like in the case of the cichlid genera *Tylochromis* Steindachner, 1895 and
604 *Oreochromis* (Klett and Meyer, 2002; Koch et al., 2007). This could explain the low level of
605 genetic intralacustrine variation of *D. lacustre* comb. nov. reported in Lake Tanganyika (0.7%
606 of uncorrected p-distance in COI) which contrasts with greater genetic variation seen in the
607 host species (2-3% of uncorrected p-distance in COI, unpublished data). However, low
608 intralacustrine genetic diversity in *D. lacustre* comb. nov. could also be caused by bottleneck
609 events that have reduced the genetic variation present in the system.

610 A third possible scenario involves a latid origin in the proto-Tanganyikan region with more
611 recent admixture of populations via lacustrine and riverine connections resulting in the
612 polyphyly of latid species in Lake Tanganyika (suggested by Otero (2004) based on
613 morphological data), as was documented for haplochromine cichlids in the lake (Meyer et al.,
614 2015; Salzburger et al., 2005). Therefore, molecular and morphological similarity of *D.*
615 *lacustre* comb. nov. in nowadays geographically isolated areas could be the result of recent
616 and maybe multiple episodes of gene flow. In any case, phylogenetic reconstruction combined
617 with the latids' fossil record is needed to discern between the above-mentioned hypotheses.

618 **Conclusions**

619 Diplectanid parasites occur primarily in marine environments. Discussion has arisen about the
620 incongruence between their morphological species delineation and the level of molecular
621 differentiation. In our study, we focused on a unique allopatric situation of diplectanid
622 parasites infecting latid species inhabiting freshwater lakes to study this incongruence. Based
623 on morphological examination, a single diplectanid species was recorded from three of the
624 four species of *Lates* from Lake Tanganyika (*L. angustifrons, L. mariae, L. microlepis*) and
625 from *L. niloticus* from Lakes Albert, Kossou, Nasser and Victoria, from the Taja River in
626 Sierra Leone and from Bahr-Sara in Tchad. Thus, similar to another monogenean species
627 infecting pelagic host species of the cichlid tribe Bathybatini in Lake Tanganyika, this
628 parasite on African *Lates* apparently failed to diverge. Results of phylogenetic reconstruction
629 combined with detailed morphological characterisation led us to propose *Dolicirroplectanum*
630 gen. nov. with *D. lacustre* comb. nov. as type species. Despite a persistent geographic barrier
631 between Lake Albert and Lake Tanganyika and speciation in the hosts, their respective
632 populations of *D. lacustre* comb. nov. infecting these lakes' latid fishes have not reached
633 species-level distinction. We suggest a link between the lack of morphological differentiation
634 between the parasite populations in both lakes, and the low rate of molecular evolution of the
635 mitochondrial COI gene, estimated at 0.5%/MY (assuming *Lates* from Lakes Albert and
636 Tanganyika diverged 9 MYA). As alternatives, scenarios proposing either more recent
637 invasion of the latid lineage into Lake Tanganyika or recent gene flow among the latid
638 lineages in Lakes Albert and Tanganyika could explain the apparently slow rate of the hosts'
639 molecular evolution and lack of parasite differentiation. Therefore, detailed studies of host
640 phylogeography, dated using the fossil record, are needed to discern between these scenarios.
641 Although species-level differentiation can be expected in the future under persisting
642 separation between the lakes, the question about the existence of genetically intermediate
643 populations of *D. lacustre* comb nov. remains.

644 **Acknowledgments**

645 The authors would like to thank: Ludmila Raisingerová, Chahrazed Rahmouni, Mulongaibalu
646 Mbalassa, Vercus Lumami Kapepula, Joseph Mbirize Ndalozibwa, Jos Snoeks and Christian
647 Sturmbauer for help with obtaining or identifying samples. Fieldwork was carried out with the
648 approval of the competent local authorities under mission statement 031/MINRST/CRH-
649 U/2016 and an export permit from the CRH-Uvira and from the National Fish and Fisheries
650 Research Institute (Nafirri, Jinja, Uganda). We also thank Tine Huyse, Nathan Vranken,
651 Emmanuel Vreven, Miguël Parrent (RMCA), Filip Volckaert (KU Leuven) and people in the
652 parasitological group, Masaryk University, Brno and Research Centre of Hydrobiology, Uvira
653 for their hospitality and the RMCA for providing access to the collections.

654 **References**

655 Bandelt, H.J., Forster, P., Rohl, A., 1999. Median-joining networks for inferring intraspecific
656 phylogenies. Mol. Biol. Evol. 16, 37–48.
657 https://doi.org/10.1093/oxfordjournals.molbev.a026036

658 Bermingham, E., McCafferty, S., Martin, A., 1997. Fish biogeography and molecular clocks:
659 perspectives from the Panamanian Isthmus., in: Kocher, T., Stepien, C. (Eds.), Molecular
660 Systematics of Fishes. Academic Press, pp. 113–128.

661 Blasco-Costa, I., Míguez-Lozano, R., Sarabeev, V., Balbuena, J.A., 2012. Molecular
662 phylogeny of species of *Ligophorus* (Monogenea: Dactylogyridae) and their affinities
663 within the Dactylogyridae. Parasitol. Int. 61, 619–627.
664 https://doi.org/10.1016/j.parint.2012.06.004

665 Boeger, W.A., Kritsky, D.C., Pie, M.R., 2003. Context of diversification of the viviparous
666 Gyrodactylidae (Platyhelminthes, Monogenoidea). Zool. Scr. 32, 437–448.
667 https://doi.org/10.1046/j.1463-6409.2003.00130.x

668 Brooks, D.R., 1979. Testing the context and extent of host-parasite coevolution. Syst. Biol.
669 28, 299–307. https://doi.org/10.1093/sysbio/28.3.299

670 Cable, J., Harris, P.D., 2002. Gyrodactylid developmental biology: historical review, current
671 status and future trends. Int. J. Parasitol. 32, 255–80.
672 https://doi.org/https://doi.org/10.1016/S0020-7519(01)00330-7

673 Capella-Gutiérrez, S., Silla-Martínez, J.M., Gabaldón, T., 2009. trimAl: a tool for automated
674 alignment trimming in large-scale phylogenetic analyses. Bioinforma. Appl. Note 25,
675 1972–1973. https://doi.org/10.1093/bioinformatics/btp348

676 Chotnipat, S., Miller, T.L., Knuckey, R.M., Hutson, K.S., 2015. Molecular and morphological

677 evidence for the widespread distribution of *Laticola paralatesi* infecting wild and farmed
678 *Lates calcarifer* in Australia. Dis. Aquat. Organ. 113, 195–205.
679 https://doi.org/10.3354/dao02848

680 Cohen, A.S., Soreghan, M.J., Scholz, C.A., 1993. Estimating the age of formation of lakes:
681 An example from Lake Tanganyika, East African Rift system. Geology 21, 511–514.
682 https://doi.org/10.1130/0091-7613(1993)021<0511:ETAOFO>2.3.CO;2

683 Coulter, G.W., 1991. Composition of the flora and fauna, in: Coulter, G.W. (Ed.), Lake
684 Tanganyika and Its Life. Natural History Musem Publications & Oxford University
685 Press, London Oxford & New York, pp. 200–274.

686 Danley, P.D., Husemann, M., Ding, B., Dipietro, L.M., Beverly, E.J., Peppe, D.J., 2012. The
687 impact of the geologic history and paleoclimate on the diversification of East African
688 cichlids. Int. J. Evol. Biol. 574851. https://doi.org/10.1155/2012/574851

689 DeSalle, R., Egan, M.G., Siddall, M., 2005. The unholy trinity: taxonomy, species
690 delimitation and DNA barcoding. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 360, 1905–
691 1916. https://doi.org/10.1098/rstb.2005.1722

692 Desdevises, Y., Morand, S., Oliver, G., 2001. Linking specialisation to diversification in the
693 Diplectanidae Bychowsky 1957 (Monogenea, Platyhelminthes). Parasitol. Res. 87, 223–
694 230. https://doi.org/10.1007/s004360000325

695 Domingues, M. V., Boeger, W.A., 2008. Phylogeny and revision of Diplectanidae Monticelli,
696 1903 (Platyhelminthes: Monogenoidea). Zootaxa 1903, 1–40.

697 Douëllou, L., 1993. Monogeneans of the genus *Cichlidogyrus* Paperna, 1960 (Dactylogyridae:
698 Ancyrocephalinae) from cichlid fishes of Lake Kariba (Zimbabwe) with descriptions of
699 five new species. Syst. Parasitol. 25, 159–186. https://doi.org/10.1007/BF00007007

700 Edgar, R.C., 2004. MUSCLE: Multiple sequence alignment with high accuracy and high
701 throughput. Nucleic Acids Res. 32, 1792–1797. https://doi.org/10.1093/nar/gkh340

702 Ergens, R., 1981. *Diplectanum lacustre* Thurston et Paperna, 1969 (Monogenea,
703 Diplectanidae) from the Egyptian *Lates niloticus*. Folia Parasitol. 28, 160.

704 Ergens, R., Lom, J., 1970. Causative agents of fish diseases. Academia, Prague.

705 Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to
706 perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10,
707 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

708 Francis, C.M., Borisenko, A. V., Ivanova, N. V., Eger, J.L., Lim, B.K., Guillén-Servent, A.,
709 Kruskop, S. V., Mackie, I., Hebert, P.D.N., 2010. The role of DNA barcodes in
710 understanding and conservation of mammal diversity in Southeast Asia. PLoS One 5,
711 e12575. https://doi.org/10.1371/journal.pone.0012575

712 Gastwirth, J.L., Gel, Y.R., Hui, W.L.W., Lyubchich, V., Miao, W., Noguchi, K., 2017.
713 lawstat: Tools for Biostatistics, Public Policy, and Law.

714 Georgieva, S., Selbach, C., Faltýnková, A., Soldánová, M., Sures, B., Skírnisson, K.,
715 Kostadinova, A., 2013. New cryptic species of the '*revolutum*' group of *Echinostoma*
716 (Digenea: Echinostomatidae) revealed by molecular and morphological data. Parasit.
717 Vectors 6, 64. https://doi.org/10.1186/1756-3305-6-64

718 Hafner, M.S., Sudman, P.D., Villablanca, F.X., Spradling, T.A., Demastes, J.W., Nadler,

719 S.A., 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites.
720 Science 265, 1087–1090.

721 Hassouna, N., Michot, B., Bachellerie, J.-P., Narbonne, D., 1984. The complete nucleotide
722 sequence of mouse 28S rRNA gene. Implications for the process of size increase of the
723 large subunit rRNA in higher eukaryotes. Nucleic Acids Res. 12, 3563–3583.

724 He, D., Chen, Y., 2007. Molecular phylogeny and biogeography of the highly specialized
725 grade schizothoracine fishes (Teleostei: Cyprinidae) inferred from cytochrome b
726 sequences. Chinese Sci. Bull. 52, 777–788. https://doi.org/10.1007/s11434-007-0123-2

727 Hebert, P.D.N., Cywinska, A., Ball, S.L., DeWaard, J.R., 2003. Biological identifications
728 through DNA barcodes. Proceedings. Biol. Sci. 270, 313–321.
729 https://doi.org/10.1098/rspb.2002.2218

730 Hubert, N., Hanner, R., Holm, E., Mandrak, N.E., Taylor, E., Burridge, M., Watkinson, D.,
731 Dumont, P., Curry, A., Bentzen, P., Zhang, J., April, J., Bernatchez, L., 2008. Identifying
732 Canadian freshwater fishes through DNA barcodes. PLoS One 3, e2490.
733 https://doi.org/10.1371/journal.pone.0002490

734 Huyse, T., Pampoulie, C., Audenaert, V., Volckaert, F.A.M., 2006. First report of
735 *Gyrodactylus* spp. (Platyhelminthes: Monogenea) in the Western Mediterranean Sea:
736 molecular and morphological descriptions. J. Parasitol. 92, 682–690.
737 https://doi.org/10.1645/GE-690R.1

738 Huyse, T., Poulin, R., Théron, A., 2005. Speciation in parasites: a population genetics
739 approach. Trends Parasitol. 21, 469–475. https://doi.org/10.1016/j.pt.2005.08.009

740 International Commission on Zoological Nomenclature, 2012. Amendment of articles 8, 9,
741 10, 21 and 78 of the International Code of Zoological Nomenclature to expand and refine
742 methods of publication. Zootaxa 3450, 1–7.

743 Jombart, T., 2008. Adegenet: A R package for the multivariate analysis of genetic markers.
744 Bioinformatics 24, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

745 Justine, J.-L., 2005. Species of *Pseudorhabdosynochus* Yamaguti, 1958 (Monogenea:
746 Diplectanidae) from *Epinephelus fasciatus* and *E. merra* (Perciformes: Serranidae) off
747 New Caledonia and other parts of the Indo-Pacific Ocean, with a comparison of
748 measurements. Syst. Parasitol. 62, 1–37. https://doi.org/10.1007/s11230-005-5480-0

749 Justine, J.-L., Euzet, L., 2006. Diplectanids (Monogenea) parasitic on the gills of the
750 coralgroupers *Plectropomus laevis* and *P. leopardus* (Perciformes, Serranidae) off New
751 Caledonia, with the description of five new species and the erection of *Echinoplectanum*
752 n. g. Syst. Parasitol. 64, 147–172. https://doi.org/10.1007/s11230-006-9028-8

753 Justine, J.-L., Henry, É., 2010. Monogeneans from *Epinephelus chlorostigma* (Val.)
754 (Perciformes: Serranidae) off New Caledonia, with the description of three new species
755 of diplectanids. Syst. Parasitol. 77, 81–105. https://doi.org/10.1007/s11230-010-9263-x

756 Kassambara, A., Mundt, F., 2017. factoextra: extract and visualize the results of multivariate
757 data analyses.

758 Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions
759 through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.

760 Klett, V., Meyer, A., 2002. What, if anything, is a Tilapia?—mitochondrial ND2 phylogeny

761 of tilapiines and the evolution of parental care systems in the African cichlid fishes. Mol.
762 Biol. Evol. 19, 865–883. https://doi.org/10.1093/oxfordjournals.molbev.a004144

763 Kmentová, N., Gelnar, M., Mendlová, M., Van Steenberge, M., Koblmüller, S., Vanhove,
764 M.P.M., 2016. Reduced host-specificity in a parasite infecting non-littoral Lake
765 Tanganyika cichlids evidenced by intraspecific morphological and genetic diversity. Sci.
766 Rep. 6, 39605. https://doi.org/10.1038/srep39605

767 Kmentová, N., Van Steenberge, M., Raeymaekers, J.A.R., Koblmüller, S., Hablützel, P.I.,
768 Muterezi Bukinga, F., Mulimbwa N'sibula, T., Masilya Mulungula, P., Nzigidahera, B.,
769 Ntakimazi, G., Gelnar, M., Vanhove, M.P.M., 2018. Monogenean parasites of sardines in
770 Lake Tanganyika: diversity, origin and intra-specific variability. Contrib. to Zool. 87,
771 105–132.

772 Koch, M., Koblmüller, S., Sefc, K.M., Duftner, N., Katongo, C., Sturmbauer, C., 2007.
773 Evolutionary history of the endemic Lake Tanganyika cichlid fish *Tylochromis polylepis*:
774 a recent intruder to a mature adaptive radiation. J. Zool. Syst. Evol. Res. 45, 64–71.
775 https://doi.org/10.1111/j.1439-0469.2006.00381.x

776 Kumar, S., Stecher, G., Tamura, K., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies,
777 J., Ludwig, W., Glockner, F., 2016. MEGA7: Molecular evolutionary genetics analysis
778 Version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.
779 https://doi.org/10.1093/molbev/msw054

780 Leigh, J.W., Bryant, D., 2015. PopART: full-feature software for haplotype network
781 construction. Methods Ecol. Evol. 6, 1110–1116.

782 Liang, K.., Leong, T.., 1991. A redescription of *Pseudorhabdosynochus latesi* (Tripathi 1955)
783 and description of *Diplectanum penangi* n. sp. from *Lates calcarifer* cultured in floating
784 cages in Malaysia and Thailand. J. Biosci. 2, 77–84.

785 Lim, S.-Y., Ooi, A.-L., Wong, W.-L., 2016. Gill monogeneans of Nile tilapia (*Oreochromis
786 niloticus*) and red hybrid tilapia (*Oreochromis* spp.) from the wild and fish farms in
787 Perak, Malaysia: infection dynamics and spatial distribution. Springerplus 5, 1609.
788 https://doi.org/10.1186/s40064-016-3266-2

789 Littlewood, D.T.J., Rohde, K., Clough, K.A., 1997. Parasite speciation within or between host
790 species? - Phylogenetic evidence from site-specific polystome monogeneans. Int. J.
791 Parasitol. 27, 1289–1297. https://doi.org/10.1016/S0020-7519(97)00086-6

792 Mannini, P., Katonda, I., Kissaka, B., Verburg, P., 1999. Feeding ecology of *Lates stappersii*
793 in Lake Tanganyika. Hydrobiologia 407, 131–139.
794 https://doi.org/10.1023/A:1003714203943

795 Meier, R., Shiyang, K., Vaidya, G., Ng, P.K.L., 2006. DNA barcoding and taxonomy in
796 Diptera: A tale of high intraspecific variability and low identification success. Syst. Biol.
797 55, 715–728. https://doi.org/10.1080/10635150600969864

798 Meinilä, M., Kuusela, J., Ziętara, M.S., Lumme, J., 2004. Initial steps of speciation by
799 geographic isolation and host switch in salmonid pathogen *Gyrodactylus salaris*
800 (Monogenea: Gyrodactylidae). Int. J. Parasitol. 34, 515–526.
801 https://doi.org/10.1016/j.ijpara.2003.12.002

802 Meyer, B.S., Indermaur, A., Ehrensperger, X., Egger, B., Banyankimbona, G., Snoeks, J.,
803 Salzburger, W., 2015. Back to Tanganyika: a case of recent trans-species-flock dispersal

804 in East African haplochromine cichlid fishes. R. Soc. open Sci. 2, 140498.
805 https://doi.org/10.1098/rsos.140498

806 Milne, I., Wright, F., Rowe, G., Marshall, D.F., Husmeier, D., McGuire, G., 2004. TOPALi:
807 software for automatic identification of recombinant sequences within DNA multiple
808 alignments. Bioinformatics 20, 1806–1807.
809 https://doi.org/10.1093/bioinformatics/bth155

810 Mohd Agos, S., Shaharom-H, F., Ihwan Zaka, M., Hassan, M., 2016. Morphological study of
811 *Cichlidogyrus mbirizei* (Ancyrocephalidae) monogenean gill parasite on red tilapia
812 (*Oreochromis* sp.) from Como River Kenyir Lake, Terengganu Malaysia. J. Fish. Aquat.
813 Sci. 11, 432–436. https://doi.org/10.3923/jfas.2016.432.436

814 Mulimbwa, N., Mannini, P., 1993. Demographic characteristics of *Stolothrissa tanganicae*,
815 *Limnothrissa miodon* and *Lates stappersii* in the Northwestern (Zairean) waters of Lake
816 Tanganyika. CIFA Occas. Pap.

817 Muss, A., Robertson, D.R., Stepien, C.A., Wirtz, P., Bowen, B.W., 2001. Phylogeography of
818 *Ophioblennius*: the role of ocean currents and geography in reef fish evolution. Evolution
819 (N. Y). 55, 561–572.

820 Ogle, D.H., Wheeler, P., Dinno, A., 2019. FSA: Fisheries Stock Analysis.

821 Ogutu-Ohwayo, R., 1995. Diversity and stability of fish stocks in Lakes Victoria, Kyoga and
822 Nabugabo after establishment of introduced species, in: Hart, P., Pitcher, T.J. (Eds.), The
823 Impact of Species Changes in African Lakes. Springer Netherlands, Dordrecht, pp. 59–
824 81. https://doi.org/10.1007/978-94-011-0563-7_5

825 Omland, K.E., 1997. Correlated rates of molecular and morphological evolution. Evolution
826 (N. Y). 51, 1381–1393.

827 Otero, O., 2004. Anatomy, systematics and phylogeny of both recent and fossil latid fishes
828 (Teleostei, Perciformes, Latidae). Zool. J. Linn. Soc. 81–133.
829 https://doi.org/10.1111/j.1096-3642.2004.00111.x

830 Paperna, I., Thurston, J.P., 1969. Monogenetic trematodes collected from cichlid fish in
831 Uganda; including the description of five new species of *Cichlidogyrus*. Rev. Zool. Bot.
832 Africaines 79, 15–33.

833 Pariselle, A., Euzet, L., 2009. Systematic revision of dactylogyridean parasites (Monogenea)
834 from cichlid fishes in Africa, the Levant and Madagascar. Zoosystema 31, 849–898.
835 https://doi.org/10.5252/z2009n4a6

836 Pariselle, A., Muterezi Bukinga, F., Van Steenberge, M., Vanhove, M.P.M., 2015.
837 Ancyrocephalidae (Monogenea) of Lake Tanganyika: IV: *Cichlidogyrus* parasitizing
838 species of Bathybatini (Teleostei, Cichlidae): reduced host-specificity in the deepwater
839 realm? Hydrobiologia 748, 99–119. https://doi.org/10.1007/s10750-014-1975-5

840 Paugy, D., Leveque, C., Teugels, G.G., 2003. The fresh and brackish water fishes of West
841 Africa - Volume 2. Royal Museum for Central Africa, Tervuren.

842 Poisot, T., Verneau, O., Desdevises, Y., Kitchener, A., Daniels, M., 2011. Morphological and
843 molecular evolution are not linked in *Lamellodiscus* (Plathyhelminthes, Monogenea).
844 PLoS One 6, e26252. https://doi.org/10.1371/journal.pone.0026252

845 Poll, M., 1953. Exploration hydrobiologique du lac Tanganika, in: Poissons Non Cichlidae.

846 Institut Royal des Sciences Naturelles de Belgique, Brussels, p. 251.

847 Poulin, R., 2002. The evolution of monogenean diversity. Int. J. Parasitol. 32, 245–254.
848 https://doi.org/10.1016/S0020-7519(01)00329-0

849 R Core Team, 2013. R: A language and environment for statistical computing. R Foundation
850 for Statistical Computing, Vienna, Austria.

851 Rahmouni, I., Řehulková, E., Pariselle, A., Rkhami, O.B., Šimková, A., 2017. Four new
852 species of *Dactylogyrus* Diesing, 1850 (Monogenea: Dactylogyridae) parasitising the
853 gills of northern Moroccan *Luciobarbus* Heckel (Cyprinidae): morphological and
854 molecular characterisation. Syst. Parasitol. 94, 575–591. https://doi.org/10.1007/s11230-
855 017-9726-4

856 Roberts, T.R., 1975. Geographical distribution of African freshwater fishes. Zool. J. Linn.
857 Soc. 57, 249–319. https://doi.org/10.1111/j.1096-3642.1975.tb01893.x

858 Rohde, K., 1980. Diversity gradients of marine Monogenea in the Atlantic and Pacific
859 Oceans. Experientia 36, 1368–1369. https://doi.org/10.1007/BF01960102

860 Rohde, K., Hayward, C., Heap, M., 1995. Aspects of the ecology of metazoan ectoparasites of
861 marine fishes. Int. J. Parasitol. 25, 945–970. https://doi.org/10.1016/0020-
862 7519(95)00015-T

863 Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Hohna, S., Larget, B.,
864 Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: Efficient Bayesian
865 phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–
866 542. https://doi.org/10.1093/sysbio/sys029

867 Salzburger, W., 2018. Understanding explosive diversification through cichlid fish genomics.
868 Nat. Rev. Genet. 1. https://doi.org/10.1038/s41576-018-0043-9

869 Salzburger, W., Mack, T., Verheyen, E., Meyer, A., 2005. Out of Tanganyika: genesis,
870 explosive speciation, key-innovations and phylogeography of the haplochromine cichlid
871 fishes. BMC Evol. Biol. 5, 17. https://doi.org/10.1186/1471-2148-5-17

872 Salzburger, W., Van Bocxlaer, B., Cohen, A.S., 2014. Ecology and evolution of the African
873 Great Lakes and their faunas. Annu. Rev. Ecol. Evol. Syst. 45, 519–545.
874 https://doi.org/10.1146/annurev-ecolsys-120213-091804

875 Schlick-Steiner, B.C., Steiner, F.M., Seifert, B., Stauffer, C., Christian, E., Crozier, R.H.,
876 2010. Integrative taxonomy: A multisource approach to exploring biodiversity. Annu.
877 Rev. Entomol. 55, 421–438. https://doi.org/10.1146/annurev-ento-112408-085432

878 Schoelinck, C., Cruaud, C., Justine, J.-L., 2012. Are all species of *Pseudorhabdosynochus*
879 strictly host specific? - A molecular study. Parasitol. Int. 61, 356–359.
880 https://doi.org/10.1016/j.parint.2012.01.009

881 Sigura, A., Justine, J.-L., 2008. Monogeneans of the speckled blue grouper, *Epinephelus*
882 *cyanopodus* (Perciformes, Serranidae), from off New Caledonia, with a description of
883 four new species of *Pseudorhabdosynochus* and one new species of *Laticola*
884 (Monogenea: Diplectanid. Zootaxa 44, 1–44.

885 Šimková, A., Dávidová, M., Papoušek, I., Vetešník, L., 2013. Does interspecies hybridization
886 affect the host specificity of parasites in cyprinid fish? Parasit. Vectors 6, 95.
887 https://doi.org/10.1186/1756-3305-6-95

888 Šimková, A., Matějusová, I., Cunningham, C.O., 2006. A molecular phylogeny of the
889 Dactylogyridae sensu Kritsky & Boeger (1989) (Monogenea) based on the D1-D3
890 domains of large subunit rDNA. Parasitology 133, 43–53.
891 https://doi.org/10.1017/S0031182006009942

892 Šimková, A., Morand, S., Jobet, E., Gelnar, M., Verneau, O., 2004. Molecular phylogeny of
893 congeneric monogenean parasites (*Dactylogyrus*): a case of intrahost speciation.
894 Evolution 58, 1001–1018. https://doi.org/10.1111/j.0014-3820.2004.tb00434.x

895 Šimková, A., Plaisance, L., Matějusová, I., Morand, S., Verneau, O., 2003. Phylogenetic
896 relationships of the Dactylogyridae Bychowsky, 1933 (Monogenea: Dactylogyridea):
897 The need for the systematic revision of the Ancyrocephalinae Bychowsky, 1937. Syst.
898 Parasitol. 54, 1–11. https://doi.org/10.1023/A:1022133608662

899 Sinnappah, N.D., Lim, L.-H.S., Rohde, K., Tinsley, R., Combes, C., Verneau, O., 2001. A
900 paedomorphic parasite associated with a neotenic amphibian host: phylogenetic evidence
901 suggests a revised systematic position for sphyranuridae within anuran and turtle
902 polystomatoineans. Mol. Phylogenet. Evol. 18, 189–201.
903 https://doi.org/10.1006/mpev.2000.0877

904 Snoeks, J., 2000. Ancient lakes: biodiversity, ecology and evolution. Adv. Ecol. Res. 31, 17–
905 38. https://doi.org/10.1016/S0065-2504(00)31005-4

906 Stamatakis, A., 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis
907 of large phylogenies. Bioinformatics 30, 1312–1313.
908 https://doi.org/10.1093/bioinformatics/btu033

909 Thomas, J.A., Welch, J.J., Lanfear, R., Bromham, L., 2010. A generation time effect on the
910 rate of molecular evolution in invertebrates. Mol. Biol. Evol. 27, 1173–1180.
911 https://doi.org/10.1093/molbev/msq009

912 Thurston, J.P., Paperna, I., 1969. *Diplectanum lacustris* sp. nov. (Dactylogyroidea:
913 Diplectanidae), a Monogenetic Trematode from the gills of the Nile Perch. Proc.
914 Helminthol. Soc. 214–218.

915 Tingbao, Y., Kritsky, D.C., Yuan, S., Jianying, Z., Suhua, S., Agrawal, N., 2006. Diplectanids
916 infesting the gills of the barramundi *Lates calcarifer* (Bloch) (Perciformes:
917 Centropomidae), with the proposal of *Laticola* n. g. (Monogenoidea: Diplectanidae).
918 Syst. Parasitol. 63, 127–41. https://doi.org/10.1007/s11230-005-9006-6

919 Vanhove, M.P.M., Pariselle, A., Van Steenberge, M., Raeymaekers, J.A.M., Hablützel, P.I.,
920 Gillardin, C., Hellemans, B., Breman, F.C., Koblmüller, S., Sturmbauer, C., Snoeks, J.,
921 Volckaert, F.A.M., Huyse, T., 2015. Hidden biodiversity in an ancient lake: phylogenetic
922 congruence between Lake Tanganyika tropheine cichlids and their monogenean
923 flatworm parasites. Sci. Rep. 5, 13669, 10.1038/srep13669.
924 https://doi.org/10.1038/srep13669

925 Vanhove, M.P.M., Tessens, B., Schoelinck, C., Jondelius, U., Littlewood, D.T.J., Artois, T.,
926 Huyse, T., 2013. Problematic barcoding in flatworms: A case-study on monogeneans and
927 rhabdocoels (Platyhelminthes). Zookeys 365, 355–379.
928 https://doi.org/10.3897/zookeys.365.5776

929 Vanhove, M.P.M., Volckaert, F.A.M., Pariselle, A., 2011. Ancyrocephalidae (Monogenea) of
930 Lake Tanganyika: I: Four new species of *Cichlidogyrus* from *Ophthalmotilapia ventralis*
931 (Teleostei: Cichlidae), the first record of this parasite family in the basin. Zool. 28, 253–

932 263. https://doi.org/10.1590/S1984-46702011000200016

933 Villar-Torres, M., Repullés-Albelda, A., Montero, F.E., Raga, J.A., Blasco-Costa, I., 2019.
934 Neither *Diplectanum* nor specific: a dramatic twist to the taxonomic framework of
935 *Diplectanum* (Monogenea: Diplectanidae). Int. J. Parasitol. 49, 365–374.
936 https://doi.org/10.1016/J.IJPARA.2018.11.003

937 Whittington, I.D., Chisholm, L.A., Rohde, K., 1999. The larvae of Monogenea
938 (Platyhelminthes). Adv. Parasitol. 44, 139–232. https://doi.org/10.1016/S0065-
939 308X(08)60232-8

940 Wickham, H., 2009. ggplot2. Springer, New York. https://doi.org/10.1007/978-0-387-98141-
941 3

942 Will, K.W., Rubinoff, D., 2004. Myth of the molecule: DNA barcodes for species cannot
943 replace morphology for identification and classification. Cladistics 20, 47–55.
944 https://doi.org/10.1111/j.1096-0031.2003.00008.x

945 Wu, W., Goodman, M., Lomax, M.I., Grossman, L.I., 1997. Molecular evolution of
946 cytochrome c oxidase subunit IV: Evidence for positive selection in simian primates. J.
947 Mol. Evol. 44, 477–491. https://doi.org/10.1007/PL00006172

948 Wu, X.Y., Chilton, N.B., Zhu, X.Q., Xie, M.Q., Li, A.X., 2005. Molecular and morphological
949 evidence indicates that *Pseudorhabdosynochus lantauensis* (Monogenea: Diplectanidae)
950 represents two species. Parasitology 130, 669–677.
951 https://doi.org/10.1017/S0031182004007152

952 Wu, X.Y., Zhu, X.Q., Xie, M.Q., Li, A.X., 2007. The evaluation for generic-level monophyly
953 of Ancyrocephalinae (Monogenea, Dactylogyridae) using ribosomal DNA sequence
954 data. Mol. Phylogenet. Evol. 44, 530–544. https://doi.org/10.1016/j.ympev.2007.03.025

955 Zahradníčková, P., Barson, M., Luus-Powell, W.J., Přikrylová, I., 2016. Species of
956 *Gyrodactylus* von Nordmann, 1832 (Platyhelminthes: Monogenea) from cichlids from
957 Zambezi and Limpopo river basins in Zimbabwe and South Africa: evidence for
958 unexplored species richness. Syst. Parasitol. 93, 679–700.
959 https://doi.org/10.1007/s11230-016-9652-x

960

961 **Funding**

962 Research was supported by the Czech Science Foundation (GBP505/12/G112 – ECIP) and

963 GA19-13573S), Belgian Science Policy (BELSPO), EMBRC Belgium - FWO project

964 GOH3817N as well as by a joint program between the Austrian agency for international

965 mobility and cooperation in education, science and research and the Ministry of Education,

966 Youth and Sports (project number 8J18AT007), the Austrian Agency for International

967 Cooperation in Education and Research (OEAD; project number CZ 08/2018; to SK) and the

968 Systematics Research Fund (SRF; to SK).

969 Table 1: An overview of host species examined for monogenean parasites with locality and

970 country.

Host species	**Locality (geographic coordinates, year)**	**Locality – subbasins** (Danley et al., 2012) **or country**	**Number of fish specimens examined (accession number in RMCA)**	**Number of infected fish specimens**	**Number of monogenean individuals***
		Lake Tanganyika			
L. angustifrons	Mpulungu (08°46'S-31°07'E, 27.7.1967)	The southern subbasin	1 (MRAC 190480)	1	2
	Mpulungu (12.4.2018)	The southern subbasin	4(-)	1	15
	Rumonge (03°58'S-29°25'E, 30.6.1967)	The northern subbasin	2 (MRAC 94069.0052-53)	2	3
	Sumbu Bay (08°31'S-30°29'E, 31.3.1947)	The southern subbasin	1 (MRAC 90850)	1	1
L. mariae	Bujumbura (03°23'S-29°22'E, 5.5.1947)	The northern subbasin	5 (MRAC 90908-912)	5	14
	Ilagala (05°12'S-29°50'E, 20.8.1993)	The northern subbasin	3 (MRAC 93152.0318-20)	2	10
	Kilomoni (04°20′S, 29°09′E, 12.8.2016)	The northern subbasin	2 (-)	1	1
	Mpulungu (27.7.1967)	The southern subbasin	2 (MRAC 190493-94)	1	3
	Mpulungu (16.4.2018)	The southern subbasin	7 (-)	0	0
	Mulembwe (06°07′S 29°16′E, 9.4.2010)	The central subbasin	7 (-)	3	19
	Nyanza (04°20'S-29°35'E, 1.1.1997)	The northern subbasin	1 (MRAC 53738)	1	23
	Rumonge (30.6.1994)	The southern subbasin	1 (MRAC 94069.0067)	0	0
	Sumbu Bay (31.3.1947)	The southern subbasin	2 (MRAC 90878-79)	2	22
L. microlepis	Bujumbura (4.5.1947)	The northern subbasin	5 (MRAC 90805-9)	5	24
	Crock Island (08°42'S-31°07'E, 16.4.2018)	The southern subbasin	8 (-)	2	13
	Edith Bay (06°30'S-29°55'E, 30.5.1947)	The southern subbasin	3 (MRAC 90833-35)	5	10
	Katukula (08°35'S-31°10'E, 14.4.2018)	The southern subbasin	5 (-)	4	22
	Moba Bay (30.12.1995)	The central subbasin	2 (MRAC 90725-6)	0	0
	Mpulungu (13.4. – 17.4. 2018)	The southern subbasin	13 (-)	6	29
	Nyanza Lac (1.1.1937)	The northern subbasin	11 (MRAC 53698-703; 53725-29)	11	181
	Sumbu Bay (9.4.1995)	The southern subbasin	3 (MRAC 95096.1192,98,99)	1	1
	Uvira (03°22′ S 29°09′E, 12.8.2016)	The northern subbasin	7 (-)	2	17
L. stappersii	Karala (05°33'S-29°28'E, 10.4.1947)	The northern subbasin	1 (MRAC P90928)	0	0

	Kasasa (08°31'S-30°42'E, 6.9.1967)	The southern subbasin	3 (MRAC 190126;35-6)	0	0
	Mpulungu (12.4.2008)	The southern subbasin	3 (-)	0	0
	Mpulungu (6.4.2018)	The southern subbasin	3 (-)	0	0
	Uvira (12.8.2016)	The northern subbasin	28 (-)	0	0
Other localities					
L. niloticus	Bahr-Sara (08°56'N-17°58'E, 1. -. 31.3.1965)	Tchad	1 (MRAC 154006)	1	2
	Kisumu, Lake Victoria (00°06'S-34°45'E, 17.12.1991)	Kenya	3 (MRAC 91104.37-39)	2	8
	Kossou (07°10'N-05°20'E, 17.12.1973)	Ivory Coast	5 (MRAC 74014.328-29; 2755-56)	4	22
	Lake Nasser (24°05'N-33°00'E, 26.2. – 11.3.1984)	Egypt	3 (MRAC 84006.0116-18)	0	0
	Lake Nasser (1.9.-30.9.1983)	Egypt	2 (MRAC 83030.0114-15)	1	1
	Luxor market (25°42'N 32°38'E, 24.11.2000)	Egypt	1 (MRAC 190480)	0	0
	Njala, riv. Taja (08°06'N-12°04'e, 12.4.1969)	Sierra Leone	5 (MRAC 73010.7057-61)	1	8
	Nyawiega, Lake Albert (01°28'N-30°56'E, 21.11.-6.12.1989)	Uganda	3 (MRAC 89059.0279)	1	2
	Nzunzu, Lake Albert (01°19'N-30°72'E, 5.4.-6.4.2017)	Uganda	1 (MRAC 2016.036.P)	1	2
	Nzunzu, Lake Albert (5.4.-6.4.2017)	Uganda	11 (MRAC 2016.036.P)	2	18

971 * Only one gill arch examined in the case of specimens retrieved from the RMCA

972

973

974

975 Table 2: Comparison of measurements performed on haptoral and genital hardparts of *Dolicirroplectanum lacustre* comb. nov. reported in Thurston

976 and Paperna (1969) from Lake Volta, in Ergens (1981) from Cairo and in this study with host species and locality (a – mean value±standard

977 deviation, b – range).

Parameters (µm)	*L. niloticus*, Lake Volta	*L. niloticus*, Cairo	*L. angustifrons*, Lake Tanganyika	*L. mariae*, Lake Tanganyika	*L. microlepis*, Lake Tanganyika
Total length	650-1000	-	544,2 (n=1)	522,0±35,4[a] (487,4 - 587,3)[b]; n=11	675,0±66,9 (588,1 - 813,1); n=13
Total width	150-250	-	188,7 (n=1)	178,8±39,5 (115,5 - 243,3); n=11	183,2±15,1 (4,7 - 204,1); n=13
Ventral anchor					
Length to notch	19-20	20-22 (n=3)	20,4 (19,5 - 21,3); n=7	19,3±1,4 (16,3 - 24,4); n=25	18,7±0,9 (16,4 - 21,2); n=61
Total length	70-80	53-57 (n=3)	43,2 (41,4 - 44,1; n=7)	42,6±2,0 (41,1 - 47,2); n=28	43,2±2,4 (40,5 - 48,3); n=62
Length to inner root	-	-	22,8 (20,9 - 24,8); n=7	22,6±1,6 (18,3 - 26,9); n=27	22,2±1,1 (18,7 - 24,9); n=52
Inner root length	10-20	10-12 (n=3)	8,7 (7,2 - 9,8); n=7	9,8±0,8 (7,7 - 11,2); n=28	9,2±1,1 (6,1 - 11,4); n=54
Outer root length	40-60	32-35 (n=3)	22,6 (20,1 - 24,4); n=7	23,7± (19,1 - 27,9); n=24	24,8±2,6 (19,2 - 30,1); n=52
Point length	5-7	9-10 (n=3)	8,2 (7,3 - 9,5); n=6	7,2±1,1 (5,4 - 9,2); n=25	7,0±1,1 (4,9 - 9,0); n=45
Dorsal anchor					
Total length	40-50	44-47 (n=3)	35,3 (32,6 - 36,6); n=6	32,3±2,0 (28,4 - 35,6); n=25	33,4±2,1 (26,0 - 38,6); n=47
Point length	-	9-10 (n=3)	7,4 (6,9 - 8,0); n=6	6,3±0,9 (4,7 - 8,1); n=13	6,0±0,9 (4,1 - 8,2); n=31
Ventral bar					
Straight length	50-60	38-44 (n=3)	44,8 (40,7 - 51,5); n=4	50,8±6,4 (39,2 - 63,3); n=30	46,6±4,5 (36,4 - 55,5); n=58
Maximum width	-	8-11 (n=3)	8,2 (7,6 - 9,2); n=6	11,4±2,1 (7,2 - 15,3); n=27	9,3±1,7 (6,1 - 13,2); n=52
Dorsal bar					
Straight length	35-40	34-38 (n=3)	31,5 (29,9 - 33,5); n=6	37,6±3,9 (29,6 - 46,1); n=29	32,8±3,5 (24,3 - 39,5); n=60
Maximum width	-	9-12 (n=3)	8,9 (7,7 - 9,8); n=5	7,2±1,4 (5,1 - 9,6); n=28	6,5±0,9 (4 - 9,0); n=59
Ventral squamodisc					
Length	30-40	38-44 (n=3)	41,6 (35,2 - 48,6); n=4	38,4±7,6 (32,3 - 57,4); n=15	38,3±6,5 (26,7 - 57,2); n=22
Width	50-70	38-51 (n=3)	47,8 (46,2 - 50,0); n=4	41,9±7,3 (29,8 - 60,4); n=16	42,5±4,4 (33,3 - 53,1); n=22
Dorsal squamodisc					
Length	-	38-44 (n=3)	35,9 (33,6 - 39,6); n=4	34,8±4,8 (29,5 - 43,4); n=6	34,9±5,8 (21,3 - 42,9); n=16
Width	-	38-51 (n=3)	42,9 (41,5 - 45,0); n=4	38,0±7,9 (33,4 - 57,2); n=8	40,4±4,3 (30,0 - 46,2); n=16
Hook			10,5 (9,5 - 11,3); n=4	10,4±0,6 (9,4 - 11,8); n=27	10,0±0,7 (7,9 - 11,7); n=49
Copulatory tube straight length	21-23	-	27,6 (24,5 - 29,2); n=3	33,9±5,2 (24,1 - 43,6); n=20	32,3±3,9 (21,4 - 41,5); n=38

Copulatory tube width	-	-	20,6 (19,6 - 21,3); n=3	20,6±4,0 (13,5 - 29,1); n=22	33,4±2,1 (26,0 - 38,6); n=47
Vagina	Not reported	Not reported	Reported in 0 out of 5 available specimens	Reported in 2 out of 24 available specimens	Reported in 0 out of 38 available specimens
Total length	-	-	-	22,1±0,8 (21,5 - 22,6); n=2	-
Tube length	-	-	-	5±0,6 (4,6 - 5,4); n=2	-
Point length	-	-	-	6±0,3 (5,8 - 6,2); n=2	-
Eyes spots					
Smaller pair distance	-	-	44,2 (40,0 - 48,4); n=2	37,3±6,3 (30,0 - 50,0); n=14	-
Larger pair distance	-	-	42,6 (42,2 - 43,0); n=2	32,9±5,1 (26,8 - 46,7); n=14	-
Pharynx length	-	-	-	34,3±9,9 (22,9 - 53,0); n=8	-
Testes					
Length	-	-	-	94,3 (n=1)	-
Width	-	-	-	42,7±13,9 (29,7 - 78,1); n=13	-
Ovary width	-	-	-	31,0±10,9 (31,0 - 64,6); n=14	-

978

979

Parameters (µm)	***L. niloticus*, Lake Albert1**	***L. niloticus*, Lake Albert2**	***L. niloticus*, Lake Victoria**	***L. niloticus*, river Taja**	***L. niloticus*, Lake Kossou**
Total length	736,5±115,3[a] (644,1 - 931,3)[b]; n=5	553,1±52,1(497,2 - 600,3); n=3	537,9±34,8 (509,2 - 576,6); n=3	662,1±113,8 (510,9 - 787,0); n=5	574,7±22,2 (550,3 - 593,7); n=3
Total width	282,0±57,6 (191,4 - 333,9); n=5	182,8±46,9 (151,7 - 236,7); n=3	220,5±11,4 (210,6 - 233,0); n=3	185,0±56,4 (141,7 - 277,8); n=5	217,3±13,6 (208,3 - 233,0); n=3
Ventral anchor					
Length to notch	21,1±1,1 (19,3 - 23,1); n=18	19,3±1,2 (18,2 - 20,6); n=3	20,5±0,6 (19,7 - 21,1); n=4	17,1±0,7 (16,2 - 18,1); n=6	20,9±1,0 (19,5 - 23,1); n=10
Total length	53,4±2,9 (50,0 - 66,4); n=18	42,1±2,1 (40,2 - 44,0); n=3	43,1±1,4 (42,7 - 45,8); n=5	34,4±1,2 (32,8 - 36,5); n=6	43,9±1,8 (40,5 - 45,9); n=10
Length to inner root	24,7±1,9 (21,0 - 29,4)	23,8±0,8 (22,9 - 24,3); n=3	24,9±1,4 (23,3 - 25,8); n=3	21,0±1,1 (19,6 - 22,5); n=5	23,7±1,2 (21,4 - 25,2); n=8
Inner root length	11,3±0,7 (10 -12,9); n=18	10,7±1,0 (9,8 - 11,8); n=3	10,5±0,9 (9,5 - 11,5)	8,9±0,7 (8,1 - 9,7); n=6	10,0±0,9 (8,0 - 11,1); n=10
Outer root length	32,5±3,3 (29,7 - 43,2); n=18	23,3±1,6 (22,0 - 25,0); n=3	24,1±1,5 (22,7 - 25,7); n=3	17,5±13,4 (15,6 - 19,4); n=6	23,5±1,3 ((21,5 - 25,7); n=10
Point length	7,9±1,1 (6 - 9,4); n=18	8,5±0,7 (7,9 - 9,3); n=3	9,5±0,9 (8,8 - 10,7)	7,4±1,1 (5,6 - 8,3); n=5	8,0±1,3 (5,4 - 9,6); n=9
Dorsal anchor					
Total length	47,3±4,0 (42,7 - 60,2); n=17	39,0±1,0 (37,6 - 39,9); n=3	40,7±0,4 (40,2 - 41,0); n=3	28,1±1,0 (27,0 - 29,6); n=6	39,9±1,8 (36,7 - 42,4); n=10
Point length	7,9±1,0 (5,9 - 9,2); n=12	7,7±1,2 (6,8 - 8,5); n=2	8,9±0,6 (8,6 - 9,6); n=3	6,6±1,4 (4,8 - 8,6); n=5	7,8±0,6 (7,3 - 8,5); n=3
Ventral bar					
Straight length	72,6±4,8 (61,5 - 78,8); n=16	59,1±11,5 (52,2 - 72,4); n=3	45,8±6,4 (36,5 - 50,0); n=4	40,0±0,9 (39,2 - 40,5); n=2	50,1±1,6 (47,8 - 52,3); n=7
Maximum width	18,5±1,9 (13,5 - 21,0); n=11	15,3±0,4 (15,0 - 15,6); n=2	15,3 (n=1)	10,2±2,7 (8,3 - 12,1); n=2	13,2±1,4 (11,2 - 14,7); n=7

Dorsal bar					
Straight length	55,4±3,2 (47,6 - 59,9); n=17	41,0±2,1 (39,5 - 42,4); n=2	39,0±2,8 (36,7 - 42,1); n=3	30,0±2,9 (26,1 - 33,5); n=6	37,4±2,5 (32,7 - 40,6); n=10
Maximum width	18,4±2,3 (13,5 - 22,0); n=15	16,5±2,7 (13,8 - 19,6); n=4	7,4±0,5 (6,9 - 7,8); n=3	6,3±2,1 (4,3 - 9,8); n=5	9,3±2,7 (6,5 - 13,9); n=8
Ventral squamodisc					
Length	64,1±7,2 (51,8 - 80,4); n=14	38,4 (n=1)	35,0±2,8 (32,4 - 39,6); n=5	32,1±5,7 (27,9 - 38,6); n=3	34,6±4,4 (29,8 - 38,6); n=3
Width	78,3±13,0 (62,5 - 118,4); n=14	44,0 (n=1)	51,8±3,5 (48,1 - 55,9); n=5	36,4±6,4 (31,5 - 43,7); n=3	50,9±3,0 (47,5 - 52,9); n=3
Dorsal squamodisc					
Length	61,4±11,1 (45,4 - 85,6); n=11	39,0±1,8 (36,9 - 40,1); n=3	32,0±2,4 (29,6 - 35,7); n=5	24,3±2,8 (21,3 - 26,8); n=3	35,3±4,3 (30,3 - 40,1); n=4
Width	63,9±6,3 (49,9 - 71,2); n=11	48,8±10,5 (42,3 - 60,9); n=3	42,9±3,5 (38,8 - 46,9); n=5	24,9±0,8 (24,2 - 25,8); n=3	41,9±2,4 (38,4 - 43,5); n=4
Hook	11,2±0,8 (10,3 - 14,0); n=18	10,4±1,0 (9,3 - 11,0); n=3	11,4± (11,0 - 12,2); n=4	9,7±0,7 (8,4 - 10,3); n=6	11,3±0,9 (10,1 - 13,2); n=11
Copulatory tube straight length	58,3±8,8 (44,5 - 83,1); n=18	44,3±3,8 (40,8 - 49,5); n=4	43,7±11,8 (36,1 - 64,6); n=5	27,3±2,9 (24,2 - 31,2); n=4	35,5±4,8 (29,2 - 42,0); n=6
Copulatory tube width	36,2±4,3 (27,2 - 44,5); n=18	27,6±5,3 (21,0 - 33,2); n=4	26,1± (24,5 - 28,0); n=5	26,1±3,6 (21,2 - 30); n=4	22,1±2,3 (18,2 - 24,5); n=6
Vagina	Reported in 18 out of 18 available specimens	Reported in 4 out of 4 available specimens	Reported in 1 out of 6 available specimens	Reported in 0 out of 6 available specimens	Reported in 3 out of 11 available specimens
Total length	41,1±4,5 (34,0 - 47,3); n=14	35,7±5,6 (30,1 - 41,3); n=3	-	-	-
Tube length	7,8±1,1 (4,8 - 9,3); n=14	6,9±1,4 (5,3 - 7,9); n=3	6,7 (n=1)	-	-
Point length	7,0±0,7 (6,0 - 8,5); n=16	8,0±1,1 (6,7 - 8,7); n=3	8.9 (n=1)	-	-
Eyes spots					
Smaller pair distance	56,7±10,2 (42,8 - 80,4); n=16	-	-	-	-
Larger pair distance	47,2±8,5 (34,9 - 64,6); n=17	-	-	-	-
Pharynx length	56,3±11,0 (39,9 - 73,2); n=10	-	-	-	-
Testes		-	-		-
Length	102,1±46,7 (57,9 - 150,9); n=3	-	-	-	-
Width	70,3±16,2 (50,5 - 103,2); n=9	-	-	-	-
Ovary width	72,7±11,1 (50,7 - 88,9); n=9	-	-	-	-

980

981

982

983

984 Table 3: Genetic intraspecific variability indices in a 412 bp portion of COI mtDNA region of *Dolicirroplectanum lacustre* comb. nov.

	Maximum uncorrected p-distance (number of individuals)	Nucleotide diversity	Haplotype diversity	Number of polymorphic sites
Lake Albert	1.2% (14)	0.0036+/-0.0026	0.8022+/-0.0936	6
Lake Tanganyika	0.7% (38)	0.0019+/-0.0016	0.5747+/-0.0713	4

985

Figure captions

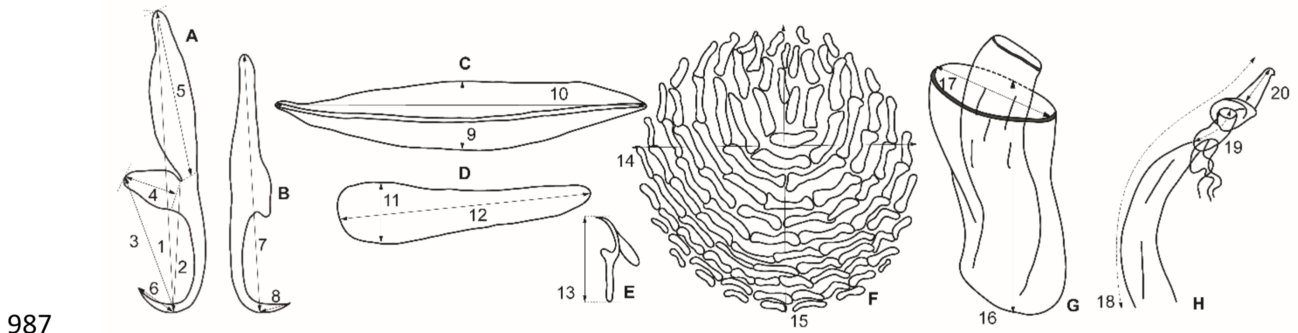
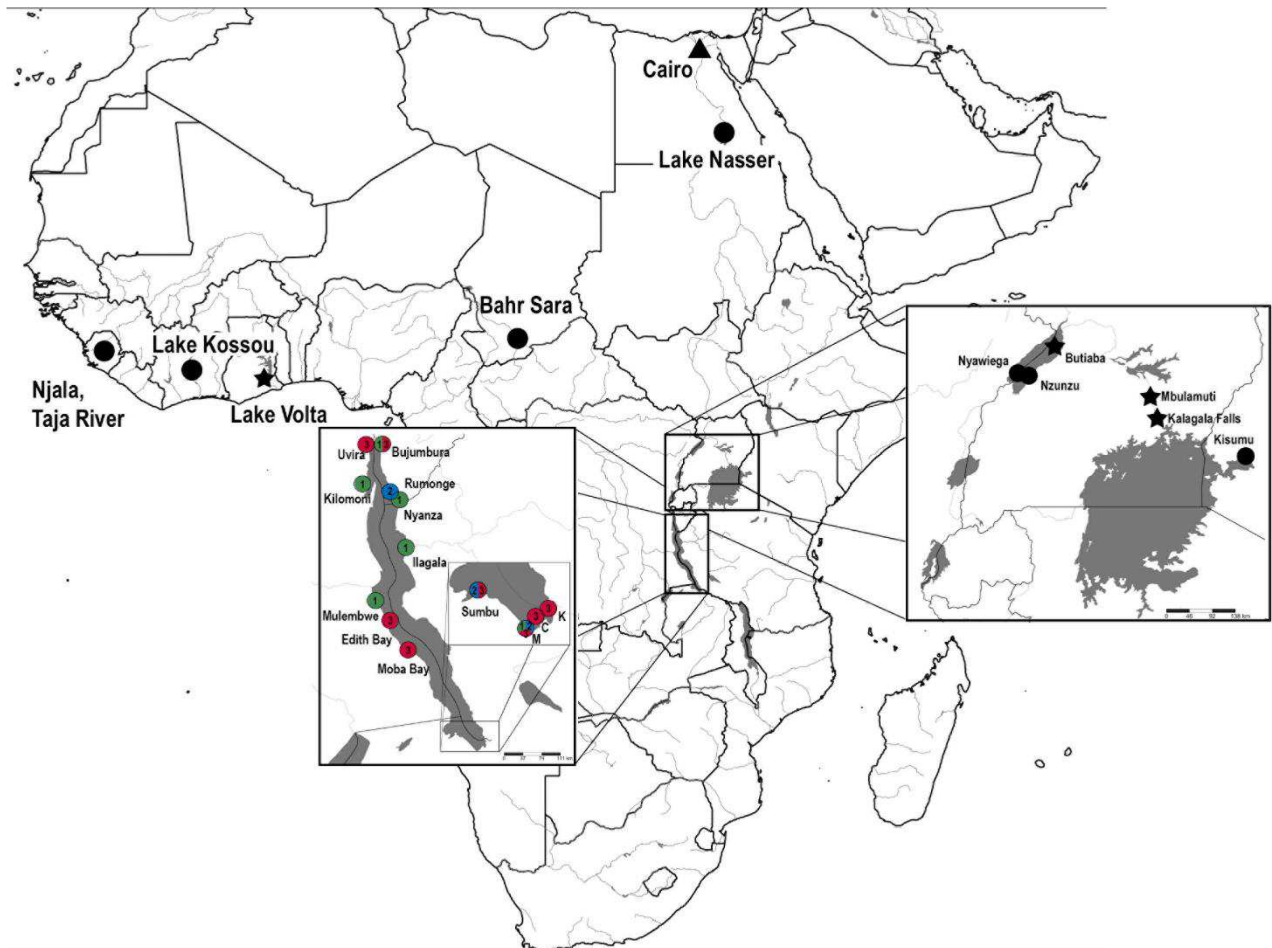


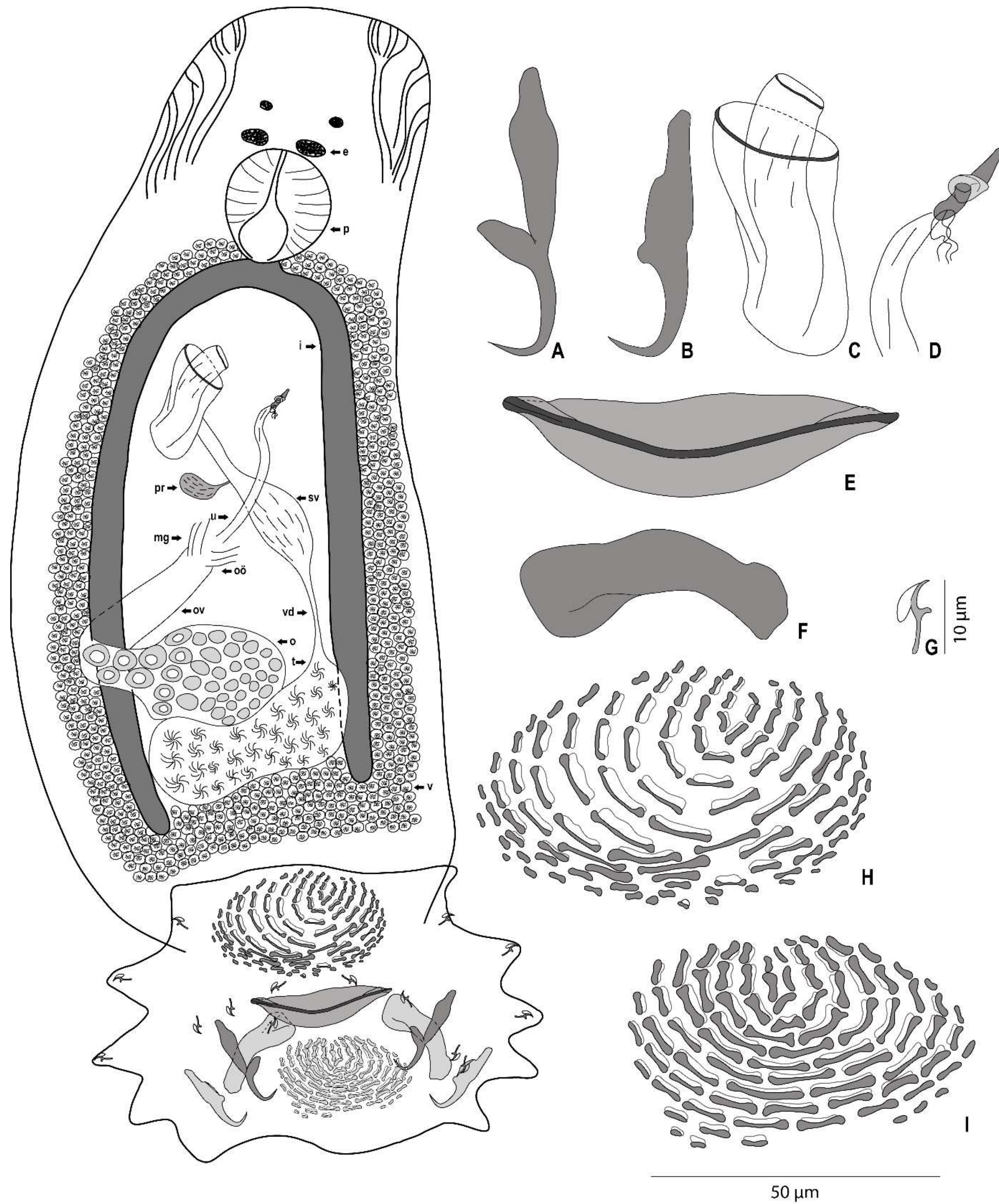
Figure 1: Measurements for sclerotized structures of haptor and reproductive organs of *Dolicirroplectanum lacustre* comb. nov. A Ventral Anchor: 1—Total length, 2—Length to notch, 3—Length to inner root, 4—Inner root length, 5—Outer root length, 6—Point length; B Dorsal anchor: 7—Total length, 8—Point length; C Ventral bar: 9—Straight length 10—Maximum width; D Dorsal bar: 11—Straight length, 12—Maximum width; E Hook: 13—Hook length; F Squamodisc: 14—Squamodisc length, 15—Squamodisc width; G Male copulatory organ: 16—Copulatory tube length, 17—Copulatory tube width; H Vagina: 18—Total length, 19—Tube length, 20—Point length.

997



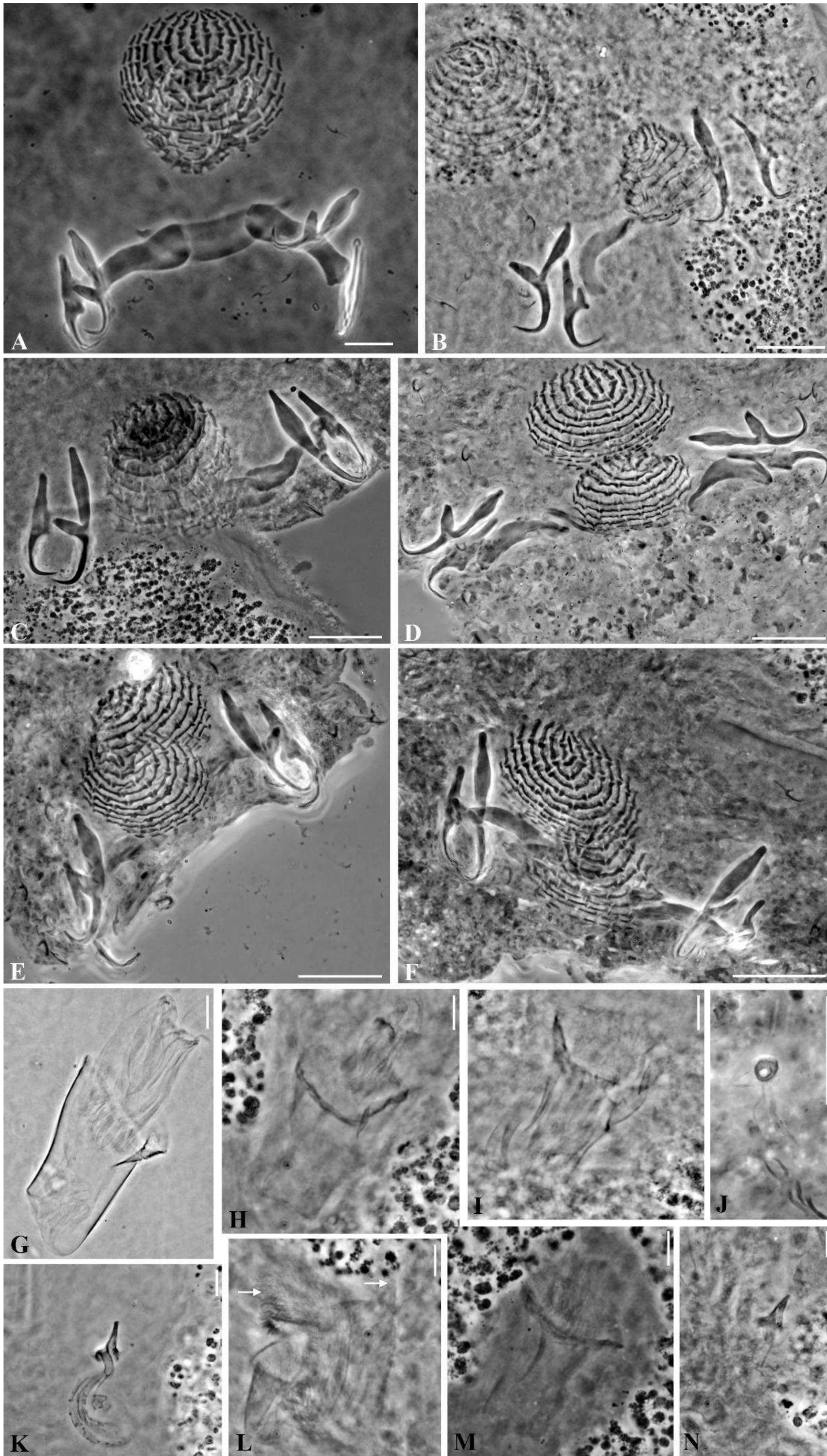
998 Figure 2: Localities with confirmed presence of *Dolicirroplectanum lacustre* comb. nov. Star
999 – localities sampled for the original description of *D. lacustre* by Thurston & Paperna, 1969,
1000 triangle – locality documented by Ergens, 1981, circle – localities sampled in this study. M –
1001 Mpulungu, C – Crocodile Island, K – Katukula. Colours denote host species: black – *Lates*
1002 *niloticus*, blue (number 2) – *L. angustifrons*, green (number 1) – *L. mariae*, red (number 3)–
1003 *L. microlepis*. Map created using SimpleMappr software v7.0.0. (available at
1004 http://www.simplemappr.net. Accessed February 25, 2018).

1005



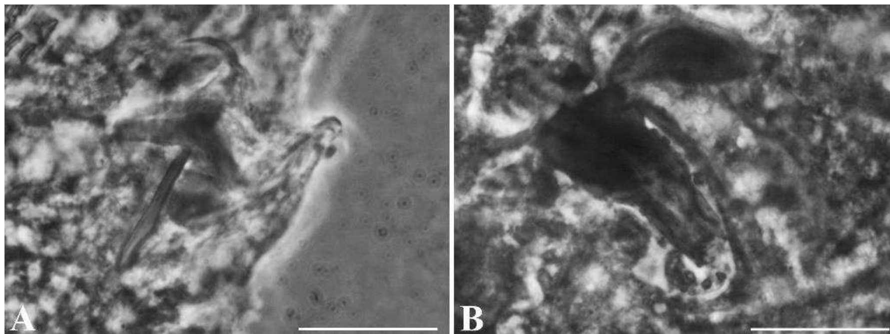
1006

1007 Figure 3: *Dolicirroplectanum lacustre* comb. nov. collected from *Lates niloticus* in Lake Albert.
1008 Specimen drawn from the ventral view. e, eye spots; i, intestine; mg, Mehlis' glands; o, ovary;
1009 oö, oötype; ov, oviduct; p, pharynx; pr, prostatic reservoir; sv, seminal vesicle; t, testes; u,
1010 uterus; v, vittelaria; vd, vas deferens, A, ventral anchor; B, dorsal anchor; C, male copulatory
1011 organ; D, vagina, E, ventral bar; F, dorsal bar; G, hook; H, ventral squamodisc; I, dorsal
1012 squamodisc.


A
B
C
D
E
F
G
H
I
J
K
L
M
N

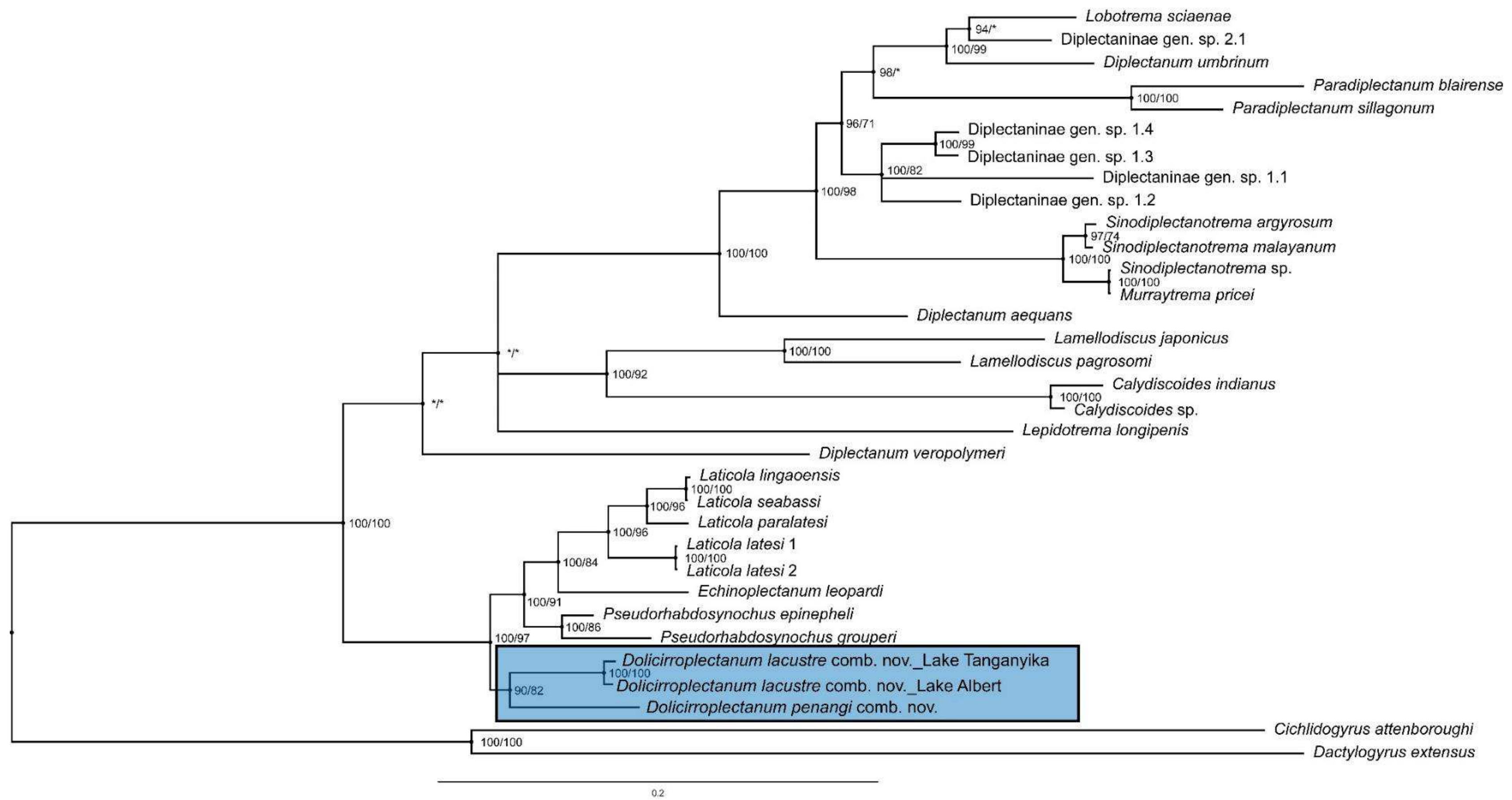
1014 Figure 4: Haptoral and male genital sclerotised structures of *Dolicirroplectanum lacustre* comb.
1015 nov. from different host species and localities collected in this study (scale bars A-F: 25 µm;
1016 G-N: 10 µm). A) Opisthaptor, *L. niloticus* in Lake Albert B) Opisthaptor, *L. niloticus* in Taja
1017 River C) Opisthaptor, *L. microlepis* in Lake Tanganyika D) Opisthaptor, *L. niloticus* in Lake
1018 Victoria E) Opisthaptor, *L. niloticus* in Lake Kossou F) Opisthaptor, *L. mariae* in Lake
1019 Tanganyika G) Male copulatory organ, *L. niloticus* in Lake Albert H) Male copulatory organ,
1020 *L. mariae* in Lake Tanganyika I) Male copulatory organ, *L. niloticus* in Lake Kossou J)
1021 Sclerotised vagina, *L. mariae* in Lake Tanganyika K) Sclerotised vagina, *L. niloticus* in Lake
1022 Albert L) Male copulatory organ, *L. niloticus* in Taja River M) Male copulatory organ, *L.*
1023 *microlepis* N) Sclerotised vagina, *L. niloticus* in Lake Victoria. Pictures were stacked.

1024



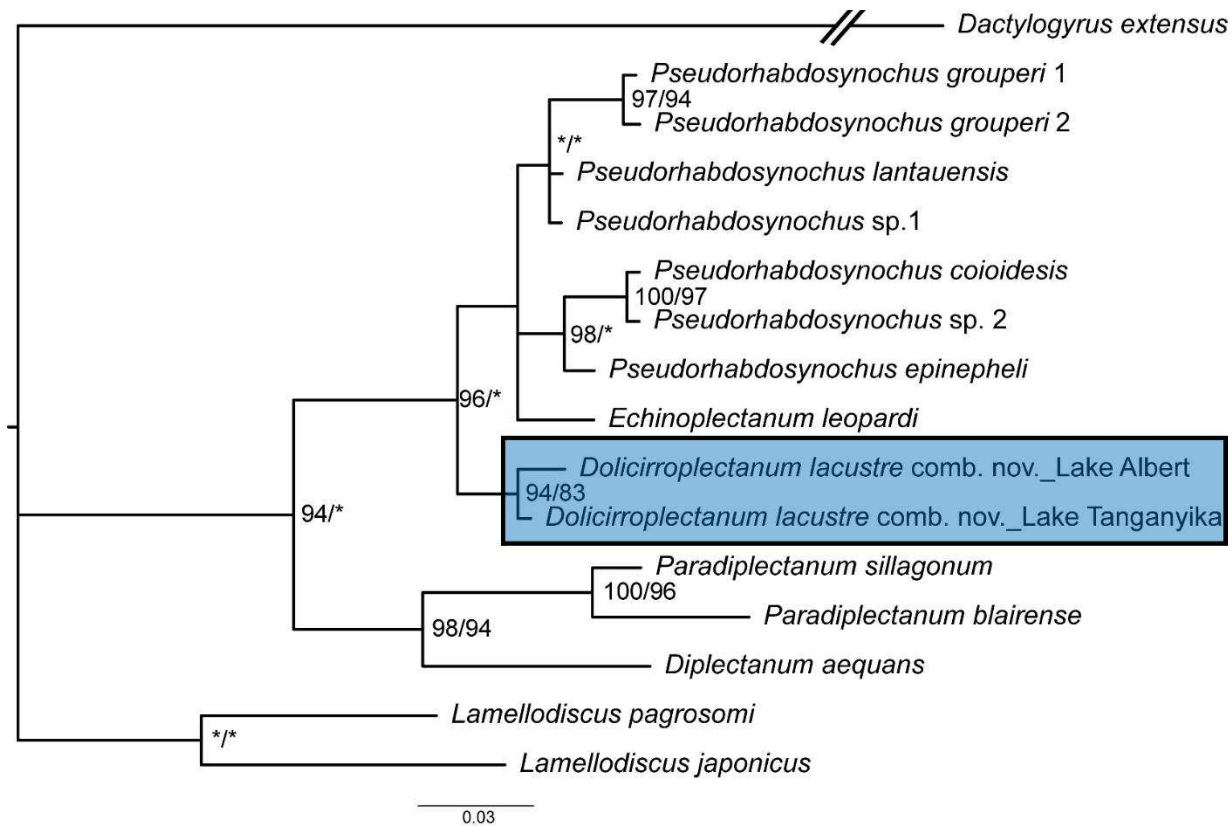
1025

1026 Figure 5: Sclerotised structures of *Dolicirroplectanum penangi* comb. nov.. A) Ventral anchor
1027 (MNHN xxx), Hainan, China B) Male copulatory organ (USNPC 180-A 6), Zhanjiang, China.
1028 Scale bar: 20 µm; several layers in the picture were combined.



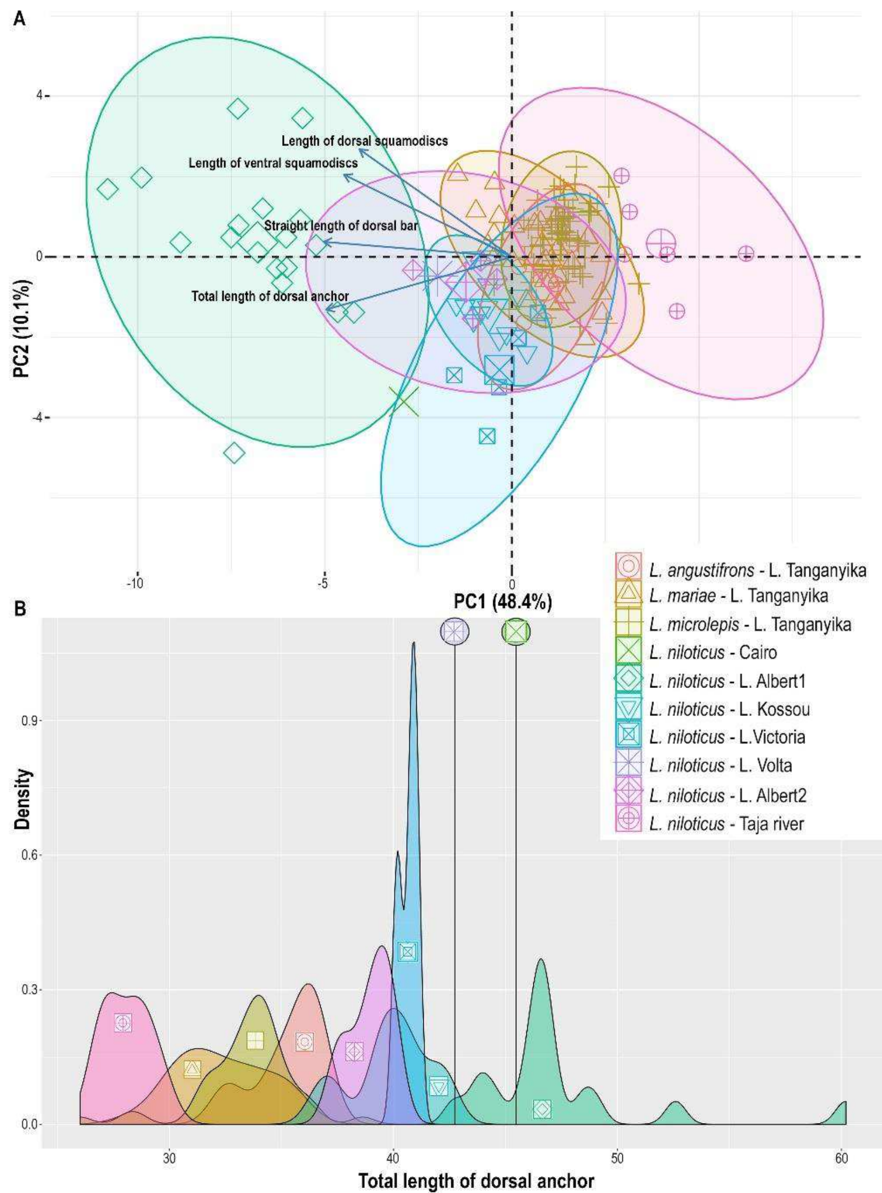
1029

1030 Figure 6: Bayesian inference phylogram based on 28S fragments from 33 haplotypes of different diplectanid species. Posterior probabilities for
1031 Bayesian inference (before slashes) and bootstrap percentages for maximum likelihood (behind slashes) are shown. The values lower than 90 of
1032 posterior probability and 80 for maximum likelihood are marked with an asterisk. The clade containing two lineages of *Dolicirroplectanum*
1033 *lacustre* comb. nov. is boxed. The scale-bar indicates the expected number of substitutions per site.



1034

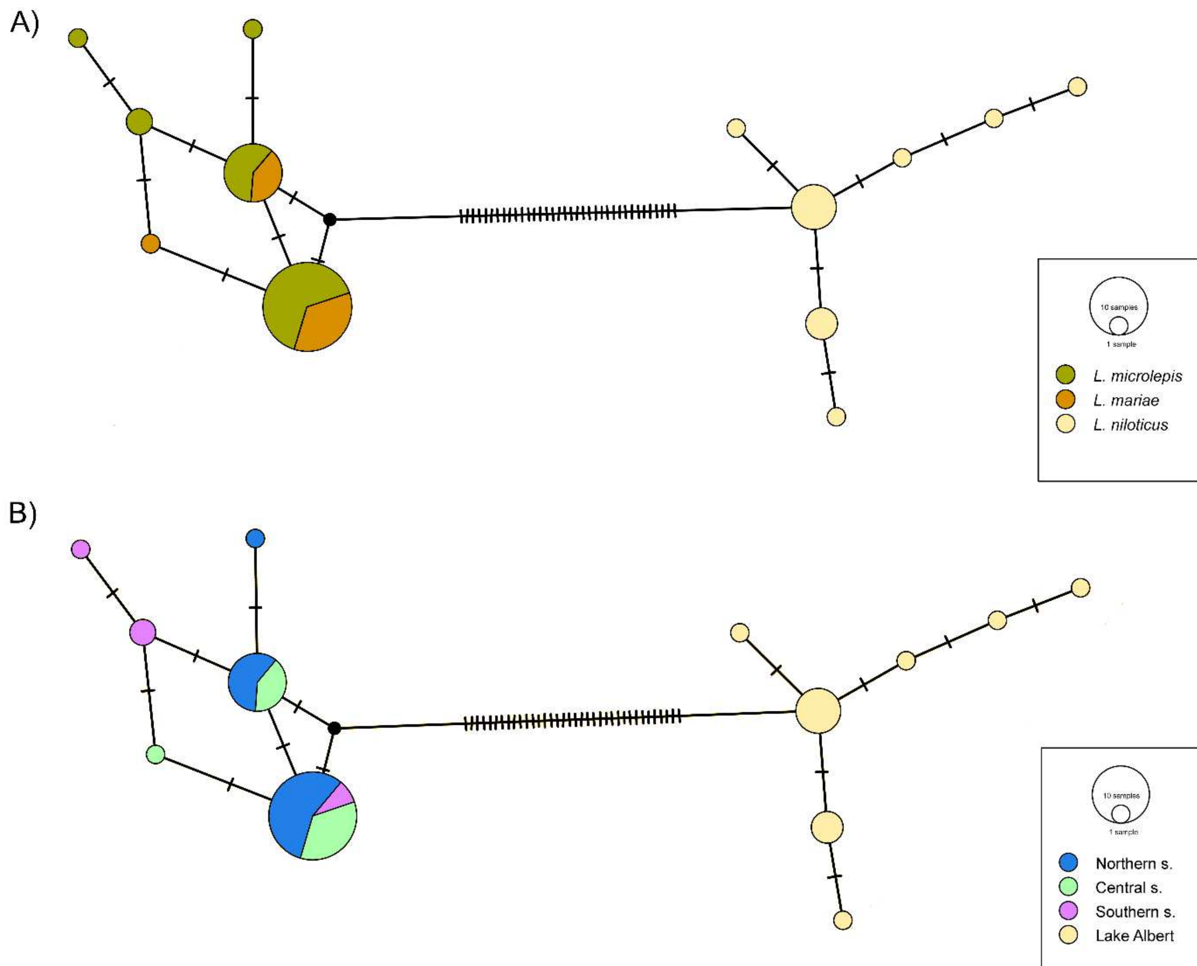
1035 Figure 7: Bayesian inference phylogram based on 18S fragments from 15 haplotypes of different diplectanid species. Posterior probabilities for
1036 Bayesian inference (before slashes) and bootstrap percentages for maximum likelihood (behind slashes) are shown. The values lower than 90 of
1037 posterior probability and 80 for maximum likelihood are marked with an asterisk. The clade containing two lineages of *Dolicirroplectanum*
1038 *lacustre* comb. nov. is boxed. The scale-bar indicates the expected number of substitutions per site.



1039

1040 Figure 8: Morphometric variability of haptoral structures of *Dolicirroplectanum lacustre* comb.
1041 nov. A) biplot of PCA (first two axes) based on measurements of haptoral sclerotized structures.
1042 B) Density plots depicting the total size of the dorsal anchor. Colours and signs denote host
1043 species and locality of specimens.

1044



1045

1046 Figure 9: Haplotype network of *Dolicirroplectanum lacustre* comb. nov. COI sequences (n =
1047 52). The circles represent different haplotypes with the size proportional to the number of
1048 individuals sharing this haplotype. Haplotypes are connected with lines, indicating the number
1049 of substitutions between haplotypes. Colours correspond to A) the host species and B)
1050 geographic origin (subbasins in Lake Tanganyika).

1051

1052

1053

1054

1055

1056

1057

1058 Table S1: List of diplectanid species obtained from GenBank with their accession numbers for

1059 the rDNA region retrieved and their host species.

Parasite species	Host species	28S rDNA	18S rDNA
Calydiscoides indianus (Yamaguti, 1953)	*Nemipterus japonicus* (Bloch, 1791)	EF100557.1	
***Calydiscoides* sp.** Young, 1969	*N. bathybius* Snyder, 1911	EF100558.1	
Diplectanocotyla gracilis Yamaguti, 1953	*Megalops cyprinoides* (Broussonet, 1782)	JN254760.1	
Diplectanum aequans (Wagener, 1857)	*Dicentrarchus labrax* L. 1758	MK203833.1	AM943816.1
D. blairense Gupta & Khanna, 1974	*Sillago sihama* (Forsskål, 1775)	AY553627	DQ537356.1
D. penangi Liang & Leong, 1991	*L. calcarifer*	DQ054821.1	
D. veropolynemi Nagibina, 1976	*Polynemus sextarius* Hora, 1926	AY553625.1	
Diplectaninae gen. sp. 1.1	*Argyrosomus regius* Asso, 1801	MK203834.1	
Diplectaninae gen. sp. 1.2	*Sciaena umbra,* L.	MK203835	
Diplectaninae gen. sp. 1.3	*A. regius*	MK203837.1	
Diplectaninae gen. sp. 1.4	*Umbrina cirrosa,* L.	MK203836.1	
Diplectaninae gen. sp. 2.1	*U. canariensis,* Valenciennes 1843	MK203838.1	
Dolicirroplectanum lacustre (Thurston & Paperna, 1969)	*Lates mariae* Steindachner, 1909*, L. microlepis* Boulenger, 1898	Nobis	Nobis
D. lacustre	*Lates niloticus* L., 1758	Nobis	Nobis
Echinoplectanum leopardi Justine & Euzet, 2006	*Plectroponus leopardus* (Lacepède, 1802)	FJ882609.1	GU121165.1
Lamelllodiscus japonicus Ogawa & Egusa, 1978	*A. s. schlegelii*	EF100561.1	EU836236.1
L. pagrosomi Murray, 1931	*Pagrus major* (Temminck & Schlegel, 1843)	EF100562.1	EU836235.1
***Laticola latesi* 1** (Tripathi, 1959)	*L. calcarifer*	DQ054824.1	
***Laticola latesi* 2**	*L. calcarifer*	AY553621.1	
Lat. lingaoensis Yang, Kritsky, Sun, Zhang, Shi & Agrawal, 2006	*L. calcarifer*	DQ054825.1	
Lat. paralatesi (Nagabina, 1976)	*L. calcarifer*	DQ054826.1	
Lobotrema sciaenae (Bychowsky & Nagibina, 1977)	*L. calcarifer*	EF100556.1	
L. seabassi Wu, Li, Zhu & Xie, 2005	*L. calcarifer*	AY553620.1	
Murraytrema pricei Bychowsky & Nagibina, 1977	*Nibea albiflora* (Richardson, 1846)	DQ157672.1	
Murraytrematoides sp. Yamaguti, 1958	*Muraenesox* sp. McClelland, 1844	JN712915.1	
Paradiplectanum sillagonum (Tripathi, 1959)	*S. sihama*	AY553626.1	AY553617.1
Paradiplectanum umbrinum (Tripathi, 1959)	*Johnius amblycephalus* (Bleeker, 1855)	EF100560.1	
Pseudorhabdosynochus. grouperi 1 (Bu, Leong, Wong, Woo & Foo, 1999)	*Epinephelus coioides* (Hamilton, 1822)	AY553628.1	AY553618.1

P. grouperi 2	*L. calcarifer* (Bloch, 1790)		FJ655782.1
P. aff. lantauensis (Beverley-Burton & Suriano, 1981)	*E. coioides*		GQ495271.1
P. lantauensis	*E. coioides*	AY553624	
P. coioidesis Bu, Leong, Wong, Woo & Foo, 1999	*E. coioides*		AY553616.1
P. epinepheli (Yamaguti, 1938)	*E. brunneus* Bloch, 1793		AY553615.1
P. latesi (Tripathi, 1955)	*L. calcarifer*	AY553621.1	
P. sp. 1 BTD-2009 Yamaguti, 1958	Epinephelinae		FJ655781.1
P. sp. 2 BTD-2009	Epinephelinae		FJ797060.1
Sinodiplectanotrema argyrosomus Zhang in Zhang, Yang & Liu, 2001	*Argyrosomus aneus* (Bloch, 1793), *N. albiflora*	DQ157673.1	
S. malayanum Lim, Tan & Gibson, 2010	*Pennahia anea* (Bloch, 1793)	GU573891.1	
S. sp. Zhang in Zhang, Yang & Liu, 2001	unknown	EF437159.1	

1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080
1081

Paper V

Kmentová N., Bray R., Koblmüller S., Artois T., De Keyzer E., Gelnar M., Vanhove M. P. M., Georgieva S. Uncharted digenean diversity in Lake Tanganyika: cryptogonimids infecting endemic lates perches. Under revision in *Parasites & Vectors* (August 2019).

NK contributed to material collection, performed the morphological characterisation, contributed to species descriptions, contributed to the phylogenetic analyses and drafted the manuscript. Overall contribution: c. 50%

1 # Uncharted digenean diversity in Lake Tanganyika: cryptogonimids infecting
2 endemic lates perches

3 **Nikol Kmentová[1,2,3*], Rodney A Bray[4], Stephan Koblmüller[5], Tom Artois[2], Els Lea R. De**
4 **Keyzer[3], Milan Gelnar[1], Maarten P. M. Vanhove[1,2,3,6] and Simona Georgieva[7]**

5 [1]Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611
6 37 Brno, Czech Republic
7 [2]Centre for Environmental Sciences, Research Group Zoology: Biodiversity & Toxicology,
8 Hasselt University, Agoralaan Gebouw D, B-3590 Diepenbeek, Belgium
9 [3]Laboratory of Biodiversity and Evolutionary Genomics, Department of Biology, University of
10 Leuven, Ch. Deberiotstraat 32, B-3000 Leuven, Belgium
11 [4]Parasitic Worms Division, Department of Life Sciences, The Natural History Museum,
12 Cromwell Road, London SW7 5BD, UK
13 [5]Institute of Biology, University of Graz, Universitätsplatz 2, A-8010 Graz, Austria
14 [6]Zoology Unit, Finnish Museum of Natural History, University of Helsinki, P.O.Box 17,
15 Helsinki FI-00014, Finland
16 [7]Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of
17 Valencia, P.O. Box 46071, Valencia, Spain
18
19 *Correspondence: kmentovan@mail.muni.cz
20
21 Emails:
22 NK: kmentovan@mail.muni.cz
23 RAB: r.bray@nhm.ac.uk
24 SK: stephan.koblmueller@uni-graz.at
25 TA: tom.artois@uhasselt.be

ELTDK: els.dekeyzer@kuleuven.be

MG: gelnar@sci.muni.cz

MPMV: maarten.vanhove@kuleuven.be

SG: simona.georgieva@gmail.com

Abstract

Background: Lake Tanganyika is considered as a biodiversity hotspot with exceptional species richness and level of endemism. Given the global importance of the lake in the field of evolutionary biology, the understudied status of its parasitological fauna is surprising with a single trematode species currently reported. Although the most famous group within the lake's fish fauna are cichlids, the pelagic zone is occupied mainly by endemic species of sardines and lates perches. In our study, we aimed to enhance our knowledge of digenean fauna in Lake Tanganyika by targeting four endemic species of lates perches, an important source for local fisheries.

Methods: A total of 85 lates perches were examined at four localities in Lake Tanganyika. Cryptogonimid digeneans were studied by means of morphological as well as molecular characterisation. Partial sequences of the nuclear 28S rRNA gene and the mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) were amplified for a representative subset of the species recovered. Phylogenetic analyses were conducted at family level under Bayesian interference.

Results: The integrated approach revealed the presence of six species within the family Cryptogonomidae. Three out of the four species of *Lates* Cuvier were recovered infected with at least one cryptogonimid species. Two out of three reported genera are new to science. Low interspecific but high intraspecific phenotypic and genetic diversity was found among *Neocladocystis* spp. Phylogenetic reconstruction based on partial 28S rDNA sequences revealed a sister group relationship for two of the newly described genera in the lake. A monophyly with species of *Acanthostomum* Looss, 1899 was further detected for the Tanganyika's representatives.

Conclusions: The present study provides the first assessment of the fish trematode diversity in Lake Tanganyika and it will serve as a baseline for future explorations on the lake's digenean diversity. Further, our study highlights the importance of multiple scope in studies to reveal the

diversification processes involved in the host-parasite system. Assessing the local biodiversity and mechanisms that underpin ecosystems' diversity are of great importance to understand the ecosystem functioning and species evolution as a consequence of functional differences among organisms.

Keywords: *Neocladocystis bembae* n. sp., *Neocladocystis biliaris* n. sp., *Tanganyikatrema* n. gen., *Grandifundilamena* n. gen., Species complexes

Background

Lake Tanganyika, the oldest African rift lake (9–12 myr) [1], has attracted scientific exploration since the mid-nineteenth century and it is recognised as an evolutionary reservoir and hotspot of diversification [2–4]. It is known for its exclusive diversity of both vertebrate and invertebrate taxa. The current knowledge on the parasite diversity in the lake is rather limited. Only a negligible portion of the fish host species have been subjected to studies [5, 6] and so far, parasitological surveys have been mainly focused on cichlid fishes and their monogenean parasites [7–12]. Currently, a total of 59 helminth parasite species are described in the lake [5, 9–14]. Lake Tanganyika is further characterised by the highest number of endemic species flocks with the greatest number of endemic non-cichlid fish species among the African Great Lakes [15]. These include the four lates perches of the genus *Lates* Cuvier (Teleostei: Latidae), i.e. *Lates mariae* Steindachner, 1909, *Lates microlepis* Boulenger, *Lates angustifrons* Boulenger and *Lates stappersii* (Boulenger), important members of the pelagic and benthopelagic lake ecosystems [16, 17] and commercial source for local fisheries [18]. Lates perches exhibit lake wide distributions, a pattern seen also in other pelagic Lake Tanganyika fish species in the lake such as sardines and cichlids [19–22]. All four species of *Lates* are top predators in the lake's open water, however, differentiation in habitat preferences can be detected [16].

Almost nothing is known on the digenean fauna infecting the endemic *Lates* spp. in Lake Tanganyika which it is in contrast to the data on *L. niloticus* (L.) and their Asian congener, *L.*

102 *calcarifer* (Bloch) (see overview on the known helminth fauna of the latid family members in
103 Table 1). The present knowledge on the parasite fauna of *Lates* spp. in the lake account records
104 for a single species of monogeneans infecting three of out four species [14], and otherwise an
105 unidentified nematode larva of the genus *Dujardinascaris* Baylis, 1947 from *L. microlepis* [13].
106 Currently, a single trematode species is known from the lake, i.e. *Neocladocystis tanganyikae*
107 (Prudhoe, 1951) (Digenea: Cryptogonimidae) originally described by Prudhoe (1951) as
108 *Cladocystis tanganyikae* Prudhoe, 1951 possibly from the poecilid *Lamprichthys tanganicanus*
109 (Boulenger, 1898). However, given the uncertainty of the host species, this record has to be
110 revalidated [5].

111 Members of the family Cryptogonimidae are parasitic in the intestine and/or pyloric
112 caeca of marine and freshwater teleosts, reptiles and amphibians [23]. Of the over 200 species of
113 64 genera reported worldwide [23] only seven species of four genera, *Acanthostomum* Looss
114 1899, *Brientrema* Dollfus, 1950, *Neocladocystis* Manter & Ritchard, 1696 and *Siphodera* Linton,
115 1910 are known from African freshwater fishes [24–31]. This is a strong emphasis on the lack of
116 research and constrains further parasitological studies on the ecology, evolution and conservation
117 of economically important fish species.

118 The present study aims to enrich the knowledge on the parasite fauna of the economically
119 important and endemic lates perches, i.e. *L. angustifrons*, *L. mariae*, *L. microlepis L. stappersii*
120 in Lake Tanganyika, and particularly on the digenean trematodes as an integral component of the
121 local food chain and ecosystem functioning [32]. Here we provide the first molecular data for
122 trematode parasites from the lake accompanied by morphological characterisation and respective
123 descriptions. Additionally, phylogenetic reconstruction is inferred evaluating the
124 interrelationships of the newly described species at family level.

125 Methods

126 Collection and fixation of specimens

127 Fish specimens of four species of *Lates*, *L. angustifrons*, *L. mariae*, *L. microlepis* and *L.*
128 *stappersii*, were either purchased from local fishermen or collected by hand nets during field
129 trips in 2016 and 2018, respectively (see Table 2). A total of 85 specimens was sampled at four
130 sampling locations: (i) at the northern part of the lake at off Uvira, Democratic Republic of the
131 Congo; and (ii) at the southern part of the lake at off Katukula, Mpulungu and Crocodile Island,
132 (all three in Zambia; see Fig. 1 for further details). Fish were examined fresh following the
133 standard protocol of Ergens & Lom [33]. The recovered digenean trematodes were rinsed and
134 cleaned in a Petri dish with saline solution; most of the saline solution was gently removed by
135 pipetting and the specimens were killed by pouring nearly boiling water. Subsequently, the
136 trematode specimens were preserved either in 4% formalin, 70% or 96% ethanol for
137 morphological and molecular studies, respectively.

138 **Morphological examination**

139 Specimens preserved in 4% formalin or 70% ethanol were stained with iron acetocarmine,
140 dehydrated through a graded ethanol series, cleared in dimethyl phthalate and examined as
141 permanent mounts in Canada balsam. All specimens for which sequence data were generated
142 were preserved in 96% ethanol and latter photographed from wet mounts in distilled water
143 using Leica Application Suite v.4.3.0. analysis software on Leica DMR light microscope
144 (Wetzlar, Germany; at magnifications of 100–1000x). Subsequently, a piece from the posterior
145 part of the specimens (the post-testicular region) was excised and used for DNA isolation. The
146 remaining anterior part of the worms was kept as molecular voucher (i.e. hologenophores
147 *sensu* Pleijel et al. [34]).

148 Specimens prepared as whole mounts were both photographed and drawn using a
149 drawing tube at a high magnification. Measurements were taken from photomicrographs using
150 Leica Application Suite v.4.3.0. analysis software. In total, 33 different characters were
151 measured and the following abbreviations were used: BL, body length; BW, body width; OSL,

152 oral sucker length; OSW, oral sucker width; VSL, ventral sucker length; VSW, ventral sucker
153 width; FBL, forebody length; HBL, hindbody length; PPH, pre-pharynx length; PHL, pharynx
154 length; PHW, pharynx width; OL, oesophagus length; IB-F, distance from anterior extremity to
155 intestinal bifurcation; IB-VS, distance from intestinal bifurcation to ventral sucker; POSTC,
156 length of post-caecal field; ATL, anterior testis length; ATW, anterior testis width; PTL,
157 posterior testis length; PTW, posterior testis width; POST, length of post-testicular field; OVL,
158 ovary length; OVW, ovary width; ABE-OV, distance from anterior body extremity to ovary; VS-
159 OV, distance from ventral sucker to ovary; OV-AT, distance from ovary to anterior testis; EL,
160 egg length; EW, egg width; POSTU, length of post-uterine field; PREVI, length of pre-vitelline
161 field; VIT, length of vitelline field; POSTVIT, length of post-vitelline field, length of seminal
162 receptacle; SRW, width of seminal receptacle. Further, the following distances were measured
163 VSL/OSL, sucker length ratio; VSW/OSW, sucker width ratio; OS/BL (%), oral sucker as a
164 proportion of body length; VS/BL (%), ventral sucker as a proportion of body length; FO/BL
165 (%), forebody as a proportion of body length; HB/BL (%), hindbody as a proportion to body
166 length; PHL/BL (%), pharynx as a proportion of body length; IB/BL (%), pre-intestinal field as
167 proportion of body length; POSTC/BL (%), post-caecal field as a proportion of body length;
168 AT/BL (%), anterior testis as a proportion of body length; PT/BL (%), posterior testis as
169 proportion of body length; POSTT/BL (%), post-testicular field as a proportion of body length;
170 ABE-OV/BL (%), anterior body extremity to ovary field as a proportion of body length; VS-
171 OV/BL (%), pre-ovarian field as a proportion of body length; OV-AT/BL (%), post-ovarian to
172 anterior testis field as a proportion of body length; OV/BL (%), ovary as a proportion of body
173 length; POSTU/BL (%), post-uterine field as a proportion of body length; PREVIT/BL (%), pre-
174 vitelline field as a proportion of body length; VIT/BL (%), vitelline field as a proportion of body
175 length; POSTVIT/BL (%), post-vitelline field as a proportion of body length; SRL/BL (%),
176 seminal receptaculum as a proportion of body length; BW/BL (%), body width as a proportion of
177 body length (Table 3). The terminology of the measured characters follows Miller & Cribb

178 [23]. The type- and voucher material are deposited at the Helminthological Collection of the
179 Natural History Museum in London, United Kingdom (NHMUK), the Royal Museum for
180 Central Africa (RMCA) in Tervuren, Belgium and in the collection of the Research Group
181 Zoology: Biodiversity and Toxicology at Hasselt University in Diepenbeek, Belgium.
182
183 **Molecular data generation**

184 The posterior portion in cases of larger specimens or complete specimens in cases of very small
185 specimens was used for genomic DNA isolation. Total genomic DNA (gDNA) isolation was
186 performed with 5% Chelex® suspension and 0.2 mg/ml of proteinase K. Genetic sequence data
187 were generated for both partial 28S ribosomal RNA (D1-D3 domains) and the mitochondrial
188 cytochrome *c* oxidase subunit 1 (*cox*1) gene using with primer combinations digl2 (forward: 5'-
189 AAG CAT ATC ACT AAG CGG-3') or LSU5 (forward: 5'-TAG GTC GAC CCG CTG AAY
190 TTA AGC A-3') and 1500R (reverse: 5'-GCT ATC CTG AGG GAA ACT TCG-3') Tkach et al.
191 [35] in the case of 28S rDNA and JB3 (forward: 5'-TTT TTT GGG CAT CCT GAG GTT TAT-
192 3'; Bowles et al. [36]) and CO1-R (reverse: 5'-CAACAAAATCATGATGCAAAAGG-3'; Miura
193 et al. [37]) in the case of *cox*1. Amplification reactions were performed in a total volume of 20 µl
194 using 2x MyFi™ DNA Polymerase Mix (Bioline Inc., Taunton, USA), *c*.50 ng of gDNA and
195 annealing temperature of 55 °C (28S) or 50 °C (*cox*1). PCR products were purified using
196 QIAquick PCR purification kit (Qiagen Ltd., Hilden, Germany). Both strands were cycle-
197 sequenced using the ABI BigDye™ 3.1 Chemistry (ABI Perkin-Elmer, London, UK) and
198 visualized on a 3730xl DNA Analyser (ABI Perkin-Elmer, London, UK) at the GATC Biotech
199 (Konstanz, Germany). The PCR primers and an additional internal primer 300F (forward: 5'-
200 CAA GTA CCG TGA GGG AAA GTT G-3'; Littlewood et al. [38] in the case of 28S rRNA
201 were used for the sequencing reactions. Contiguous sequences were assembled using Geneious
202 v.8 (http://www.geneious.com/; Kearse et al. [39]); and submitted to GenBank under accession

203 number XXX-XXX (28S rDNA) and XXX-XXX (cox1) (see Table S1 for provenance data and
204 accession number details).

205 **Sequence alignments and phylogenetic analyses**

206 Two main alignments for the partial 28S rDNA sequences were built to infer the
207 robustness and phylogenetic position of the African cryptogonimids: (i) a set for 52 taxa within
208 Cryptogonimidae Ward, 1917 (843 bp); and (ii) a restricted dataset for 10 species of
209 *Acanthostomum* Looss, 1899 (489 bp). The alignments were constructed in MAFFT v.7 [40] on
210 the EMBL-EBL bioinformatics web platform (http://www.ebi.ac.uk/Tools/msa/mafft/) under the
211 default setting with a gap opening penalty of 1.53 and gap extension penalty of 0.123 over 1,000
212 cycles of iterative refinement incorporating local pairwise alignment information [40]. Highly
213 variable parts of the alignments were determined and excluded by Gblocks [41] as implemented
214 in SeaView v.4 [42] under less stringent parameters and refined by eye. Uncorrected pairwise
215 distances were calculated in MEGA v.7 [43]. jModelTest v.2 [44] was used to select the 'best-
216 fitting' models of sequence evolution under the Bayesian information criterion.

217 Phylogenetic relationships were inferred under Bayesian inference (BI) inMrBayes v3.2.0
218 [45]. Two independent runs were performed for 10,000,000 generations and sampled every
219 1,000th generation. The 'burn-in' was set for the first 25% of the sampled trees. Bayesian analyses
220 were executed online on the CIPRES Science Gateway v. 3.3 [46]. Parameter convergence and
221 run stationarity were assessed in Tracer v1.6 [47]. The outgroup choices were informed on
222 broader phylogenies of the Digenea [48]. The resulting trees were visualised in FigTree v.1.4.3
223 (http://tree.bio.ed.ac.uk/software/figtree/). All species included in the phylogenetic analyses
224 together with their GenBank accession numbers are listed in Additional file 1: Table S1.

225 The *cox*1 sequence alignment comprised only newly-generated sequences for three of the
226 cryptogonimids recovered in the lates perches from Lake Tanganyika. The examined matrix
227 consisted of 795 bp of nine terminals.

228 # Results

229 ## Digenean diversity in Lake Tanganyika's lates perches

230 Examination of 85 individuals of lates perches from four localities in Lake Tanganyika (all four
231 endemic species were included in our dataset, Table 2) revealed a total of 32 infections with
232 cryptogonimid trematodes. Three of four *Lates* species examined, i.e. *L. angustifrons*, *L. mariae*
233 and *L. microlepis*, were infected with at least one species of cryptogonimid trematode. No
234 digenean specimens were recovered from *L. stappersii* in neither of the two localities where the
235 species had been sampled. Distribution and infection parameters are listed in Table 2. Adult
236 cryptogonimids were detected in the fish intestine, pyloric caeca and gallbladder while immature
237 specimens were recovered only in the intestine. Sequence data were successfully generated for
238 representatives of five out of the six species recovered. The newly recovered cryptogonimids
239 exhibited specific morphological and molecular features when compared with other members of
240 the family. The taxonomy proposed here is based on a combined morphological and molecular
241 approach which resulted in the description of six new species and erection of two new genera.

242 ## Taxonomy and species description

243

244 **Superfamily Opisthorchioidea Looss, 1899**

245 **Family Cryptogonimidae Ward, 1917**

246

247 **Genus *Neocladocystis* Manter & Pritchard, 1969**

248 ***Neocladocystis bemba* n. sp. Georgieva, Kmentová & Bray**

249 ***Type-host*: *Lates microlepis* Boulenger (Perciformes: Latidae).**

250 ***Other-host:* *Lates angustifrons* Boulenger (Perciformes: Latidae).**

251 ***Type-locality*****:** Lake Tanganyika at Mpulungu, Zambia (8°46'S, 31°07'E).

252 ***Other-localities*****:** Lake Tanganyika at Mutondwe Island, Zambia (8°42'S, 31°07'E), Lake
253 Tanganyika at Katukula, Zambia (8°36'S, 31°11'E).

254 ***Site in host*****:** Immature specimens in intestine and eggs-bearing specimens in pyloric caeca.

255 ***Type-specimens*****:** The holotype (xxx) and xxx paratypes (xxx) were deposited Helminthological
256 Collection of the Natural History Museum in London, United Kingdom, xxx paratypes (xxx) at
257 the Hasselt University, in Diepenbeek, Belgium and xxx paratypes () at the Royal Museum for
258 Central Africa in Tervuren, Belgium.

259 ***Representative DNA sequences*****:** GenBank accession numbers: xxx (partial (D1-D3 domains)
260 28S rRNA gene); xxx (*cox*1).

261 ***ZooBank registration*****:** XXXXXXX.

262 *Etymology*: The specific name *bemba* is in honour of the Bemba people inhabiting the North-
263 Eastern part of Zambia where the type-locality of *N. bemba* n. sp. is situated.

264 **Description**

265 [Based on 21 specimens including 6 immature individuals; Fig. 2a, Additional file 2: Fig. S1,
266 Table 3]. Body irregularly oval, flattened. Tegument spined, spines reach close to posterior
267 extremity, posterior to level of caeca, largest in mid-body level. Oral sucker spherical, sub-
268 terminal. Ventral sucker pre-equatorial, rounded, may be completely obscured by eggs, distinctly
269 smaller than oral sucker. Pre-pharynx short or undistinguishable. Pharynx oval, muscular, longer
270 than wider. Oesophagus often not detectable, occasionally short. Intestinal bifurcation in about
271 mid-forebody, anterior to ventral sucker. Caeca relatively wide, reach into post-testicular region.

272 Testes two, entire or slightly lobed, oblique, contiguous or slightly separated, in posterior
273 half of hindbody. Seminal vesicle sacculotubular, dextral, naked, between ovary and ventral
274 sucker. Gonotyl absent. Genital pore median, just anterior to ventral sucker.

275 Ovary small, sub-spherical or irregular, entire or slightly lobed, dextral, intercaecal, pre-
276 testicular at distance from posterior testis. Mehlis' gland and Laurer's canal not observed.
277 Seminal receptacle spherical or saccular, post-ovarian, immediately posterior to ovary or
278 partially overlapping it dorsally. Uterine coils extend from level of testes to intestinal bifurcation,
279 mostly intercaecal. Eggs numerous, noticeably variable, tanned, operculate. Vitellarium
280 follicular, in two lateral fields, extend anteriorly from about level of ventral sucker to post-
281 testicular field close to posterior extremity of body, overlapping caeca dorsally and ventrally.

282 Excretory vesicle Y-shaped, bifurcates just posterior to ventral sucker (seen on immature
283 specimens). Excretory vesicle narrower posteriorly, widens and reaches at least to uterus,
284 anterior may reach at about level of pharynx. Excretory pore terminal.

285 **Remarks**

286 Currently, only two species of *Neocladocystis* are known from Africa, i.e. *N. tanganyikae*
287 (Prudhoe, 1951) and *N. congoensis* Manter & Pritchard, 1969. *Neocladocystis tanganyikae* was
288 described from 'residus de fixations des poissons' from Lake Tanganyika. These fishes were
289 taken in a small bay south of Cape Tembwe in Lake Tanganyika on the Congolese side of Lake
290 Tanganyika and apparently included *Lamprichthys tanganicanus* and several species of cichlids.
291 Unfortunately, it is not possible to state which of the fishes collected is the type-host of *N.*
292 *tanganyikae* [26]. *Neocladocystis congoensis* has been reported in *Parauchenoglanis monkei*
293 (Keilhack, 1910) from Ebogo near the River Nyong in Cameroon [29] and from 'an unidentified
294 siluroid fish near Kisangani (Stanleyville)' in Democratic Republic of Congo [49]. Unlike in *N.*
295 *bemba* n. sp. and the other newly described species *N. biliaris* n. sp. (description follows bellow),
296 in neither species does the vitellaria enter the forebody. A third congener species, *N. intestinalis*

297 (Vaz, 1932), was reported from the South American characiform *Salminus brasiliensis* (Cuvier,
298 1817) in Paraná River, Argentina with several fish species as second intermediate hosts [50, 51].
299 *Neocladocystis bemba* n. sp. is distinguished from *N. congoensis* and *N. tanganyikae* by a
300 combination of characters including the relative position of ovary and seminal receptacle, size of
301 eggs and relative size of oral sucker together with the presence of entire ovary and testes and the
302 distribution of the vitelline fields which may extend from about the level of the ventral sucker to
303 the post-testicular region almost to the posterior body extremity.

304

305 ***Neocladocystis biliaris* n. sp. Georgieva, Kmentová & Bray**

306 ***Type-host*: *Lates mariae*** Steindachner (Perciformes: Latidae)

307 ***Type-locality*:** Lake Tanganyika at Uvira, DRC (4°20′S, 29°09′E)

308 ***Type-specimens*:** The holotype (xxx) and two paratypes (xxx) were deposited at the
309 Helminthological Collection of the Natural History Museum in London, United Kingdom and
310 one paratype (xxx) at the Hasselt University, in Diepenbeek, Belgium.

311 ***Site in host*:** Gallbladder

312 ***Representative DNA sequences*:** GenBank accession numbers: xxx (partial (D1-D3 domains)
313 28S rDNA gene).

314 ***ZooBank registration*:** XXXXXXX.

315 ***Etymology*:** The specific name *biliaris* is derived from the Latin *vesica biliaris*, meaning
316 gallbladder, referring to the infection site of this species in the gallbladder.

317

318 **Description**

319 [Based on 6 specimens; Fig. 1b, Additional file S2: Fig. S2, Table 3]. Body irregular oval,
320 flattened. Tegument smooth. Oral sucker spherical, sub-terminal. Ventral sucker pre-equatorial,
321 spherical, may be completely obscured by eggs, distinctly smaller than oral sucker. Pre-pharynx
322 very short, not visible some specimens. Pharynx oval, longer than wider. Oesophagus often not
323 detectable, occasionally short. Intestinal bifurcation just posterior to pharynx. Caeca blind,
324 narrow, reach into posterior end of posterior testis.

325 Testes two, slightly lobed, oblique, contiguous or slightly separated, in posterior half of
326 hindbody. Seminal vesicle naked, entire, dextral, at level of ventral sucker, posterior end
327 obscured by eggs. Gonotyl absent. Genital pore median, immediately anterior to ventral sucker.

328 Ovary entire, pre-testicular, at distance from anterior testis. Uterus fills much of body
329 from anterior extremity to mid-testicular region, most intercaecal. Eggs numerous, noticeably
330 variable, tanned, operculate. Vitellarium follicular in two lateral fields, reach from just pre-
331 ventral sucker region to close to posterior extremity, overlapping caeca dorsally and ventrally.

332 Excretory vesicle Y-shaped, narrower posteriorly, anteriorly widens and reaches uterus;
333 bifurcates, arms extent to forebody, mostly obscured by eggs. Excretory pore terminal.

334 **Remarks**

335 *Neocladocystis bemba* n. sp. *Neocladocystis biliaris* n. sp. differs from its congener by a
336 combination of characters including the larger body length, entire seminal vesicle, relative length
337 of post-testicular region and the unique microhabitat exploited in the fish host, i.e. the
338 gallbladder.

339

340 ***Neocladocystis* sp.**

341 ***Host:*** *Lates angustifrons* Boulenger (Perciformes: Latidae).

342 ***Locality*:** Lake Tanganyika at Mpulungu, Zambia (8°46'S, 31°07'E).

343 ***Site in host*:** Intestine.

344 ***Representative DNA sequences*:** GenBank accession numbers: xxx (partial (D1-D3 domains)
345 28S rRNA gene).

346 **Description**

347 As only a single immature specimen of *Neocladocystis* n. sp. lacking species-specific characters
348 was obtained, full morphological description was not possible (Additional file 2: Fig. S3).
349 Comparative sequence analysis of 28S rDNA confirmed the distinct status of this specimen as
350 another species of *Neocladocystis* (the electronic photographic voucher of the specimen used in
351 molecular characterisation is provided in Additional file 2: Fig. S3).

352 **Amended diagnosis** (based on Manter & Pritchard, 1969)
353 Body oval, widest at level of ventral sucker, length:width ratio *c*.1.5–3. Oral sucker subterminal,
354 spherical. Circumoral spines lacking. Tegumental spines present or absent. Ventral sucker
355 unspecialized, small, pre-equatorial, not obviously embedded in ventrogenital sac. Sucker-width
356 ratio *c*.1.7. Forebody *c*.25–35% of body-length. Pre-pharynx short, narrow. Oesophagus short or
357 indistinguishable. Intestinal bifurcation immediately anterior to ventral sucker. Caeca blind,
358 narrow, end at level of testes. Testes two, symmetrical to slightly oblique at posterior body
359 extremity. Seminal vesicle tubular. Gonotyl absent. Ovary lobed, immediately anterior to testes.
360 Uterus in hindbody between testes and ventral sucker, may extend to intestinal bifurcation in
361 mid-forebody. Vitellarium follicular. Vitelline follicles in two lateral fields, mainly in hindbody,
362 from level of testes, may reach level of intestinal bifurcation. Arms of excretory vesicle may
363 almost reach level of pharynx, sometimes do not exceed intestinal bifurcation. In intestine and
364 gallbladder of freshwater fishes (Cichlidae, Characidae, Bagridae, Latidae); Africa and South
365 America. Type-species: *Neocladocystis tanganyikae* (Prudhoe, 1951) Manter & Pritchard, 1969.

366 **Differential diagnosis**

367 Species of *Neocladocystis* morphologically and genetically resemble members of
368 *Acanthostomum* Looss, 1899, consisting of cosmopolitan parasites in fishes and reptiles, in
369 having a round oral sucker opening sub-terminally and a short oesophagus. They differ in
370 possessing blind caeca and the absence of circumoral spines. *Brientrema* Dollfus, 1950 with
371 members infecting freshwater fishes (Malapteruridae, Citharinidae) in Africa resembles
372 *Neocladocystis* by the possession of a nearly spherical oral sucker, a very short pre-pharynx and
373 oesophagus, blind caeca and two slightly oblique testes. However, the two genera differ in the
374 presence *versus* absence of circumoral spines and in vitelline fields reaching about level of the
375 ventral sucker in *Neocladocystis*.

376

377 ***Tanganyikatrema* n. gen. Kmentová, Georgieva & Bray**

378 **Diagnosis**

379 Body elongate, fusiform, widest at level of ventral sucker or mid-body, length:width ratio *c.*1.7–
380 2.7. Tegument armed with minute spines extending to level to posterior margin of posterior
381 testis. Eye spots lacking. Oral sucker infundibuliform, muscular, lacking circumoral spines,
382 opens terminally. Ventral sucker sub-spherical, unspecialised. Sucker-width ratio *c.*1-1.7.
383 Forebody occupies 32-62% of body length. Pre-pharynx of variable length. Pharynx elongate,
384 muscular. Oesophagus indistinguishable, bifurcates just pre-pharyngeal. Caeca reach posterior
385 testis. Testes two, tandem, oval, contiguous or slightly overlapping, entire, in posterior hindbody,
386 close to posterior extremity. Cirrus and cirrus-sac absent; seminal vesicle tubule-saccular, dorso-
387 sinistral to ventral sucker. Genital pore sinistral in posterior forebody. Gonotyl absent. Ovary
388 pre-testicular, dextral, entire, close to anterior testis. Uterus restricted to hindbody anterior to
389 mid-level of anterior testis. Seminal receptacle saccular, relatively large, in region of anterior

390 testis and ovary. Eggs numerous, small, elliptical, tanned, operculate. Vitellarium follicular,
391 follicles in two lateral groups between ventral sucker and level of ovary or anterior testis;
392 excretory pore terminal, excretory vesicle obscured. *Type-species*: *Tanganyikatrema fusiforma* n.
393 sp.

394 *Etymology:* The genus epithet is proposed in reference to the ecosystem of Lake Tanganyika to
395 honour this biodiversity hotspot and appended to the commonly used ending -*trema*. It is to be
396 treated as feminine.

397 **Differential diagnosis**

398 The only digenean genus parasitic in fish reported in Lake Tanganyika, *Neocladocystis*, differs
399 from *Tanganyikatrema* n. gen. in the presence of rounded *versus* infundibuliform oral sucker,
400 short *versus* pre-pharynx variable in length and long oesophagus, and slightly oblique *versus*
401 tandem testes. *Neocladocystis* includes species parasitic in cichlid and bagrid fishes in Africa and
402 characid fishes in South America. *Tanganyikatrema* n. gen. morphologically resembles
403 *Claribula* Overstreet, 1969, a monotypic genus proposed for species parasitic in marine fishes of
404 the families Albulidae and Sphyraenidae off Florida, USA by the possession of fusiform body
405 and a cup-shaped oral sucker but differs in the presence of spined tegument, pre-pharynx,
406 oesophagus (in mature individuals), intestinal bifurcation anterior to ventral sucker and testes
407 located in the posterior third of the hindbody. *Isocoelium* Ozaki, 1927 is a genus whose
408 representatives parasitise in marine uranoscopid fishes. It resembles the new genus in the
409 presence of tegumental spines, oblique to slightly tandem testes and the uterus reaching no
410 further posteriorly then the testicular zone but differs by having a deeply lobed ovary at mid-
411 hindbody and shorter forebody in proportion.

412

413 ***Tanganyikatrema fusiforma* n. sp. Kmentová, Georgieva & Bray**

414 ***Type-host*****:** *Lates microlepis* Boulenger (Perciformes: Latidae).

415 ***Other-host:*** *Lates angustifrons* Boulenger (Perciformes: Latidae).

416 ***Type-locality*****:** Lake Tanganyika at Katukula, Zambia (8°36'S, 31°11'E).

417 ***Other-localities*****:** Lake Tanganyika at Mutondwe Island, Zambia (8°42'S, 31°07'E) and at
418 Mpulungu, Zambia (8°46'S, 31°07'E).

419 ***Type-specimens*****:** The holotype (xxx) and xx paratypes (xxx) were deposited at the
420 Helminthological Collection of the Natural History Museum in London, United Kingdom, xxx
421 paratypes (xxx) at the Hasselt University, in Diepenbeek, Belgium and xxx paratypes (xxx) at
422 the Royal Museum for Central Africa in Tervuren, Belgium.

423 ***Site in host*****:** Intestine.

424 ***Representative DNA sequences*****:** GenBank accession number: xxx (partial (D1-D3 domains) 28S
425 rRNA); xxx (*cox*1).

426 ***ZooBank registration*****:** XXXXXXX.

427 ***Etymology*****:** The specific name is derived from the Latin *fusiformis* meaning fusiform and
428 referring to body shape wide at the middle and tapered at forebody as well as tail.

429 **Description**

430 [Based on 12 specimens including 3 immature individuals; Fig. 3a, Additional file 2: Fig. S4,
431 Table 3]. Body elongate, fusiform, narrow, longer than wide. Tegument spined, spines reach to
432 posterior margin of posterior testis. Eye-spot pigment absent. Oral sucker infundibuliform
433 (distorted in larger worm figured) or cup-shaped, massive, relatively large, longer than wide,
434 squared-off posteriorly, aperture terminal. Circumoral spines absent. Ventral sucker pre-
435 equatorial, rounded, unspecialised, embedded in ventrogenital sac. Pre-pharynx long. Pharynx
436 oval, muscular. Oesophagus shorter than pre-pharynx. Intestinal bifurcation in posterior forebody

437 just anterior to ventral sucker. Caeca end blindly, reach into post-testicular region close to
438 posterior body extremity.

439 Testes two, entire, tandem, contiguous or overlapping, in posterior third of hindbody,
440 anterior testis oval, posterior testis sub-triangular. Post-testicular region short. Cirrus and cirrus
441 sac absent. Seminal vesicle tubulosaccular, naked, long, bipartite, convoluted, sinistral to ventral
442 sucker, posterior extent obscured by eggs. No prostatic cells evident. Common genital pore
443 median, immediately antero-sinistral to ventral sucker. Gonotyl absent.

444 Ovary regularly oval, pre-testicular, anterior or overlapping to anterior testis. Uterus fills
445 much of hindbody from anterior testis anteriorly, passes dorsally to ventral sucker, mostly
446 intercaecal. Mehlis' gland and Laurer's canal not observed. Seminal receptacle saccular,
447 contiguous with anterior testis and dorsal to ovary. Eggs numerous, elliptical, malformed in
448 larger worms, operculated, tanned. Vitellarium follicular, in two lateral fields, extends from
449 about level anterior to ovary to ventral sucker; laterally overlapping caeca from both sides
450 dorsally and ventrally. Seminal receptacle saccular, dorsal and posterior to ovary.

451 Excretory system not clearly visible, pore terminal, vesicle not clearly detected.

452

453 ***Tanganyikatrema* sp. 'elongata'**

454 ***Type-host*:** *Lates angustifrons* Boulenger (Perciformes: Latidae)

455 ***Other-host:*** *Lates microlepis* Boulenger (Perciformes: Latidae).

456 ***Type-locality*:** Lake Tanganyika at Mpulungu, Zambia (8°46'S, 31°07'E)

457 ***Type-specimens*:** The holotype (xxx) and two paratypes (xxx) were deposited at the
458 Helminthological Collection of the Natural History Museum in London, United Kingdom.

459 ***Site in host*:** Intestine.

460 ***Representative DNA sequences*****:** GenBank accession numbers: xxx (partial (D1-D3 domains)
461 28S rRNA gene).

462 ***ZooBank registration*****:** XXXXXXX.

463 ***Etymology*****:** We distinguish *Tanganyikatrema* sp. 'elongata' from *Tanganyikatrema fusiforma*
464 with the epithet 'elongata' derived from the Latin as a combination of *elongatae* and *forma*
465 referring to the elongated forebody of the species. *Disclaimer:* this does not intend to be a
466 nomenclatural act and this name should not be interpreted as a species name.

467 **Description**

468 [Based on 3 specimens including 2 immature individuals; Fig. 3b, Additional file 2: Fig. S5,
469 Table 3]. Body elongate, narrow. Tegument spined, spines scale-like at anterior body extremity
470 diminishing in size posteriorly, extending close to posterior body extremity. Oral sucker
471 infundibuliform, shallow, muscular, aperture terminal, lacking circumoral spines. Ventral sucker
472 pre-equatorial, oval, muscular. Pre-pharynx long. Pharynx oval, small. Oesophagus, intestinal
473 bifurcation and caeca ending not clearly visible.

474 Primordial testes entire, small, tandem, overlapped, in posterior third of hindbody, both
475 testes squared. Seminal vesicle not observed.

476 Primordial ovary small, regularly oval, pretesticular. Uterus and vitelline fields restricted
477 to hindbody. Mehlis' gland, Laurer's canal not observed and seminal receptacle not visible.

478 **Remarks**

479 Two species of *Tanganyikatrema* n. gen. are distinguished from each other by length:width ratio
480 of oral sucker (bigger in *Tanganyikatrema fusiforma* n. sp.) as well as forebody:hindbody ratio
481 with forebody being elongated up to 65% of body length in *Tanganyikatrema* sp. 'elongata'
482 compared to 41% in immature and 32% in egg-bearing individuals *Tanganyikatrema fusiforma*
483 n. sp.

484

485

486 ***Grandifundilamena* n. gen. Bray, Kmentová & Georgieva**

487 **Diagnosis**

488 Body elongate, relatively narrow, widest at level of oral sucker, length:width ratio *c*.10.4–11.6.
489 Tegument lacks spines. Eye-spots absent. Oral sucker broadly infundibuliform, lacking
490 circumoral spines, opens terminally. Ventral sucker sub-spherical, in anterior quarter of body,
491 distinctly smaller than oral sucker. Sucker-width ratio *c*.2.5-2.9. Forebody occupies 23-24% of
492 body length. Pre-pharynx relatively short. Pharynx oval, relatively large. Oesophagus short or
493 indistinguishable; intestinal bifurcation in posterior-forebody. Caeca reach to the end of posterior
494 testis. Nine testes, in tandem series in posterior third of body, reaching close to posterior
495 extremity. Cirrus and cirrus-sac absent. Seminal vesicle long, narrow, tubular, mainly in
496 hindbody. Genital pore immediately anterior to ventral sucker. Gonotyl absent. Vitellarium
497 follicular, two lateral fields from posterior level of seminal vesicle to level of posterior testis.
498 Ovary lobed, in posterior third of hindbody. Seminal receptacle saccular, in ovarian region.
499 Uterus mainly in hindbody, pretesticular. Excretory pore terminal, vesicle not detected. *Type-*
500 *species*: *Grandifundilamena novemtestes* n. sp.

501 *Etymology*: The specific epithet is derived from the Latin *grandis* meaning grand and
502 combination of *infundbuli* and *vitulamena* referring to the funnel shape of oral sucker. It is to be
503 treated feminine.

504 **Differential diagnosis**

505 *Grandifundilamena* n. gen. is distinguished from the other cryptogonimid genera by a
506 combination of infundibuliform oral sucker, the pharynx being larger than the ventral sucker, the
507 vitelline fields extending from nearly the posterior body extremity to about mid-hindbody level,

508 the entire ovary, the seminal vesicle anterior to ovary and nine tandem testes. Several
509 cryptogonimids are reported to possess multiple testes, e.g. *Polyorchitrema* Srivastava, 1939
510 (Sparidae), *Iheringtrema* Travassos, 1947 (Pimelodidae), *Siphodera* (many families, primarily
511 Lujanidae), *Siphomutabilis* Miller & Cribb, 2013 (Lutjanidae), *Novemtestis* Yamaguti, 1942
512 (metacercariae in Mullidae, host of adults unknown), *Acanthosiphodera* Madhavi, 1976
513 (Lutjanidae). The common difference between *Grandifundilamena* n. gen. and species of
514 *Polyorchitrema*, *Iheringtrema*, *Siphodera*, *Siphomutabilis* as well as *Acanthosiphodera* lies in
515 the presence of infundibuliform *versus* round oral sucker and larger body length:width ratio.
516 Additionally, position of vitellaria fields restricted to the forebody and the absence of oral spines
517 distinguish *Grandifundilamena* n. gen. from species of *Novemtestis*. *Grandifundilamena* n. gen.
518 resembles *Mitotrema anthostomatum* Manter, 1963, a species infecting serranid fishes in the
519 Pacific Ocean, in the presence of infundibuliform sucker combined with elongated body but
520 differs in the proportional length of forebody (<10%) and number of testes (2). Miller & Cribb
521 [52] using molecular evidence have shown that closely related species may have two or multiple
522 testes. In *Siphomutabilis* the type-species, *Siphomutabilus gurukun* (Machida, 1986) Miller &
523 Cribb, 2013, possess nine testes arranged in a longitudinal row, as recovered in
524 *Grandifundilamena* n. gen. *Siphomutabilus aegyptensis* (Hassanine & Gibson, 2005) Miller &
525 Cribb, 2013 [53] has been reported with nine testes distributed as in a ring, and *S. raritas* Miller
526 & Cribb, 2013 and *S. bitesticulatus* Miller & Cribb, 2013 have been reported both with two
527 testes [52–54].

528

529 ***Grandifundilamena novemtestes* n. sp. Bray, Kmentová & Georgieva**

530 ***Type-host*: *Lates angustifrons* Boulenger (Latidae).

531 ***Other-host*: *Lates microlepis* Boulenger (Latidae).

532 ***Type-locality*****:** Lake Tanganyika at Mpulungu, Zambia (8°46'S, 31°07'E).

533 ***Type-specimens*****:** The holotype (xxx) and 3 paratypes (xxx) were deposited at the

534 Helminthological Collection of the Natural History Museum in London, United Kingdom.

535 ***Site in host*****:** Intestine.

536 ***ZooBank registration*****:** XXXXXXX.

537 ***Etymology*****:** The specific name is derived from the Latin as a combination of *novem* and *testes*

538 referring to the nine testes present in *Grandifundilamena novemtestes* n. sp.

539

540 **Description**

541 [Based on 4 specimens including 1 immature; Fig. 4, Additional file 2: S6, Table 3]. Body long,

542 relatively narrow, widest at oral sucker, soma widest in anterior forebody (for width

543 measurements), tapering gradually, trends to take up curved position on slides and is difficult to

544 mount fully dorso-ventrally. Tegumental spines not detected. Oral sucker massive, broadly

545 infundibuliform may extend as triangle posteriorly, aperture wide, terminal. Ventral sucker pre-

546 equatorial, rounded, much smaller than oral sucker. Pre-pharynx short, narrow. Pharynx broadly

547 oval, larger than ventral sucker. Oesophagus absent. Intestinal bifurcation in posterior forebody.

548 Caeca wide, end blindly, reach into post-testicular region close to posterior body extremity.

549 Testes nine, transversely oval, entire, small, in tandem row, reaching from just posterior

550 to seminal receptacle to close to posterior body extremity; contiguous, in posterior third of

551 hindbody. Seminal vesicle naked, long, convoluted, posteriorly extents, obscured by eggs.

552 Genital pore median, immediately anterior to ventral sucker.

553 Ovary irregularly sub-triangular, pre-testicular at distance from anterior testis. Seminal

554 receptacle saccular, anterior to ovary. Uterus narrow, reaches between ventral sucker and

555 anterior testis, passes dorsally to ventral sucker, most intercaecal. Eggs small, tanned, operculate.
556 Vitellarium follicular, fields reach from about level of posterior testis to level of posterior end of
557 seminal vesicle, post-vitelline field short.

558 Excretory pore terminal, vesicle not traced beyond posterior testis.

559

560 Detailed reciprocal morphological comparison of the newly described genera with already
561 described morphologically similar cryptogonimid genera is listed in Table 4.

562 **Molecular characterisation and phylogeny**

563 The newly obtained sequences of 28S rDNA region (1,240 bp) represented five distinct
564 genotypes, which correspond with morphologically distinct species recovered in this study,
565 confirming the presence of five species. Interspecific sequence divergence ranged between 1 and
566 25 bp (0.1–2.1%; see Table 5 for further details). Replicate specimens of the two most abundant
567 genotypes of *N. bemba* n. sp. (five specimens ex *L. microlepis* and a two specimens ex *L.*
568 *angustifrons*) and *Tanganyikatrema fusiforma* n. sp. (three specimens ex *L. microlepis* and two
569 isolates ex *L. angustifrons*) shared identical 28S rDNA sequences. A single sequence of *N.*
570 *biliaris* n. sp. recovered from *L. mariae* at Uvira differed by a single nucleotide from *N. bemba*
571 n. sp. An individual of *Tanganyikatrema* sp 'elongata' for which the molecular characterisation
572 was recovered differed from *Tanganyikatrema fusiforma* n. sp. by four nucleotides in 28S rDNA
573 gene portion. Representative single genotypes per species were used in the phylogenetic
574 analyses. Two of the recovered genotypes were shared between parasites of *L. microlepis* and *L.*
575 *angustifrons* (i.e. represented by seven and five isolates, respectively) while the remaining three
576 genotypes represented unique sequences recovered from three of the host species, i.e. *L. mariae*,
577 *L. microlepis* and *L. angustifrons*.

578 Partial *cox*1 sequences were obtained for nine isolates from three of the six
579 cryptogonimid trematode species, i.e. seven *Neocladocystis bemba* n. sp. ex *L. microlepis* and a
580 single sequence for *Neocladocystis* sp. and *Tanganyikatrema fusiforma* n. sp., both recovered
581 from *L. microlepis*. Comparative sequence analysis revealed high levels of intra- and
582 interspecific genetic divergence. The intraspecific genetic divergence for the isolates of *N.*
583 *bemba* n. sp. ranged between 1.3 and 4.2% (10–33 bp difference). All isolates of *N. bemba* n. sp.
584 represented unique haplotypes. Sequence divergence between *N. bemba* n. sp. and *N.* n. sp.
585 ranged between 15.5 and 16.2% (123–129 bp divergence) and 17.1–18.5% (136–147 bp
586 difference) between *N. bemba* n. sp. and *Tanganyikatrema fusiforma* n. sp. Further, the
587 sequences of *Neocladocystis* n. sp. and *Tanganyikatrema fusiforma* n. sp. differed considerably,
588 i.e. 19.4% (154 bp difference).

589 Phylogenetic relationships among representatives of the Cryptogonimidae were assessed
590 based on a dataset including 52 taxa (Fig. 5a). Overall they clustered into two major clades, i.e.
591 (i) one formed by the freshwater representatives of *Acanthostomum* and (ii) a second major clade
592 including the remaining currently available sequences for cryptogonimids, all reported from
593 marine fishes except for *Caecincola parvulus* Marchal & Gilbert, 1905 which was recovered
594 from the freshwater centrarchid *Micropterus salmoides* (Lacépède, 1802) in the USA. *Mitotrema*
595 *anthostomatum* Manter, 1963 diverged earlier among the marine cryptogonimids Despite the
596 large number of sequences available for marine cryptogonimids, BI analysis does not lend much
597 statistical support for the major nodes and indicated a lack of phylogenetic resolution. The newly
598 obtained sequences from Lake Tanganyika clustered with species of *Acanthostomum* reported
599 from Asia in a strongly supported clade.

600 Relationships among the newly sequenced isolates from Lake Tanganyika were further
601 assessed based on a restricted dataset including only the currently available isolates of
602 *Acanthostomum* (Fig. 5b). The novel isolates from Tanganyika formed a strongly supported

603 clade sister to a clade comprising sequences for *A. burminis* (Bhalerao, 1926) from India and
604 Thailand and an otherwise unidentified digenean labelled as *Acanthostomum* sp. VVT-2013 ex
605 the gastropod *Mieniplotia scabra* (Müller). The African isolates from *Lates* spp. formed two
606 strongly supported sister clades: (i) a clade comprised by species of *Tanganyikatrema* n. gen. and
607 (ii) a clade consisting of *Neocladocystis* spp. The remaining two isolates for *Acanthostomum* cf.
608 *americanum* (Vigueras, 1957) and *A. loossi* (Vigueras, 1957) clustered as earlier divergent taxa
609 to the clade of *A. burminis* with the novel isolates from Lake Tanganyika.

610 Discussion

611 The present study provides the first estimates of the trematode diversity in lates perches in Lake
612 Tanganyika. Employing thorough morphological characterisation and phylogenetic inference
613 based on sequence data of the 28S rRNA gene the presence of six distinct cryptogonimid species
614 parasitic in three of the four species of *Lates* endemic to Lake Tanganyika was revealed. All of
615 the recovered cryptogonimid trematodes represent species new to science. The presence of
616 *Neocladocystis* in the lake first reported by Prudhoe, 1951 was confirmed, with three new
617 species being recovered. The unique morphological characters of some of the species described
618 in the present study and their phylogenetic distinctiveness required the erection of two new
619 genera.

620 Cryptogonimid trematodes in Africa

621 All specimens in the present study possessed typical morphological characters of cryptogonimid
622 digeneans: testes at distance from posterior extremity, extensive uterus, gonolyl absent, common
623 genital pore opening just anterior to ventral sucker, a Y-shaped excretory vesicle, tanned eggs
624 and a lack of cirrus and cirrus-sac. In total, six cryptogonimid species from three genera are
625 described from three latid hosts, including the erection of two new genera. Although, Lake
626 Tanganyika has been studied for several decades, the present study is the first to provide
627 molecular data for digenean trematodes in this biodiversity hotspot. Furthermore, only three

628 species of the family Latidae, i.e. *L. niloticus* (western Africa), *L. calcarifer* (South-East Asia)
629 and *Psammoperca waigiensis* (Cuvier) (Indo-West Pacific) have been previously screened for
630 endohelminth infections (see Table 1 and references therein). Therefore, our study significantly
631 increases the knowledge on the parasite fauna in lates perches, an economically important group
632 for fisheries worldwide.

633 Currently, only seven cryptogonimid species of four genera, *Acanthostomum*,
634 *Brientrema*, *Neocladocystis* and *Siphodera*, have been reported from African freshwater fishes
635 [25, 26, 28, 29]. Of these, two species *Acanthostomum absconditum* (Looss, 1901) and *A.*
636 *spiniceps* (Looss, 1896) were recorded from a bagrid fish host, two from claroteids
637 (*Neocladocystis congoensis)* and *Siphodera ghanensis* Fischthal & Thomas, 1968), and a single
638 species from gymnorchiid (*A. gymnarchi* (Dollfus, 1950), malapterurid (*Brientrema*
639 *malapterurid* Dollfus, 1950) and otherwise unidentified cichlid fish hosts (*Neocladocystis*
640 *tanganyikae*), respectively [24–31].

641 To date, only three species of *Neocladocystis* have been known worldwide of which two,
642 *N. congoensis* and *N. tanganyikae*, were described from African freshwater fishes [26, 29].
643 Interspecific variability is seen mainly in the mutual position of the bifurcation of the caeca and
644 the ventral sucker and in the extent of the vitelline follicles and the sucker ratios [26, 29, 51].
645 Combined morphological and molecular characterisation of the cryptogonimids recovered in
646 Lake Tanganyika allowed us to assign three of them to *Neocladocystis*. Interestingly,
647 intraspecific phenotypic variability of *N. bemba* n. sp. was combined with a high morphological
648 homogeneity among the three new species of *Neocladocystis* indicating for presence of species
649 complex and recent speciation event. In the present case, host species and geographic origin as
650 well as localisation in the host could be the driving force for their diversification between *N.*
651 *bemba* n. sp. and *N. biliaris* n. sp. This is comparable with the evolution of other
652 cryptogonimids, *Retrovarium formosum* Miller & Cribb, 2007 and *Retrovarium exiguiformosum*
653 Miller & Cribb, 2007 both infecting the chinamanfish, *Symphorus nematophorus* (Bleeker)

654 (Lutjanidae: Perciformes) and reported from distant geographical areas in the Great Barrier Reef
655 [54]. A similar evidence for high levels of morphological homogeneity was previously
656 documented for the species of *Euryakaina* Miller, Adlard, Bray, Justine & Cribb, 2010
657 (Cryptogonimidae), though these species are by notably higher distances in the 28S rDNA [55].
658 Unfortunately, as only a single immature individual of the third putative new species of
659 *Neocladocystis*, *Neocladocystis* sp., collected from *L. angustifrons* was available. This did not
660 allow us to provide a full species description. However, its distinct species status was confirmed
661 by a difference of six and seven base pairs, respectively, when compared *Neocladocystis* sp. and
662 *N. bemba* n. sp. and *N. biliaris* n. sp. in the 28S rDNA sequences.

663 Although, the cryptogonimids typically have a three-host life-cycle with adults that are
664 localised in the intestine or pyloric caeca [56], adult specimens of *N. biliaris* n. sp. were localised
665 in the gallbladder of *L. mariae*. Therefore, localisation outside the digestive tract, more
666 specifically in the gallbladder, was added to the general characterisation of the genus. Unlike the
667 others, individuals of *N. bemba* n. sp. exhibited an intrahost migration with immature individuals
668 in the lumen of the intestine with adults being localised in the pyloric caeca.

669 A difference of four bp in the 28S rDNA gene was recovered between the
670 morphologically distinct species of *Tanganyikatrema* n. gen. indicative for interspecific variance.
671 Morphologically, the two species mainly differed in the relative position of the ventral sucker.
672 Unlike in the case of *N. bemba* n. sp. and *N. biliaris* n. sp., the two species of *Tanganyikatrema*
673 n. gen. were collected from the same host species and locality.

674 *Grandifundilamena novemtestes* n. gen. n. sp., possessed unique morphological
675 characters not only among the cryptogonimids discovered in the present study but also among
676 the currently known cryptogonimid trematodes. The presence of multiple testes, a character
677 rarely seen not only among the cryptogonimids [23] but also among the digenetic trematodes,
678 and a wide, strongly muscular and infundibular oral sucker which supported the erection of the

679 new genus. Unfortunately, the limited number of specimens collected prevented us of conducting
680 molecular characterisation and phylogenetic positioning of *Grandifundilamena novemtestes* n.
681 sp.

682 There are more species yet to be discovered in the largely unexplored fish fauna in Lake
683 Tanganyika. Collecting novel material from distinct localities along the lake would reveal the
684 real magnitude of the trematode species diversity in the lates perches. Additional material is
685 needed to reveal actual geographical species distribution in the lake. Further, clarification of host
686 species of *N. tanganyikae* along with the sequence data generation is needed to improve the
687 generic diagnosis.

688 **Biodiversity in Lake Tanganyika's pelagic zone**

689 The biodiversity in Lake Tanganyika is concentrated mainly in the littoral zone which
690 offers unique opportunities for within-lake diversification currently documented for a number of
691 vertebrate and invertebrate species such as cichlid fishes [55], crustaceans [56], poriferans [57]
692 and gastropods [3, 58]. Cryptogonimid digeneans are known to parasitise gastropod invertebrates
693 as first intermediate hosts and fishes as second and definitive hosts. The metacercarial stage is
694 trophically transmitted to the definitive host. Considering that trematode parasites are largely
695 dependent on the local food web and the species interactions involved, the reported species here
696 could therefore provide a link between the highly biodiverse littoral lake zone and the wide
697 pelagic habitat. Unfortunately, there have been studies on the larval trematode diversity in the
698 lake. The lake's pelagic zone is inhabited by less diverse fish assemblages including lates
699 perches, clupeids and some cichlid tribes [14, 19, 20, 22, 59]. Parasites recovered from meso-
700 and/or bathy-pelagic freshwater and marine hosts tend to show low host specificity and limited
701 diversity [9, 60–62]. However, as overall endohelminth biodiversity in the lake's littoral habitat
702 is unknown, it remains unclear how it compares to digenean diversification in the pelagic zone.

703 Despite the relatively high number of examined fish individuals, neither digenean nor
704 monogenean, cestode or acanthocephalan parasites [14] were recovered in *L. stappersii* so far.
705 This might be related to the different life histories and diet preferences compared to its
706 congeners. *Lates mariae* and *L. microlepis* are known to switch from littoral juvenile form to
707 exclusively pelagic top predator with night migration upon prey differentiated mainly by
708 preferred depth of occurrence as *L. mariae* tend to be found in higher depths [16, 63]. *Lates*
709 *angustifrons* is characterised by his preference of specific inshore rocky habitat and predominant
710 solitary and more sedentary lifestyle compared to above mentioned species. Unlike other
711 congeners in the lake, *L. stappersii* (Boulenger, 1914) exhibits truly pelagic lifestyle forming
712 large groups upon clupeid prey [16, 64]. Consequently, closer contact and/or habitat sharing with
713 gastropods, as intermediate hosts for the trematode parasites is rather limited [59].

714

715 **Phylogenetic reconstruction**

716 The phylogeny of the Cryptogonimidae has been a subject of a number of studies with more
717 extensive surveys conducted in the Indo-Pacific region at the Great Barrier Reef [52,65–67, and
718 reference therein]. Despite the limited sequence data available for freshwater cryptogonimid
719 species, our study demonstrates that freshwater parasitism within the family occurs in at least
720 two independent lineages (see Fig. 5a). Apart from the above mentioned earlier diverging clade
721 of *Acanthostomum* spp. including the novel sub-lineage from Lake Tanganyika, just a single
722 species, *Caecincola parvulus* Marshall & Gilbert, 1905 reported from the freshwater centrarchid
723 *Micropterus salmoides* (Lacepède) in the USA, is the only other freshwater cryptogonimid but
724 clustered within the major marine clade. Further, the monophyly of species parasitic in
725 caesionid, haemulid, lutjanid and nemipterid fish hosts was rejected, possibly indicating multiple
726 switching events between major definitive host groups through their evolution.

727 The analysis based on the 28S rDNA sequences confirmed the distinct status of both new
728 genera for which sequence data were obtained. In this respect the recognition of *Neocladocystis*
729 as a distinct clade and the erection of *Tanganyikatrema* n. gen. are justified based on both
730 morphological and molecular evidence. Contrasting patterns of diversification have been
731 revealed in the three new genera described here. The diversification events were associated with
732 morphological divergence indicating that similar environmental/microhabitat contexts do not
733 always imply similar outcomes of diversification [68]. Similarly, despite the striking
734 morphological differences among the species recovered in the lates perches from Lake
735 Tanganyika, the phylogenetic analyses recovered two of them to form a strongly supported
736 monophyletic clade sister to *Acanthostomum* (Fig. 5a). This further highlights the importance of
737 taxon-dependent factors on the processes involved in their diversification. Three representatives
738 of *Acanthostomum* are known to infect a wide range of fish species as definitive hosts. These
739 include members of distinct families such as Bagridae, Gymnarchidae and Latidae in Africa,
740 with *L. niloticus* reported as a host of two species of *Acanthostomum* in Egypt [27, 31]. Rather
741 recent diversification processes within this digenean lineage indicated by subtle differentiation
742 between the reported congeners both at morphological and DNA sequence levels correspond
743 with the assumed recent invasion and subsequent diversification of the lates perches in Lake
744 Tanganyika (own unpublished data). However, the lack of parasitological data, especially for
745 endohelminths, in the lake prevents any further conclusions regarding the host specificity of this
746 digenean lineage.
747

748 ## Conclusions

749 Six cryptogonimid trematode species belonging to three genera were reported from the endemic
750 lates perch hosts in Lake Tanganyika. Substantial intraspecific phenotypic variability combined
751 with interspecific morphological similarity and contrasting with clear genetic differentiation has

752 been recognised in the recovered species of *Neocladocystis*. Therefore, rather a recent speciation
753 driven by the host species preference and/or geographically dependent diversification is
754 hypothesised. Future investigations based on more abundant material and faster evolving
755 molecular markers is needed to assess the real levels of intraspecific variation in the
756 Tanganyika's cryptogonimid trematodes. The novel molecular data gathered here indicated the
757 existence of an exclusively freshwater clade within the cryptogonimid genera. The present
758 results highlight the importance of concerted efforts and application of an integrated approach to
759 the assessment of the real biodiversity in this unique ecosystem.

760 **Abbreviations**

761 NHMUK **-** Helminthological Collection of the Natural History Museum in London, United
762 Kingdom; RMCA - Royal Museum for Central Africa; *cox*1 - cytochrome *c* oxidase subunit 1

763 **Acknowledgments**

764 We would like to thank Holger Zimmermann and Aneesh P. H. Bose who helped to collect host
765 samples. We are also grateful to F. Muterezi Bukinga, T. Mulimbwa N'sibula, P. Masilya
766 Mulungula, Joseph Mbirize, D. Muzumani Risasi, L. Makasa and the entire teams of Research
767 Centre of Hydrobiology in Uvira and Fisheries Research Unit in Mpulungu for their hospitality.

768 **Declarations**

769 **Ethics declarations**

770 Ethics approval and consent to participate
771 This study was approved by Animal Care and Use Committee in Faculty of Science, Masaryk
772 University in Brno (Czech Republic).

773 **Consent for publication**

774 Not applicable

Availability of data and materials

The type- and voucher material are deposited at the Helminthological Collection of the Natural History Museum in London, United Kingdom (NHMUK), the Royal Museum for Central Africa (RMCA) in Tervuren, Belgium and in the collection of the Research Group Zoology: Biodiversity and Toxicology at Hasselt University in Diepenbeek, Belgium. DNA sequences generated as a part of this study were deposited to the NCBI nucleotide database.

Competing interests

The authors declare that they have no competing interests.

Funding

Research has been supported by the Czech Science Foundation (P505/12/G112 (ECIP) and GA19-13573S), EMBRC Belgium - FWO project GOH3817N as well as by a joint program between the Austrian agency for international mobility and cooperation in education, science and research and the Ministry of Education, Youth and Sports (project number 8J18AT007), the Austrian Agency for International Cooperation in Education and Research (OEAD; project number CZ 08/2018; to SK) and the Systematics Research Fund (SRF; to SK). MPMV was co-financed by institutional funding of the Finnish Museum of Natural History and the Belgian Directorate-General for Development Cooperation and Humanitarian Aid (CEBioS program). ELRDK was supported by the Belgian Development Cooperation through VLIR-UOS (VLADOC scholarship NDOC2016PR006 to ELRDK and South Initiative project CD2018SIN218A101). Fieldwork was carried out with the approval of the competent local authorities under mission statement 031/MINRST/CRH-U/2016 and the permission of the Fisheries Department of Zambia and under a study permit issued by the government of Zambia (SP 008732). SG benefited from a postdoctoral fellowship 'Juan de la Cierva-Formación' of the MICINN (FJCI-2016–29535), Spain.

Authors' contributions

800 NK performed the morphological characterisation, described the species, contributed to the
801 phylogenetic analyses and drafted the manuscript. RAB contributed to the morphological
802 characterisation, species descriptions and revised the manuscript. SG carried out the sequencing,
803 performed the phylogenetic analyses and took part in the morphological identification of the
804 isolates, discussed the results and helped drafted the manuscript and supervised the study. SK
805 identified fish species, contributed to sampling and provided scientific background on Lake
806 Tanganyika and its ichthyofauna. ELRDK contributed to sampling and revised the manuscript.
807 SK and MPMV revised the manuscript. MPMV and NK designed the study. MG provided
808 scientific background in the field of parasite ecology. All authors read and approved the final
809 version of the manuscript.

810

811 **References**

812 1. Cohen AS, Soreghan MJ, Scholz CA. Estimating the age of formation of lakes: An example
813 from Lake Tanganyika, East African Rift system. Geology. 1993;21:511–4.

814 2. Nishida M. Lake Tanganyika as an evolutionary reservoir of old lineages of East African
815 cichlid fishes: Inferences from allozyme data. Experientia. 1991;47:974–9.

816 3. Wilson AB, Glaubrecht M, Meyer A. Ancient lakes as evolutionary reservoirs: evidence from
817 the thalassoid gastropods of Lake Tanganyika. Proc R Soc London Ser B Biol Sci.
818 2004;271:529–36.

819 4. Snoeks J. Ancient lakes: biodiversity, ecology and evolution. Adv Ecol Res. Elsevier;
820 2000;31:17–38.

821 5. Coulter GW. Composition of the flora and fauna. In: Coulter GW, editor. Lake Tanganyika its
822 life. London Oxford & New York: Natural History Musem Publications & Oxford University
823 Press; 1991. p. 200–74.

824 6. Scholz T, Vanhove MPM, Jayasundera Z, Gelnar M. A guide to the parasites of African
825 freshwater fishes. Scholz T, Vanhove MPM, Jayasundera Z, Gelnar M, editors. Abc Taxa; 2018.

826 7. Pariselle A, Van Steenberge M, Snoeks J, Volckaert FAM, Huyse T, Vanhove MPM.
827 Ancyrocephalidae (Monogenea) of Lake Tanganyika: Does the *Cichlidogyrus* parasite fauna of
828 *Interochromis loocki* (Teleostei, Cichlidae) reflect its host's phylogenetic affinities? Contrib to
829 Zool. 2015;84:25–38.

830 8. Pariselle A, Muterezi Bukinga F, Van Steenberge M, Vanhove MPM. Ancyrocephalidae
831 (Monogenea) of Lake Tanganyika: IV: *Cichlidogyrus* parasitizing species of Bathybatini
832 (Teleostei, Cichlidae): reduced host-specificity in the deepwater realm? Hydrobiologia.
833 2015;748:99–119.

834 9. Kmentová N, Gelnar M, Mendlová M, Van Steenberge M, Koblmüller S, Vanhove MPM.
835 Reduced host-specificity in a parasite infecting non-littoral Lake Tanganyika cichlids evidenced
836 by intraspecific morphological and genetic diversity. Sci Rep. 2016;6:39605.

837 10. Kmentová N, Van Steenberge M, Raeymaekers JAR, Koblmüller S, Hablützel PI, Muterezi
838 Bukinga F, et al. Monogenean parasites of sardines in Lake Tanganyika: diversity, origin and
839 intra-specific variability. Contrib to Zool. 2018;87:105–32.

840 11. Rahmouni C, Vanhove MPM, Šimková A. Underexplored diversity of gill monogeneans in
841 cichlids from Lake Tanganyika: eight new species of *Cichlidogyrus* Paperna, 1960 (Monogenea:
842 Dactylogyridae) from the northern basin of the lake, with remarks on the vagina and the heel of
843 the male copu. Parasit Vectors. 2017;10:591.

844 12. Rahmouni C, Vanhove MPM, Šimková A. Seven new species of *Cichlidogyrus* Paperna,
845 1960 (Monogenea: Dactylogyridae) parasitizing the gills of Congolese cichlids from northern
846 Lake Tanganyika. PeerJ. 2018;6:e5604.

847 13. Baylis HA. Some parasitic worms, mainly from fishes, from Lake Tanganyika. Ann Mag Nat

848 Hist. 1928;1:552–62.

849 14. Kmentová N, Koblmüller S, Van Steenberge M, Artois T, Muterezi Bukinga, F. Mulimbwa
850 N'sibula, T. Muzumani Risasi, D. Masilya Mulungula P, Gelnar M, et al. Failure to diverge in
851 African Great Lakes: the case of *Dolicirroplectanum lacustre* comb. nov. (Monogenea,
852 Diplectanidae) infecting latid hosts. J Great Lakes Res. under review.

853 15. Salzburger W, Van Bocxlaer B, Cohen AS. Ecology and evolution of the African Great
854 Lakes and their faunas. Annu Rev Ecol Evol Syst. 2014;45:519–45.

855 16. Coulter GW. The biology of *Lates* species (Nile perch) in Lake Tanganyika, and the status of
856 the pelagic fishery for *Lates* species and *Luciolates stappersii* (Blgr.). J Fish Biol. 1976;9:235–
857 59.

858 17. Plisnier PD, Mgana H, Kimirei I, Chande A, Makasa L, Chimanga J, et al. Limnological
859 variability and pelagic fish abundance (*Stolothrissa tanganicae* and *Lates stappersii*) in Lake
860 Tanganyika. Hydrobiologia. 2009;625:117–34.

861 18. Lowe-McConnell RH. Fish faunas of the African Great Lakes: origins, diversity, and
862 vulnerability. Conserv Biol. 1993;7:634–43.

863 19. Coulter GW. Pelagic Fish. In: Coulter GW, editor. Lake Tanganyika its life. London Oxford
864 & New York: Natural History Musem & Oxford 1991. p. 111–50.

865 20. Koblmüller S, Odhiambo EA, Sinyinza D, Sturmbauer C, Sefc KM. Big fish, little
866 divergence: phylogeography of Lake Tanganyika's giant cichlid, *Boulengerochromis microlepis*.
867 Hydrobiologia. 2015;748:29–38.

868 21. De Keyzer ELR, De Corte Z, Van Steenberge M, Raeymaekers JAM, Calboli FCF,
869 Kmentová N, et al. First genomic study on Lake Tanganyika sprat *Stolothrissa tanganicae*: a
870 lack of population structure calls for integrated management of this important fisheries target
871 species. BMC Evol Biol. 2019;19:6.

872 22. Koblmüller S, Zangl L, Börger C, Daill D, Vanhove MPM, Sturmbauer C, et al. Only true
873 pelagics mix: comparative phylogeography of deepwater bathybatine cichlids from Lake
874 Tanganyika. Hydrobiologia. 2019;832:93–103.

875 23. Miller TL, Cribb TH. Family Cryptogonimidae Ward, 1917. In: Bray RA, Gibson DI,]ones
876 A, editors. Keys to Trematoda Vol 3. London: CAB International and Natural History Museum;
877 2008. p. 51–112.

878 24. Looss A. Ueber die Fasciolidengenera *Stephanochasmus, Acanthochasmus* und einige
879 andere. Centrbl Bak. 1901;29:595–661.

880 25. Dollfus RP. Trematodes récolths au Congo Belge par le Professeur Paul Brien (mai-août
881 1937). Ann Mus Congo Beig. 1950;1–135.

882 26. Prudhoe S. Trematoda, Cestoda and Acanthocephala. Résultats Scientifiques de l'Exploration
883 Hydrobiologique du Lac Tanganika 1951;3:1–10.

884 27. Issa GI. Description of *Acanthostomum niloticum* n. sp. and *Acanthostomum spiniceps*
885 knobus (Trematoda, Acanthostomidae) from the river Nile, Egypt. Wildliji Dis Asoc Mirro-card.
886 1962;31.

887 28. Fischthal JH, Thomas JD. Digenetic trematodes of some freshwater and marine fishes from
888 Ghana. Proc. Helminthol. Soc. 1968;35:126–40.

889 29. Manter HW, Pritchard MH. Some digenetic trematodes of Central Africa chiefly from fishes.
890 Rev Zool Bot Africaines. 1969;80:51–61.

891 30. Moravec F. On two acanthostomatid trematodes, *Acanthostomum spiniceps* (Looss, 1896)
892 and *A. absconditum* (Looss, 1901), from African bagrid fishes. Folia Parasitol. 1976;23:201–6.

893 31. Brooks DR. Revision of the Acanthostominae Poche, 1926 (Digenea: Cryptogonimidae).
894 Zool J Linn Soc. 1980;70:313–82.

895 32. Marcogliese DJ. Parasites of the superorganism: Are they indicators of ecosystem health? Int
896 J Parasitol. 2005;35:705–16.

897 33. Ergens R, Lom J. Causative agents of fish diseases. Prague: Academia; 1970.

898 34. Pleijel F, Jondelius U, Norlinder E, Nygren A, Oxelman B, Schander C, et al. Phylogenies
899 without roots? A plea for the use of vouchers in molecular phylogenetic studies. Mol Phylogenet
900 Evol. 2008;48:369–71.

901 35. Tkach V V., Littlewood DTJ, Olson PD, Kinsella JM, Swiderski Z. Molecular phylogenetic
902 analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). Syst Parasitol. 2003;56:1–
903 15.

904 36. Bowles J, Blair D, McManus DP. Genetic variants within the genus *Echinococcus* identified
905 by mitochondrial DNA sequencing. Mol Biochem Parasitol. 1992;54:165–73.

906 37. Miura O, Kuris AM, Torchin ME, Hechinger RF, Dunham EJ, Chiba S. Molecular-genetic
907 analyses reveal cryptic species of trematodes in the intertidal gastropod, *Batillaria cumingi*
908 (Crosse). Int J Parasitol. 2005;35:793–801.

909 38. Littlewood DTJ, Curini-Galletti M, Herniou EA. The interrelationships of Proseriata
910 (Platyhelminthes: Seriata) tested with molecules and morphology. Mol Phylogenet Evol.
911 2000;16:449–66.

912 39. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic:
913 An integrated and extendable desktop software platform for the organization and analysis of
914 sequence data. Bioinformatics. 2012;28:1647–49.

915 40. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7:
916 Improvements in performance and usability. Mol Biol Evol. 2013;30:772–80.

917 41. Castresana J. Selection of conserved blocks from multiple alignments for their use in

918 phylogenetic analysis. Mol Biol Evol. 2000;17:540–52.

919 42. Gouy M, Guindon S, Gascuel O. SeaView Version 4: A multiplatform graphical user
920 interface for sequence alignment and phylogenetic tree building. Mol Biol Evol. 2010;27:221–
921 24.

922 43. Kumar S, Stecher G, Tamura K, Gerken J, Pruesse E, Quast C, et al. MEGA7: Molecular
923 evolutionary genetics analysis Version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870–74.

924 44. Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics
925 and parallel computing. Nat Methods. 2012;9:772.

926 45. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, et al. MrBayes
927 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space.
928 Syst Biol. 2012;61:539–42.

929 46. Miller MA, Pfeiffer W, Schwartz T. Creating the CIPRES Science Gateway for inference of
930 large phylogenetic trees. 2010 Gatew Comput Environ Work. IEEE; 2010. p. 1–8.

931 47. Rambaut A, Suchard MA, Drummond AJ. Tracer v1.6. 2014. Available from
932 http://beast.bio.ed.ac.uk/Tracer.

933 48. Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DTJ. Phylogeny and classification of
934 the Digenea (Platyhelminthes: Trematoda). Int J Parasitol. 2003;33:733–55.

935 49. Khalil LF, Thurston JP. Studies on the helminth parasites of freshwater fishes of Uganda
936 including the descriptions of two new species of digeneans. Rev Zool Bot afr. 1973;87:209–48.

937 50. Vaz Z. Contribuiçao ao conhecimento dos Trematoides de peixes fluviaes do Brasil. Ina
938 Diss. 1932;47.

939 51. Quintana MG, Ostrowski de Núñez M. The life cycle of *Neocladocystis intestinalis* (Vaz,
940 1932) (Digenea: Cryptogonimidae), in *Aylacostoma chloroticum* (Prosobranchia: Thiaridae), and

941 *Salminus brasiliensis* (Characiformes: Characidae), in Argentina. Parasitol Res. 2016;115:2589–
942 95.

943 52. Miller TL, Cribb TH. Dramatic phenotypic plasticity within species of *Siphomutabilus* n. g.
944 (Digenea: Cryptogonimidae) from Indo-Pacific caesionines (Perciformes: Lutjanidae). Syst
945 Parasitol. 2013;86:101–12.

946 53. Hassanine RME-S, Gibson DI. Trematodes of Red Sea fishes: *Hexangium brayi* n. sp.
947 (Angiodictyidae Looss, 1902) and *Siphodera aegyptensis* n. sp. (Cryptogonimidae Ward, 1917),
948 with a review of their genera. Syst Parasitol. 2005;61:215–22.

949 54. Machida M. Monorchiidae (Trematoda, Digenea) from fishes of Japanese and adjacent
950 waters. Bull Natl Sci Mus, Tokyo Ser A Zool. 2005;31:123–36.

951 55. Sefc K, Mattersdorfe K, Ziegelbecker A, Neuhüttler N, Steiner O, Goessler W, et al. Shifting
952 barriers and phenotypic diversification by hybridization. Ecol Lett. 2017;20:651–62.

953 56. Fryer G. Evolution in ancient lakes: radiation of Tanganyikan atyid prawns and speciation of
954 pelagic cichlid fishes in Lake Malawi. Hydrobiologia. 2006;568:131–42.

955 57. Meixner MJ, Lüter C, Eckert C, Itskovich V, Janussen D, von Rintelen T, et al. Phylogenetic
956 analysis of freshwater sponges provide evidence for endemism and radiation in ancient lakes.
957 Mol Phylogenet Evol. 2007;45:875–86.

958 58. Glaubrecht M. Adaptive radiation of thalassoid gastropods in Lake Tanganyika, East Africa:
959 morphology and systematization of a paludomid species flock in an ancient lake. Zoosystematics
960 Evol. 2008;84:71–122.

961 59. Hecky RE. The pelagic ecosystem. In: COULTER GW, editor. Lake Tanganyika its life.
962 London Oxford & New York: Natural History Musem & Oxford University Press; 1991. p. 91–
963 110.

964 60. Rohde K, Hayward C, Heap M. Aspects of the ecology of metazoan ectoparasites of marine
965 fishes. Int J Parasitol. 1995;25:945–70.

966 61. Luque JL, Mouillot D, Poulin R. Parasite biodiversity and its determinants in coastal marine
967 teleost fishes of Brazil. Parasitology. 2004;128:671–82.

968 62. Klimpel S, Palm HW, Busch MW, Kellermanns E, Rückert S. Fish parasites in the Arctic
969 deep-sea: Poor diversity in pelagic fish species vs. heavy parasite load in a demersal fish. Deep
970 Sea Res Part I Oceanogr Res Pap. 2006;53:1167–81.

971 63. Mölsä H, Reynolds JE, Coenen EJ, Lindqvist OV. Fisheries research towards resource
972 management on Lake Tanganyika. Hydrobiologia. 1999;407:1–24.

973 64. Mannini P, Katonda I, Kissaka B, Verburg P. Feeding ecology of *Lates stappersii* in Lake
974 Tanganyika. Hydrobiologia. 1999;407:131–9.

975 65. Miller TL, Cribb TH. Coevolution of *Retrovarium* n. gen. (Digenea: Cryptogonimidae) in
976 Lutjanidae and Haemulidae (Perciformes) in the Indo-West Pacific. Int J Parasitol.
977 2007;37:1023–45.

978 66. Miller T, Cribb T. Eight new species of *Siphoderina* Manter, 1934 (Digenea,
979 Cryptogonimidae) infecting Lutjanidae and Haemulidae (Perciformes) off Australia. Acta
980 Parasitol. 2008;53:344–64.

981 67. Miller TL, Cutmore SC, Cribb TH. Two species of *Neometadena* Hafeezullah & Siddiqi,
982 1970 (Digenea: Cryptogonimidae) from Moreton Bay, Australia, including the description of
983 *Neometadena paucispina* n. sp. from Australian Lutjanidae. Syst Parasitol. 2018;95:655–64.

984 68. Poulin R. Variation in the intraspecific relationship between fish length and intensity of
985 parasitic infection: biological and statistical causes. J Fish Biol. 2000;56:123–37.

986 69. Emere MC. Parasitic Infection of the Nile Perch *Lates niloticus* (L) in River Kaduna . J

987 Aquat Sci. 2000;15:51–4.

988 70. Abdel-Gaber R, Abdel-Ghaffar F, Mehlhorn H, Al Quraishy S, Morsy K, Maher S. Light
989 microscopic study of four plagiorchiid trematodes infecting marine fish in the south-eastern
990 Mediterranean Sea, Alexandria City, with descriptions of two new species. Parasitol Res.
991 2018;117:1341–56.

992 71. Hamouda AH, Sorour SS, El-habashi NM, Adam EA. Parasitic infection with emphasis on
993 *Tylodelphys* spp . as new host and locality records in Nile Perch ; *Lates niloticus* from Lake
994 Nasser , Egypt. 2018;8:19–33.

995 72. Klapper R, Meyer C, Kuhn T, Karl H. Food safety aspects of fresh Nile perch (*Lates*
996 *niloticus*) fillets from Lake Victoria imported to the European market: Helminth parasites and
997 microbiological status. Food Control. 2017;78:311–6.

998 73. Bray RA, Cribb TH. Species of *Stephanostomum* Looss, 1899 (Digenea: Acanthocolpidae)
999 from fishes of Australian and South Pacific waters, including five new species. Syst Parasitol.
1000 2003;55:159–97.

1001 74. Chatterji PN. On a new species of the genus *Psilostomum* Looss, 1899. Indian J. Helminthol.
1002 1958.

1003 75. Smith JW. The blood flukes (Digenea: Sanguinicolidae and Spirorchidae) of cold-blooded
1004 vertebrates and some comparison with the schistosomes. Helminthol Abstr Ser A, Anim Hum
1005 Helminthol. 1972;41:161–204.

1006 76. Herbert BW, Shaharom FM. A new blood fluke, *Parasanguinicola vastispina* gen. nov., sp.
1007 nov. (Trematoda: Sanguinicolidae), from sea bass, *Lates calcarifer* (Centropomidae) cultured in
1008 Malaysia. Parasitol Res. 1995;81:349–54.

1009 77. Arthur JR, Lumanlan-Mayo S. Checklist of the parasites of fishes of the Philippines. FAO
1010 Fisheries Technical Paper. 1997.

1011 78. Rückert S, Palm HW, Klimpel S. Parasite fauna of seabass (*Lates calcarifer*) under
1012 mariculture conditions in Lampung Bay, Indonesia. J Appl Ichthyol. 2008;24:321–7.

1013 79. Bilqees FM. Teaching and research in marine parasitology - problems and solution. 1995.

1014 80. Bilqees FM. A new trematode genus *Pseudohypertrema* from fishes of the karachi coast
1015 pakistan. Nor J Zool. 1976;24:201–3.

1016 81. Bray RA, Cribb TH, Barker SC. Hemiuridae (Digenea) from marine fishes of the Great
1017 Barrier Reef, Queensland, Australia. Syst Parasitol. 1993;25:37–62.

1018 82. Yamaguti S. Parasitic Worms mainly from Celebes. Part 1. New Digenetic Trematodes of
1019 Fishes. Acta Med Okayama. Okayama University Medical School; 1952;8.

1020 83. Rodgers LJ, Burke JB. Aetiology of "red spot" disease (vibriosis) with special reference to
1021 the ectoparasitic digenean *Prototransversotrema steeri* (Angel) and the sea mullet, *Mugil*
1022 *cephalus* (Linnaeus). J Fish Biol. 1988;32:655–63.

1023 84. Cribb T, Bray R, Barker S. A review of the family Transversotrematidae (Trematoda :
1024 Digenea) with the description of a new genus, *Crusziella*. Invertebr Syst. 1992;6:909.

1025 85. Bray RA, Cribb TH. *Ningalooia psammopercae* n. g., n. sp. (Digenea: Acanthocolpidae)
1026 from the Waigieu seaperch *Psammoperca waigiensis* (Cuvier) (Perciformes: Latidae) on the
1027 Ningaloo Reef, Western Australia. Syst Parasitol. 2007;66:131–5.

1028

1029 Abbreviations: GBR, Great Barrier Reef; SWA, South-West Asia, USA, the United States of
1030 America

1031

Table 1 List of digenean species described from members of family Latidae

Host	Digenean species	Family	Reference
Lates niloticus (L.)	*Euclinostomum* sp.	Clinostomidae	[69]
	Acanthostomum knobus (Issa, 1962)	Cryptogonimidae	[27, 31]
	Acanthostomum niloticum Issa, 1962		[27, 31]
	Acanthostomum spiniceps (Looss, 1896)		[70]
	Tylodelphys sp. (metacercaria)	Diplostomidae	[71]
	Echinostoma sp.	Echinostomatidae	[72]
Lates calcarifer (Bloch)	*Stephanostomum cloacum* (Srivastava, 1938)	Acanthocolpidae	[73]
	Allocreadium fasciatusi Kakaji, 1969[a]	Allocreadiidae	[74]
	Cardicola sp.	Aporocotylidae	[75]
	Cruoricola lates Herbert, Shaharom-Harrison & Overstreet, 1994		[75, 76]
	Parasanguinicola vastispina Herbert & Shaharom, 1995		[75, 76]
	Prosorhynchus luzonicus Velasquez, 1959	Bucephalidae	[77]
	Prosorhynchus sp.		[78]
	Callodistomum minutus Zaidi & Khan, 1977	Callodistomidae	[79]
	Pseudometadena celebesensis Yamaguti, 1952	Cryptogonimidae	[77]
	Pseudometadena sp.		[77]
	Proctoeces maculatus (Looss, 1901)[b]	Fellodistomidae	[79]
	Pseudohypertrema karachiense Bilqees, 1976		[80]
	Erilepturus hamati (Yamaguti, 1934)[c]	Hemiuridae	[81]
	Lecithochirium sp.		[77]
	Opecoelus piriformis Yamaguti, 1952	Opecoelidae	[82]
	Psilostomum sp.	Psilostomidae	[77]
	Sanguinicolidae gen. sp.	Sanguinicolidae	[78]
	Prototransversotrema steeri Angel, 1969	Transversotrematidae	[83]
	Transversotrema patialense (Soparkar, 1924)		[77, 84]
Psammoperca waigiensis (Cuvier)	*Ningalooia psammopercae* Bray & Cribb, 2007	Acanthocolpidae	[85]

[a] Reported as *Psilostomum chilkai* Chatterji, 1956; [b] *Complexobursa magna* Bilqees, 1979; [c] *Lecithochirium neopacificum* Velasquez, 1962

Table 2 Distribution and infection parameters of cryptogonimid species recovered in this study.

Host species	**Locality**	**Geographic coordinates**	**Locality – sub-basins** (Danley **et al.**, 2012)	**Date of collection**	**Number of fish specimens examined**	**Number of fish specimens infected**
L. angustifrons	Mpulungu	8°46'S, 31°07'E	Southern sub-basin	12.4.2018	7	2/0/1/2/1/1
L. mariae	Uvira	3°22′S, 29°09′E	Northern sub-basin	12.8.2016	2	0/1/0/0/0/0
	Mpulungu	8°46'S, 31°07'E	Southern sub-basin	16.4.2018	11	0/0/0/0/0/0
L. microlepis	Mutondwe Island	8°42'S, 31°07'E	Southern sub-basin	16.4.2018	8	3/0/0/2/0/0
	Katukula	8°43'S, 30°57'E	Southern sub-basin	14.4.2018	5	3/0/0/3/0/0
	Mpulungu	8°46'S, 31°07'E	Southern sub-basin	13.4.2018	14	8/0/0/3/1/1
	Uvira	3°22′S, 29°09′E	Northern sub-basin	12.8.2016	7	0/0/0/0/0/0
L. stappersii	Mpulungu	8°46'S, 31°07'E	Southern sub-basin	6.4.2018	3	0/0/0/0/0/0
	Uvira	3°22′S, 29°09′E	Northern sub-basin	12.8.2016	28	0/0/0/0/0/0

Infection parameters are provided in the following order: *Neocladocystis bemba* n. sp./*Neocladocystis biliaris* n. sp./*Neocladocystis* sp./*Tanganyikatrema fusiforma* n. sp./*Tanganyikatrema* sp. 'elongata'/*Grandifundilamena novemtestes* n. sp.

Table 3 Comparative morphometric data for the newly described species of *Neocladocystis*, *Tanganyikatrema* n. gen. and *Grandifundilamena novemtestes* n. gen.

	***Neocladocystis bemba* n. sp.**	***Neocladocystis bemba* n. sp. Imm**	***Neocladocystis biliaris* n. sp.**	***Tanganyikatrema fusiforma* n. sp.**	***Tanganyikatrema fusiforma* n. sp. Imm**	***Tanganyikatrema* sp. 'elongata'**	***Grandifundilamena novemtestes* n. sp.**
BL	1,329 (1067–1823)[a]	597 (255–1,028)[j]	2286 (1,940–3,074)[j]	990 (632–1,574)[j]	456 (335–525)[m]	558 (437–679)[n]	3,948 (3,458–4,585)[m]
BW	609 (475–745)[a]	273 (114–401)[j]	1000 (944–1201)[j]	249 (146–387)[j]	133 (103–171)[m]	95,5 (95–96)[n]	362 (303–414)[m]
OSL	141 (114–176)[b]	99 (81–160)[j]	225 (198–272)[g]	157 (119–206)[j]	108 (100–115)[m]	108,5 (107–110)[n]	415 (355–482)[m]
OSW	154 (128–189)[b]	108 (68–168)[j]	231 (206–269)[g]	115 (70–152)[i]	72,6 (65–77)[m]	80 (69–92)[m]	510 (425–612)[m]
VSL	92 (71–114)[c]	57 (47–84)[j]	109 (93–121)[m]	105 (62–134)[i]	64 (51–84)[m]	56 (54–58)[n]	187 (165–206)[m]
VSW	98 (75–124)[d]	57 (51–81)[j]	121 (106–136)[m]	107 (54–127)[i]	68 (54–91)[m]	53,5 (53–54)[n]	189 (168–216)[m]
FBL	422 (349–514)[c]	207 (83–369)[g]	689 (679–698)[n]	306 (220–494)[i]	189 (128–237)[m]	304,5 (262–419)[n]	927 (787–1,063)[m]
HBL	874 (719–1,210)[c]	333 (125–575)[g]	1274 (1,140–1,408)[n]	510 (320–765)[d]	205 (156–253)[m]	205,5 (202–209)[n]	2,883 (2,506–3,316)[m]
PPH	18 (10–38)[j]	not detectable	18 (15–20)[n]	53 (20–122)[l]	not detectable	100 (80–120)[n]	170 (80–264)[m]
PHL	70 (61–96)[e]	54 (38–73)[g]	98 (93–107)[l]	87 (64–122)[c]	35[o]	45 (39–50)[n]	247 (223–278)[m]
PHW	60 (47–94)[e]	46 (34–56)[l]	86 (79–89)[l]	52 (35–76)[c]	39[o]	27 (25–29)[n]	189 (160–224)[m]
OL	18 (5–38)[j]	not detectable	18 (15–20)[l]	74 (43–100)[m]	not detectable	60[o]	absent
IB-F	223 (159–271)[a]	103 (70–132)[l]	341 (313–364)[l]	270 (146–412)[c]	185 (148–222)[n]	270[o]	734 (632–820)[m]
IB-VS	223 (201–249)[g]	68 (50–95)[g]	330 (313–346)[n]	45 (0–82)[g]	6,5 (0–13)[n]	0[o]	189 (121–265)[m]
POSTC	158 (89–250)[a]	55 (26–72)[g]	248 (116–303)[g]	36 (18–59)[c]	36 (12–60)[n]	not detectable	77 (53–101)[m]
ATL	237 (181–326)[f]	139 (100–206)[g]	379 (287–430)[j]	129 (82–183)[j]	52 (38–66)[n]	53[o]	121 (81–160)[m]
ATW	183 (135–255)[f]	98 (78–113)[g]	304 (278–359)[j]	143 (75–214)[d]	61 (56–66)[n]	47[o]	141 (99–176)[m]
PTL	254 (167–394)[h]	138 (95–197)[g]	416 (348–450)[j]	136 (99–171)[c]	51 (37–65)[n]	5[o]	146 (120–160)[m]
PTW	226 (135–306)[e]	90 (67–128)[g]	309 (248–416)[j]	127 (88–187)[c]	63,5 (56–71)[n]	58[o]	130 (95–157)[m]
POST	205 (132–401)[h]	110 (67–162)[g]	201 (80–323)[g]	85 (44–126)[c]	19 (16–22)[n]	29[o]	128 (55–209)[m]
OVL	176 (140–296)[c]	92 (70–113)[g]	242 (213–284)[m]	83 (47–120)[i]	46 (28–66)[m]	not detectable	181 (166–196)[m]
OVW	185 (144–247)[i]	81 (65–109)[g]	241 (186–319)[l]	73 (53–102)[i]	43 (41–47)[m]	not detectable	140 (102–177)[m]
ABE-OV	608 (510–720)[c]	261 (243–296)[l]	1344 (1,073–1,868)[m]	695 (487–1,124)[c]	209[o]	not detectable	2,676 (1,978–3,355)[m]
VS-OV	145 (73–200)[j]	28 (8–78)[g]	295[o]	257 (120–496)[i]	35,5 (30–41)[n]	not detectable	1,560 (1,026–2,077)[m]
OV-AT	123 (65–220)[j]	58 (30–80)[g]	141 (113–173)[l]	0[i]	0[n]	not detectable	220 (100–355)[m]
EL	37 (34–40)[e]	not detectable	35,3 (33–38)[d]	31 (27–39)[c]	not detectable	29[o]	27 (26–28)[m]

EW	16 (12–19)[e]	not detectable	15,9 (13–18)[d]	16 (13–20)[c]	not detectable	15[o]	11 (10–11)[m]
POSTU	353 (176–478)[f]	203 (120–300)[g]	587 (457–683)[j]	281 (171–397)[c]	115,3 (79–140)[m]	not detectable	732 (636–828)[m]
PREVI	318 (228–455)[f]	not detectable	447 (139–578)[j]	396 (320–583)[d]	280[o]	not detectable	2043 (1,879–2,208)[m]
VIT	944 (714–1,206)[k]	not detectable	1518 (1,356–1,648)[l]	359 (226–483)[d]	120[o]	not detectable	2160 (2,100–2,200)[m]
POSTVIT	96 (59–150)[k]	not detectable	134 (80–213)[d]	355 (198–603)[c]	125[o]	not detectable	229 (166–310)[m]
SRL	139 (79–220)[j]	not detectable	264 (205–323)[j]	86 (52–156)[l]	40,5 (40–41)[n]	not detectable	172 (129–214)[m]
SRW	136 (82–184)[d]	not detectable	173 (119–251)[j]	58,8 (42–98)[l]	25,5 (18–33)[n]	not detectable	190 (156–224)[m]
VSL/OSL	1,59 (1,2–2,1)[c]	1,7 (1,5–1,9)[j]	1,7 (1,7–1,8)[n]	1,5 (1,2–1,9)[i]	1,8 (1,4–2,2)[m]	1,9 (1,7–2,0)[n]	2,2 (2,15–2,3)[m]
VSW/OSW	1,65 (1,5–1,8)[d]	1,9 (1,3–2,1)[j]	1,7 (1,5–1,9)[n]	1,1 (0,8–1,4)[i]	1,1 (0,8–1,3)[m]	1,6 (1,5–1,7)[n]	2,7 (2,5–2,9)[m]
OS/BL (%)	10,75 (8,06–12,67)[a]	18,5 (12,7–32,5)[j]	9,7 (8,8–11,0)[g]	17,2 (10,2–22,2)[i]	24,8 (19,7–32,8)[m]	19,3 (15,8–22,9)[n]	10,4 (10,2–10,5)[m]
VS/BL (%)	6,6 (5,4–8,0)[c]	10,7 (6,9–18,4)[j]	5,3 (4,6–6,2)[m]	12,6 (6,0–19,1)[i]	14,2 (11,4–16,0)[m]	10,6 (7,9–13,3)[n]	12,8 (12,3–13,3)[m]
FO/BL (%)	30,65 (26,0–35,4)[c]	34,6 (28,7–38,5)[g]	33,1 (31,2–35,0)[n]	31,3 (23,0- 40,0)[i]	41,0 (38,2–46,7)[m]	60,8 (59,9–61,7)[n]	23,2 (22,8–24,2)[m]
HB/BL (%)	62,73 (57,55–67,23)[c]	54,4 (49,0–64,4)[g]	60,8 (58,8–62,9)[n]	57,7 (50,6–65,5)[d]	45,1 (40,8–48,2)[m]	38,8 (29,7–47,8)[n]	72,1 (71,7–72,3)[m]
PHL/BL (%)	5,29 (3,95–6,64)[a]	8,5 (5,2–11,8)[g]	4,6 (4,2–5,0)[l]	9,5 (5,7–16,3)[c]	10,4[o]	5,7[o]	6,2 (6,1–6,4)[m]
IB/BL (%)	16,22 (12,08–21,02)[i]	23,6 (22,1–25,1)[m]	15,9 (14,2–18,2)[l]	27,8 (11,3–42,8)[j]	43,9 (43,7–44,2)[n]	not detectable	18,4 (17,9–18,3)[m]
POSTC/BL (%)	11,85 (8,34–15,57)[k]	10,8 (5,0–14,2)[g]	10,5 (6–12,7)[g]	4,2 (2,2–7,9)[c]	7,5 (3,6–11,4)[n]	not detectable	1,9 (1,5–2,2)[m]
AT/BL ()	18,46 (15,23–23,17)[i]	20,9 (18,2–22,9)[g]	18,8 (14,9–21,3)[g]	11,5 (9,6–14,1)[j]	12,2 (11,3–13,0)[n]	7,8[o]	3,0 (2,3–3,5)[m]
PT/BL (%)	19,46 (15,0–28,0)[k]	20,7 (19,2–24,4)[g]	20,2 (17,5–22,3)[g]	12,5 (9,9–14,6)[c]	15,3 (14,0–16,7)[n]	8,5[o]	3,7 (3,0–4,6)[m]
POSTT/BL (%)	16,20 (12,6–22,0)[a]	16,7 (12,8–20,4)[g]	9,2 (4,0–14,4)[g]	13,5 (10,9–17,4)[c]	11,9 (11,0–12,8)[n]	7,4[o]	3,2 (1,6–4,6)[m]
ABE-OV/BL (%)	47,02 (39,0–54,9)[c]	45,9 (40,3–49,5)[l]	54,9 (48,5–60,8)[m]	66,9 (57,1–71,4)[c]	62,4[o]	not detectable	68,2 (57,2–73,2)[m]
VS-OV/BL (%)	10,85 (6,29–14,5)[j]	3,5 (1,6–7,6)[g]	15,2[o]	25,1 (19,0–31,5)[i]	8,5 (8,1–9,0)[n]	not detectable	40,0 (29,7–45,3)[m]
OV-AT/BL (%)	9,6; (5,3–19,2)[j]	11,6 (9,1–16,2)[m]	6,5 (5,0–7,9)[l]	0[c]	0[m]	not detectable	5,2 (2,9–7,7)[m]
OV/BL (%)	14,0 (10,7–25,9)[d]	14,1 (11,0–16,2)[g]	10,2 (9,2–11,9)[m]	12,5 (9,9–14,6)[c]	15,3 (14,0–16,7)[n]	8,5[o]	4,6 (4,3–4,8)[m]
POSTU/BL (%)	28,2 (20,6–36,7)[a]	27,6 (24,3–31,0)[n]	26,3 (22,2–31,0)[g]	28,2 (23,5–34,6)[d]	25,1 (23,6–26,7)[m]	not detectable	18,5 (18,1–18,4)[m]
PREVIT/BL (%)	21,9 (16,8–28,8)[k]	not detectable	22,3 (16,0–26,7)[g]	38,0 (31,2–46,4)[d]	53,3[o]	not detectable	51,1 (47,6–48,2)[m]
VIT/BL (%)	70,3 (65,1–77,1)[k]	not detectable	70,4 (65,8–74,9)[l]	34,9 (29,9–55,3)[j]	22,9[o]	not detectable	54,0 (48,4–53,2)[m]
POSTVIT/BL (%)	7,3 (5,5–10,0)[i]	not detectable	6,5 (3,2–11,0)[j]	35,4 (23,6–39,8)[d]	23,8[o]	not detectable	5,9 (4,8–6,8)[m]
SRL/BL (%)	10,85 (7,4–15,5)[g]	not detectable	12,8 (10,2–14,7)[g]	11,0 (9,9–12,1)[n]	not detectable	not detectable	4,3 (2,8–5,4)[m]
BW/BL (%)	2,21 (1,65–2,70)[a]	2,2 (1,9–2,6)[j]	2,3 (2,1–2,6)[g]	4,0 (3,2–5,7)[i]	3,4 (3,1–4,0)[m]	5,8 (4,6–7,1)[n]	11,1 (10,4–11,6)[m]

Notes: [a]n=11; [b]n=15; [c]n=8; [d]n=7; [e]n=14; [f]n=12; [g]n=5; [h]n=13; [i]n=9; [j]n=6; [k]n=10; [l]n=4; [m]n=3; [n]n=2; [o]n=1

Abbreviations: Imm, immature specimen; BL, body length; BW, body width; OSL, oral sucker length; OSW, oral sucker width; VSL, ventral sucker length; VSW, ventral sucker width; FBL, forebody length; HBL, hindbody length; PPH, pre-pharynx length; PHL, pharynx length; PHW, pharynx width; OL, oesophagus length; IB-F, distance from anterior extremity to intestinal bifurcation; IB-VS, distance from intestinal bifurcation to ventral sucker; POSTC, length of post-caecal field; ATL, anterior testis length; ATW, anterior testis width; PTL, posterior testis length; PTW, posterior testis width; POST, length of post-testicular field; OVL, ovary length; OVW, ovary width; ABE-OV, distance from anterior body extremity to ovary; VS-OV, distance from ventral sucker to ovary; OV-AT, distance from ovary to anterior testis; EL, egg length; EW, egg width; POSTU, length of post-uterine field; PREVI, length of pre-vitelline field; VIT, length of vitelline field; POSTVIT, length of post-vitelline field; SRL, length of seminal receptacle; SRW, width of seminal receptacle; VSL/OSL, sucker length ratio; VSW/OSW, sucker width ratio; OS/BL (%), oral sucker as a proportion of body length; VS/BL (%), ventral sucker as a proportion of body length; FO/BL (%), forebody as a proportion of body length; HB/BL (%), hindbody as a proportion to body length; PHL/BL (%), pharynx as a proportion of body length; IB/BL (%), pre-intestinal field as proportion of body length; POSTC/BL (%), post-caecal field as proportion of body length; AT/BL (%), anterior testis as proportion of body length; PT/BL (%), posterior testis as proportion of body length; POSTT/BL (%), post-testicular field as proportion of body length; ABE-OV/BL (%),anterior body extremity to ovary field as proportion of body length; VS-OV/BL (%), pre-ovarian field as proportion of body length; OV-AT/BL (%), post-ovarian to anterior testis field as proportion of body length; OV/BL (%), ovary as proportion of body length; POSTU/BL (%), post-uterine field as proportion of body length; PREVIT/BL (%), pre-vitelline field as proportion of body length; VIT/BL (%), vitelline field as proportion of body length; POSTVIT/BL (%), post-vitelline field as proportion of body length; SRL/BL (%), seminal receptaculum as proportion of body length; BW/BL (%), body width as proportion of body length.

Table 4 Comparison of morphological characters among the selected cryptogonimid genera based on [23] and this study.

	Neocladocystis Manter & Pritchard, 1969 (amended diagnosis)	*Tanganyikatrema* n. gen.	*Grandifundilamena novemtestes* n. gen. n. sp.	*Acanthostomum* Looss, 1899	*Brientrema* Dollfus, 1950	*Claribulla* Overstreet, 1969	*Iheringtrema* Travassos, 1947	*Isocoelium* Ozaki, 1927	*Siphodera* Linton, 1910
Body	Irregular oval	Elongate, fusiform	Long, relatively narrow	Elongate-oval to distinctly elongate	Fusiform	Elongate with distinct constriction immediately posterior to oral sucker	Elongate-oval	Very elongate	Oval, enlongate-oval
BL/BW	*c*.1.5–2.5	*c*.4–7	*c*.11	*c*.2–3.5	*c*.2–2.5	*c*.6	*c*.3	*c*.11–16	*c*.2–3.5
OS	Almost round, opens subterminally	Infindibular or cup-shaped, massive, relatively large, longer than wide, squared-off posteriorly, opens terminally	Massive, broadly infundibular, opens terminally	Funnel-shaped, opens terminally	Nearly round, opens terminally	Funnel- or cup-shaped, opens almost terminally	Almost round, opens subterminally	Longer than wide, opens subterminally	Nearly round, opens almost terminally
Circumoral spines	Absent	Absent	Absent	Enlarged (rarely without)	Enlarged	Absent	Absent	Absent	Absent

VS	Pre-equatorial, rounded, unspecialised, not obviously embedded in ventrogenital sac	Pre-equatorial, rounded, unspecialised, embedded in ventrogenital sac	Pre-equatorial, rounded, much smaller than oral sucker.	Pre-equatorial, rounded, unspecialized, not obviously embedded in ventrogenital sac	Pre-equatorial, rounded, unspecialised, embedded in ventrogenital sac	Pre-equatorial, rounded unspecialised, deeply embedded in ventrogenital sac	Pre-equatorial, rounded, unspecialised, not obviously embedded in ventrogenital sac	Pre-equatorial, rounded, unspecialised, not obviously embedded in ventrogenital sac	Pre-equatorial, rounded unspecialised, embedded in ventrogenital sac, usually smaller than VS
OSW/VSW	*c*.1.5–2.1	*c*.0.8–1.7	*c*.2.7	*c*.1–2.5	*c*.1.5–2.5	*c*.2.5	*c*.2.0–2.5	*c*.2.0	*c*.1–2.5
Tegument	Smooth or spined, spines reach to posterior extremity, largest in mid-region	Spined, spines can reach at about level of ovary	Spines not observed	Smooth or spined, spines reach to posterior extremity	Spined, spines reach to posterior extremity	Spines not observed	Spines not observed	Spines not observed	Spined
Forebody	*c*.26–40% of body-length	*c*.30–62% of the body-length	*c*.24 % of body length	*c*.5–40% of body length	*c*.20–25% of body-length	*c*.40% of body length	*c*.20% of body-length	*c*.20–25% of body-length	*c*.20–33% of body length
Pre-pharynx	Short or absent	Variable in length	Broadly oval, larger than ventral sucker	Short	Very short	Very short	Very short	Short	Short
Oesophagus	Short or indistinct	Shorter than Pre-pharynx	Absent	Short	Very short	Short	Very short	Long	Short
Intestinal bifurcation	In about mid-forebody, at level of ventral sucker	In posterior forebody just anterior to VS	In posterior forebody	Immediately anterior to VS	Immediately anterior to or dorsal to VS	In anterior forebody	In mid-forebody	In posterior forebody	In mid-forebody
Caeca	Blind, end at level of testes or at post-testicular region	Blind, reach into post-testicular region	Blind, reach into post-testicular region	Opens *via* separate ani close to posterior extremity	Blind, end close to posterior extremity	Blind, end posteriorly to testes	Blind, end close to posterior extremity	Blind, end close to posterior extremity	Blind, end close to posterior extremity
Testes	2, symmetrical or oblique, lobed, contiguous or slightly separated	2, entire, tandem, contiguous, in posterior third of hindbody; anterior testis oval, posterior testis sub-triangular	9, transversely oval entire, in tandem row, reaching from just posterior to testes close to posterior extremity, contiguous, in posterior third of hindbody	2, entire, tandem, contiguous, in posterior third of hindbody	2, symmetrical to slightly oblique close to posterior extremity	2, strongly oblique to tandem, in mid-hindbody	9, irregular, in posterior hindbody	2, mostly intercaecal, distinctly elongate, strongly oblique to slightly tandem	Usually 9, in two lateral groups, or one large group in hindbody
Seminal vesicle	Tubular, naked	Tubulosaccular, naked, long, convoluted	Naked, long, convoluted	Tubular	Tubulosaccular	Tubulosaccular, in forebody	Tubular	Tubular	Tubusaccular

Gonotyl	Absent	Absent	Absent	Absent	A spined muscular structure immediately anterior to VS	Absent	Absent	Absent	Absent
Genital pore	Median, immediately anterior to VS	Median, immediately anterior to VS	Median, immediately anterior to VS	Median, immediately anterior to VS	Median, immediately anterior to VS	Median, at mid-level of intestinal bifurcation and VS	Sinistral to ventral sucker	Median, immediately posterior to ventral sucker	Median, posterior and slightly sininstral to VS
Ovary	Dextral, pretesticular, regularly lobed	Regularly oval, pre-testicular, overlapping anterior testis	Irregularly sub-triangular, pre-testicular	Entire, pre-testicular; in posterior hindbody	Entire, anterior to testes in posterior hindbody	Entire, immediately anterosinistral to anterior testis	Deeply lobed, median, immediately anterior to testes	Deeply lobed, well separated form anterior testis, occupies full width of body in mid-hindbody	Deeply lobed, immediately anterior to testes
Uterus	Uterus fills much of body from bifurcal region to anterior testicular region, most intercaecal	Fills much of hindbody from anterior testis anteriorly, passes dorsally to VS, most intercaecal	Narrow, reaches between VS and anterior testis, passes dorsally to ventral sucker, most intercaecal	In hindbody between ovary and VS	In hindbody between ovary and VS	In hindbody, extends close to posterior extremity	In hindbody, between gonads and VS	In hindbody, between VS and anterior testis	In hindbody, extends close to posterior extremity
Vitelline follicles	In 2 lateral groups from just post-bifurcal to close to posterior extremity, lateral, just overlapping caeca	In 2 lateral groups, reach from VS to level distinctly anterior to ovary, lateral just overlapping caeca dorsally and ventrally	In 2 lateral groups, reach from about halfway between VS and ovary to level of posterior testis	In 2 lateral groups, in hindbody, from level of anterior testis to mid-body slightly posterior to VS	In 2 lateral groups, in hindbody, from posterior extremity to slightly anterior to ovary	In 2 restricted lateral groups between VS and gonads	In 2 lateral groups, confluent posteriorly, from posterior extremity to pharynx	In two bands, occupy full body width from midway between ventral sucker to ovary and ovary to anterior testis	In 2 lateral groups, may extend from level of testes into forebody
Excretory vesicle	Y-shaped, bifurcates lateral to ovary, narrow posteriorly, widens and reaches uterus			Y-shaped, bifurcated	Bifurcated	Bifurcated	Unknown	Bifurcated	Y-shaped, bifurcated
Arms	May extend to the level of pharynx, excretory pore	Not clear	Not clear	Reach level of pharynx	Reach pharynx	Reach pharynx	Unknown	Reach pharynx	Reach level of pharynx

	terminal at posterior end of body								
Parasitic in	Freshwater fish: Latidae: *L. angustifrons, L. mariae, L. microlepis*	Freshwater fish: Latidae: *L. angustifrons, L. microlepis*	Freshwater fish: Latidae: *L. angustifrons, L. microlepis*	Freshwater and marine fishes, reptiles	Freshwater fishes: Malapteruridae, Citharinidae and pelicans (probable pseudoparasitism)	Marine fishes: Albulidae, Sphyraenidae	Freshwater fishes: Pimelodidae	Marine fishes: Uranoscopidae	Marine fishes
Site in host	Immature specimens in intestine and eggs-bearing specimens in pyloric caeca, gallbladder	Intestine	Intestine	Intestine	Intestine	Intestine	Intestine	Intestine	Intestine
Distribution	Africa: Lake Tanganyika	Africa: Lake Tanganyika	Africa: Lake Tanganyika	Cosmopolitan	Africa	North America: Florida, US	South America: Brazil	Japan	Atlantic, Indian and Pacific Oceans
	N. tanganyikae (Prudhoe, 1951) Manter & Pritchard, 1969	*Tanganyikatrema fusiforma* n. sp.	*Grandifundilamena novemtestes* n. gen. n. sp.	*A. spiniceps* Looss, 1899	*B. pelecani* Dollfus, 1950	*C. longula* Overstreet, 1969	*I. iheringi* Travassos, 1947	*I. mediolecithale* Ozaki, 1927	*S. vinaledwardsii* (Linton, 1901)

Abbreviations: BL, body length; BW, body width; OS, oral sucker; OSW, oral sucker width; VSW, ventral sucker width; VS, ventral sucker.

Table 5 Total pairwise differences among partial 28S rDNA sequences of cryptogonimid species reported in this study.

Species		1	2	3	4	5
1	*Neocladocystis bemba* n. sp.	–	0.1	0.5	1.9	1.9
2	*Neocladocystis biliaris* n. sp.	1	–	0.6	2.0	2.0
3	*Neocladocystis* sp.	6	7	–	1.6	1.8
4	*Tanganyikatrema fusiforma* n. sp.	24	25	20	–	0.3
5	*Tanganyikatrema* sp. 'elongata'	24	25	22	4	–

Uncorrected pairwise differences (below the diagonal) and mean divergence (uncorrected p-distance in % above the diagonal) among the newly crytogonimid species recovered from *Lates* spp. in Lake Tanganyika.

Figure captions

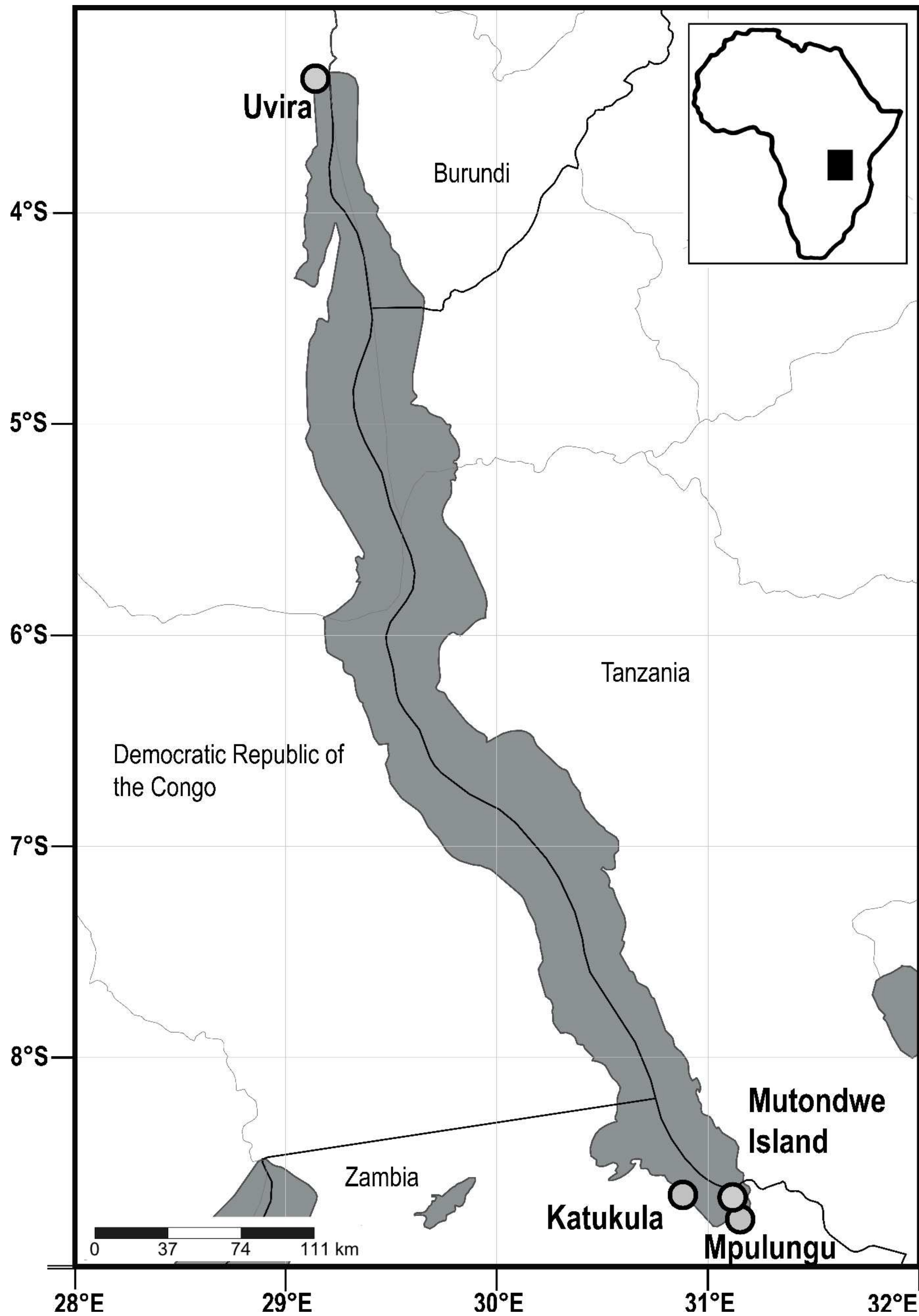


Fig. 1 Map of the Lake Tanganyika with the sampling locations of *Lates* spp. The map was created using SimpleMappr software v7.0.0. (available at http://www.simplemappr.net. Accessed March 5, 2019)

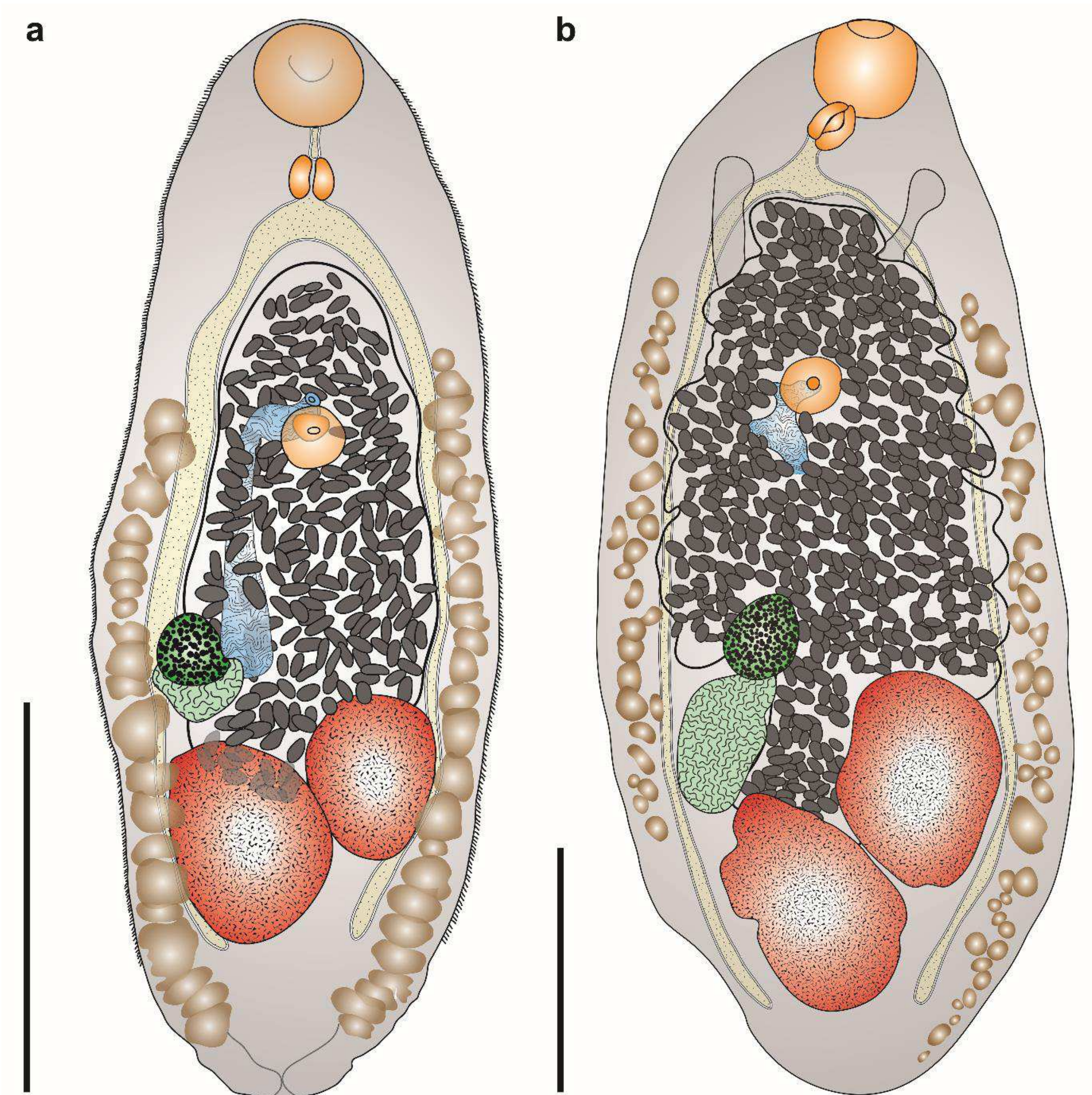


Fig. 2 Line drawings of the paragenophores of *Neocladocystis* spp. described in present study. a *Neocladocystis bemba* n. sp. recovered in the pyloric caeca of *Lates microlepis* off Mutondwe Island, Lake Tanganyika. b *Neocladocystis biliaris* n. sp. from the liver of *L. mariae* off Uvira fish market, Lake Tanganyika. Scale-bars: 500 µm

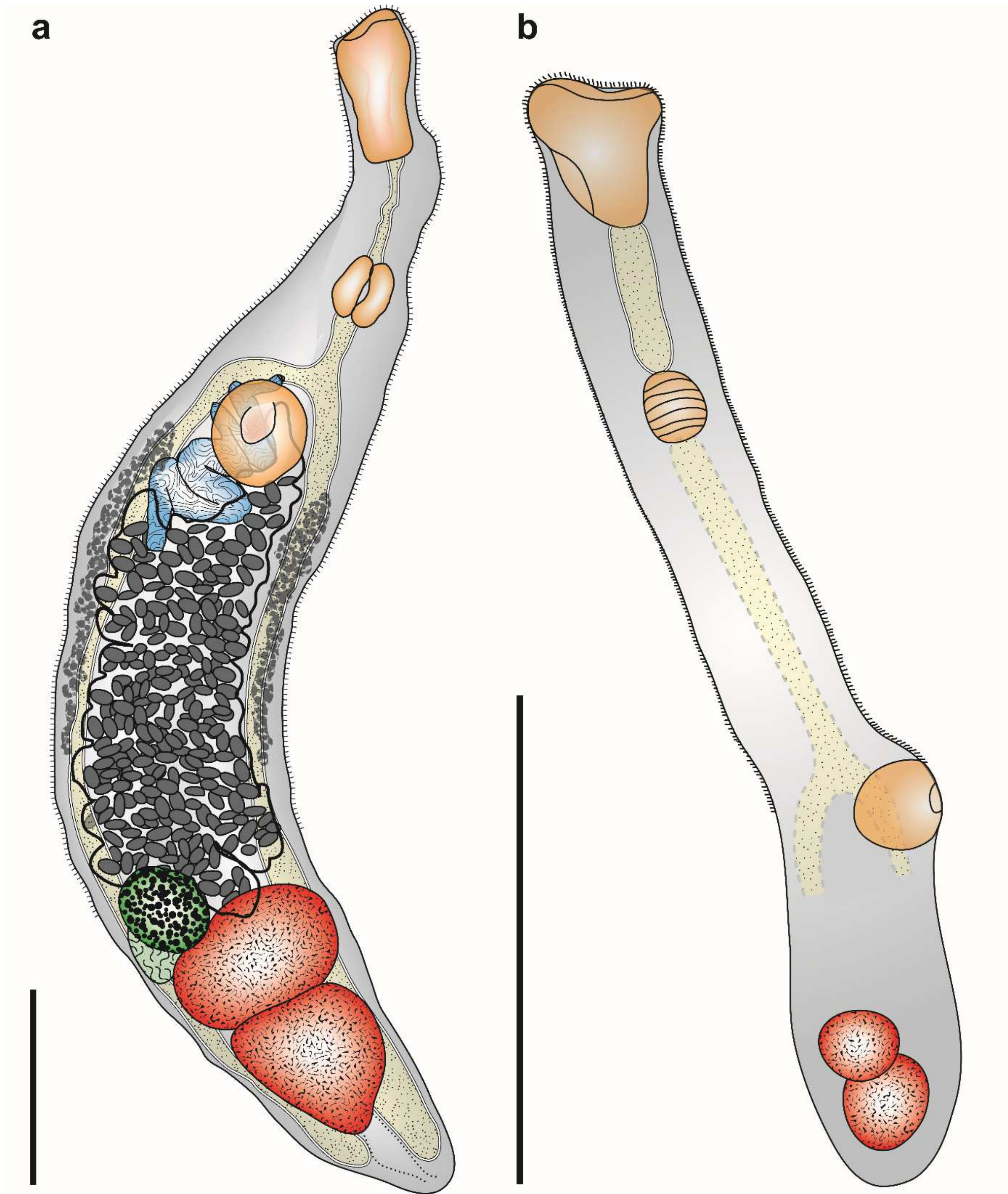


Fig. 3 Line drawings of the paragenophores of *Tanganyikatrema* n. gen. described in present study. a *Tanganyikatrema* fusiforma n. sp. recovered from the intestine of Lates microlepis off Katukula Bay, Lake Tanganyika. b *Tanganyikatrema* sp. 'elongata' recovered from the intestine of *L. angustifrons* off Mpulungu. Scale-bars: 500 µm

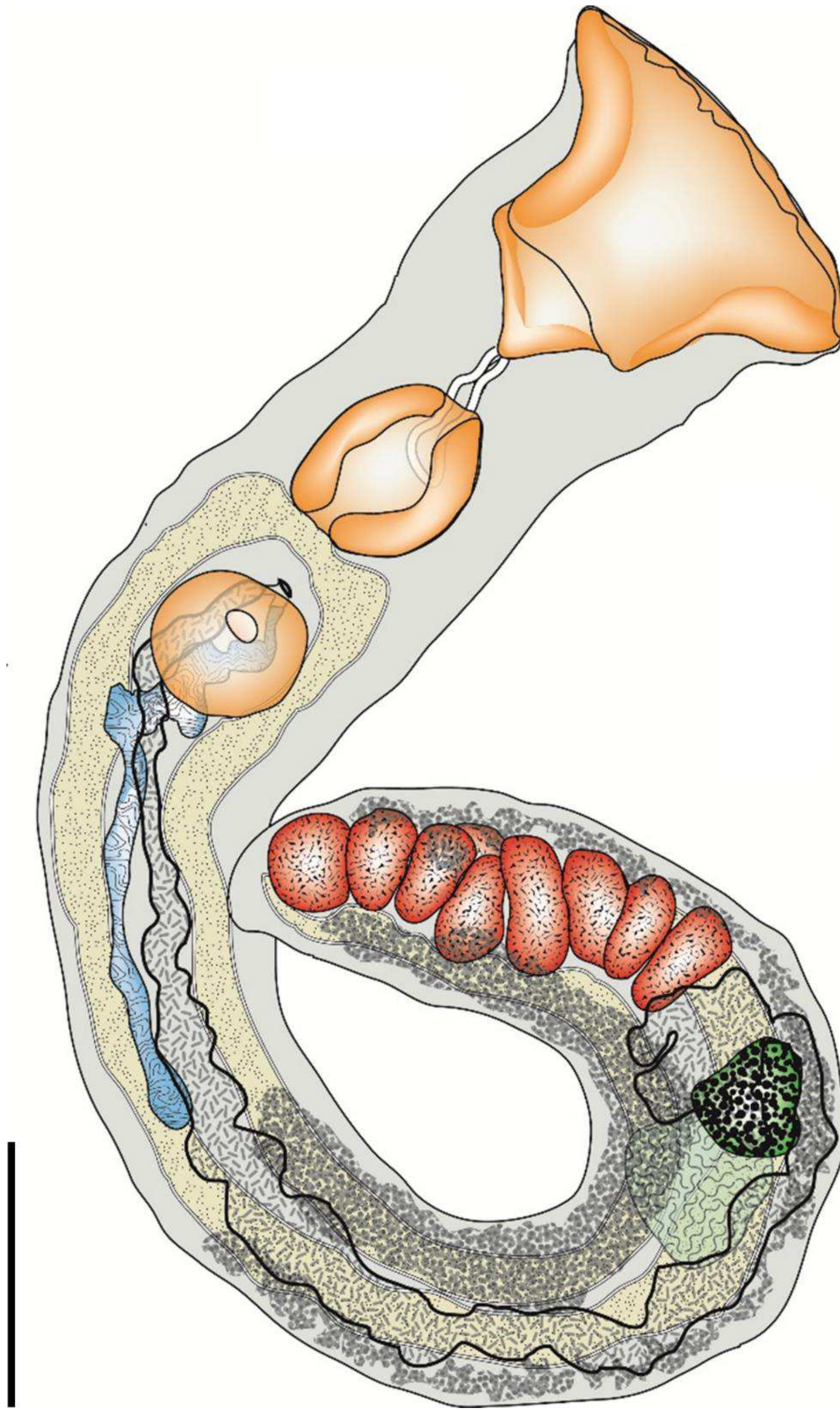
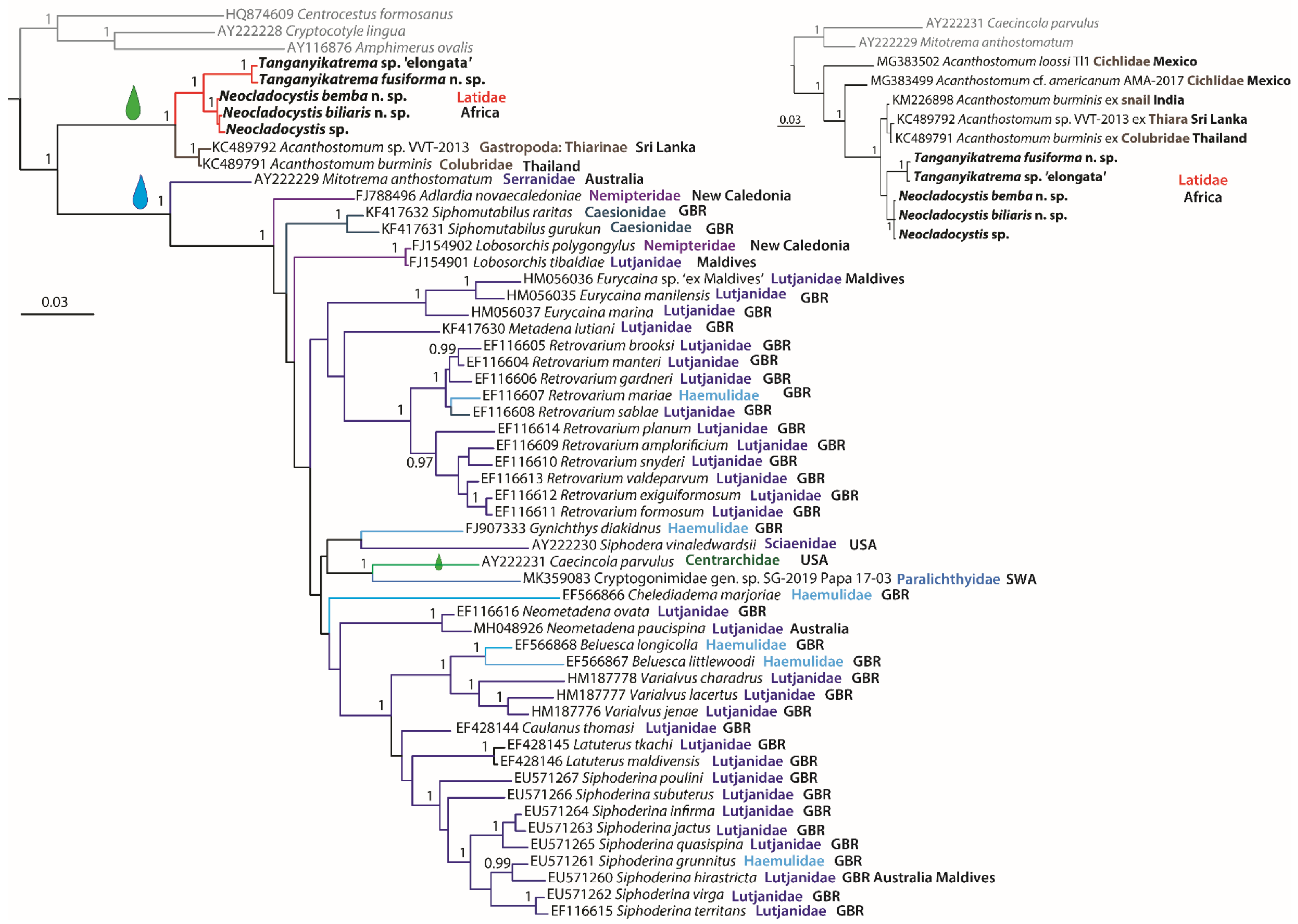


Fig. 4 Line drawings of the paragenophores of *Grandifundilamena novemtestes* n. sp. Specimen recovered in the intestine of *Lates angustifrons* off Mpulungu fish market, Lake Tanganyika. Scale-bars: 500 µm



HQ874609 Centrocestus formosanus
AY222228 Cryptocotyle lingua
AY116876 Amphimerus ovalis
Tanganyikatrema sp. 'elongata'
Tanganyikatrema fusiforma n. sp.
Neocladocystis bemba n. sp.
Neocladocystis biliaris n. sp.
Neocladocystis sp.
Latidae
Africa
KC489792 Acanthostomum sp. VVT-2013 Gastropoda: Thiarinae Sri Lanka
KC489791 Acanthostomum burminis Colubridae Thailand
AY222229 Mitotrema anthostomatum Serranidae Australia
FJ788496 Adlardia novaecaledoniae Nemipteridae New Caledonia
KF417632 Siphomutabilus raritas Caesionidae GBR
KF417631 Siphomutabilus gurukun Caesionidae GBR
FJ154902 Lobosorchis polygongylus Nemipteridae New Caledonia
FJ154901 Lobosorchis tibaldiae Lutjanidae Maldives
HM056036 Eurycaina sp. 'ex Maldives' Lutjanidae Maldives
HM056035 Eurycaina manilensis Lutjanidae GBR
HM056037 Eurycaina marina Lutjanidae GBR
KF417630 Metadena lutiani Lutjanidae GBR
EF116605 Retrovarium brooksi Lutjanidae GBR
EF116604 Retrovarium manteri Lutjanidae GBR
EF116606 Retrovarium gardneri Lutjanidae GBR
EF116607 Retrovarium mariae Haemulidae GBR
EF116608 Retrovarium sablae Lutjanidae GBR
EF116614 Retrovarium planum Lutjanidae GBR
EF116609 Retrovarium amplorificium Lutjanidae GBR
EF116610 Retrovarium snyderi Lutjanidae GBR
EF116613 Retrovarium valdeparvum Lutjanidae GBR
EF116612 Retrovarium exiguiformosum Lutjanidae GBR
EF116611 Retrovarium formosum Lutjanidae GBR
FJ907333 Gynichthys diakidnus Haemulidae GBR
AY222230 Siphodera vinaledwardsii Sciaenidae USA
AY222231 Caecincola parvulus Centrarchidae USA
MK359083 Cryptogonimidae gen. sp. SG-2019 Papa 17-03 Paralichthyidae SWA
EF566866 Chelediadema marjoriae Haemulidae GBR
EF116616 Neometadena ovata Lutjanidae GBR
MH048926 Neometadena paucispina Lutjanidae Australia
EF566868 Beluesca longicolla Haemulidae GBR
EF566867 Beluesca littlewoodi Haemulidae GBR
HM187778 Varialvus charadrus Lutjanidae GBR
HM187777 Varialvus lacertus Lutjanidae GBR
HM187776 Varialvus jenae Lutjanidae GBR
EF428144 Caulanus thomasi Lutjanidae GBR
EF428145 Latuterus tkachi Lutjanidae GBR
EF428146 Latuterus maldivensis Lutjanidae GBR
EU571267 Siphoderina poulini Lutjanidae GBR
EU571266 Siphoderina subuterus Lutjanidae GBR
EU571264 Siphoderina infirma Lutjanidae GBR
EU571263 Siphoderina jactus Lutjanidae GBR
EU571265 Siphoderina quasispina Lutjanidae GBR
EU571261 Siphoderina grunnitus Haemulidae GBR
EU571260 Siphoderina hirastricta Lutjanidae GBR Australia Maldives
EU571262 Siphoderina virga Lutjanidae GBR
EF116615 Siphoderina territans Lutjanidae GBR
0.03
AY222231 Caecincola parvulus
AY222229 Mitotrema anthostomatum
MG383502 Acanthostomum loossi TI1 Cichlidae Mexico
MG383499 Acanthostomum cf. americanum AMA-2017 Cichlidae Mexico
KM226898 Acanthostomum burminis ex snail India
KC489792 Acanthostomum sp. VVT-2013 ex Thiara Sri Lanka
KC489791 Acanthostomum burminis ex Colubridae Thailand
Tanganyikatrema fusiforma n. sp.
Tanganyikatrema sp. 'elongata'
Neocladocystis bemba n. sp.
Neocladocystis biliaris n. sp.
Neocladocystis sp.
Latidae
Africa
0.03

Fig. 5 Bayesian inference phylogram based on partial 28S rDNA sequences (D1-D3 domains) for (a) the Cryptogonimidae and (b) *Acanthostomum* spp. + the newly sequenced cryptogonimid representatives from Lake Tanganyika. Both phylograms were constructed under the GTR+Γ model of nucleotide substitution. Posterior probability values above 0.95 are displayed only. The newly described herein cryptogonimid species are highlighted in bold. Freshwater and marine origin of the species is indicated by green and blue drop, respectively. Host family specification together with place of origin of the ingroup taxa is indicated. The scale-bar represents number of nucleotide substitutions per site.

Additional file 1

Table S1. Summary data for 28S rDNA sequences retrieved from the GenBank database for species used in the phylogenetic analyses

Digenean species	Host species	Locality, country	GenBank accession number	Reference
Acanthostomum burminis (Bhalerao; 1926)	*Xenochrophis piscator* (Schneider)	Kanchanaburi Province, Thailand	KC489791	[1]
A. burminis	“snail“	Tiruchendur Beach, India	KM226898	[2]
A. cf. *americanum* AMA-2017	*Cichlasoma urophthalmum* (Günther)	Ria Celestun Biosphere Reserve, Yucatan Peninsula, Mexico	MG383499	[3]
Acanthostomum loossi (Vigueras, 1957)	*C. urophthalmum*	Ria Celestun Biosphere Reserve, Yucatan Peninsula, Mexico	MG383502	[3]
Acanthostomum sp. VVT-2013	*Thiara scabra* (Müller)	Peradeniya, Sri Lanka	KC489792	[1]

Adlardia novaecaledoniae Miller, Bray, Goiran, Justine & Cribb, 2009	*Nemipterus furcosus* (Valenciennes)	Baie Maa, New Caledonia	FJ788496	[4]
Amphimerus ovalis Barker, 1911	*Trionyx muticus* (Lesueur)	Mississippi, USA	AY116876	[5]
Beluesca littlewoodi Miller & Cribb, 2007	*Plectorhinchus gibbosus* (Lacepède)	Lizard Island, Great Barrier Reef, Australia	EF566867	[6]
B. longicolla Miller & Cribb, 2007	*P. gibbosus*	Heron Island, Great Barrier Reef, Australia	EF566868	[6]
Caecincola parvulus Marshall & Gilbert, 1905	*Micropterus salmoides* (Lacepède)	USA	AY222231	[5]
Caulanus thomasi Miller & Cribb, 2007	*Lutjanus bohar* (Forsskål)	Lizard Island & Heron Island, Great Barrier Reef, Australia	EF428144	[7]
Centrocestus formosanus (Nishigori, 1924) Price, 1932	*Mesocricetus auratus* Waterhouse	Thailand	HQ874609	[8]
Chelediadema marjoriae Miller & Cribb, 2007	*Diagramma labiosum* Macleay	Lizard Island & Heron Island, Great Barrier Reef, Australia	EF566866	[7]
Cryptocotyle lingua (Creplin, 1825) Fischoeder, 1903	*Littorina littorea* (L.)	Isle of Sylt, North Sea, Germany	AY222228	[5]
Cryptogonimidae gen. sp. SG-2019	*Paralichthys patagonicus* Jordan	San Matias Gulf, Argentina	MK359083	[9]
Euryakaina manilensis (Velasquez, 1961) Miller, Adlard, Bray, Justine & Cribb, 2010	*Lutjanus vitta* (Quoy & Gaimard); *L. quinquelineatus* (Bloch)	Off Luzon Island, Philippines	HM056035	[10]
Eurycaina marinum (Hafeezullah & Siddiqi) 1970 Miller, Adlard, Bray, Justine & Cribb, 2010	*Lutjanus carponotatus* (Richardson); *L. fulviflamma* (Forsskål); *L. monostigma* (Cuvier); *L. russellii* (Bleeker)	Off Tuticorin, India	HM056037	[10]

Euryakaina sp.	*Lutjanus kasmira* (Forsskål)	Off Rasdhoo Atoll, Maldives	HM056036	[10]
Gynichthys diakidnus Miller & Cribb, 2009	*Plectorhinchus gibbosus*	Lizard Island & Heron Island, Great Barrier Reef, Australia	FJ907333	[11]
Latuterus maldivensis Miller & Cribb, 2007	*L. bohar*	Rasdhoo Atoll, Lizard Island, Great Barrier Reef, Australia	EF428146	[7]
L. tkachi Miller & Cribb, 2007	*L. bohar*	Lizard Island, Great Barrier Reef, Australia	EF428145	[7]
Lobosorchis polygongylus Miller, Downie & Cribb, 2009	*Nemipterus furcosus* (Valenciennes)	Lizard Island, Great Barrier Reef, Australia	FJ154902	[12]
L. tibaldiae Miller & Cribb, 2005	*L. fulviflamma*	Heron Island, Great Barrier Reef, Australia	FJ154901	[12]
Metadena lutiani (Yamaguti, 1942) Miller & Cribb, 2008	*L. bohar*	off the Great Barrier Reef, Australia	KF417630	[4]
Mitotrema anthostomatum Manter, 1963	*Cromileptes altivelis* (Valenciennes)	Australia	AY222229	[5]
Tanganyikatrema fusiforma n. sp.	*Lates microlepis* Boulenger,	Crock Island; Katukula; Mpulungu, Lake Tanganyika, Zambia	XXX	Nobis
Tanganyikatrema sp. 'elongata'	*L. angustifrons, L. microlepis*	Mpulungu, Lake Tanganyika, Zambia	XXX	Nobis
Neocladocystis bemba n. sp. Georgieva, Kmentová & Bray	*L. microlepis*, *L. angustifrons*	Crock Island; Katukula; Mpulungu, Lake Tanganyika, Zambia	XXX	Nobis
N. biliaris n. sp. Georgieva, Kmentová & Bray	*L. mariae* Steindachner	Uvira, Lake Tanganyika, DRC	XXX	Nobis
Neocladocystis sp.	*L. angustifrons*	Mpulungu, Lake Tanganyika, Zambia	XXX	Nobis

Neometadena paucispina Miller, Cutmore & Cribb, 2018	*L. fulviflamma, L. russellii*	Off North Stradbroke Island, Moreton Bay, Australia	MH048926	[13]
Neometadena ovata (Yamaguti, 1952) Miller & Cribb, 2008	*L. carponotatus*	Off Lizard Island, Great Barrier Reef, Australia	EF116616	[14]
Retrovarium amplorificium Miller & Cribb, 2007	*Symphorus nematophorus* (Bleeker, 1860)	Lizard Island & Heron Island, Great Barrier Reef, Australia	EF116609	[14]
R. brooksi Miller & Cribb, 2007	*L. bohar, L. fulviflamma, L. gibbus* (Forsskål)	Heron Island, Rasdhoo Atoll, Moorea, Great Barrier Reef, Australia	EF116605	[14]
R. exiguiformosum Miller & Cribb, 2007	*S. nematophorus*	Lizard Island & Heron Island, Great Barrier Reef, Australia	EF116612	[14]
R. formosum Miller & Cribb, 2007	*S. nematophorus*	Lizard Island, Great Barrier Reef, Australia	EF116611	[14]
R. gardneri Miller & Cribb, 2007	*Lutjanus sebae* (Cuvier)	Heron Island, Great Barrier Reef, Australia	EF116606	[14]
R. manteri Miller & Cribb, 2007	*Lutjanus argentimaculatus* (Forsskål)	Lizard Island & Heron Island, Great Barrier Reef, Australia	EF116604	[14]
R. mariae Miller & Cribb, 2007	*D. labiosum*	Heron Island, Great Barrier Reef, Australia	EF116607	[14]
R. planum Miller & Cribb, 2007	*S. nematophorus*	Lizard Island & Heron Island, Great Barrier Reef, Australia	EF116614	[14]
R. sablae Miller & Cribb, 2007	*Aprion virescens* Valenciennes	Heron Island, Rasdhoo Atoll, Moorea, Great Barrier Reef, Australia	EF116608	[14]
R. snyderi Miller & Cribb, 2007	*S. nematophorus*	Lizard Island & Heron Island, Great Barrier Reef, Australia	EF116610	[14]

R. valdeparvum Miller & Cribb, 2007	*S. nematophorus*	Lizard Island & Heron Island, Great Barrier Reef, Australia	EF116613	[14]
Siphodera vinaledwardsii (Linton, 1901) Linton, 1910	*Sciaenops ocellatus* (L.)	Gulf of Mexico, South of Horn Island, Mississippi, USA	AY222230	[5]
Siphoderina grunnitus Miller & Cribb, 2008	*Plectorhinchus gibbosus* (Lacepède)	Lizard Island, Great Barrier Reef, Australia	EU571261	[15]
S. hirastricta (Manter, 1963) Miller & Cribb, 2008	*L. argentimaculatus*	Off Lizard Island, Great Barrier Reef, Queensland, Australia; Ningaloo Reef, Western Australia; Rasdhoo Atoll, Maldives.	EU571260	[15]
S. infirma Miller & Cribb, 2008	*L. russelli*	Lizard Island, Great Barrier Reef, Australia	EU571264	[15]
S. jactus Miller & Cribb, 2008	*L. fulviflamma*	Heron Island, Great Barrier Reef, Australia	EU571263	[15]
S. poulini Miller & Cribb, 2008	*L. argentimaculatus*	North Stradbroke Island, Moreton Bay, Queensland, Australia	EU571267	[15]
S. quasispina Miller & Cribb, 2008	*L. fulviflamma*	Heron Island, Great Barrier Reef, Australia	EU571265	[15]
S. subuterus Miller & Cribb, 2008	*Lutjanus adetii* (Castelnau)	Heron Island, Great Barrier Reef, Australia	EU571266	[15]
S. territans Miller & Cribb, 2008	*Lutjanus carponotatus* (Richardson)	Heron Island, Great Barrier Reef, Australia	EF116615	[15]
S. virga Miller & Cribb, 2008	*L. russelli*	North Stradbroke Island, Moreton Bay, Queensland, Australia	EU571262	[15]
Siphomutabilis gurukun (Machida, 1986) Miller & Cribb, 2013	*Caesio cuning* (Bloch); *Caesio caerulaurea* (Lacepède)	Off Lizard Island, Great Barrier Reef, Queensland, Australia; Ningaloo Reef, Western Australia; Rasdhoo Atoll, Maldives	KF417631	[4]

S. raritas Miller & Cribb, 2013	*C. cuning*	Off Lizard Island, Great Barrier Reef, Queensland, Australia; Ningaloo Reef, Western Australia; Rasdhoo Atoll, Maldives	KF417632	[4]
Varialvus charadrus Miller, Bray, Justine & Cribb, 2010	*L. vitta, L. bohar, L. carponotatus, L. fulviflamma, L. fulvus, L. gibbus, L. kasmira, L. quinquelineatus*	Lizard Island, Great Barrier Reef, Australia	HM187778	[16]
V. jenae Miller, Bray, Justine & Cribb, 2010	*L. carponotatus*	Lizard Island, Great Barrier Reef, Australia	HM187776	[16]
V. lacertus Miller, Bray, Justine & Cribb, 2010	*L. quinquelineatus; L. fulvus*	Lizard Island, Great Barrier Reef, Australia	HM187777	[16]

References

1. Jayawardena UA, Tkach V V., Navaratne AN, Amerasinghe PH, Rajakaruna RS. Malformations and mortality in the Asian Common Toad induced by exposure to pleurolophocercous cercariae (Trematoda: Cryptogonimidae). Parasitol Int. 2013;62:246–52.

2. Arya LK, Rathinam SR, Lalitha P, Kim UR, Ghatani S, Tandon V. Trematode fluke *Procerovum varium* as cause of ocular inflammation in children, south India. Emerg Infect Dis. 2016;22:192–200.

3. Martínez-Aquino A, Vidal-Martínez VM, Aguirre-Macedo ML. A molecular phylogenetic appraisal of the acanthostomines *Acanthostomum* and *Timoniella* and their position within Cryptogonimidae (Trematoda: Opisthorchioidea). PeerJ. 2017;5:e4158.

4. Miller TL, Cribb TH. Dramatic phenotypic plasticity within species of *Siphomutabilus* n. g. (Digenea: Cryptogonimidae) from Indo-Pacific caesionines (Perciformes: Lutjanidae). Syst Parasitol. 2013;86:101–112.

5. Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DTJ. Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). Int J

Parasitol. 2003;33:733–55.

6. Miller T, Cribb T. Two new cryptogonimid genera *Beluesca* n. gen. and *Chelediadema* n. gen. (Digenea: Cryptogonimidae) from tropical Indo-West Pacific Haemulidae (Perciformes). Zootaxa. 2007;1543:45–60.

7. Miller T, Cribb T. Two new cryptogonimid genera (Digenea, Cryptogonimidae) from *Lutjanus bohar* (Perciformes, Lutjanidae): analyses of ribosomal DNA reveals wide geographic distribution and presence of cryptic species. Acta Parasitol. 2007;52:104–113.

8. Wongsawad C, Wongsawad P, Sukontason K, Maneepitaksanti W, Nantarat N. Molecular phylogenetics of *Centrocestus formosanus* (Digenea: Heterophyidae) originated from freshwater fish from Chiang Mai Province, Thailand. Korean J Parasitol. 2017;55:31–7.

9. Hernández-Orts JS, Georgieva S, Landete DN, Scholz T. Heterophyid trematodes (Digenea) from penguins: A new species of *Ascocotyle* Looss, 1899, first description of metacercaria of *Ascocotyle (A.) patagoniensis* Hernández-Orts et al. (2012), and first molecular data. Int J Parasitol Parasites Wildl. 2019;8:94–105.

10. Miller TL, Adlard RD, Bray RA, Justine J-. L, Cribb TH. Cryptic species of *Euryakaina* n. g. (Digenea: Cryptogonimidae) from sympatric lutjanids in the Indo-West Pacific. Syst Parasitol. 2010;77:185–204.

11. Miller TL, Cribb TH. *Gynichthys diakidnus* n. g., n. sp. (Digenea: Cryptogonimidae) from the grunt *Plectorhinchus gibbosus* (Lacépède, 1802) (Perciformes: Haemulidae) off the Great Barrier Reef, Australia. Syst Parasitol. 2009;74:103–112.

12. Miller TL, Downie AJ, Cribb TH. Morphological disparity despite genetic similarity; new species of *Lobosorchis* Miller & Cribb, 2005 (Digenea: Cryptogonimidae) from the Great Barrier Reef and the Maldives. Zootaxa. 2009;1992:37–52.

13. Miller TL, Cutmore SC, Cribb TH. Two species of *Neometadena* Hafeezullah & Siddiqi, 1970 (Digenea: Cryptogonimidae) from Moreton Bay, Australia, including the description of *Neometadena paucispina* n. sp. from Australian Lutjanidae. Syst Parasitol. 2018;95:655–64.

14. Miller TL, Cribb TH. Coevolution of *Retrovarium* n. gen. (Digenea: Cryptogonimidae) in Lutjanidae and Haemulidae (Perciformes) in the Indo-West Pacific. Int J Parasitol. 2007;37:1023–45.

15. Miller T, Cribb T. Eight new species of *Siphoderina* Manter, 1934 (Digenea, Cryptogonimidae) infecting Lutjanidae and Haemulidae (Perciformes) off Australia. Acta Parasitol. 2008;53:344–64.

16. Miller T, Bray R, Justine J-L, Cribb T. *Varialvus* gen. nov. (Digenea, Cryptogonimidae), from species of Lutjanidae (Perciformes) off the Great Barrier Reef, New Caledonia and the Maldives. Acta Parasitol. 2010;55:327–39.

Additional file 2

Figure S1. Photomicrographs of a paragenophore specimen of the *Neocladocystis bemba* n. sp. **a** Ventral view. **b** Anterior body extremity with oral sucker. **c** Mid-body. **d** Ovarian and testicular region. **e** Posterior part of hindbody. *Scale-bar*: 100 µm.

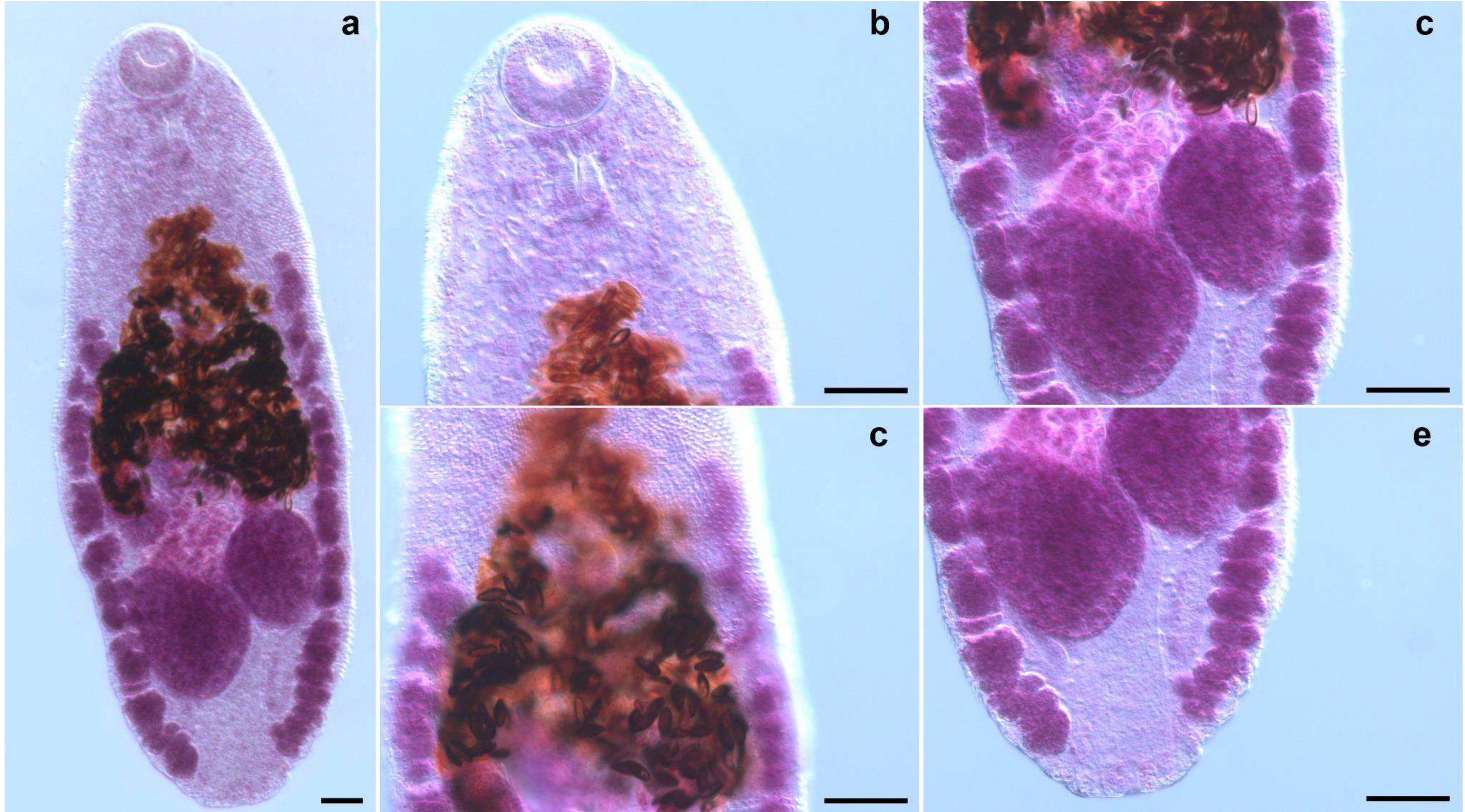
Figure S2. Photomicrographs of a paragenophore specimen of the *Neocladocystis biliaris* n. sp. a Ventral view. **b** Anterior body extremity with oral sucker. **c** Mid-body. **d** Ovarian and testicular region. **e** Posterior part of hindbody. *Scale-bar*: 200 µm

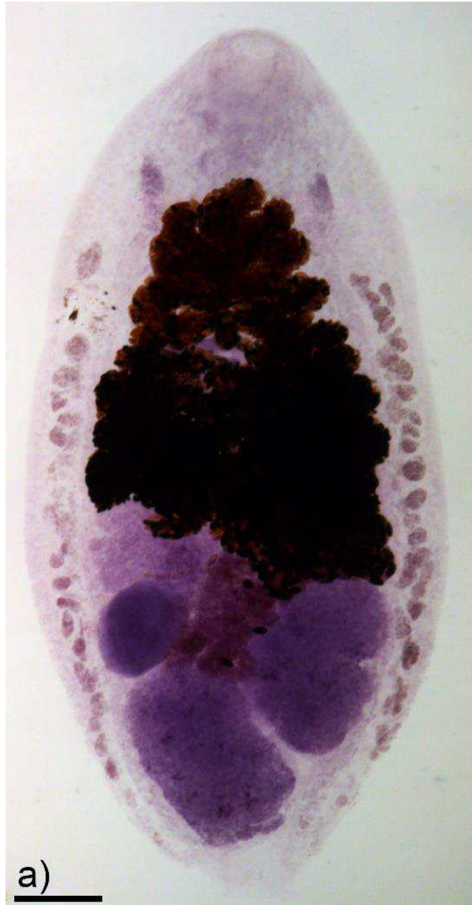
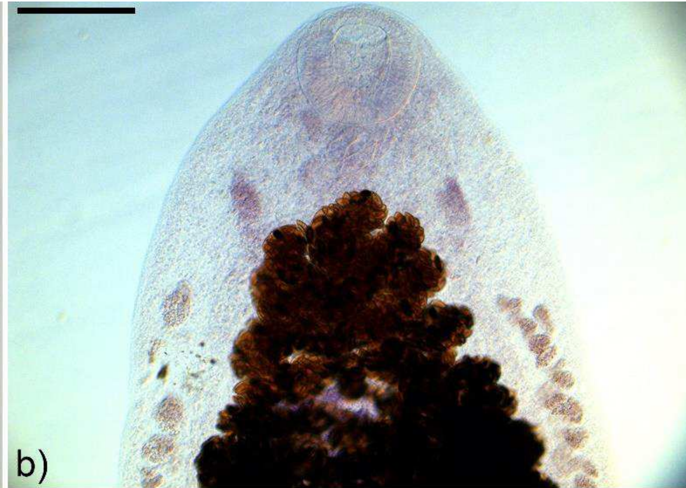
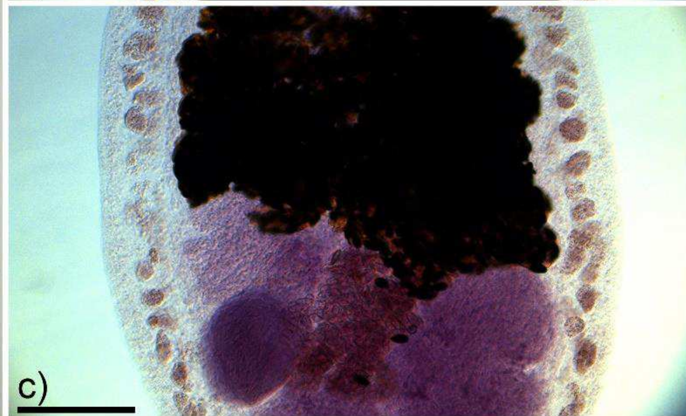
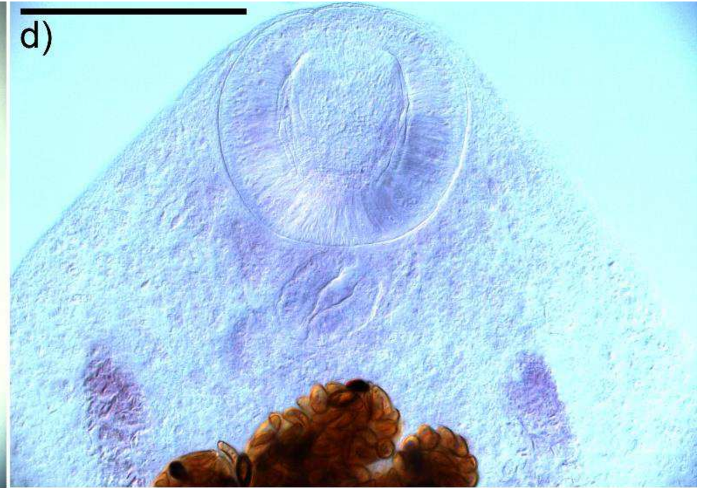
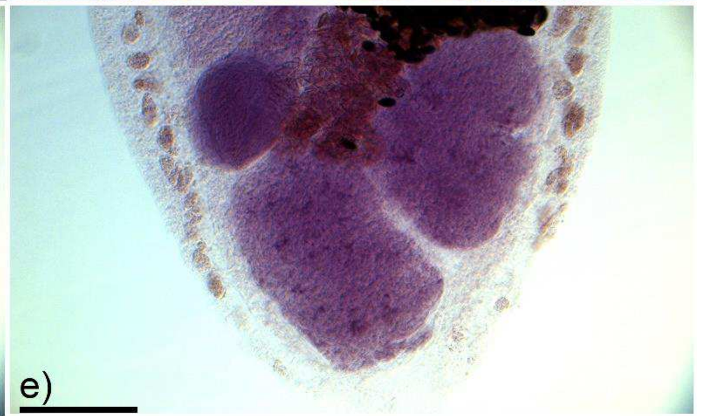
Figure S3. Photomicrographs of a paragenophore specimen of the *Neocladocystis* sp. Ventral view *Scale-bar*: 200 µm

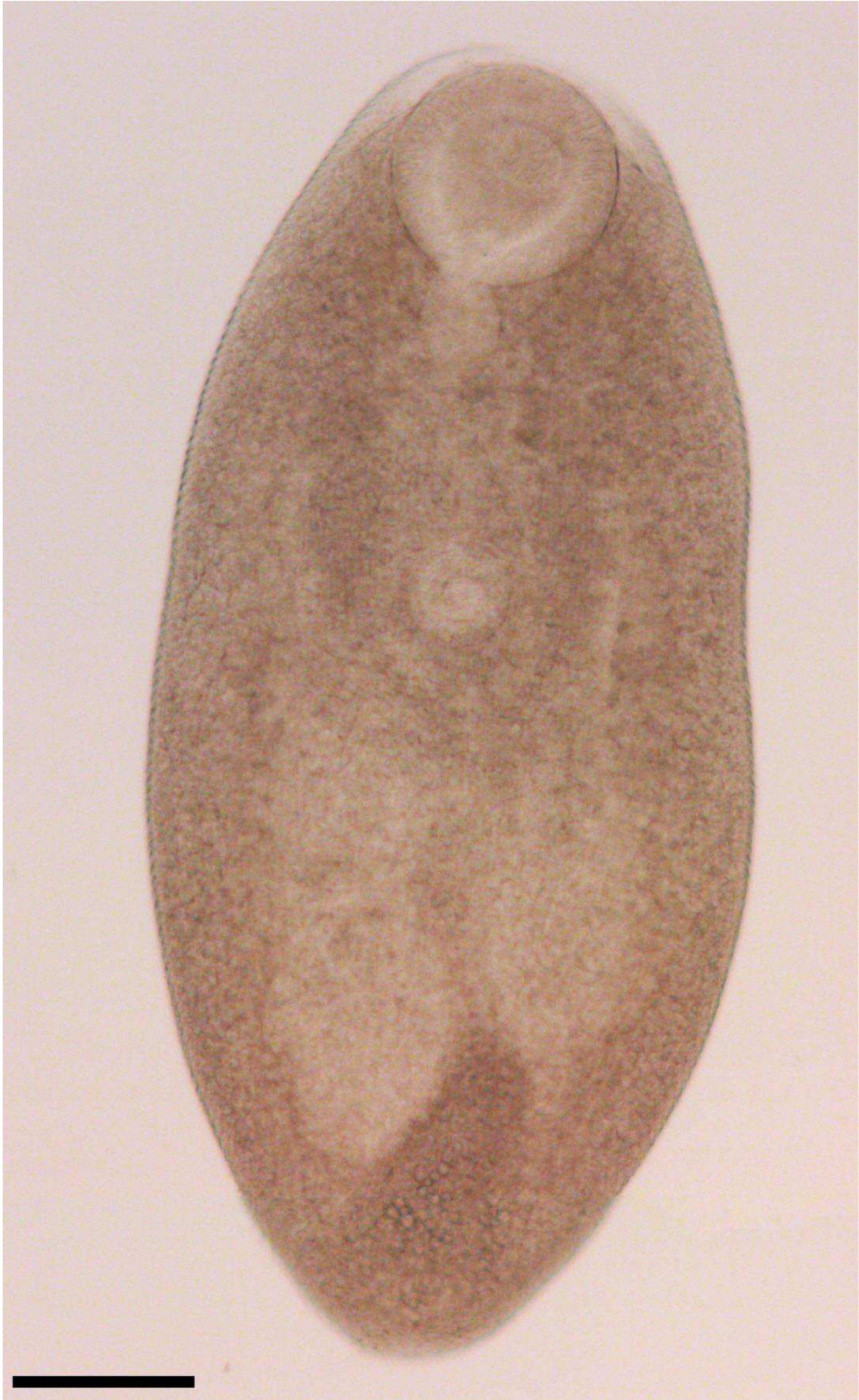
Figure S4. Photomicrographs of a paragenophore specimen of the *Mutabiliprepharynga fusiforma* n. sp. **a** Ventral view. **b** Anterior body extremity with oral sucker. c Mid-body. **d** Posterior part of hindbody. *Scale-bar*: 100 µm

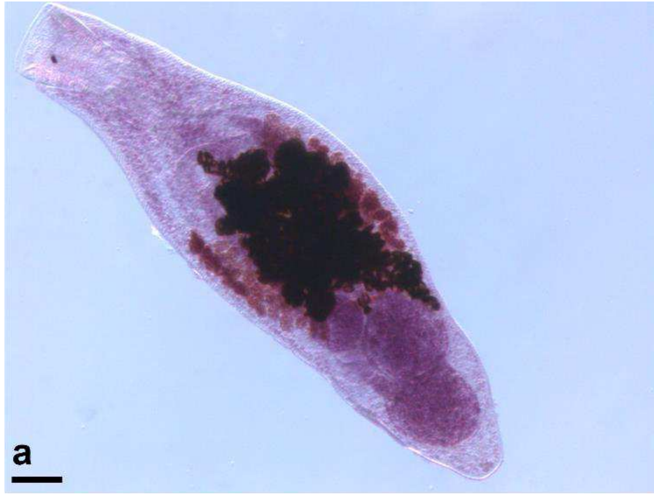
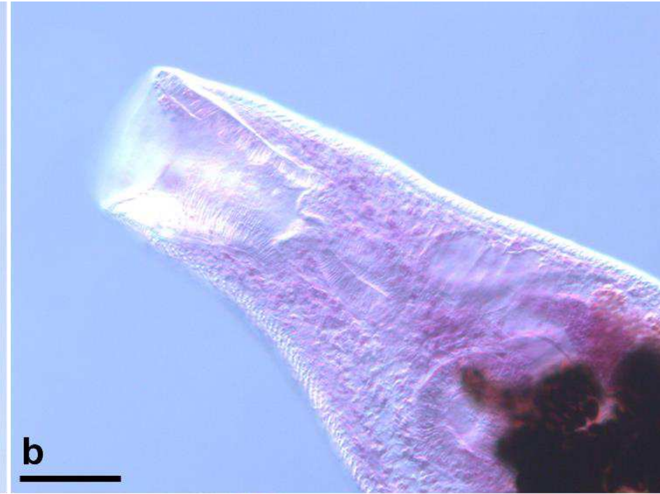
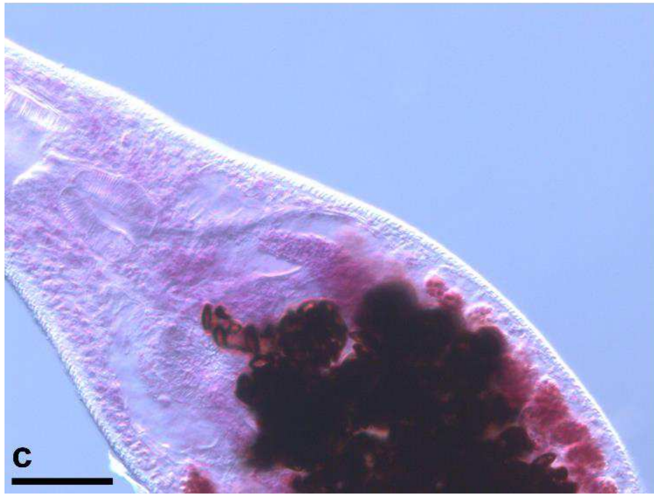
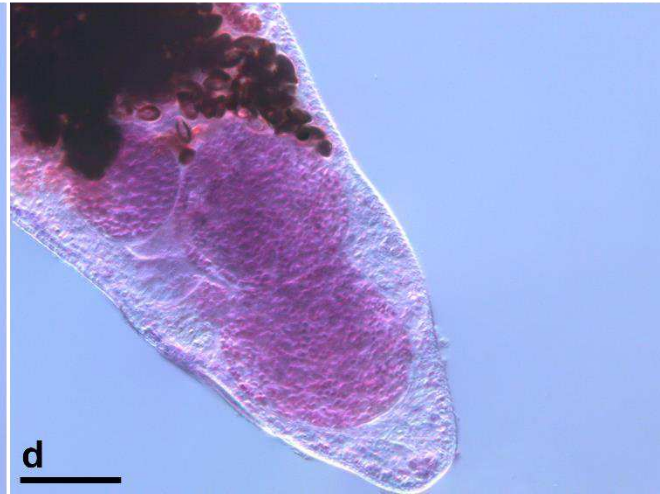
Figure S5. Photomicrographs of a paragenophore specimen of the *Mutabiliprepharynga* sp. 'elongata'. **a** Ventral view. **b** Anterior body extremity with oral sucker. **c** Mid-body. **d** Posterior part of hindbody. *Scale-bar*: 100 µm

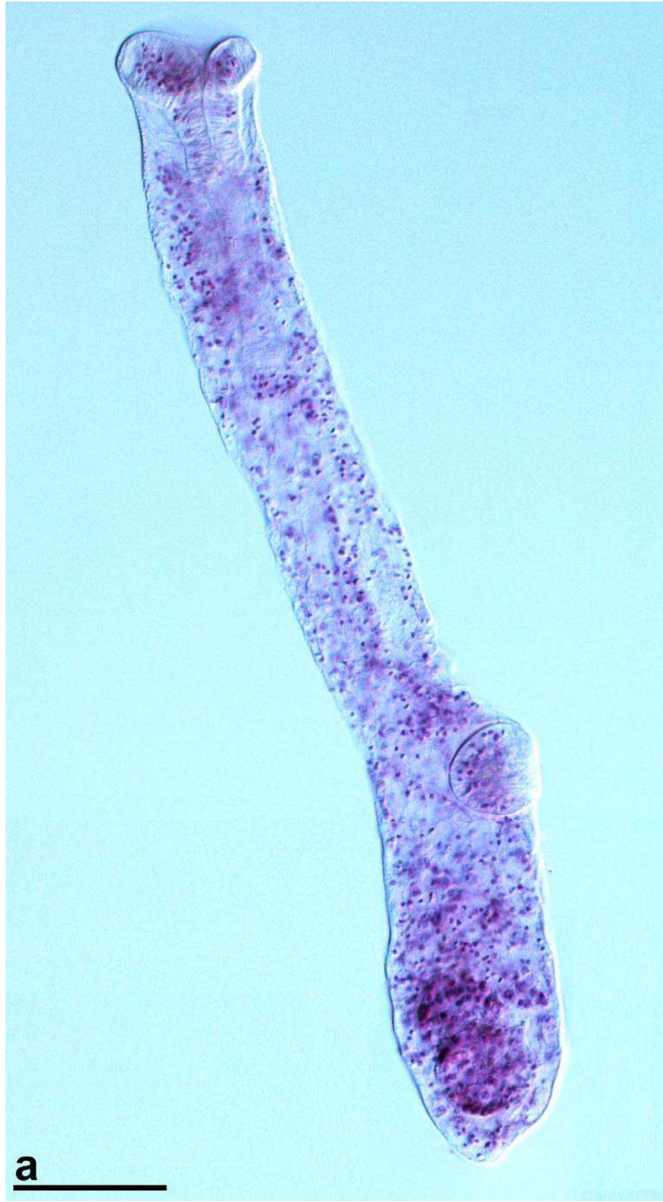
Figure S6. Photomicrographs of a paragenophore specimen of the *Grandifundilamena novemtestes* n. sp. **a** Ventral view. **b** Ovarian and testicular region. **c, d** Anterior body part. *Scale-bar*: 500 µm

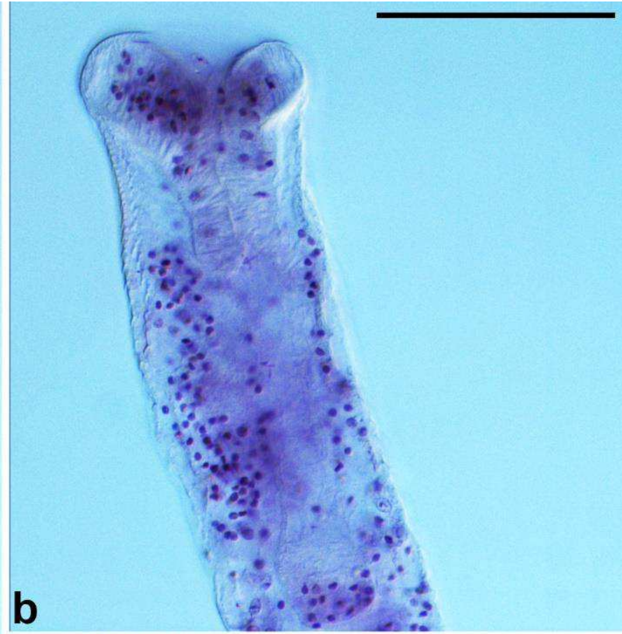

a
b
c
c
e

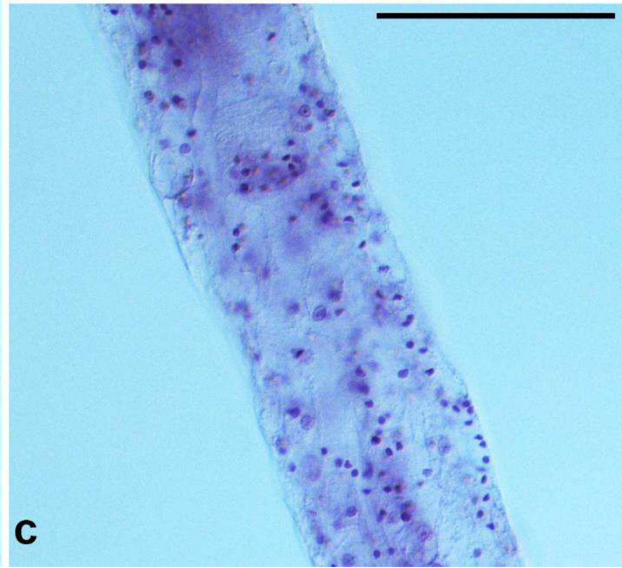

a)

b)

c)

d)

e)

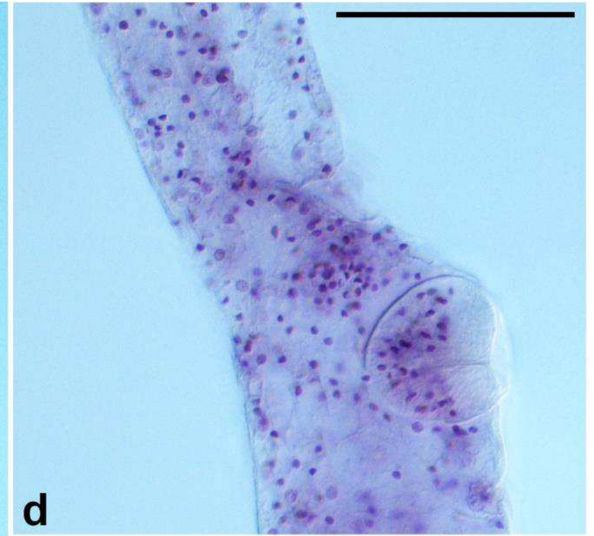


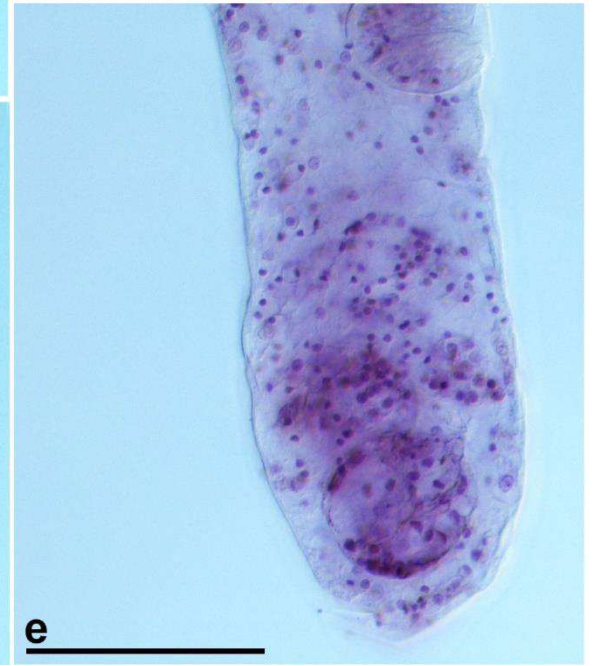

a

b

c

d

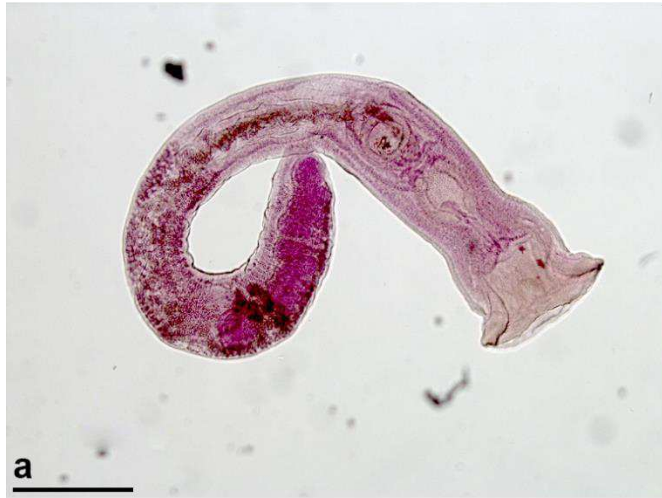

a

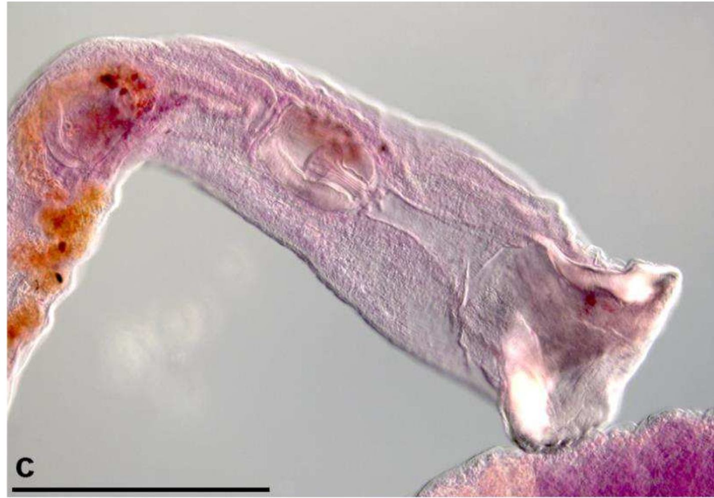

b

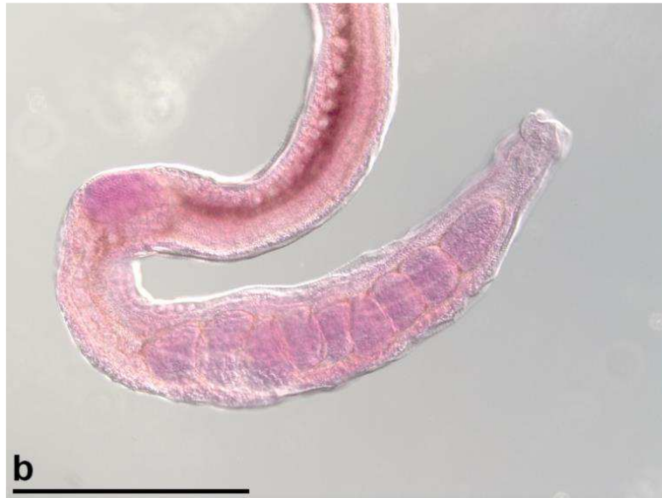

c

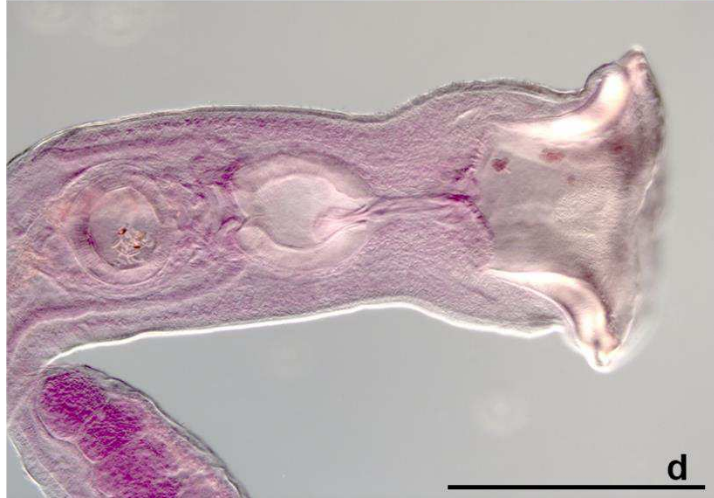

d


e


a


c


b


d

10 APPENDIX

In this section, list of scientific publications co-authored by Nikol Kmentová as a participation in research project outside the topic of this Ph.D. thesis. Papers are listed chronologically.

De Keyzer E. L. R., De Corte Z., Van Steenberge M., Raeymaekers J. A. M., Calboli F. C. F., Kmentová N., Mulimbwa N'Sibula T., Virgilio M., Vangestel C., Masilya Mulungula P., Volckaert F. A. M., Vanhove M. P. M. 2019. First genomic study on Lake Tanganyika sprat *Stolothrissa tanganicae*: a lack of population structure calls for integrated management of this important fisheries target species. *BMC Evolutionary Biology* 19:6. DOI: 10.1186/s12862-018-1325-8 [Q2, IF (2018) = 3.045].

Publication is available at:

https://bmcevolbiol.biomedcentral.com/articles/10.1186/s12862-018-1325-8

De Keyzer et al. BMC Evolutionary Biology (2019) 19:6
https://doi.org/10.1186/s12862-018-1325-8

BMC Evolutionary Biology

RESEARCH ARTICLE **Open Access**

First genomic study on Lake Tanganyika sprat *Stolothrissa tanganicae*: a lack of population structure calls for integrated management of this important fisheries target species

Els L. R. De Keyzer[1,2*†], Zoë De Corte[3,4†], Maarten Van Steenberge[1,3,4], Joost A. M. Raeymaekers[1,5], Federico C. F. Calboli[1], Nikol Kmentová[6], Théophile N'Sibula Mulimbwa[7], Massimiliano Virgilio[3], Carl Vangestel[4], Pascal Masilya Mulungula[7], Filip A. M. Volckaert[1] and Maarten P. M. Vanhove[1,2,6,8,9]

Abstract

Background: Clupeid fisheries in Lake Tanganyika (East Africa) provide food for millions of people in one of the world's poorest regions. Due to climate change and overfishing, the clupeid stocks of Lake Tanganyika are declining. We investigate the population structure of the Lake Tanganyika sprat *Stolothrissa tanganicae,* using for the first time a genomic approach on this species. This is an important step towards knowing if the species should be managed separately or as a single stock. Population structure is important for fisheries management, yet understudied for many African freshwater species. We hypothesize that distinct stocks of *S. tanganicae* could be present due to the large size of the lake (isolation by distance), limnological variation (adaptive evolution), or past separation of the lake (historical subdivision). On the other hand, high mobility of the species and lack of obvious migration barriers might have resulted in a homogenous population.

Results: We performed a population genetic study on wild-caught *S. tanganicae* through a combination of mitochondrial genotyping (96 individuals) and RAD sequencing (83 individuals). Samples were collected at five locations along a north-south axis of Lake Tanganyika. The mtDNA data had low global FST and, visualised in a haplotype network, did not show phylogeographic structure. RAD sequencing yielded a panel of 3504 SNPs, with low genetic differentiation ($F_{ST} = 0.0054$; 95% CI: 0.0046–0.0066). PCoA, fineRADstructure and global F_{ST} suggest a near-panmictic population. Two distinct groups are apparent in these analyses ($F_{ST} = 0.1338$ 95% CI: 0.1239,0.1445), which do not correspond to sampling locations. Autocorrelation analysis showed a slight increase in genetic difference with increasing distance. No outlier loci were detected in the RADseq data.

(Continued on next page)

* Correspondence: els.dekeyzer@kuleuven.be
†Els L. R. De Keyzer and Zoë De Corte contributed equally to this work.
[1]Laboratory of Biodiversity and Evolutionary Genomics, KU Leuven, Charles Deberiotstraat 32, B-3000 Leuven, Belgium
[2]Capacities for Biodiversity and Sustainable Development (CEBioS), Operational Directorate Natural Environment, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000, Brussels, Belgium
Full list of author information is available at the end of the article

© The Author(s). 2019 **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

(Continued from previous page)

Conclusion: Our results show at most very weak geographical structuring of the stock and do not provide evidence for genetic adaptation to historical or environmental differences over a north-south axis. Based on these results, we advise to manage the stock as one population, integrating one management strategy over the four riparian countries. These results are a first comprehensive study on the population structure of these important fisheries target species, and can guide fisheries management.

Keywords: Fish, Freshwater, High-throughput sequencing, RAD sequencing, SNP, Panmixis, Population genomics, East Africa, Great Lakes, Stock management

Introduction

Freshwater ecosystems support more species per unit area than any other ecosystem. Yet, they currently suffer from fast declines in species richness [1]. The decline in biodiversity reduces the resilience of aquatic ecosystems, decreasing their ability to provide ecosystem services such as food, drinking water, climate regulation, and social and health benefits [2]. As freshwater habitats play an important role in fisheries with almost 13% of the world's aquatic catches [3], and one third of African fish catches [4], this decrease in resilience jeopardizes the future of human communities [5]. Therefore, it is unfortunate that freshwater fisheries have been less well studied compared to marine fisheries and are often overlooked in policy and regulation matters [6].

The sustainable exploitation of freshwater ecosystem services benefits from science-based management, based on sound biological knowledge of the system and its species. An important component of biological information is related to the structure of fish populations. The genetic structure of fish populations can be used to support the delineation of demographic units [7, 8], commonly referred to as stocks. Knowledge about stocks allows to preserve genetic variation and to decide on the size of meaningful management units [9]. Currently, most fisheries management units are not sufficiently supported by information on the population structure of the target species [10, 11]. Lack of scientifically supported management entails a risk for overfishing, and loss of population densities [12], especially when catch effort is not spread homogeneously [11].

In tropical systems, the biological knowledge on fisheries target species is less advanced and information on the population structure is often lacking. Hence, the scope for science-based management is small. This also holds for the Great Lakes of East Africa, in spite of their ecological, economic and social significance. Lake Tanganyika (LT) is the oldest African Great Lake, in which unique and very diverse aquatic communities have evolved [13]. It is situated in the western range of the Great African rift valley, measures almost 680 km in length and 50 km in width, and contains more than 1.89×10^7 km^3 of water [14]. The oxygenated layer is deeper in the South (180 m) than in the North (120 m), as recorded during a dry season sampling [15]. The prevailing south-eastern winds cause an inclination of the thermocline, causing the upper water column to be somewhat warmer in the North (average annual temperature 25.8 °C), than in the South (average annual temperature 24 °C) [15, 16]. These differences are more pronounced in the dry season from May until September [16]. The lake is divided into three subbasins, which have been intermittently disconnected during periods of low water levels during its 6 million year history, forming distinct palaeolakes. The presumed prolonged division of the lake into these palaeolakes, approximately 1 million years ago, had profound influences on the lake's diverse benthic fauna [17]. Lake levels continued to rise and fall, but it is assumed that since 106.000 years ago (106 kya), the subbasins of Lake Tanganyika have remained connected [18].

The fishery of LT plays an invaluable role in food security in one of the poorest regions in the world. Many people living near the lakeshore depend on artisanal fishing for their protein supply [19]. The Lake's pelagic fisheries have a huge importance to local communities by providing almost 200,000 tons of fish yearly [20]. Pelagic catches are composed of mainly three species. The clupeids *Stolothrissa tanganicae* (Lake Tanganyika sprat; Clupeidae; Actinopterygii) and *Limnothrissa miodon* (Lake Tanganyika sardine; Clupeidae; Actinopterygii) provide 65% of the catch (by weight) [20]; a perciform predator, *Lates stappersii* (sleek lates; Latidae; Actinopterygii), provides 30% of the catch [20]. Additionally, *S. tanganicae* serves as an important food source for *L. miodon* and *L. stappersii* [21]. In the northern part of LT, *S. tanganicae* dominates the catches of artisanal fishermen [22]. In the South, the species is less abundant and catches are dominated by *L. stappersii* [23]. *Stolothrissa tanganicae* has a life style that is reminiscent of that of marine clupeids. It forms schools that differ in size and density throughout the day [24]. The species migrate deeper into the lake at dawn and back to the surface at dusk, probably following their zooplankton prey [21] and escaping their predators. The fish live up to 1.5 years, reach maturity at about 70 mm standard length (SL) [25] and their maximum SL is

about 100 mm [23]. *Stolothrissa tanganicae* spawns throughout the year, with peaks in February–May [26] or August–September [27] in the North of the lake and in August–December [28], and possibly April–July [29] in the South. Eggs are spawned pelagically, sink and hatch one to 1.5 days later before they have reached the anoxic zone [24]. Feeding habits have mostly been studied in the northern part of the lake, where *S. tanganicae* feeds on zooplankton, mainly the calanoid copepod *Tropodiaptomus simplex* [30].

Observations at landing sites have shown a decrease of clupeid catches in LT [31, 32]. Hence, multiple calls for better management of this unique resource have been made [33, 34]. Yet, prior to this, it is necessary to understand the genetic structure of the two species, as it is unclear if they should be treated as single stocks or to be managed as different populations. A collapse of the clupeid fisheries would threaten the food security of millions of people. Additionally, loss of clupeid fisheries will also harm the biodiversity in LT as people will turn to fishing less resilient species, such as littoral cichlids. Furthermore, agriculture could increase further to compensate for the loss of protein source, which will cause runoff, destroying important habitats. Overall, the fisheries of LT are data-poor, which hampers the assessment of the exploitation status of the targeted stocks [35]. Clupeids can be considered very resistant to fisheries collapses because of their early age at maturity, pelagic lifestyle (reducing the risk of habitat destruction) and their absence in the bycatch of other species [36]. Nevertheless, there are many examples of pelagic species that were thought to be resilient against population collapses, yet collapsed under excessive fishing pressure. Among these examples are clupeids like the Pacific sardine (*Sardinops sagax*) [37, 38], and the Atlantic herring (*Clupea harengus*) [39].

Previous attempts to reveal the population genetic structure of *S. tanganicae* and *L. miodon* are scarce. In *S. tanganicae,* the only genetic study conducted so far suggested a single panmictic stock [40], while in *L. miodon*, no clear large-scale geographic structure could be identified [41]. However, the genetic markers used in these studies (RAPD markers in *S. tanganicae*; allozyme markers and mtDNA Restriction Fragment Length Polymorphism (RFLP) of the ND 5/6 gene in *L. miodon*), may lack the sensitivity to detect genetic structure in highly dispersive organisms. Recent developments in sequencing technologies, such as Restriction site Associated DNA markers (RAD sequencing) allow to infer population structure based on numerous single nucleotide polymorphisms (SNPs) [42]. The accuracy of RAD sequencing in detecting low levels of genetic differentiation therefore exceeds the accuracy of molecular techniques based on other marker types, even at small sample sizes [40, 43]. Although commonly used to detect population structure in pelagic marine species, RAD sequencing has less often been used in pelagic freshwater species.

In this study, we combine an analysis of mitochondrial DNA (mtDNA) haplotypes with a genomic analysis of nuclear DNA to assess population structure of *S. tanganicae* along a north-south axis in LT. A heterogeneous population genetic structure is possible for three reasons. First, the distance between the northern and southern end of the lake is large, compared to the assumed migration distances of this species, so levels of mixing might decrease with distance (hypothesis of isolation by distance). Second, there are limnological differences between the North and the South of the lake, to which *S. tanganicae* might have distinct adaptations (hypothesis of adaptive evolution). Since the eggs slowly sink to a depth of 150 m in the South [20], and need to hatch before reaching the anoxic zone, we assume this leaves less time for the eggs in the North to develop. The inclination of the thermocline could have an effect on larval development and productivity. Finally, fluctuations in the lake water level stands [41] have affected connectivity in the lake. Lower connectivity limits migration, which could lead to population structuring (hypothesis of historical subdivision). Alternatively, since migration distances are not exactly known and these sardines are highly mobile and may have large effective population size, the species could be panmictic across the entire north-south axis. This panmixia would fit with observations in other sardines and anchovies, which often show low population differentiation [44].

Material and methods

Sampling and DNA extraction

We selected five sampling sites along a latitudinal gradient covering the three subbasins of LT (Fig. 1). Two sites were selected in the northern basin (Uvira and Uvira 2), one in the central basin (Kalemie), and two in the southern basin (Mpulungu and Kalambo Lodge) (Table 1). This allowed us to evaluate population structure at the level of the entire lake, as well as among nearby sampling sites in the North and South of the lake. All samples were bought in the morning between August 11th and 20th 2016 (Table 1) from local fishermen who operated in a small range around the landing site. Since fishermen do not recast nets after they have been filled up by a passing school, all individuals within a sample belonged to the same school. To minimize the probability that a migrating school was sampled twice, the fish were bought on the same day for the two locations in the North, and on consecutive days for the two locations in the South (Table 1). For each sample, a finclip was stored in 99% ethanol. All individuals where measured and 32 were sexed of which 4 were male, 12 were female and 16 were not mature thus sex could not be identified.

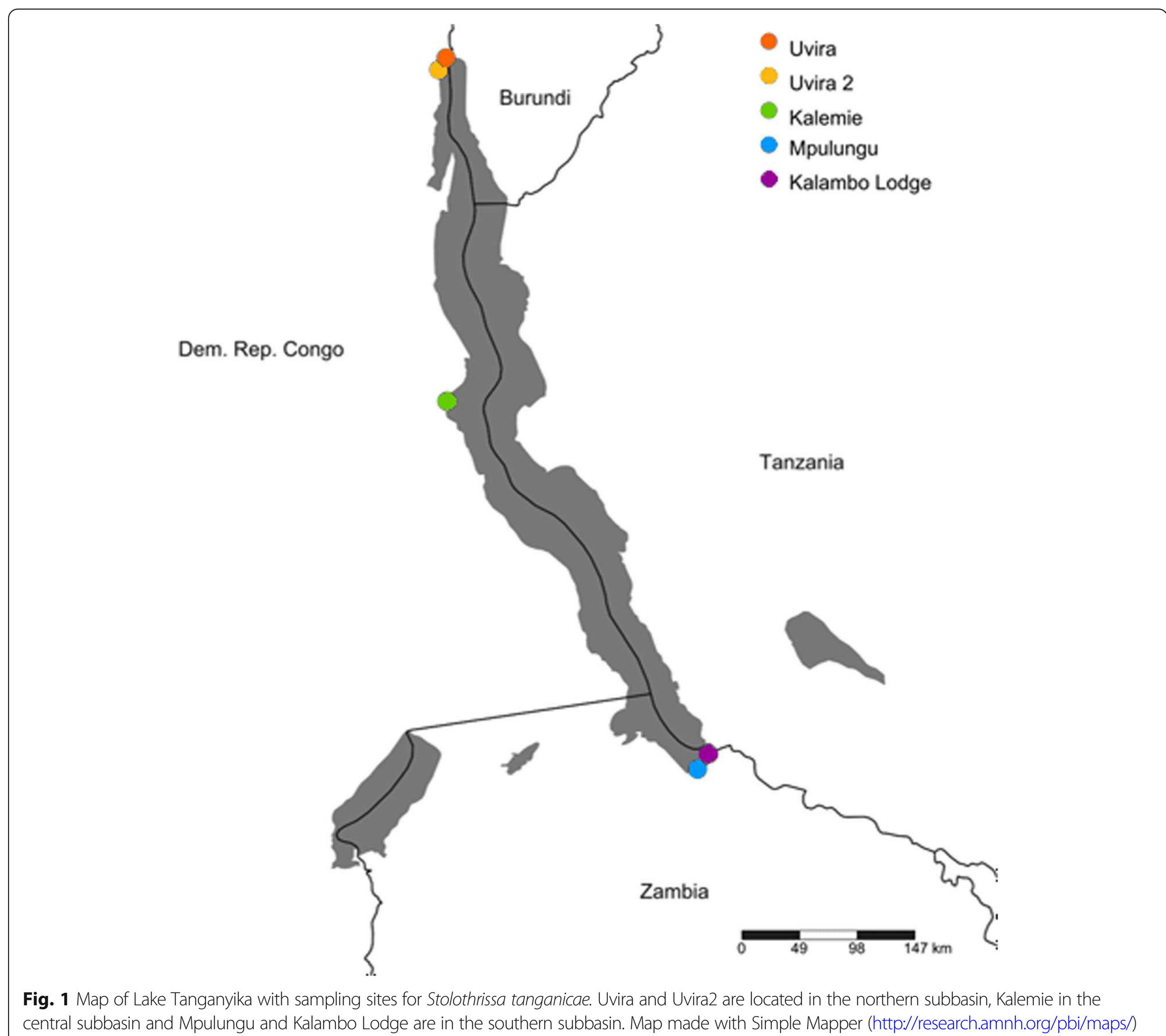


Fig. 1 Map of Lake Tanganyika with sampling sites for *Stolothrissa tanganicae*. Uvira and Uvira2 are located in the northern subbasin, Kalemie in the central subbasin and Mpulungu and Kalambo Lodge are in the southern subbasin. Map made with Simple Mapper (http://research.amnh.org/pbi/maps/)

In total 96 individuals were used for the analysis of mitochondrial data and for the RAD library construction. DNA was extracted from finclips, using the NucleoSpin Tissue kit (Macherey-Nagel GmBH) according to the manufacturer's instructions.

Table 1 Sampling information on *Stolothrissa tanganicae*

Site	n	Subbasin	Date	Longitude	Latitude
Uvira	16	northern	11/08/2016	−3.333539	29.189359
Uvira2	16	northern	11/08/2016	−3.395340	29.162933
Kalemie	32	central	12/08/2016	−5.947490	29.196633
Mpulungu	16	southern	19/08/2016	−8.762340	31.110506
Kalambo Lodge	16	southern	20/08/2016	−8.653927	31.195447

Sample size (n), subbasin, date of sampling and coordinates for the five sampling site. Sites represent the landing sites where fresh fish were purchased

Mitochondrial sequence data

The mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified using the universal primer combination HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3′) and LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3′) [45]. The PCR mix consisted of 1 μL of template DNA, 2.5 μL PCR buffer, 0.75 μL Platinum $MgCl_2$ (50 mM), 0.5 μL of dNTPs (10 mM), 1 μL of both primers (10 μM), 0.15 μL Platinum *Taq* polymerase (5 units/μL) and 18.1 μL of milli-Q water, totaling 25 μL. The PCR cycling profile consisted of 3 min at 94 °C, followed by 35 cycles at 94 °C for 45 s, 52 °C for 40 s, 72 °C for 90 s, 10 min at 72 °C and cooling to 4 °C. PCR products were purified by means of GFX purification columns (GE Healthcare, Chicago, IL, USA), subjected to sequencing reactions using the BigDye v3.1

cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and sequenced using the LCO1490 primer, with an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Sequence quality was verified with Geneious v11 [46] and MEGA v7.0 [47] by checking each SNP for base quality, assuming a reading error if a SNP is rare and quality is low. We checked for mutations recorded on the second position in a codon, which did not occur. Sequences were aligned with MUSCLE [48] using the default settings (Gap penalties: open = – 400; extend = 0, clustering method UPGMB, λ = 24). Before analyses, primers were trimmed out and sequences translated into amino acids to check for the absence of internal stop codons. Given the absence of gaps, the alignment was straightforward. The mitochondrial sequence data were used (a) to double-check the morphological identification of voucher specimens via DNA barcoding (data not shown) and (b) to assess possible genetic structure across individuals from different sampling sites. For this, a Median Joining Network [49] was made with PopART 1.7 [50], with ε = 0. Differentiation among individuals from the different sampling sites was estimated by global F_{ST} and pairwise F_{ST} between sampling sites in the diveRsity package [51] in R, using 100 bootstraps to calculate bias corrected 95% confidence intervals. We calculated number of haplotypes and Tajima's D statistic, using DnaSP v6 [52].

RAD library preparation

Six RAD libraries, each including 16 individually indexed specimens, were prepared according to the protocol described in Baird et al. [53] and Etter et al. [54]. Individual DNA samples were digested using restriction enzyme *SbfI-HF* (NEB, cut site 5'-CCTGCA^GG-3′). In silico digestion of the genome of the related Atlantic herring (*Clupea harengus*) [55] revealed 21,544 RAD loci with SbfI. Samples were individually barcoded with P1 adapters ligated to the fragment's overhanging end. The RAD libraries were sheared to a size of 350 base pairs (bp) and the fragments between 200 and 700 bp selected by gel size selection. A second, library-specific barcoded adapter (P2), was ligated to the DNA fragments for identification of the samples. RAD libraries were sequenced 101 bp paired-end on an Illumina HiSeq1500 platform at the Medical Centre for Genetics of the University of Antwerp, Belgium.

Processing of RAD data

Overall read quality was assessed using the FastQC software v0.11.5 [56]. Raw sequence data was demultiplexed using the *process_radtags* module in Stacks v1.46 [57, 58], while reads characterized by ambiguous barcodes, ambiguous cut sites or low quality scores were discarded. PCR duplicates were removed via the *clone_filter* module and SNPs were called using the *denovo_map* pipeline, both implemented in Stacks. We screened a range of parameter combinations and selected a minimum coverage of ten reads per stack (m = 10) and a maximum number of five base pair differences between stacks within (M = 5) and between (n = 5) individuals. This parameter setting allowed us to retain a sufficient number of orthologues at a considerable depth. Individuals with insufficient raw reads (< 0.8 million), a high proportion of missing data (> 50%) and low depth (< 9.7) were removed. A final round of filtering was performed using VCFtools v0.1.14 [59] in order to discard sites characterized by heterozygosity excess (p-value < 0.01), a minimum allele frequency of less than 0.05, and more than 20% of missing data.

Neutral population structure

Genetic variation of each sample was assessed by expected and observed heterozygosity and allelic richness, using the diveRsity v1.9.90 package in R v3.4.1. Using the same package, estimates of global F_{ST} were calculated, for two geographic scales, lake-wide and between nearby locations. To make lake-wide comparisons, we pooled the two northern (Uvira + Uvira2) and the two southern (Mpulungu + Kalambo Lodge) sampling sites. Pairwise F_{ST} was calculated across the sampling sites, with 95% confidence intervals based on 100 bootstrap iterations over loci.

Population structure was inspected with the R package ADEGENET v2.1.0 [60] to perform a non-centered, non-scaled Principal Coordinates Analysis (PCoA) based on Euclidean distances between specimens. Missing data in this analysis were replaced by the mean allele frequencies. In addition, we performed a Discriminant Analysis of Principal Components (DAPC) [61] with default settings. DAPC reduces the variation within the sampling sites, while maximizing the variation between them. As the amount in explained variance showed a continuous gradual decline with most important PCs, no optimal cutoff of number of PCs could be identified. Therefore, the DAPC was based on 28 PCs, the largest number of informative PCs [60].

Population structure was assessed using an MCMC method to infer recent shared ancestry based on patterns of genomic similarity implemented in fineRADstructure [62], which is a modification of fineSTRUCTURE [63] for RAD data. As this analysis showed to be highly sensitive to missing data, we retained only SNPs scored in more than 90% of all individuals. The RAD tags were ordered according to linkage disequilibrium with the sampleLD.R script provided in fineRADstructre. Subsequently, the co-ancestry matrix was calculated and used to identify populations by a clustering algorithm. This approach is robust for missing RAD alleles and is sensitive to subtle

population structure. The MCMC chain ran with a burnin of 100,000, 100,000 iterations and a thinning interval of 1000. We further explored whether genetic similarity between individuals decreased with geographical distance by conducting a spatial autocorrelation analysis over the five sampling sites in GenAlEx v6.501 [64–66]. Correlation coefficients between individuals were depicted as a function of increasing inter-individual geographical distance and confidence intervals based on 1000 bootstraps.

Genome scan of outlier loci

Putative signatures of natural selection were assessed using three different approaches to detect outlier loci. First, we assessed the distribution of the global F_{ST} values among loci, at the lake-wide scale and between all five of the locations, to identify possible candidates for outliers, using the diveRsity v1.9.90 package of R. Secondly, we performed a Bayesian outlier detection method in BayeScan v2.1 [67] which incorporates locus- and population-specific F_{ST} effects [67–69]. For each level, three replicate runs were executed with default parameter settings. False discovery rate (FDR) threshold was 0.05, and only loci consistently identified as outliers in each of three independent runs were considered as true outliers. Finally, we assessed the possible occurrence of adaptation along the latitudinal gradient. We applied an individual-based latent fixed mixed model (LFMM) in which SNP frequencies were associated to latitudinal variation, while accounting for neutral population structure [70]. The number of latent factors was set to one. We ran the model ten times, using 20,000 sweeps for burn-in and 40,000 additional sweeps as run-length and calculated the median Z-value of all replicated runs for each locus separately. We applied a correction by dividing the raw *p*-value by a genomic inflation factor, corresponding to the median of the square z-value divided by the median of the chi-square distribution [71]. To correct for the multiple tests, SNPs that were considered as non-neutral, were characterized by a q-value of 0.05 or less [71]. LFMM analyses were performed using the LEA package in R [72].

Results

Phylogeography based on mitochondrial sequence data

The Median Joining Network based on mitochondrial COI fragments of a length of 643 bp, does not suggest separation either between the five sampling sites, or between the three subbasins (Fig. 2). The global F_{ST} value between sampling sites is 0.0026. Pairwise F_{ST} values (Table 2) do not significantly differ from zero. Values range from -0.027 (95% CI: - 0.072, 0.0641) (Mpulungu – Uvira 2) to 0.0327 (95% CI: -0.0358, 0.1498) (Kalemie – Uvira). In these 96 samples, there are 47 different haplotypes and Tajima's D is significantly negative (D = -2.414, $p < 0.01$).

Quality of RAD genotyping

Due to a low number of reads (< 0.8 million), a high percentage of missing reads (> 50%) and low depth (< 9.7), 12 individuals were discarded. Two individuals from the Mpulungu sampling site were very similar, indicating possible contamination. To solve this, one of these individuals was removed. This resulted in 83 retained individuals, at least 15 per sampling site, with the number of reads per specimen ranging from 0.9 to 3.7 million (average per specimen = 1.96 million). Filtering produced a final dataset containing 3504 SNPs distributed across these 83 individuals, with a mean depth per individual of 29.66 (minimum of 9.7 and maximum of 57.9) and a mean missing per individual of 12% (minimum of 0.007% and maximum of 48%). Detailed information on missing data per individual can be found in Additional file 1.

Nuclear genetic diversity and neutral population structure

Observed and expected heterozygosity values were similar among the sampling sites, with expected heterozygosity ranging from 0.2088 (Kalemie) to 0.2605 (Uvira) and observed heterozygosity ranging from 0.1920 (Kalemie) to 0.2619 (Uvira) (Table 3). Allelic richness across the different sampling sites ranged from 1.7831 (Kalemie) to 1.9047 (Mpulungu) (Table 3).

Genetic differentiation estimated by global F_{ST} was relatively low, but significantly different from zero, with a F_{ST} value of 0.0068 (95% CI: 0.0057–0.0079) between sampling sites and a F_{ST} value of 0.0054 (95% CI: 0.0046–0.0066) between the northern, central and southern basin. Similarly, pairwise F_{ST} values between sampling sites are low, ranging from -0.0012 (95% CI: -0.002–0.0001) (Kalambo Lodge – Uvira) to 0.0250 (95% CI: 0.0215–0.0281) (Kalemie – Uvira) (Table 2).

The PCoA revealed no clustering based on the geographic origin of the samples (Fig. 3a). Individuals are separated on PC1 (9.30% explained variation) in one large cluster of 63 individuals and one smaller cluster of 20 individuals, regardless of the sampling site. PC2 and PC3 explained 1.72 and 1.71% of the variation respectively. PCoA was repeated with only the individuals in the larger cluster, to check if there is no hidden structure (Fig. 3b). Here, PC1 explains 2.47% of the variation and PC2 explains 2.44%. F_{ST} between the large and smaller cluster is significantly different from zero: 0.1338 [0.1239,0.1445]. The DAPC analysis shows no obvious pattern of genetic structuring across sampling sites, although some degree of separation on the diagonal is visible, with the samples from the central basin placed between those from the North and those from the South (Fig. 4, Additional file 2). For the visualization of patterns of haplotype similarity with fineRADstructure (Fig. 5), a reduced dataset of 1255 SNPs was used, to correct for

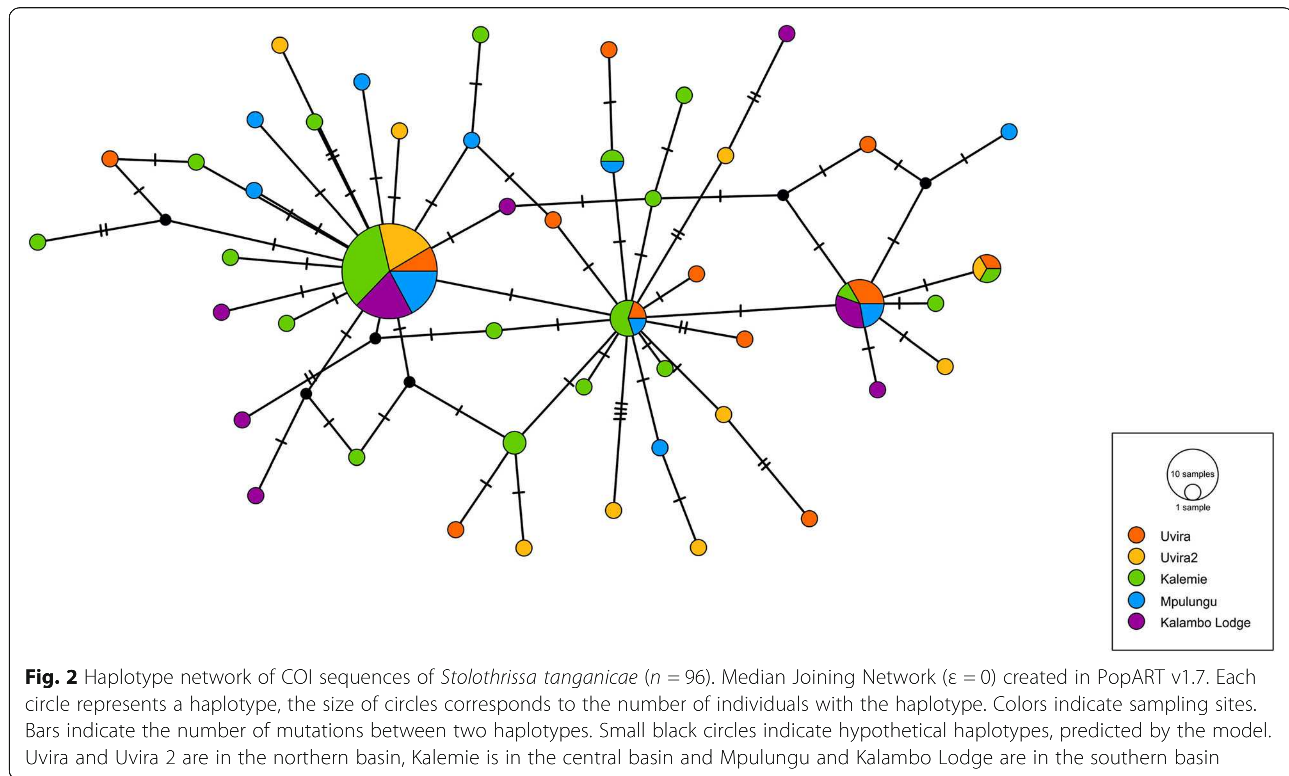


Fig. 2 Haplotype network of COI sequences of *Stolothrissa tanganicae* ($n = 96$). Median Joining Network ($\varepsilon = 0$) created in PopART v1.7. Each circle represents a haplotype, the size of circles corresponds to the number of individuals with the haplotype. Colors indicate sampling sites. Bars indicate the number of mutations between two haplotypes. Small black circles indicate hypothetical haplotypes, predicted by the model. Uvira and Uvira 2 are in the northern basin, Kalemie is in the central basin and Mpulungu and Kalambo Lodge are in the southern basin

effects of unevenly distributed levels of missing data. The structure provided by the fineRADstructure analysis corroborated with the results of the PCoA analysis, placing the same individuals in the same two clusters. These two groups are irrespective of sex or sampling site.

Autocorrelation analysis shows a low but significant level of genetic structuring in the five sampling sites along a north-south axis of LT, indicating a difference in populations in the North compared to the South. At a distance of 400 km, random processes like stochastic drift seem to overcome the homogenizing effect of gene flow (Fig. 6).

Outlier loci

Patterns in global F_{ST} at each SNP are concordant with previous results as the majority of F_{ST} values are clustered around zero, indicating low levels of genetic structuring (Additional file 3). Only 32 SNPs are characterized with a F_{ST} higher than 0.1 according to sampling site and 12 SNPs according to subbasin. The highest F_{ST} value is 0.21. None of these are identified as significant outliers by BayeScan (Additional file 4) or LFMM (Additional file 5) at a FDR threshold of 0.05.

Discussion

Population structure of *Stolothrissa tanganicae*

The population structure of *Stolothrissa tanganicae* was explored over five sampling sites in the three subbasins of LT using mitochondrial COI sequences and RAD sequencing data, to verify the existence of biologically meaningful management units. This species showed for both marker types a very weak genetic structure, suggesting a near-panmictic population. For both markers, the difference between samples from the different subbasins is not larger than the difference within subbasins. This pattern is obvious in both

Table 2 Pairwise genetic differentiation (F_{ST}) between sampling sites of *Stolothrissa tanganicae*

F_{ST}	Uvira	Uvira 2	Kalemie	Mpulungu	Kalambo lodge
Uvira		0.0077 [– 0.0621, 0.1302]	0.0316 [– 0.0368, 0.1496]	0.0206 [– 0.0579, 0.1609]	0.03267 [– 0.0358, 0.1498]
Uvira 2	0.0044 [0.0023, 0.0066]		−0.0200 [– 0.0650, 0.0551]	– 0.0275 [– 0.0720, 0.0641]	−0.0247 [– 0.0787, 0.0803]
Kalemie	0.0250 [0.0215, 0.0281]	0.0045 [0.0029,0.0066]		−0.0136[– 0.051, 0.0463]	−0.0035[– 0.0485, 0.0524]
Mpulungu	0.0166 [0.0140, 0.0194]	0.0017 [-0.0001, 0.0041]	0.0014 [-0.0005, 0.0030]		−0.0219 [– 0.0782, 0.0584]
Kalambo Lodge	-0.0012 [-0.0028, 0.0001]	-0.0005 [-0.0024, 0.0010]	0.0100 [0.0085, 0.0010]	0.0031 [0.0014, 0.0051]	

Values below the diagonal are from the nuclear DNA, above the diagonal from mitochondrial data. The values in brackets represent 95% confidence intervals based on 100 bootstraps over loci

Table 3 Nuclear genetic diversity of *Stolothrissa tanganicae* by sampling site

	Sample size	H_e (mean ± SE)	H_o (mean ± SE)	AR (mean ± SE)
Uvira	15	0.2605 ± 0.0024	0.2619 ± 0.0029	1.8716 ± 0.0041
Uvira2	15	0.2301 ± 0.0024	0.2213 ± 0.0025	1.8401 ± 0.0044
Kalemie	22	0.2088 ± 0.0025	0.1920 ± 0.0026	1.7831 ± 0.0046
Mpulungu	15	0.2529 ± 0.0023	0.2565 ± 0.0026	1.9047 ± 0.0033
Kalambo Lodge	16	0.2232 ± 0.0025	0.2181 ± 0.0027	1.8408 ± 0.0040

Expected and observed heterozygosity (H_e and H_o) and allelic richness (AR) by sampling site. Sample size is the number of individuals used for the analysis of the RADseq data, after exclusion of low quality samples. SE: standard error

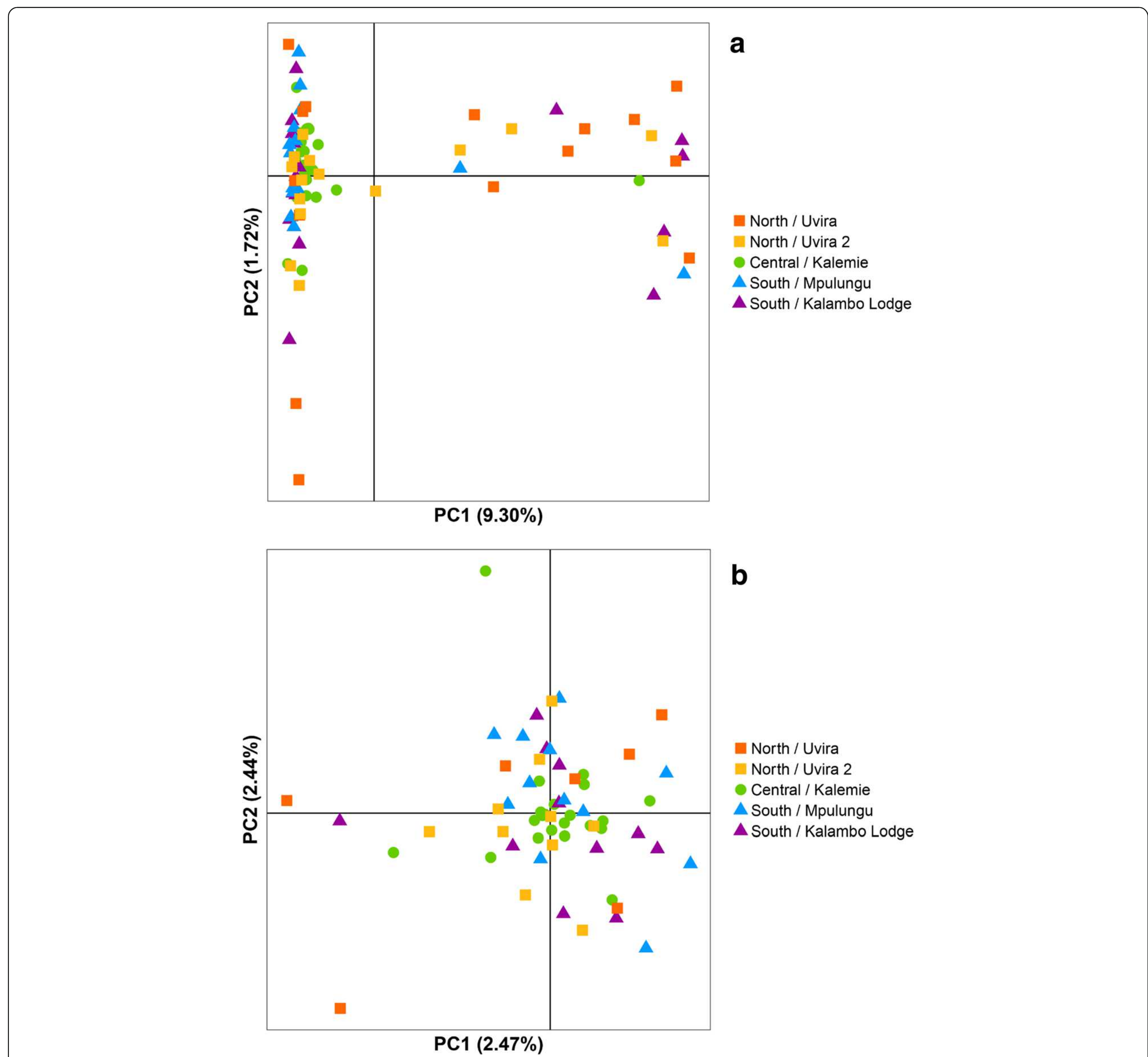


Fig. 3 PCoA based on kinship of nuclear DNA. Each dot represents one *S. tanganicae* individual. Dots that are closer together have more similar genotypes. Colors represent the five sampling sites. **a**. all individuals, PC1 explains 9.30% of the variation and PC2 1.72% of the variation. **b**. Plot with only the individuals from the larger cluster, PC1 2.47% explains of the variation and PC2 explains 2.44% of the variation. Made with ADEGENET v2.1.0 package in R

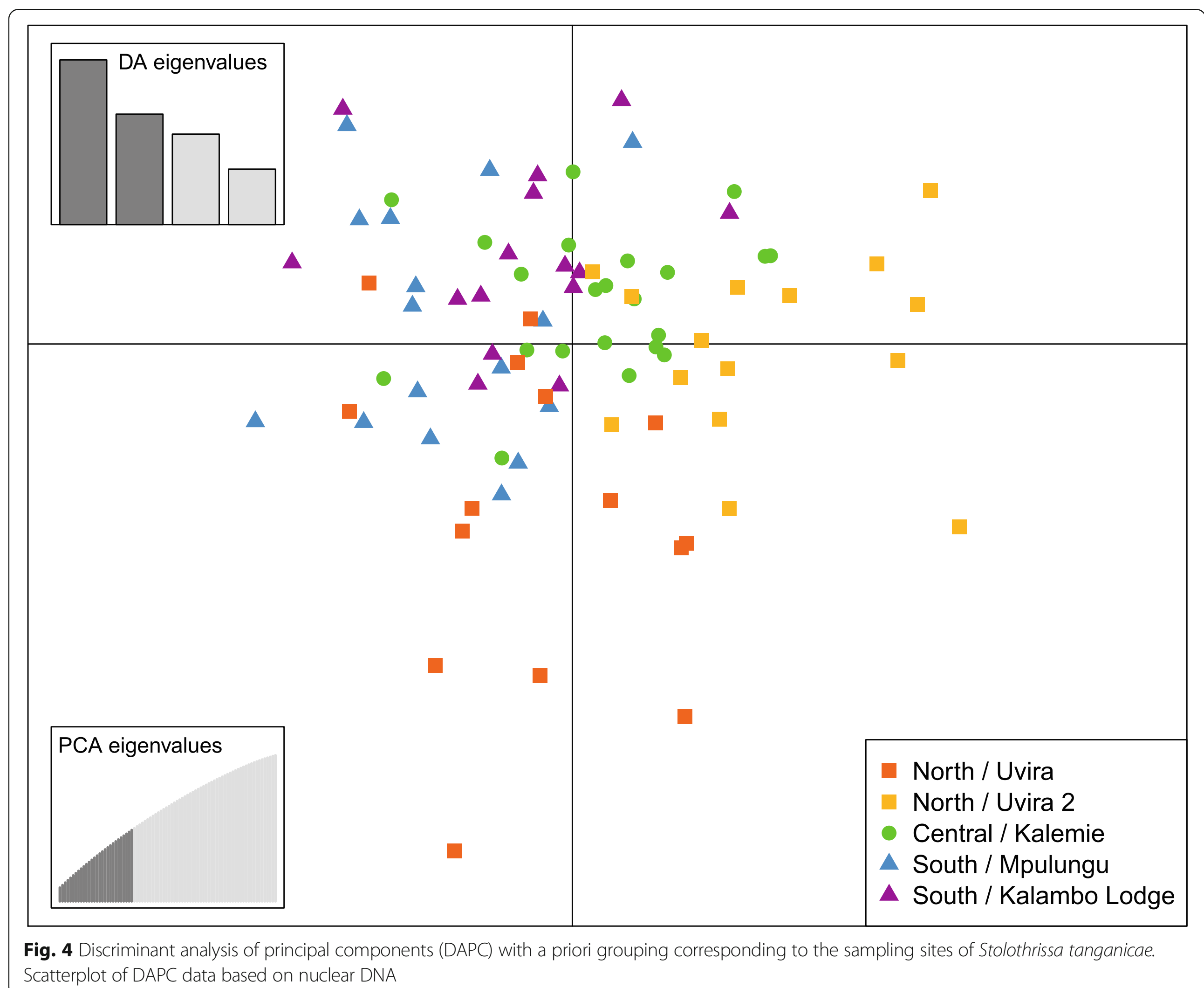


Fig. 4 Discriminant analysis of principal components (DAPC) with a priori grouping corresponding to the sampling sites of *Stolothrissa tanganicae*. Scatterplot of DAPC data based on nuclear DNA

the PCoA and fineRADstructure analysis of the RAD data and the haplotype network based on the mitochondrial DNA. The PCoA plot (Fig. 3) and the Median Joining Network (Fig. 2), show no genetic structuring according to sampling site or subbasin. Autocorrelation analysis revealed that there is a limitation to long-distance migration, as at a distance of 400 km, a decline in gene flow becomes apparent. The high number of different mitochondrial haplotypes (47), suggests many different maternal lineages.

The diverse set of lineages and the overall weak genetic structure confirm the conclusions of a previous population genetic study on *S. tanganicae*, which suggested a single panmictic stock [40]. However, this study was based on random amplified polymorphic DNA (RAPD) markers. RAPD markers are often difficult to interpret, and the results are not always reproducible. Our confirmation of the results based on a large set of high-quality SNPs represents an important benchmark, and indicates that *S. tanganicae* has been near-panmictic since the 1990s. No other population genetic studies on *S. tanganicae* are available, but a study by Sako et al. [43] revealed significant differences in otolith chemistry between populations from the northern and southern basin. This difference suggests that populations from the North and South of the lake spend most of their lifetime in different environments and implies that long-distance migrations must be rare. This seems to contradict the genetic patterns. However, a few migrants per generation are usually sufficient to maintain a near-panmictic population at the level of the entire lake.

Some of our analyses suggested the existence of two separate groups, independent of geographical origin. This is apparent in the PCoA plot (Fig. 3a), where we found a separation along the first axis. FineRADstructure analysis revealed the same two clusters. It is unclear what difference there is between these two groups, which differ in size. Missing data were equally distributed among the groups, so this is not the origin of the separation. The two groups could point to the

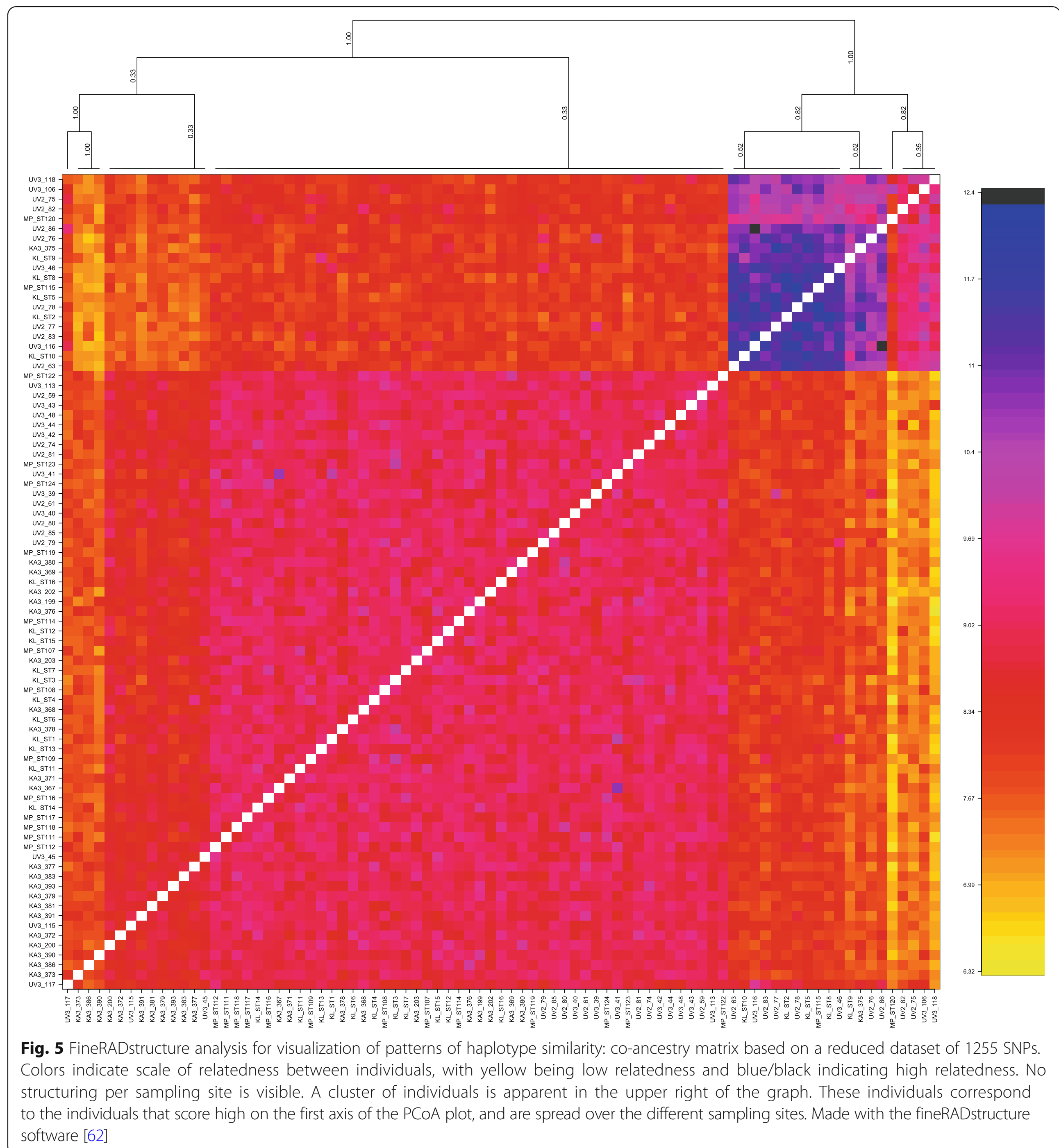


Fig. 5 FineRADstructure analysis for visualization of patterns of haplotype similarity: co-ancestry matrix based on a reduced dataset of 1255 SNPs. Colors indicate scale of relatedness between individuals, with yellow being low relatedness and blue/black indicating high relatedness. No structuring per sampling site is visible. A cluster of individuals is apparent in the upper right of the graph. These individuals correspond to the individuals that score high on the first axis of the PCoA plot, and are spread over the different sampling sites. Made with the fineRADstructure software [62]

two different sexes, yet for the 16 individuals that have been sexed in this study, male and female individuals show up in both groups in both analysis. Separate groups may as well arise because of a difference in spawning times. There are currently no indications for this in *S. tanganicae*, but it has been shown for Atlantic herring where spring-spawning and autumn spawning individuals were genetically differentiated [73]. Another possibility is that *S. tanganicae* frequently hybridizes with the other endemic clupeid, *L. miodon,* since F_{ST} between both groups is very large ($F_{ST} = 0.1338$ [0.1239,0.1445]). A larger sample size, as well as individuals of both species, are required to test the identity of the two separate groups. Individuals from the two groups have been found in all the sampling sites, so they do not alter the conclusions of the various analyses in this study.

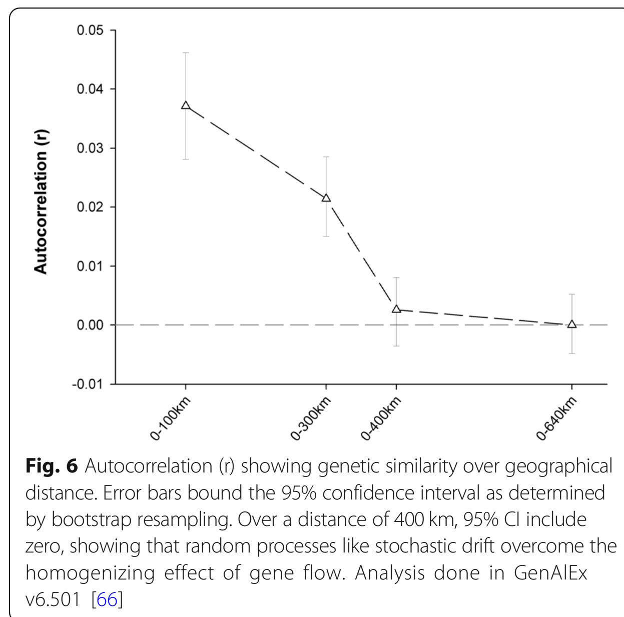


Fig. 6 Autocorrelation (r) showing genetic similarity over geographical distance. Error bars bound the 95% confidence interval as determined by bootstrap resampling. Over a distance of 400 km, 95% CI include zero, showing that random processes like stochastic drift overcome the homogenizing effect of gene flow. Analysis done in GenAlEx v6.501 [66]

The lakescape of pelagic fish

It is worthwhile to speculate what may cause the weak geographical population genetic structure of *Stolothrissa tanganicae*. At the start of this study, we hypothesized that population structure could arise due to isolation by distance, adaptive evolution, or the distinct history of the subbasins. We also considered the possibility of a homogeneous population because of large effective population sizes and high mobility of the species and the long period during which obvious migration barriers were absent. Our data did not show genetic differentiation between the different sampling locations over a north-south axis of the lake.

First, the data revealed a very weak pattern of isolation by distance, which was detected with the autocorrelation analyses. In 1970, Coulter stated, based on his observations in the northern and southern basins, that there were no reports of large clupeid migrations, and that there was no reason to assume there were any [25]. Yet, as stated above, some migration either individually or in schools may cause sufficient gene flow to keep the population structure near-panmictic. Similar to the marine environment, the pelagic zone of LT does not contain many barriers for migration. The frequent algal blooms in LT attract zooplankton, which in turn attracts the sprats. These algal blooms occur in the South of the lake in May–June, due to upwelling of nutrient rich water caused by tilting of the epilimnion because of strong south-east winds. After the winds cease around September, currents reverse and an algal bloom occurs in the North in October–November [16]. Migrations follow these blooms, as indicated by a positive correlation between *S. tanganicae* abundance and measures of chlorophyll *a* [23]. Catch statistics indicate a peak in *S. tanganicae* catches during phytoplankton blooms in the North [23, 74] and the South [75] of the lake. These seasonal migrations may contribute to the mixing of populations.

We found no traces of local adaptation to different conditions in the North and the South of LT. The number of loci in this study may have been too low to detect genomic regions involved in adaptive processes. There are some limnological differences along a north-south axis that could trigger local adaptation. For instance, the timing of major spawning events in *S. tanganicae* differ across the lake [21, 25, 28], but it is unknown if this difference in spawning time is an adaptive trait or linked to phenotypic plasticity in response to the timing of the plankton blooms [28] and depth of the oxygenated layer. Little is known about spawning areas and mating behaviour of the sprat. There is little information on how the eggs are fertilized and deposited and about dispersal of eggs, both possible facilitators of population mixing, as has been shown for marine species [76]. Expanding this limited knowledge is needed for good monitoring and conservation of the stock, and could help in explaining why the population remains homogeneous.

Our results do not show signatures of a population that differentiated because of historical barriers, which would have caused greater differences in genotypes between our samples. At times of extreme low-stands of the water levels of LT, the lake would be divided into three separate lakes, according to the three subbasins. It is assumed that the differentiation in cichlids was triggered by this isolation [77–79]. It is unclear if *S. tanganicae* also differentiated into different populations in the isolated subbasins as a result of low water levels. This lack of observed differentiation could be due to the pelagic life style of the sprat, enabling dispersal throughout the lake, similar to the benthopelagic Lake Tanganyika's giant cichlid (*Boulengerochromis microlepis*) [80] and two eupelagic *Bathybates* species (*B. fasciatus* and *B. leo*) [81] whose populations also do not show any phylogeographic structure.

A possible explanation for the homogeneous structure found here for *S. tanganicae* is that these populations could have passed through a bottleneck and quickly expanded again. This assumption is supported by a significant negative value of Tajima's D statistic, showing that observed heterozygosity is lower than expected heterozygosity due to inbreeding. Clupeids are known to have highly fluctuating population sizes, with large declines in numbers and fast expansions [82], leading to traceable bottlenecks [44]. Fishing pressure, poor recruitment or limited food availability could have significantly reduced the number of remaining sprats. Lake Tanganyika sprat is an r-selected species [83] with a short lifespan, many offspring and reaching an age of

maturity within a few months [84]. Furthermore, schooling reduces the effort to find a mate. This makes *S. tanganicae* excellently equipped for rapid population expansions [20].

Just like *S. tanganicae* in this study, sardines worldwide, often assessed over greater geographic distances, show below-average levels of population differentiation in comparison to other marine fishes. This is generally explained by their pelagic lifestyle, limited proportion of the population that contributes to the next generation, overharvesting and population bottlenecks [44, 85, 86]. In some cases, population genetic structure was detected [87], for example in the presence of physical barriers such as ocean currents [88] or over large geographical distances [89]. In other cases, subtle levels of ecological adaptation have been detected, for example between Atlantic herring (*Clupea harengus*) from the North Sea [90] and the Baltic Sea [91].

Implications for fisheries management and future research

The weak genetic structure in *S. tanganicae* over a north-south axis of LT, emphasises the need for integrated management of the entire stock. On the one hand, a single homogeneous stock might be easier to manage, since local extinctions can be countered by migrations from other populations. The adaptive potential and chance of survival of a metapopulation is bigger than that of an isolated subpopulation. On the other hand, managing such a homogeneous population has its own difficulties: Lake Tanganyika is bordered by four countries, each with its own legislation, law enforcement and economic reality. As the geographically unstructured sprat stocks do not correspond to international borders, each local management regime influences the stock available to the neighbouring countries. Our findings also underpin the importance of locating and protecting the spawning areas of *S. tanganicae*, since degradation of a spawning area could impact the stock in a wider area. Illegal fishing of clupeid fry in the spawning areas forms a huge burden on the stocks [92, 93]. It is also important to have more knowledge on which parts of the lake serve as sources and which as sinks for the *S. tanganicae* population. This information is vital to delineate spawning areas and source populations as protected areas.

Future research on the pelagic species in Lake Tanganyika remains necessary to provide information for management and conservation. More information on migrations of these pelagic clupeids would be beneficial for more directed management. The availability of a reference genome would be a step towards interpretation of adaptive traits if outlier SNPs would be detected. It will also be vital towards discovering genomic signatures of overfishing. There is also a need to look at the population structure of the two other major fisheries target species in Lake Tanganyika, *L. miodon* and *L. stappersii*. They both have a more littoral lifestyle than *S. tanganicae* [21], hence their populations might be more structured. Also, *L. stappersii* has a very different life history than the clupeids: these predators are bigger and live longer, which might affect their population structuring. This type of research can be useful in many other systems. It can be expanded to pelagic fish of the other African Great Lakes, and beyond. There are many lake ecosystems where a small, fast growing, pelagic fish species forms the link between zooplankton and piscivorous animals, just like the clupeids of Lake Tanganyika. Many of these systems would benefit from having information about the population structure of their pelagic fisheries targets. In some of these lakes, for example Lake Victoria, the pelagic fishes are becoming more important in the ecosystem due to overfishing of the larger fish species.

Conclusion

Our study confirms previous findings on the population structure of *S. tanganicae* in Lake Tanganyika. A near-panmictic population structure was detected over a north-south axis of the lake, with slightly increasing genetic distance over increasing geographical distance. This homogeneity in the stock of one of the major fisheries target species in LT underscores the need for integrated stock management between the four nations bordering Lake Tanganyika.

Additional files

Additional file 1: Sequencing quality and information on missing data per individual. Table shows the sampling site, individual ID, number of SNPs, number of missing SNPs, frequency of missing SNPs, mean read depth and number of raw reads per individual. (PDF 370 kb)

Additional file 2: Density plot of DAPC. Densities of individuals on the first discriminant function of the DAPC shown in Fig. 4. (PDF 28 kb)

Additional file 3: Frequency distribution of global F_{ST} of *Stolothrissa tanganicae* per SNP. Grouping by sampling site and subbasin. (PDF 5 kb)

Additional file 4: Outlier analysis based on BayeScan v2.1. A. F_{ST}-Log10 posterior probability for two levels (sampling site and subbasin) for each of the three replicates (R1, R2, R3). B. Q value for grouping according sampling site and subbasin with each of the three replicates (R1, R2, R3). (PDF 169 kb)

Additional file 5: Individual-based latent fixed mixed model (LFMM) analysis. Distribution of adjusted *p*-values, corrected with the genomic inflation factor. Made with the LEA package in R. (PDF 4 kb)

Abbreviations

bp: Base pairs; CI: Confidence interval; COI: Cytochrome *c* oxidase subunit I; FDR: False discovery rate; Kya: Thousand years ago; LFMM: Latent fixed mixed model; LT: Lake Tanganyika; mtDNA: Mitochondrial DNA; NGS: Next Generation Sequencing; RAD: Restriction site associated DNA; RFLP: Restriction fragment length polymorphism; SE: Standard error; SL: Standard length; SNP: Single nucleotide polymorphism

Acknowledgements

We thank Pierre-Denis Plisnier for his contribution to the research plan. We are grateful to all who helped us obtain clupeid samples: Fabrizia Ronco, Adrian Indermaur, Walter Salzburger, Donatien Muzumani Risasi, Fidel Muterezi

Bukinga, Simon Kambale Mukeranya, Joseph Mbirize Ndalozibwa and Elias Bilali Mela. We thank the LBEG team for advice, discussion and support troughout the project. Michiel Jorissen contributed to the figures. We thank three anonymous reviewers for helping us improve the manuscript.

Funding
Research has been supported by the JEMU pilot project PopGenSprat (BELSPO funding) and the Belgian Development Cooperation through VLIR-UOS (VLADOC scholarship NDOC2016PR006 to ELRDK). MPMV was supported by the Belgian Directorate-General for Development Cooperation and Humanitarian Aid (CEBioS program). MPMV and NK are supported by the Czech Science Foundation (P505/12/G112 (ECIP)). These funding sources had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Availability of data and materials
Fish vouchers and tissue samples were deposited in the ichthyology collection of the Royal Museum for Central Africa (Tervuren, Belgium) under collection number 2016.20. Mitochondrial sequences were deposited in NCBI GenBank under accession numbers MH290064 - MH290159 and SNP data gained through RAD sequencing is submitted to Mendeley Data and can be retrieved via doi:https://doi.org/10.17632/hhd3mz3myd.1 .

Authors' contributions
ELRDK wrote the manuscript and contributed to lab work and data analysis. ZDC performed lab work and analysis and contributed to the manuscript. Contribution of these two authors to the project is equal. NK, NM, PMM and MPMV carried out fieldwork. MVS, JAMR,FCFC, CV, MPMV and FAMV contributed to data analysis and data interpretation. NK and MV contributed to lab work. NK, CV, MV and NM contributed to interpretation of results and aided in writing the manuscript. MVS, JAMR, PMM and MPMV devised and oversaw the study, contributed to interpretation of results and critically revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Collection of specimens used in the study complied with institutional, national, and international guidelines. Fieldwork in D.R. Congo was carried out with the approval of the CRH – Uvira, which falls under the Congolese Ministry for science and technology ("Ministère National de la Recherche Scientifique et Technologie") under mission statement 031/MINRST/CRH-U/2016. Samples were exported with an export permit from the CRH-Uvira. Sampling in Zambia was carried out with the approval of the Zambian Department of Fisheries under a memorandum of understanding between the University of Basel, the University of Zambia and the Zambian Department of Fisheries. An export permit was obtained from the Department of Fisheries at Mpulungu. Samples were imported in Belgium under import permit CONT/IEC/CHM/1365428 to the RMCA of the Belgian Federal Agency for the safety of the food chain ('FAVV'). No animals were killed for this study, dead specimens were obtained from local fish markets.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
[1]Laboratory of Biodiversity and Evolutionary Genomics, KU Leuven, Charles Deberiotstraat 32, B-3000 Leuven, Belgium. [2]Capacities for Biodiversity and Sustainable Development (CEBioS), Operational Directorate Natural Environment, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000, Brussels, Belgium. [3]Joint Experimental Molecular Unit & Biology Department, Royal Museum for Central Africa, Leuvensesteenweg 13, B-3080 Tervuren, Belgium. [4]Joint Experimental Molecular Unit & Operational Directorate Taxonomy and Phylogeny, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium. [5]Faculty of Bioscience and Aquaculture, Nord University, Universitetsalléen 11, N-8026 Bodø, Norway. [6]Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, CZ-611 37 Brno, Czech Republic. [7]Département de Biologie, Centre de Recherche en Hydrobiologie, B.P. 73, Uvira, Democratic Republic of Congo. [8]Zoology Unit, Finnish Museum of Natural History, University of Helsinki, P.O.Box 17, FI-00014 Helsinki, Finland. [9]Hasselt University, Centre for Environmental Sciences, Research Group Zoology: Biodiversity & Toxicology, Agoralaan Gebouw D, B-3590 Diepenbeek, Belgium.

Received: 4 April 2018 Accepted: 11 December 2018
Published online: 08 January 2019

References
1. Kopf RK, Finlayson CM, Humphries P, Sims NC, Hladyz S. Anthropocene baselines: assessing change and managing biodiversity in human-dominated aquatic ecosystems. Bioscience. 2015;65:798–811.
2. Chapin FS III, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, et al. Consequences of changing biodiversity. Nature. 2000;405:234–42.
3. FAO. The State of World Fisheries and Aquaculture; 2016. p. 2016.
4. de Graaf GJ, Garibaldi L. The value of African fisheries. FAO. Fish. Aquac. In: Circ; 2014.
5. Ripple WJ, Wolf C, Galetti M, Newsome TM, Alamgir M, Crist E, et al. World scientists' warning to humanity: a second notice. Bioscience. 2017;67:1026–8.
6. Cooke SJ, Allison EH, Beard TD, Arlinghaus R, Arthington AH, Bartley DM, et al. On the sustainability of inland fisheries: finding a future for the forgotten. Ambio. Springer Netherlands. 2016;45:753–64.
7. Stephenson RL. Stock complexity in fisheries management: a perspective of emerging issues related to population sub-units. Fish Res. 1999;43:247–9.
8. Hauser L, Carvalho GR. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish Fish. 2008;9:333–62.
9. Ovenden JR, Berry O, Welch DJ, Buckworth RC, Dichmont CM. Ocean's eleven: a critical evaluation of the role of population, evolutionary and molecular genetics in the management of wild fisheries. Fish Fish. 2015;16: 125–59.
10. Reiss H, Hoarau G, Dickey-Collas M, Wolff WJ. Genetic population structure of marine fish: mismatch between biological and fisheries management units. Fish Fish. 2009;10:361–95.
11. Guan W, Cao J, Chen Y, Cieri M, Quinn T. Impacts of population and fishery spatial structures on fishery stock assessment. Can J Fish Aquat Sci. 2013;70: 1178–89.
12. Kerr LA, Hintzen NT, Cadrin SX, Clausen LW, Dickey-collas M, Goethel DR, et al. Lessons learned from practical approaches to reconcile mismatches between biological population structure and stock units of marine fish. ICES J Mar Sci. 2016;74:1708–22.
13. Salzburger W, Van Bocxlaer B, Cohen AS. Ecology and evolution of the African Great Lakes and their faunas. Annu Rev Ecol Evol Syst. 2014;45: 519–45.
14. Huttula T. Flow, thermal regime and sediment transport studies in Lake Tanganyika. Huttula T, editor. Kuopio University Publications C. Natural and Environ Sci 73; 1997.
15. De Wever A, Muylaert K, Van Der Gucht K, Pirlot S, Cocquyt C, Descy J, et al. Bacterial community composition in Lake Tanganyika: vertical and horizontal heterogeneity. Appl Environ Microbiol. 2005;71:5029–37.
16. Plisnier PD, Chitamwebwa D, Mwape L, Tshibangu K, Langenberg V, Coenen E. Limnological annual cycle inferred from physical-chemical fluctuations at three stations of Lake Tanganyika. Hydrobiologia. 1999;407:45–58.
17. Danley PD, Husemann M, Ding B, DiPietro LM, Beverly EJ, Peppe DJ. The impact of the geologic history and paleoclimate on the diversification of east African cichlids. Int J Evol Biol. 2012;2012:1–20.
18. McGlue MM, Lezzar KE, Cohen AS, Russell JM, Tiercelin JJ, Felton AA, et al. Seismic records of late Pleistocene aridity in Lake Tanganyika, tropical East Africa. J Paleolimnol. 2008;40:635–53.
19. Van der Knaap M, Katonda KI, De Graaf GJ. Lake Tanganyika fisheries frame survey analysis: assessment of the options for management of the fisheries of Lake Tanganyika. Aquat Ecosyst Heal Manag. 2014;17:4–13.
20. Mölsä H, Reynolds JE, Coenen EJ, Lindqvist OV. Fisheries research towards resource management on Lake Tanganyika. Hydrobiologia. 1999;407:1–24.
21. Coulter GW. Lake Tanganyika and its life. In: British museum (natural history); 1991.

22. Kimirei IA, Mgaya YD. Influence of environmental factors on seasonal changes in clupeid catches in the Kigoma area of Lake Tanganyika. African J Aquat Sci. 2007;32:291–8.
23. Plisnier PD, Mgana H, Kimirei I, Chande A, Makasa L, Chimanga J, et al. Limnological variability and pelagic fish abundance (*Stolothrissa tanganicae* and *Lates stappersii*) in Lake Tanganyika. Hydrobiologia. 2009;625:117–34.
24. Matthes H. Preliminary investigations into the biology of the Lake Tanganyika Clupeidae. Fish Res Bull. 1967;4:1965–6.
25. Coulter G. Population changes within a group of fish species following their exploitation. J Fish Biol. 1970;2:329–53.
26. Roest FC. Stolothrissa tanganicae: population dynamics, biomass evolution and life history in the Burundi waters of Lake Tanganyika. UN, FAO, CIFA Tech Pap. 1977:42–63.
27. Mulimbwa N, Sarvala J, Raeymaekers JAM. Reproductive activities of two zooplanktivorous clupeid fish in relation to the seasonal abundance of copepod prey in the northern end of Lake Tanganyika. Belgian J Zool. 2014; 144:77–92.
28. Chapman DW. Van well P. growth and mortality of *Stolothrissa tanganicae*. Trans Am Fish Soc. 1978;107:523–7.
29. Ellis CMA. The size at maturity and breeding seasons of sardines in southern Lake Tanganyika. AfrJTropHydrobiolFish. 1971;1:59–66.
30. Mgana HF, Herzig A, Mgaya YD. Diel vertical distribution and life history characteristics of *Tropodiaptomus simplex* and its importance in the diet of *Stolothrissa tanganicae*, Kigoma, Tanzania. Aquat Ecosyst Health Manag. 2014;17:14–24.
31. Coenen EJ, Nikomeze E. Lake Tanganyika, Burundi, results of the 1992–93 catch assessment surveys; 1994.
32. Van Der Knaap M, Kamitenga DM, Many LN, Tambwe AE, De Graaf GJ. Lake Tanganyika fisheries in post-conflict Democratic Republic of Congo. Aquat Ecosyst Health Manag. 2014;17:34–40.
33. Fryer G. Conservation of the Great Lakes of East Africa: a lesson and a warning. Biol Conserv. 1972;4:256–62.
34. Britton AW, Day JJ, Doble CJ, Ngatunga BP, Kemp KM, Carbone C, et al. Terrestrial-focused protected areas are effective for conservation of freshwater fish diversity in Lake Tanganyika. Biol Conserv. 2017;212:120–9.
35. Vasconcellos M, Cochrane K. Overview of world status of data-limited fisheries : inferences from landings statistics. In: Kruse GH, Gallucci VF, Hay DE, Perry RI, Peterman RM, Shirley TC, et al., editors. Fish assess Manag data-limited situations. Anchorage: Alaska Sea Grant College Program University of Alaska Fairbanks Lowell; 2005. p. 1–20.
36. Hutchings JA, Reynolds JD. Marine fish population collapses: consequences for recovery and extinction risk. Bioscience. 2004;54:297-309.
37. Murphy GI. Vital statistics of the Pacific sardine (*Sardinops Caerulea*) and the population consequences. Ecology. 1967;48:731–6.
38. Zwolinski JP, Emmett RL, Demer DA. Predicting habitat to optimize sampling of Pacific sardine (Sardinops sagax). ICES J Mar Sci. 2011;68: 867–79.
39. Overholtz WJ. The Gulf of Maine - Georges Bank Atlantic herring (*Clupea harengus*): spatial pattern analysis of the collapse and recovery of a large marine fish complex. Fish Res. 2002;57:237–54.
40. Hess JE, Matala AP, Narum SR. Comparison of SNPs and microsatellites for fine-scale application of genetic stock identification of Chinook salmon in the Columbia River basin. Mol Ecol Resour. 2011;11:137–49.
41. Cohen AS, Talbot MR, Awramik SM, Dettman DL, Abell P. Lake level and paleoenvironmental history of Lake Tanganyika, Africa, as inferred from late Holocene and modern stromatolites. Bull Geol Soc Am. 1997; 109:444–60.
42. Mesnick SL, Taylor BL, Archer FI, Martien KK, Treviño SE, Hancock-Hanser BL, et al. Sperm whale population structure in the eastern and central North Pacific inferred by the use of single-nucleotide polymorphisms, microsatellites and mitochondrial DNA. Mol Ecol Resour. 2011;11:278–98.
43. Puckett EE, Eggert LS. Comparison of SNP and microsatellite genotyping panels for spatial assignment of individuals to natal range: a case study using the American black bear (Ursus americanus). Biol Conserv. 2016;193: 86–93.
44. Grant WS, Bowen BW. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for coservation. J Hered. 1998;89:415–26.
45. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol. 1994;3:294–9.
46. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012;28: 1647–9.
47. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870–4.
48. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004;32:1792–7.
49. Bandelt H-J, Forster P, Röhl A. Median-joining networks for inferring intraspecific phylogenies. Mol Biol. 1999;16:37–48.
50. Leigh JW, Bryant D. POPART: full-feature software for haplotype network construction. Methods Ecol Evol. 2015;6:1110–6.
51. Keenan K, Mcginnity P, Cross TF, Crozier WW, Prodöhl PA. diveRsity: an R package for the estimation and exploration of population genetics parameters and their associated errors. Methods Ecol Evol. 2013;4:782–8.
52. Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, et al. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol. 2017;34:3299–302.
53. Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, et al. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One. 2008;3:1–7.
54. Etter PD, Bassham S, Hohenlohe PA, Johnson EA, Cresko WA. SNP discovery and genotyping for evolutionary genetics using RAD sequencing. Methods Mol Biol. 2011;772:157–78.
55. Barrio AM, Lamichhaney S, Fan G, Rafati N, Pettersson M, Zhang H, et al. The genetic basis for ecological adaptation of the Atlantic herring revealed by genome sequencing. elife. 2016;5:1–32.
56. Andrews S. FastQC: a quality control tool for high throughput sequence data; 2010.
57. Catchen JM, Amores A, Hohenlohe P, Cresko W, Postlethwait JH, De Koning D-J. Stacks: building and genotyping loci de novo from short-read sequences. G3journall. 2011;1:171–82.
58. Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA. Stacks: an analysis tool set for population genomics. Mol Ecol. 2013;22:3124–40.
59. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, et al. The variant call format and VCFtools. Bioinformatics. 2011;27:2156–8.
60. Jombart T. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics. 2008;24:1403–5.
61. Jombart T, Devillard S, Balloux F. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet. 2010;11:1–15.
62. Malinsky M, Trucchi E, Lawson DJ, Falush D. RADpainter and fineRADstructure: population inference from RADseq data. Mol Biol Evol. 2018;35:1284–90.
63. Lawson DJ, Hellenthal G, Myers S, Falush D. Inference of population structure using dense haplotype data. PLOS Genet Public Library of Science. 2012;8:1–16.
64. Peakall R, Ruibal M, Lindenmayer DB, Url S. Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. Evolution (N Y). 2003;57:1182–95.
65. Vekemans X, Hardy OJ. New insights from fine-scale spatial genetic structure analyses in plant populations. Mol Ecol. 2004;13:921–35.
66. Peakall R, Smouse PE. GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. Mol Ecol Notes. 2006;6:288–95.
67. Foll M, Gaggiotti O. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. Genetics. 2008;180:977–93.
68. Narum SR, Hess JE. Comparison of FST outlier tests for SNP loci under selection. Mol Ecol Resour. 2011;11:184–94.
69. Pérez-Figueroa A, García-Pereira MJ, Saura M, Rolán-Alvarez E, Caballero A. Comparing three different methods to detect selective loci using dominant markers. J Evol Biol. 2010;23:2267–76.
70. Frichot E, Schoville SD, Bouchard G, François O. Testing for associations between loci and environmental gradients using latent factor mixed models. Mol Biol Evol. 2013;30:1687–99.
71. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A. 2003;100:9440–5.
72. Frichot E, François O. LEA: an R package for landscape and ecological association studies. Methods Ecol Evol. 2015;6:925–9.
73. Lamichhaney S, Fuentes-pardo AP, Rafati N, Ryman N, Mccracken GR. Parallel adaptive evolution of geographically distant herring populations

on both sides of the North Atlantic Ocean. Proc Natl Acad Sci. 2017; 114:3452–61.
74. van Zwieten PAM, Roest FC, Machiels MAM, van Densen WLT. Effects of inter-annual variability , seasonality and persistence on the perception of long-term trends in catch rates of the industrial pelagic purse-seine fishery of northern Lake Tanganyika (Burundi). Fish Res. 2002;54:329–48.
75. Phiri H, Shirakihara K. Distribution and seasonal movement of pelagic fish in southern Lake Tanganyika. Fish Res. 1999;41:63–71.
76. Sinclair MM, Illes TD. Population regulation and speciation in the oceans. J du Cons Int Explor la Mer. 1989;45:165–75.
77. Verheyen E, Rüber L, Snoeks J, Meyer A. Evolution on islands - Mitochondrial phylogeography of rock-dwelling cichlid fishes reveals evolutionary influence of historical lake level fluctuations of Lake Tanganyika, Africa. Philos Trans R Soc London Ser B Biol Sci. 1996;351:797 LP–805.
78. Koblmüller S, Salzburger W, Obermüller B, Eigner E, Sturmbauer C, Sefc KM. Separated by sand, fused by dropping water: habitat barriers and fluctuating water levels steer the evolution of rock-dwelling cichlid populations in Lake Tanganyika. Mol Ecol. 2011;20:2272–90.
79. Sturmbauer C, Baric S, Salzburger W, Rüber L, Verheyen E. Lake level fluctuations synchronize genetic divergences of cichlid fishes in African lakes. Mol Biol Evol. 2001;18:144–54.
80. Koblmüller S, Odhiambo EA, Sinyinza D, Sturmbauer C, Sefc KM. Big fish, little divergence: phylogeography of Lake Tanganyika's giant cichlid, *Boulengerochromis microlepis*. Hydrobiologia. 2015;748:29–38.
81. Koblmüller S, Zangl L, Börger C, Daill D, Vanhove MPM, Sturmbauer C, et al. Only true pelagics mix: comparative phylogeography of Deepwater bathybatine cichlids from Lake Tanganyika. Hydrobiologia. 2018;3:1-11.
82. Whitehead PJP. FAO Species catalogue: Vol. 7 Clupeoid fishes of the world. FAO fish. synopsis. 1985.
83. Thompson AB. Simulation of reproductive rate, prey selection and the survival of pelagic fish of the African Great Lakes. Hydrobiologia. 1999;407:207–18.
84. Mulimbwa N, Shirakihara K. Growth, recruitment and reproduction of sardines (*Stolothrissa tanganicae* and *Limnothrissa miodon*) in northwester Lake Tanganyika. Tropics. 1994;4:57–67.
85. Kinsey ST, Orsoy T, Bert TM, Mahmoudi B. Population structure of the Spanish sardine *Sardinella aurita*: natural morphological variation in a genetically homogeneous population. Mar Biol. 1994;118:309–17.
86. García-Rodríguez FJ, García-Gasca SA, La C-AJD, Cota-Gómez VM. A study of the population structure of the Pacific sardine *Sardinops sagax* (Jenyns, 1842) in Mexico based on morphometric and genetic analyses. Fish Res. 2011;107:169–76.
87. Sebastian W, Sukumaran S, Zacharia PU, Gopalakrishnan A. Genetic population structure of Indian oil sardine, *Sardinella longiceps* assessed using microsatellite markers. Conserv Genet Springer Netherlands. 2017;18:951–64.
88. Atarhouch T, Rami M, Naciri M, Dakkak A. Genetic population structure of sardine (*Sardina pilchardus*) off Morocco detected with intron polymorphism (EPIC-PCR). Mar Biol. 2007;150:521–8.
89. Gonzalez EG, Zardoya R. Relative role of life-history traits and historical factors in shaping genetic population structure of sardines (*Sardina pilchardus*). BMC Evol Biol. 2007;7:197.
90. Limborg MT, Helyar SJ, De Bruyn M, Taylor MI, Nielsen EE, Ogden R, et al. Environmental selection on transcriptome-derived SNPs in a high gene flow marine fish, the Atlantic herring (*Clupea harengus*). Mol Ecol. 2012;21:3686–703.
91. Corander J, Majander KK, Cheng L, Merilä J. High degree of cryptic population differentiation in the Baltic Sea herring *Clupea harengus*. Mol Ecol. 2013;22:2931–40.
92. Mulimbwa N, Sarvala J, Micha J-C. The larval fishery on *Limnothrissa miodon* in the Congolese waters of Lake Tanganyika: impact on exploitable biomass and the value of the fishery. Fish Manag Ecol. 2018:1-7.
93. McLean KA, Byanaku A, Kubikonse A, Tshowe V, Katensi S, Lehman AG. Fishing with bed nets on Lake Tanganyika: a randomized survey. Malar J. 2014;13:395.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

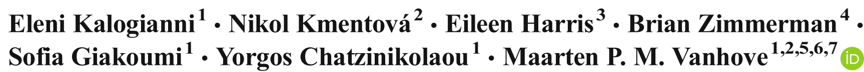
Kalogianni E., Kmentová N., Harris E., Zimmerman B., Giakoumi S., Chatzinikolaou Y., Vanhove M. P. M. 2017. Occurrence and effect of trematode metacercariae in two endangered killifishes from Greece. *Parasitology Research*: 116(11):3007-3018. DOI: 10.1007/s00436-017-5610-z [Q2, IF (2017) = 2.255].

Publication is available at: https://link.springer.com/article/10.1007%2Fs00436-017-5610-z

Parasitol Res (2017) 116:3007–3018
DOI 10.1007/s00436-017-5610-z

ORIGINAL PAPER

Occurrence and effect of trematode metacercariae in two endangered killifishes from Greece



Eleni Kalogianni[1] **· Nikol Kmentová**[2] **· Eileen Harris**[3] **· Brian Zimmerman**[4] **· Sofia Giakoumi**[1] **· Yorgos Chatzinikolaou**[1] **· Maarten P. M. Vanhove**[1,2,5,6,7]

Received: 1 July 2017 / Accepted: 4 September 2017 / Published online: 13 September 2017
© Springer-Verlag GmbH Germany 2017

Abstract We report digeneans (Diplostomidae, Crassiphialinae) in the endangered freshwater fishes *Valencia letourneuxi* and *Valencia robertae*, endemics of Western Greece. Digenean metacercariae occurred in two forms in the abdominal cavity, excysted and encysted, the latter attached to the gonads, liver and alimentary tract. Parasites were, using morphological and molecular techniques, identified as two representatives of Crassiphialinae, specifically part of the *Posthodiplostomum-Ornithodiplostomum* clade. The spatial, seasonal, and age class variation in parasite prevalence was examined. Autumn parasite prevalence varied between the six populations sampled (18.2 to 100%). Seasonal prevalence at the two sites sampled quadannually peaked in autumn and reached its lowest value in spring; prevalence increased with size to 100% in young adult fish. We did not find a correlation between prevalence and host sex. Overall parasites' weight averaged 0.64% of the host's, while parasite weight increased with host weight. A comparison of relative condition and hepatosomatic and gonadosomatic indices of infected and metacercariae-free specimens showed that infection did not have a significant effect on host body condition and reproduction. Regarding the parasite's life cycle, planorbid gastropods are proposed as potential first intermediate hosts in view of the host's diet and occurrence data of molluscs in the ecosystem. This is the first record of a diplostomid digenean in valenciid fishes and of representatives of the *Posthodiplostomum-Ornithodiplostomum* clade in a native Greek freshwater fish. Our findings are discussed in conjunction to fish conservation interventions, since parasites may contribute to the decline of endangered species.

Keywords Digenea · Parasite prevalence · Host body condition · Planorbidae · Platyhelminthes · Valenciidae

✉ Maarten P. M. Vanhove
maarten.vanhove@kuleuven.be

[1] Institute of Marine Biological Resources and Inland Waters, Hellenic Centre for Marine Research, 46.7 km Athinon – Souniou Av., P.O. Box 712, 190 13 Anavyssos, Greece

[2] Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

[3] Division of Parasites and Vectors, Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK

[4] Zoological Society of London, Regent's Park, London NW1 4RY, UK

[5] Laboratory of Biodiversity and Evolutionary Genomics, Department of Biology, University of Leuven, Ch. Deberiotstraat 32, 3000 Leuven, Belgium

[6] Capacities for Biodiversity and Sustainable Development, Operational Directorate Natural Environment, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1000 Brussels, Belgium

[7] Centre for Environmental Sciences, Research Group Zoology: Biodiversity and Toxicology, Hasselt University, Agoralaan Gebouw D, 3590 Diepenbeek, Belgium

Introduction

The critically endangered freshwater fish *Valencia letourneuxi* (Sauvage, 1880) (Valenciidae) is an endemic of Western Greece and southern Albania (Crivelli 2006). Its sister species, the recently described *Valencia robertae* Freyhof, Kärst and Geiger, 2014, endemic of Central Greece, encompasses the most southern populations previously included in *V. letourneuxi*. Both species are characterized by a fragmented geographical distribution, narrow ecological requirements, and low population densities (Barbieri et al. 2000; Kalogianni et al. 2010a). In the last 30 years, both species' geographical range has been reduced, with some populations now being extinct

and others in a precarious state (2005 survey data, Kalogianni et al. 2010b; see also Economidis 1991; Bianco et al. 1996; Barbieri et al. 2002a, b, 2015). Their decline has been attributed to the degradation of their habitats due to anthropogenic activities, as well as to predation and competition with the introduced Eastern mosquitofish *Gambusia holbrooki* Girard, 1859 (Bianco and Miller 1989; Barbieri et al. 2000, b; Kottelat and Freyhof 2007). The parasite fauna of these Greek killifishes has never been studied. Parasitization, however, of individuals of a native fish species, either by non-native parasites transmitted from introduced fish (Prenter et al. 2004) or by native parasites, can also potentially contribute to its decline, by influencing host behavior, survival, growth, and fecundity, as well as host population dynamics (Marcogliese 2004). Parasitic organisms are often neglected in the management and conservation of biological resources and ecosystems (Marcogliese 2004). Research on the parasites of endangered species can, however, provide information about their host organisms and the ecological interactions between these organisms (e.g., Whiteman et al. 2007). Parasite community composition can provide valuable information for the management and conservation of aquatic species and habitats, e.g., by contributing to understanding introduction routes (e.g., Huyse et al. 2015). Furthermore, the assemblage of parasites within a host organism potentially reflects that host's trophic position in the food web, as well as the presence in the ecosystem of various other organisms that participate in the life cycles of these parasites. Parasite populations and communities could also be useful indicators of environmental stress, such as eutrophication or acidification, as well as of biodiversity (Marcogliese 2005; Vidal-Martinez et al. 2010).

A preliminary examination of the abdominal cavity of *V. letourneuxi* and *V. robertae* revealed the presence of digenean trematode metacercariae, possibly belonging to Diplostomidae Poirier, 1886. Among parasites, trematodes are the dominant group that causes retarded growth, morbidity, and mortality, especially in juvenile fishes (Shareef and Abidi 2015). Digenean trematodes are widespread around the globe and are characterized by a complex life cycle, often involving a mollusk as first intermediate host; a fish, an amphibian, or occasionally a mammal as second intermediate host; and piscivorous birds or mammals as definitive hosts (Niewiadomska 2002; Cribb et al. 2003). Digeneans have been shown to induce behavioral changes in their fish secondary host, such as decreasing swimming performance (Coleman 1993) or decreasing predator avoidance (Poulin 1993) resulting in increased predation of the host (Ondráčková et al. 2006). They have also been shown to cause damage to fish host tissues resulting in blindness, inflammatory reactions and perforations in some cases (Sharriff et al. 1980; Niewiadomska 2002; Vianna et al. 2005), though there are also studies reporting no effect of digenean parasitism on fish host condition (e.g., Silva-Souza and Ludwig 2005).

In this study, we further examined the abovementioned metacercariae retrieved from *V. letourneuxi* and *V. robertae* in order to identify these parasites using morphological and molecular techniques; to examine the variation in prevalence between seasons, locations, and host sexes and size classes; and to assess the effect of the metacercariae on host condition and reproduction.

Materials and methods

Sampling methodology and phenotypic characterization of hosts and parasites

Samples were collected from six sites in Western Greece, in the autumn of 2005, 2006, and 2009; one site hosts *V. robertae* and the other five sites *V. letourneuxi* (Fig. 1 and Table 1). To explore seasonal variation in parasitization, seasonal samplings (July, September, January, May) were conducted at two sites, Mornos and Acheron (sites 4 and 5, hosting *V. robertae* and *V. letourneuxi* respectively, Table 1). All sampling sites were located at lowland semi-lotic streams or canals (elevation range 0–6 m), associated with springs, while distance from sea ranged from 0.4 to 13.5 km. Site depth ranged between 0.9 and 1.7 m and site width between 4 and 13 m. All sites were thickly vegetated (surface aquatic vegetation ranged between 40 and 95%). Salinity varied between 0.1 and 6.5 ppt and temperature between 14.5 and 25 °C.

This study was conducted within the frame of a wide scope research program during 2005–2009, targeting *V. letourneuxi* (*V. robertae* had not yet been described as a separate species at the time). This research included dietary studies, studies on the effect of *G. holbrooki* Girard, 1859 on *V. letourneuxi* and genetic studies. Fish were collected with a D-shaped net (2-mm mesh) from the stream banks at the six sites described above (Fig. 1 and Table 1). In five of the six sites sampled (sites 1, 2, 3, 5, and 6; Table 1), representatives of *Valencia* are found in association with the introduced *G. holbrooki* (Kalogianni et al. 2010a). After identification, a total of 296 specimens of *Valencia* were anesthetized with quinaldine and preserved in 4% formaldehyde for further laboratory analyses. In the laboratory, for each fish, total and standard length (TL and SL, nearest 0.1 mm) were measured, and total and net host weight, before and after evisceration respectively (HW and NW, nearest 0.01 mg) as well as liver and gonad weight (LW and GW, nearest 0.01 mg after blotting dry) were also recorded. External surfaces, viscera, and musculature of the fish were examined under a Olympus SZX7 stereo microscope for parasitic infection. Parasites were removed and blotted dry and their weight was recorded (PW, nearest 0.01 mg, weighed for all metacercariae of one host specimen together).

Parasite prevalence (number of infected fish per total fish examined) was calculated for all metacercariae together (i.e.,

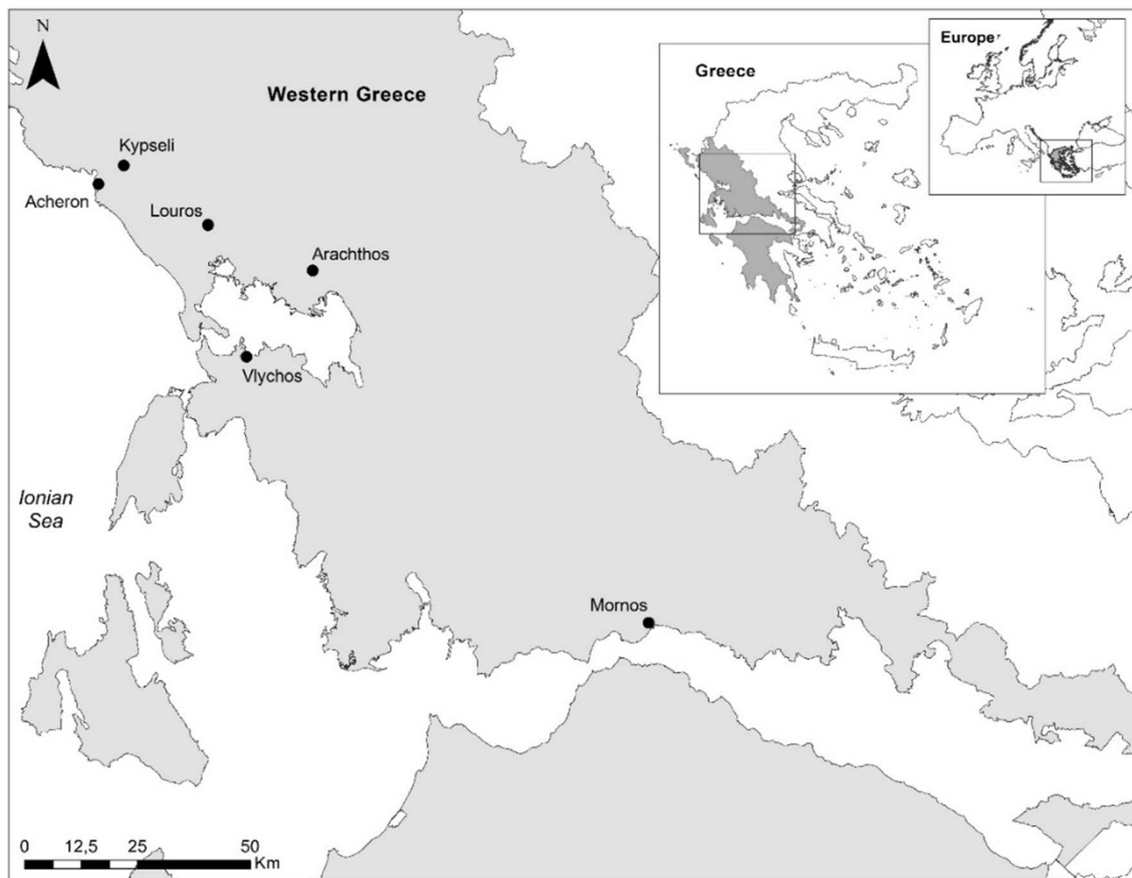


Fig. 1 Location of sampling sites in Western Greece. Mornos hosts *Valencia robertae*, while Vlychos, Arachthos, Louros, Kypseli, and Acheron host *V. letourneuxi*

no species-level morphological identification of the digeneans could be made and these prevalence values are hence not parasite species specific). Differences in parasite prevalence between sexes and 2 mm size classes (larval size range < 13 mm, juvenile range ≥ 13 and ≤ 17 mm, and adults > 17 mm; see Barbieri et al. 2000 and Kalogianni et al. 2010b) were examined pooling data from all the autumn samples. The effect of host sex on the prevalence of parasites was tested by applying chi-square test. Differences in mean standard length between infected and metacercariae-free specimens were examined using a *t* test. The relationship between total PW and HW was examined with Pearson's correlation.

Finally, ANCOVA was used to investigate potential effects of parasitization on the condition and reproduction of the female and male fish hosts, after calculation of the relative condition (NW/SL), the hepatosomatic (LW/NW), and the

Table 1 Features of the six sites sampled in autumn, number of fish examined, standard length range, and parasite prevalence (%) in the total sample, in females and males

Site	Date	Latitude	Longitude	Veg (%)	Temp (°C)	Salinity (ppt)	*N* fish	SL range (mm)	Parasite prevalence (%)		
									% total	% ♀	% ♂
1 Vlychos	Oct 2005	38° 54′ 54″ N	20° 52′ 32″ E	50	21.4	3.5	20	8.8–24.8	20.00	27.27	12.50
2 Louros	Oct 2005	39° 10′ 31″ N	20° 45′ 53″ E	95	15.8	0.2	16	9.7–24.8	50.00	33.33	71.43
3 Arachthos	Oct 2005	39° 05′ 30″ N	21° 02′ 13″ E	70	17.8	0.4	11	10.0–24.5	18.18	16.67	20.00
4 Mornos*	Sep 2006	38° 24′ 26″ N	21° 55′ 03″ E	80	25.0	0.3	23	16.5–56.0	100.00	100.00	100.00
5 Acheron*	Sep 2006	39° 14′ 55″ N	20° 28′ 50″ E	40	17.2	3.2	16	12.7–20.0	100.00	100.00	100.00
6 Kypseli	Sep 2009	39° 17′ 15″ N	20° 32′ 35″ E	60	16.9	0.1	9	7.0–29.2	55.56	40.00	75.00

The two sites that were also sampled seasonally are marked with an asterisk. Sites 1, 2, 3, 5, and 6 host *V. letourneuxi*; site 4 hosts *V. robertae*

Veg vegetation cover

gonadosomatic (GW/NW) indices of infected and metacercariae-free specimens. Fish standard length was used as a covariate to account for possible size effects. Prior to statistical analysis, values of the above indices were log10 transformed. ANCOVA was conducted with the PASW 17 software.

Parasite identification

Parasite identification was done using combined morphological and molecular methods. The digenean metacercariae, excysted when necessary, were stained using paracarmine, dehydrated through a series of alcohols, and cleared in beechwood creosote. They were mounted in Canada balsam on glass microscope slides and examined under a Olympus BH2 high-power microscope with interference phase.

Additional samples, used for the genetic characterization of the parasites, were collected from the Acheron site (site 5) in June 2008. We extracted DNA from individual (artificially or naturally) excysted metacercariae found in the abdominal cavity with the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's instructions. Polymerase Chain Reaction was performed using a GeneAmp PCR system 9700 thermocycler (Applied Biosystems) and Illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare), adding 1 μL of each primer (20 μM) (Sigma-Aldrich), 2 μL of template DNA and 21 μL of double-distilled, autoclaved, and filter-sterilized water, for a total reaction volume of 25 μL. We amplified fragments of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene and of the nuclear rDNA region (ITS-1, 5.8, ITS-2). Primer combinations were MplatCOX1dF (5′-TGTAAAACGACGGCCAGTTT WCITTRGATCATAAG-3′) and MplatCOX1dR (5′-CAGG AAACAGCTATGACTGAAAYAAYAIIGGATCICCACC-3′) (Moszczynska et al. 2009) for COI and the combinations of D1 (5′-AGGAATTCCTGGTAAGTGCAAG-3′) with D2 (5′-CGTTACTGAGGGAATCCTGG-3′) (Hillis and Dixon 1991) and 81_f (5′-GTAACAAGGTTTCCGTAGGTGAA-3′) (Gustinelli et al. 2010) with ITS2.S_r (5′-CCTG GTTAGTTTCTTTTCCTCCGC-3′) (Cribb et al. 1998) for rDNA. These regions (ITS-1, 5.8 and ITS-2) are commonly used for species identification in flatworms (Vanhove et al. 2013; Stoyanov et al. 2017). After an initial denaturation of 2 min at 94 °C, samples were subjected to 35 cycles (40 for 81f–ITS2sr) of 30 s at 94 °C, 30 s (40 s for 81f–ITS2sr) at 50 °C, and 60 s (90s for 81f–ITS2sr) at 72 °C. After a final elongation of 10 min (5 min for 81f–ITS2sr) at 72 °C, samples were cooled to 4 °C. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) following the manufacturer's protocol. Sequencing of both strands was carried out using the same primers as above with an Applied Biosystems 3730 DNA analyzer and BigDye version 1.1. Sequences were deposited in NCBI GenBank under accession numbers KY320571-3. Voucher specimens for the genetically characterized parasite population were deposited in the Natural History Museum (London, United Kingdom) (NHMUK 2015.12.2.1) (parasite) and the Natural History Museum Rijeka (Croatia) (PMR VP 3140-2) (host). Sequences were visually corrected and aligned in MEGA v6 (Tamura et al. 2013) with the MUSCLE algorithm and UPGMB clustering method (Edgar 2004) under default conditions. The best fitting substitution model describing molecular evolution of the sequences was selected by TOPALi v2.5 (Milne et al. 2009) based on the Bayesian information criterion. The GTR model (Rodríguez et al. 1990) was used for the rDNA region. Pairwise deletion was used to construct a distance matrix. The phylogenetic position of the collected parasite haplotypes within Diplostomidae (GenBank accession numbers in Table 2, representatives of available diplostomid genera were selected) was inferred based on the rDNA combining the results of a maximum likelihood tree search performed in RAxML 8.7.4. (Stamatakis 2014) with bootstrap values calculated using 1000 replicates and Bayesian interference performed in MrBayes 3.2 (Ronquist et al. 2011). Posterior probabilities were approximated for 10,000,000 generations, sampled at each 1000th generation and with a burn-in of 10% in two separate runs. Chain stationarity and parameter convergence were checked in Tracer 1.6 (Rambaut et al. 2014). Because of its position in a different but related digenean family (Clinostomidae), *Clinostomum complanatum* (Rudoplhi, 1814) collected from *Barbus barbus* (L.) was used as an out-group. File conversion was carried out using ALTER (Glez-Peña et al. 2010). Phylogenetic trees were rendered by FigTree 1.4.2 (Rambaut and Drummond 2009) and edited in Adobe Photoshop CS6.

Results

Parasite identification

Digenean metacercariae were found in the abdominal cavity of *V. letourneuxi* and *V. robertae*, while isolated cysts were also found in some fishes beneath the lens of the eye. Parasites were identified as members of Diplostomidae, subfamily Crassiphialinae. This was based on the morphological characters of the "neascus" (a characteristic type of diplostomid metacercariae, see Niewiadomska 2002), such as the presence of a bipartite body, with a reserve bladder consisting of a ramified median and two lateral canals forming a net-like structure in the forebody, and a developed hindbody with unconnected excretory canals. Since only immature gonads were present, further identification to genus or species level was not possible, because the classification is based on the size of testes and the absence/presence of an ejaculatory pouch (Niewiadomska 2002).

For the genetic identification of the parasites, sequences from nine metacercariae (only specimens from the abdominal cavity were available for molecular work) were obtained and

Table 2 List of digenean species (Diplostomatidae) obtained from GenBank with their accession numbers for the ITS1-5.8S-ITS2 rDNA region and for the COI sequences retrieved, their host species and the country where the species were collected

Parasite species	Host species	Country	ITS1-5.8S-ITS2 rDNA	COI mtDNA
Alaria mustelae Bosma, 1931	*Mustela frenata* Lichtenstein, 1831	USA	JF820609.1	KT254032.1
Austrodiplostomum ostrowskiae Dronen, 2009	*Dorosoma cepedianum* (Lesueur, 1818)	USA	KT728782.1	KR271028.1
Bolbophorus confusus (Krause, 1914)	*Pelecanus onocrotalus* Linné, 1758	Israel	AY242851.1	–
Clinostomum complanatum Rudolphi, 1814	*Triturus carnifex* (Laurenti, 1768)	Italy	KM518257.1	JF718595.1
Diplostomum mergi Dubois, 1932	*Radix auricularia* Linnaeus, 1758	Czech Republic	KR149499.1	KR149528.1
Diplostomum paracaudum (Iles, 1959)	*Gadus morhua* Linnaeus, 1758	Denmark/Germany	KJ889013.1	JQ639176.1
Diplostomum spathaceum Rudolphi, 1819	*Larus ridibundus* Linné, 1766	Czech Republic	KR269765.1	JX986895.1
Ornithodiplostomum sp. 1 Dubois, 1936	*Percina caprodes* (*Rafinesque*, *1818*)	Canada	HM064937.1	HM064748.1
Ornithodiplostomum sp. 2 Dubois, 1936	*Notemigonus crysoleucas* (Mitchill, 1814)	Canada	HM064939.1	HM064764.1
Ornithodiplostomum sp. 3 Dubois, 1936	*Pimephales promelas* (Rafinesque, 1820)	Canada	HM064942.1	HM064784.1
Ornithodiplostomum sp. 4 Dubois, 1936	*Pimephales promelas*	Canada	HM064945.1	HM064785.1
Ornithodiplostomum sp. 5 Dubois, 1936	*Notemigonus crysoleucas* (Mitchill, 1814)	Canada	FJ469595.1	KT831368.1
Ornithodiplostomum sp. 6 Dubois, 1936	*Pimephales promelas* (Rafinesque, 1820)	Canada	HM064946.1	HM064790.1
Ornithodiplostomum scardinii (Schulman in Dubinin, 1952)	*Scardinius erythrophthalmus* (Linnaeus, 1758)	Czech Republic	KX931443.1	KX931425.1
Posthodiplostomum brevicaudatum 1 (von Nordmann, 1832)	*Perca fluviatilis* (Linnaeus, 1758)	Czech Republic	KX931428.1	KX931418.1
Posthodiplostomum brevicaudatum 2	*Gasterosteus aculeatus* (Linnaeus, 1758)	Bulgaria	KX931429.1	KX931419.1
Posthodiplostomum centrarchi 1 (Hoffman, 1958)	*Lepomis gibbosus* (Linnaeus, 1758)	Bulgaria	KX931441.1	KX931421.1
Posthodiplostomum centrarchi 2	*Lepomis gibbosus*	Slovakia	KX931442.1	KX931423.1
Posthodiplostomum sp. 1 Dubois, 1936	*Channa punctata* (Bloch, 1793)	India/Canada	KF738447.1	HM064795.1
Posthodiplostomum sp. 2 Dubois, 1936	*Channa argus* (Cantor, 1842)	Japan/Canada	AB693170.1	HM064798.1
Posthodiplostomum sp. 3 Dubois, 1936	*Lepomis gibbosus*	Canada	HM064957.1	HM064821.1
Posthodiplostomum sp. 4 Dubois, 1936	*Morone americana* (Gmelin, 1789)	Canada	HM064960.1	HM064844.1
Posthodiplostomum sp. 5 Dubois, 1936	*Lepomis gibbosus*	Canada	HM064958.1	HM064857.1
Posthodiplostomum sp. 6 Dubois, 1936	*Micropterus salmoides* Lacepède, 1802	Canada	HM064962.1	HM064864.1
Posthodiplostomum sp. 7 Dubois, 1936	*Perca flavescens* Mitchill, 1814	Canada	HM064961.1	HM064865.1
Tetracotyle xenentodoni Chakrabarti, 1970	*Tetracotyle xenentodoni* (Hamilton, 1822)	India/Canada	KU316948.1	HM064876.1
Tylodelphys aztecae García-Varela, Sereno-Uribe, Pinacho-Pinacho, Hernández-Cruz, Pérez-Ponce de León, 2015	*Podilymbus podiceps* (Linnaeus, 1758)	Mexico	KT175388.1	KT175369.1
Tylodelphys mashonensis (Beverley-Burton, 1963)	*Clarias gariepinus* Burchell, 1822	Tanzania	KC685363.1	KR863382.1

two different rDNA haplotypes were recorded, with a length of 1159 base pairs and a pairwise difference of 4.8%. These haplotypes did not correspond with the distinction between encysted and excysted metacercariae. Only one corresponding COI haplotype was recorded with a length of 531 base pairs, due to low amplification success. The pairwise distances in the entire dataset ranged from 0.5 to 37.8% in the rDNA regions and from 0.5 to 33.2% in the COI region (pairwise deletion). Phylogenetic analyses of rDNA did not cluster the two haplotypes of metacercariae infecting Greek killifishes together. Genetic distances between both haplotypes surpassed those between other sequences considered to belong to different species. This indicates the presence of two parasite species. They clustered with representatives of *Posthodiplostomum* Dubois, 1936 and *Ornithodiplostomum* Dubois, 1936 placed among other basal lineages of this clade. Both methods produced the same tree topology for rDNA (Fig. 2). The analyses confirmed the previously observed polyphyly of *Posthodiplostomum*.

Host-parasite ecology

Metacercariae occurred in two forms (encysted and excysted; Fig. 3), the encysted form usually in groups attached to the

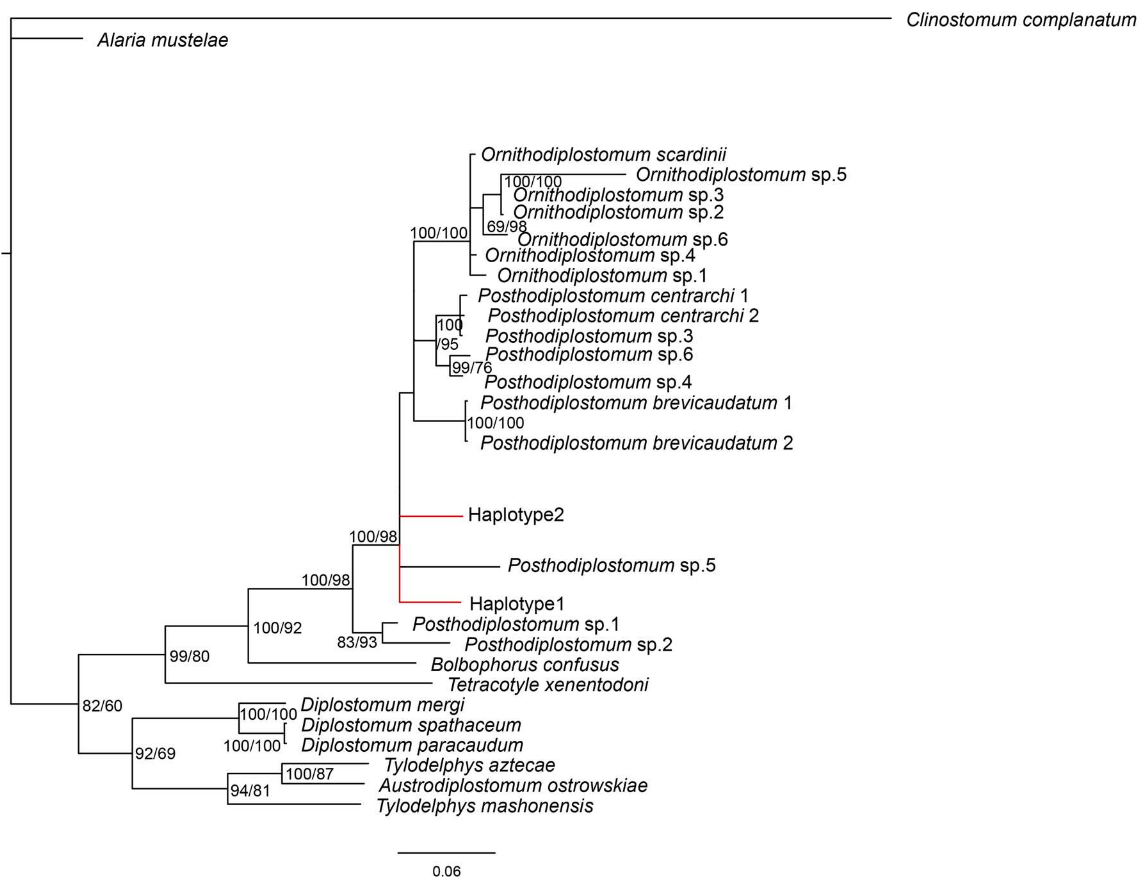


Fig. 2 Phylogenetic tree based on rDNA fragments from 29 haplotypes of Diplostomatidae. Posterior probabilities for Bayesian inference, (before slash) and bootstrap percentages for maximum likelihood (behind slash) are shown. Clades that neither yield a support value higher than 80 nor of 50 under BI or ML, respectively, are collapsed. The haplotypes obtained in this study are called haplotypes 1 and 2. Branch lengths show the number of expected nucleotide substitutions per site under BI

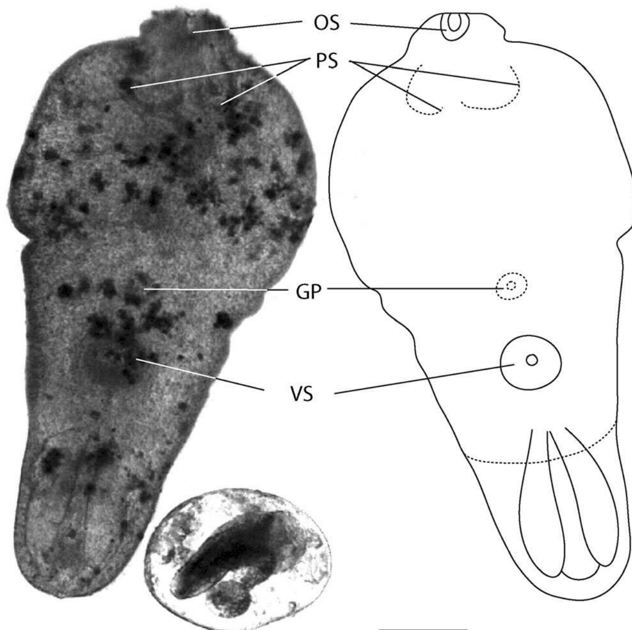


Fig. 3 Encysted and excysted metacercariae of diplostomatid digenean from the abdominal cavity of *V. letourneuxi* and *V. robertae* (scale bar 250 μm), OS—oral sucker; PS—pseudosuckers; GP—genital pore; VS—ventral sucker

anterior end of the gonad, adjacent to the mesentery, and at the area between the liver and the digestive tract, with parasites varying in size. Of a total of 296 fishes, 219 were found to be parasitized, corresponding to a high overall metacercaria prevalence of 73.99% (not separated between parasite forms).

Parasite prevalence, in the six *Valencia* populations studied, varied in autumn from 18.18 to 100% (Table 1; maximum prevalence was 100% for both *V. robertae* and *V. letourneuxi*). Seasonal prevalence remained high throughout the year at the two sites sampled quadannually (Table 1) ranging between 74.3 and 100% (Fig. 4). In these two sites, prevalence peaked in autumn with all fish being parasitized (100%) and then decreased in the winter to 82.3%, due to the appearance of a group of metacercariae-free fish, ranging in size from 13 to 36 mm SL (juveniles and adults, see Kalogianni et al. 2010b). In the spring, prevalence further decreased to 74.3%, reaching its lowest value. Parasite prevalence in function of the host's sex (ratio 5:4 in favor of females), was 52.88 and 63.15% in host females ($n = 52$) and males ($n = 42$), respectively, but this difference was not statistically significant ($\chi^2 = 0.792$; $df = 1$; $P = 0.374$).

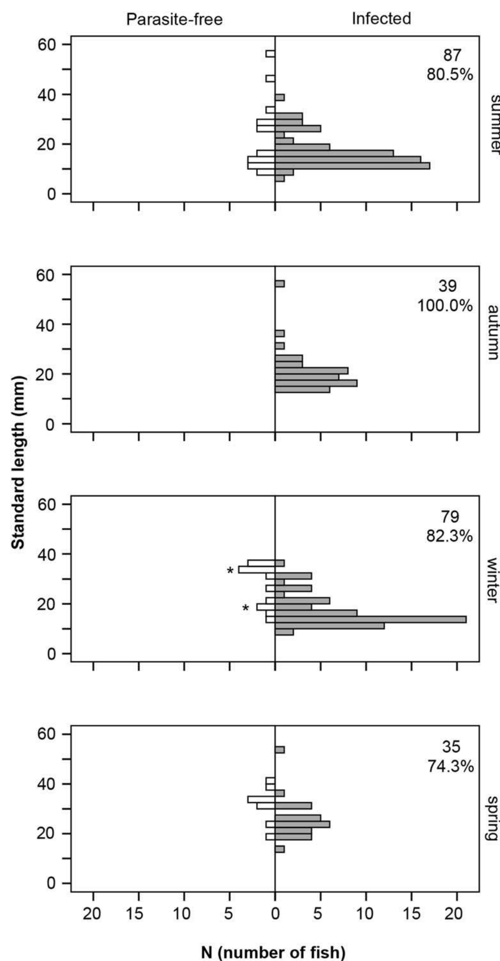


Fig. 4 Seasonal variation of the size frequency distribution of infected and metacercariae-free specimens in Mornos and Acheron habitats that host *V. robertae* and *V. letourneuxi*, respectively. Number of specimens and parasite prevalence values (%) are also shown. The two groups of metacercariae-free fish of the winter sample are marked with asterisks

Parasite prevalence increased gradually with host size (SL, Fig. 5), from a prevalence of 0% for the larval length classes 6–8 and 8–10 mm to a maximum prevalence value of 100% in the 22–24 mm adult length class (larval size range < 13 mm, juvenile range ≥ 13 and ≤ 17 mm, and adults > 17 mm, see Barbieri et al. 2000 and Kalogianni et al. 2010b). For fish lengths > 24 mm, these prevalence values remained stable, with one exception for the adult length class 24–26 mm (prevalence 75%; Fig. 5). Mean SL values for infected and metacercariae-free fish differed, i.e., mean SL of infected fish was 19.70 ± 0.92 S.E. ($n = 58$); mean SL of metacercariae-free fish was 13.64 ± 0.55 S.E. ($n = 36$); this difference was statistically significant ($t = -4.846$, $p < 0.0001$).

Mean parasite-host weight ratio was 0.0064 ± 0.0010 S.E., with parasite weight averaging 0.64% of the host weight. Parasite weight and host weight were positively correlated ($n = 9$, rho correlation = 0.882, $p = 0.002$; see Fig. 6) for fish < 600 mg, with a mean parasite-host weight ratio of 0.0076 ± 0.0009 S.E. and percentage mean 0.76%; however, there was no correlation for the larger specimens (mean ± S.E. parasite-host weight ratio of 0.0009 ± 0.0007 and percentage mean 0.09%, $n = 2$).

The study of the condition, gonadosomatic and hepatosomatic indices, showed no statistically significant differences between infected and metacercariae-free specimens, in either males or females ($p > 0.05$).

Discussion

Parasite identification

This study is the first record of valenciids as second intermediate hosts for diplostomid parasites, being heavily infected by their metarcercariae. No other endoparasites were found in this study. (Dactylogyridean monogenean gill parasites were also found—at very low prevalence and infection intensity—but fall outside of the scope of this study.) Dominance of (immature) endoparasites in general, and of trematode metacercariae in particular, in killifishes has been previously observed. It was suggested to demonstrate the importance of these fishes as intermediate or paratenic hosts (Nezhybová et al. 2017). Pairwise uncorrected genetic distances between the retrieved rDNA haplotypes (4.8%) suggest the existence of two different diplostomid species (Georgieva et al. 2013; Stoyanov et al. 2017). Unfortunately, the metacercarial lifestage does not allow morphology-based species-level distinction. Therefore, parasite haplotypes could not be linked with specific phenotypic characters; neither was there a link with being encysted or not. The collected parasite species are considered as representatives of *Posthodiplostomum* or *Ornithodiplostomum* based on the results of phylogenetic analysis (Fig. 2). Interestingly, haplotype 1 takes a basal position within the *Posthodiplostomum-Ornithodiplostomum* clade and most likely represents a hitherto unsequenced phylogenetic lineage. Low maximum likelihood bootstrap values of deeper nodes compared to the Bayesian inference posterior probabilities highlight the unresolved position of several diplostomid genera and an insufficient number of already published sequences. Moreover, the tree also indicates that *Posthodiplostomum* is not monophyletic and in need of revision (Locke et al. 2010b; Athokpam and Tandon 2014; García-Varela et al. 2016). However, according to Stoyanov et al. (2017), the uncertain phylogenetic positions of *Posthodiplostomum* species is often the result of incorrect morphological identification due to low quality of specimens, creating confusion even in available molecular data. Although previous studies based on barcoding approaches reported cryptic diversity in the family (Locke et al. 2010a, b; Georgieva et al. 2013), complicated morphological identification and the lack of information about adult stages makes formal species description challenging as it depends on high

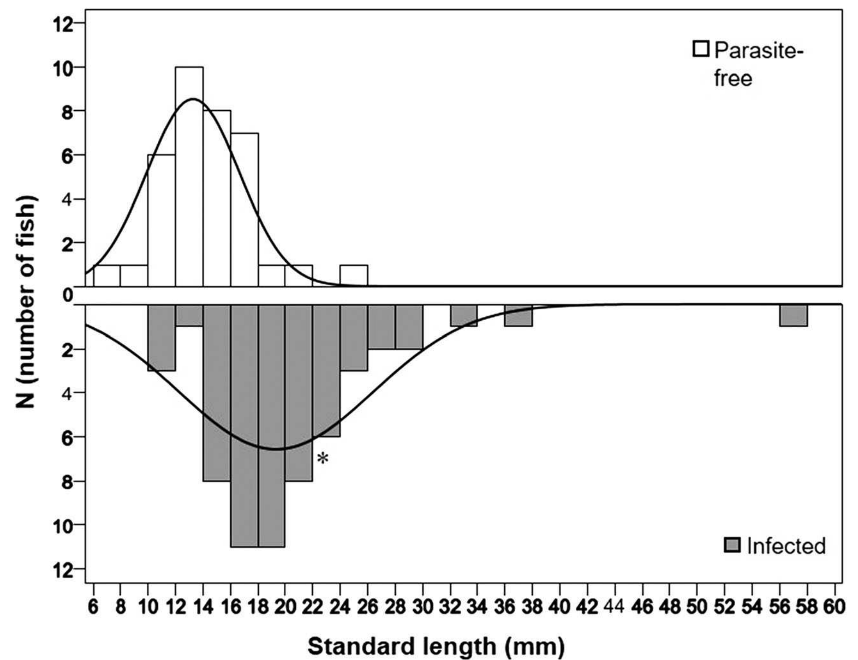


Fig. 5 Size frequency distribution of metacercariae-free and infected *V. robertae* and *V. letourneuxi* specimens of the autumn samplings ($n = 94$). Maximum parasite prevalence (100%) was first observed at the 22–24 mm length class (marked with asterisk)

quality stained material. Four species of *Posthodiplostomum* (*P. brevicaudatum* (von Nordmann, 1832); *P. centrarchi* Hoffman, 1958; *P. cuticola* (von Nordmann, 1832); *P. minimum* (MacCallum, 1921)) and one of *Ornithodiplostomum* (*O. scardinii* (Schulman in Dubinin, 1952)) respectively, have been reported from more than 70 freshwater fish hosts, mostly cyprinids and cobitids, in Europe (Sonin 1986; Stoyanov et al. 2017). To the best of our knowledge, this is the first report of a freshwater species native to Greece found infected by either of both abovementioned diplostomid genera (see, e.g., Stoyanov et al. 2017). Our results enrich the list of the known digenean fauna in Greece, following the record of *Diplostomum spathaceum* (Rudolphi, 1819) from ten different fish host species in lakes Volvi and Vistonis (Kalfa-Papaioannou and Sinis 1985).

Host-parasite ecology

Within the framework of this study, differences were found in the prevalence of the reported diplostomid infection of fish between the various *Valencia* populations studied. These cannot probably be attributed to variation in habitat features, such

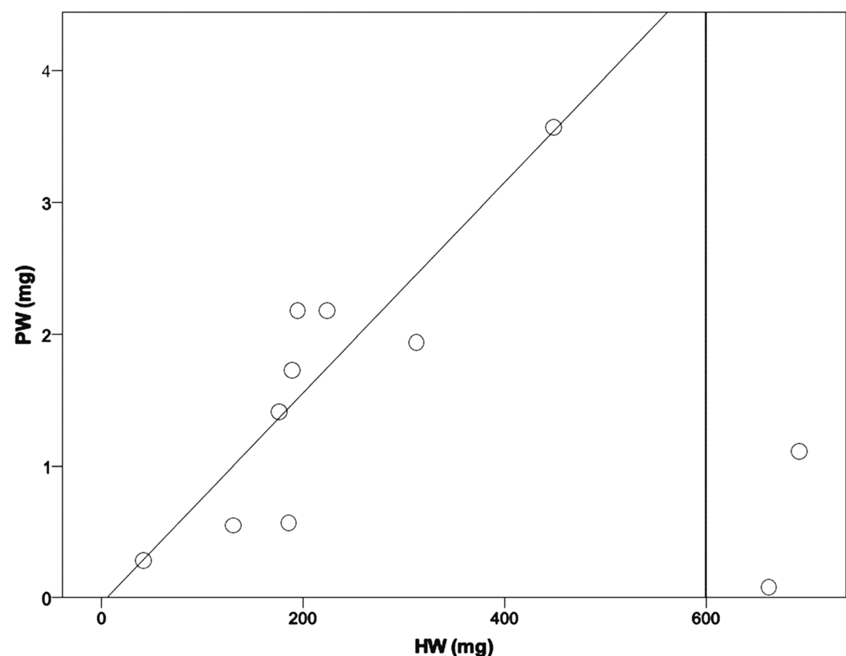


Fig. 6 Parasite weight (PW) and host weight (HW, i.e., total fish host weight) positive relationship for fish < 600 mg

as temperature, salinity, or surface vegetation cover, as all the habitats of the target species are spring-fed streams and wetlands that are rather stable both hydrologically and thermally, nor to a variation of fish population densities, evident from data published elsewhere (Kalogianni et al. 2010a). Therefore, we assume that this spatial variation in the parasite prevalence could be related to a spatial variation of the primary host (gastropod) densities, as well as of that of the definitive host. Parasite prevalence also remained high throughout the year, a range similar to that reported elsewhere for *Posthodiplostomum cuticula* (Ondráčková et al. 2004a) or other digeneans (Mbokane et al. 2015; Kondo et al. 2016). Maximum prevalence (100%) was observed in autumn, and then prevalence decreased in winter, due to the presence of both juvenile and adult metacercariae-free fish. This trend, common to both species of *Valencia*, is thought to be attributed to water temperature as an important factor for the emergence of cercariae from the snail, corresponding to the highest propagation of second intermediate hosts (Chubb 1979; Ondráčková et al. 2004b). Alternatively, the metacercariae-free juvenile fish in the winter samples could be the product of late recruitment at the end of autumn (the reproductive period of both target species extends to late October, see Barbieri et al. 2000; Kalogianni et al. 2010a) not yet parasitized due to limited exposure time. The presence of metacercariae-free mature adults (over 30 mm) in the winter samples of both species, on the other hand, could be attributed to parasite mortality induced by an adaptive immune response of the fish host (for a review of immune responses induced in teleost fish by digenean metacercariae, see Alvarez-Pellitero 2008). Parasite prevalence may also be related to gastropod availability that is at its lowest in winter and spring, as it has been shown in a seasonal dietary study on *V. letourneuxi* that also included benthic data (Kalogianni et al. 2010b).

The linear correlation between parasite weight and host weight indicates that infection occurs early in the life of the fish and then the parasites grow with the host and/or fish accumulate metacercariae as they grow. The positive relation between the length of the host and the prevalence of the parasite observed in the target species is most likely a result of temporal accumulation of parasitization, as larger fish could be exposed repeatedly to infection for a longer time than younger fish (Saad-Fares and Combes 1992; Paes et al. 2010). This is also supported by the presence of different stages of digenean metacercariae in the target species, as suggested also in various cyprinids or cobitids (Ondráčková et al. 2004a). Finally, there were no significant differences in the susceptibility of infection between males and females, as reported also for other freshwater fish species infected with diplostomid digeneans (Flores and Semenas 2002; Machado et al. 2005). Digenean metacercariae have been also found, in various freshwater species, subcutaneously in the trunk region and head, fins, gills, eyes, and muscle tissue, as well as in viscera (Sonin 1986; Niewiadomska 2002). In the two host species of this study, metacercariae were found mostly in the visceral cavity, in association with the gonads and the digestive tract and liver, but also beneath the lens of the eye. Hence, it seems a systemic infection. This distribution of the parasite therefore corresponds with previous studies and it is correlated with the high infection level reported (Kvach et al. 2017).

Previous studies have shown that the natural definitive hosts of *Posthodiplostomum* and *Ornithodiplostomum* are piscivorous birds, with planorbid or lymnaeid gastropods as the most common first intermediate hosts (Miller 1954; Niewiadomska 2002; Faltýnková et al. 2008; Nguyen et al. 2012). Planorbids and lymnaeids were the only gastropods that were found at both Louros, Mornos, and Acheron *Valencia* habitats during a benthic faunal study conducted in summer 2009 (unpublished data). However, only planorbid availability reflected the variation of parasite prevalence between these three populations, with the Louros habitat having both lower planorbid availability and lower parasite prevalence. Furthermore, benthic macroinvertebrate data collected seasonally at the Mornos habitat to assess food availability for a dietary study on *V. robertae* (then *V. letourneuxi*) showed that planorbids were the only gastropod taxon available throughout the year (Kalogianni et al. 2010b). In addition, that study showed that the target species consumed only three gastropod prey categories (Planorbidae, Valvatidae, and Physidae), with Lymnaeidae being absent from its diet and Planorbidae being the most frequent and abundant gastropod in the species diet. Since the planorbids reflect variation in metacercariae prevalence and figure as preferable prey of valenciid fishes, we assume high contact rates. We therefore suggest planorbids as the first intermediate host of the collected digenean species.

Parasite effects on the host species and conservation implications

The results of the current study show that there was no correlation between the diplostomid infection of the target species and the condition and reproduction of the hosts, as reported also elsewhere (Paes et al. 2010; Gholami et al. 2011), though there are studies that have shown a negative correlation between the abundance of *Posthodiplostomum* sp. and the relative condition factor of its hosts (Lucký 1970).

The absence of any detectable differences on fish condition and reproduction between infected and metacercariae-free *Valencia* specimens leads us to tentatively assume that this endoparasite is not pathogenic to its host. Given the short lifespan of the target species (2 or 3 years in the wild, Barbieri et al. 2002b) and the fact that trematode species may live for more than a year in the fish host and even for the whole lifespan of the host (Kalantan et al. 1987; Dias et al. 2006), it appears that this host-parasite relationship bears the characteristics of a strategy in which the parasite does not

affect fish survival, fitness, and reproduction. However, the observed eye infection by diplostomid metacercariae could affect fish vision and thus increase its predation by birds, as reported for other diplostomids (Seppälä et al. 2005).

Parasites are a potential risk factor in conservation initiatives targeting native species, such as population enhancement, assisted migration, or reintroduction actions. Digenean colonization of non-native areas depends on the strategy of larval stages, highly productive asexual reproduction, host specificity, level of virulence in intermediate hosts, and measure of similarity of environmental conditions between source or recipient localities (see Bauer 1991; Kennedy 1993). Based on the enemy release hypothesis, introduced endangered host species could profit of parasite loss (Genner et al. 2008) or be affected by spill-back of parasites from alien hosts in an introduction locality (McCallum and Dobson 1995; Daszak et al. 2000; Holt et al. 2003). On the other hand, a scenario suggesting a greater pathogenetic effect of co-introduced parasites on native hosts was also documented (naïve host hypothesis) (Anderson and May 1992; McCallum and Dobson 1995; Hudson et al. 1998). In this respect, translocations of endangered species to reestablish or to help recover populations could introduce parasites, harmless to the reintroduced population, but pathogenic to the already present naïve conspecifics or other sympatric species (see Daszak et al. 2000; Britt et al. 2004). Therefore, even the seemingly harmless diplostomid digeneans reported here should be considered carefully in the context of the conservation-related release or translocation of *Valencia* populations. Finally, no specimens of the introduced mosquitofish *G. holbrooki*, examined in two water systems where the species is sympatric with *Valencia* species (Acheron and Louros, unpublished data), were found infected by the same diplostomid metacercariae. This indicates that the parasites were not introduced locally through the mosquitofish nor that it could pose a threat to the native species, acting as a reservoir for these parasites. A similar absence of metacercariae of digenean parasites has been also reported in the only available study on mosquitofish parasites in Europe, namely in *G. holbrooki* from eight Mediterranean river mouths in Spain and France (Benejam et al. 2009).

Conclusion

This study showed no negative effect of metacercaria infection on *Valencia* species. Furthermore, seasonal differences in digenean prevalence and parasite accumulation over fish age were documented, with no differences in infection parameters between host sexes. It also confirmed the need for a revision of the complicated taxonomy of diplostomids and their unresolved phylogenetic classification. While we suggest planorbids to be potential first intermediate hosts, further investigations reconstructing the life cycle of the here reported parasites are required, in order to understand the ecological parameters of infection of their secondary host, as well as to identify the other host taxa. Such information is important to the understanding of parasite-host interactions, as well as to the planning or implementation of appropriate conservation measures for the endangered fish species, targeted in this study, as well as other vulnerable fish hosts of these parasites.

Acknowledgements This research was conducted in the frame of a wider study on the ecology, biology, genetics, and diet of *V. letourneuxi* undertaken by scientists of HCMR and was partially funded by the European Union of Aquarium Curators (EUAC) in the frame of the project "Rapid Assessment of the Status of *Valencia letourneuxi*, the Greek Killifish" (2005-2009). This research received support from the SYNTHESYS Project (http://www.synthesys.info/) (GB-TAF-2984) which is financed by European Community Research Infrastructure Action under the FP7 Integrating Activities Programme. N.K. and M. P.M.V. are supported by the Czech Science Foundation [P505/12/G112 (ECIP)]. The authors wish to thank Andrea Waeschenbach and D. Tim J. Littlewood for the advice regarding the molecular work, Rod A. Bray for the advice regarding parasite identification, the staff of the Wolfson Wellcome Biomedical Laboratories at the Natural History Museum for their technical support, Nicholas Koutsikos for producing the map with the sampling sites' location, and Marcelo Kovačić for curatorial services. For this study, HCMR had secured all necessary permits for fish collection from the Greek Ministry of Environment, Energy and Climate Change (permit numbers 97429/4350 and 85404/130).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

Alvarez-Pellitero P (2008) Fish immunity and parasite infections: from innate immunity to immunoprophylactic prospects. Vet Immunol Immunopathol 126(3–4):171–198

Anderson RM, May RM (1992) Infectious diseases of humans: dynamics and control. Oxford University Press, Oxford

Athokpam VD, Tandon V (2014) Morphological and molecular characterization of *Posthodiplostomum* sp. (Digenea: Diplostomidae) metacercaria in the muscles of snakeheads (*Channa punctata*) from Manipur, India. Helminthologia 51(2):141–152

Barbieri R, Daoulas C, Psarras T, Stoumboudi MT, Economou AN (2000) The biology and ecology of *Valencia letourneuxi* Sauvage, 1880 (Valenciidae)—prospects for conservation. Mediterr Mar Sci 1:75–90

Barbieri R, Economou AN, Stoumboudi MT, Economidis PS (2002a) Freshwater fishes of Peloponnese (Greece): distribution, ecology and threats. In: Collares-Pereira MJ, Cowx IG, Coelho MM (eds) Conservation of freshwater fishes: options for the future. Fishing News Books, Blackwell Science, Oxford, pp 55–64

Barbieri R, Stoumboudi MT, Economou AN (2002b) Threatened fishes of the world: *Valencia letourneuxi* Sauvage, 1880 (Valenciidae). Environ Biol Fish 65:46

Barbieri R, Zogaris S, Kalogianni E, Stoumboudi M, Chatzinikolaou Y, Giakoumi S, Kapakos Y, Kommatas D, Koutsikos N, Tachos V, Vardakas L, Economou AN (2015) Freshwater fishes and lampreys

of Greece: an annotated checklist. HCMR Monographs on Marine Sciences 8, Hellenic Centre for Marine Research, Athens

Bauer ON (1991) Spread of parasites and diseases of aquatic organisms by acclimatization: a short review. J Fish Biol 39:679–686

Benejam L, Alcaraz C, Sasal P, Simon-Levert G, García-Berthou E (2009) Life history and parasites of an invasive fish (*Gambusia holbrooki*) along a latitudinal gradient. Biol Invasions 11:2265–2277

Bianco PG, Miller RR (1989) First record of *Valencia letourneuxi* (Sauvage, 1880) in Peloponnese (Greece) and remarks on the Mediterranean family Valenciidae (Cyprinodontiformes). Cybium 13:385–387

Bianco PG, Ahnelt H, Economidis P (1996) The freshwater fishes from eastern, and large Mediterranean islands with comments on their safety status. Acta Univ Carol Biol 40:45–60

Britt A, Welch C, Katz A, Iambana B, Porton I, Junge R, Crawford G, Williams C, Haring D (2004) The re-stocking of captive-bred ruffed lemurs (*Varecia variegata variegata*) into the Betampona Reserve, Madagascar: methodology and recommendations. Biodivers Conserv 13(3):635–657

Chubb JC (1979) Seasonal occurrence of helminths in freshwater fishes. Part II. Trematoda. Adv Parasitol 17:141–313

Coleman FC (1993) Morphological and physiological consequences of parasites encysted in the bulbus arteriosus of an estuarine fish, the sheepshead minnow, *Cyprinodon variegatus*. J Parasitol 79:247–254

Cribb TH, Anderson GR, Adlard RD, Bray RA (1998) A DNA-based demonstration of a three-host life-cycle for the Bivesiculidae (Platyhelminthes: Digenea). Int J Parasitol 28:1791–1795

Cribb TH, Bray RA, Olson PD, Littlewood DTJ (2003) Life cycle evolution in the digenea: a new perspective from phylogeny. Adv Parasitol 54:197–254

Crivelli AJ (2006) *Valencia letourneuxi*. The IUCN Red List of Threatened Species 2006:e.T22830A9393054. doi:https://doi.org/10.2305/IUCN.UK.2006.RLTS.T22830A9393054.en. Accessed 27 June 2017

Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife—threats to biodiversity and human health. Science 287(5452):443–449

Dias MLGG, Minte-Vera CV, Eiras JC, Machado MH, Souza GTR, Pavanelli GC (2006) Ecology of *Clinostomum complanatum* Rudolphi, 1814 (Trematoda: Clinostomidae) infecting fish from the floodplain of the high Paraná River, Brazil. Parasitol Res 99:675–681

Economidis PS (1991) Check list of freshwater fishes of Greece. Recent status of threats and protection. Hellenic Society for the Protection of Nature, Athens

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797

Faltýnková A, Našincová V, Kablásková L (2008) Larval trematodes (Digenea) of planorbid snails (Gastropoda: Pulmonata) in Central Europe: a survey of species and key to their identification. Syst Parasitol 69:155–178

Flores V, Semenas L (2002) Infection patterns of *Tylodelphys barilochensis* and *T. crubensis* (Trematoda: Diplostomatidae) metacercariae in *Galaxias maculatus* (Osmeriformes: Galaxiidae) from two Patagonian lakes and observations on their geographical distribution in the Southern Andean Region, Argentina. J Parasitol 88(6):1135–1139

García-Varela M, Sereno-Uribe AL, Pinacho-Pinacho CD, Domínguez-Domínguez O, Pérez-Ponce de León G (2016) Molecular and morphological characterization of *Austrodiplostomum ostrowskiae* Dronen, 2009 (Digenea: Diplostomatidae), a parasite of cormorants in the Americas. J Helminthol 90(2):174–185

Genner MJ, Michel E, Todd JA (2008) Resistance of an invasive gastropod to an indigenous trematode parasite in Lake Malawi. Biol Invasions 10:41–49

Georgieva S, Soldánová M, Pérez-del-Olmo A, Dangel DR, Sitko J, Sures B, Kostadinova A (2013) Molecular prospecting for European *Diplostomum* (Digenea: Diplostomidae) reveals cryptic diversity. Int J Parasitol 43:57–72

Gholami Z, Mobedi I, Esmaeili HR, Kia EB (2011) Occurrence of *Clinostomum complanatum* in *Aphanius dispar* (Actinoptrygii: Cyprinodontidae) collected from Mehran River, Hormuzgan Province, south of Iran. Asian Pac J Trop Biomed 1(3):189–192

Glez-Peña D, Gómez-Blanco D, Reboiro-Jato M, Fdez-Riverola F, Posada D (2010) ALTER: program-oriented format conversion of DNA and protein alignments. Nucleic Acids Res 38(suppl. 2):W14–W18

Gustinelli A, Caffara M, Florio D, Otachi EO, Wathuta EM, Fioravanti ML (2010) First description of the adult stage of *Clinostomum cutaneum* Paperna, 1964 (Digenea: Clinostomidae) from grey herons *Ardea cinerea* L. and a redescription of the metacercaria from the Nile tilapia *Oreochromis niloticus niloticus* (L.) in Kenya. Syst Parasitol 76(1):39–51

Hillis DM, Dixon MT (1991) Ribosomal DNA: molecular evolution and phylogenetic inference. Q Rev Biol 66:411–426

Holt RD, Dobson AP, Begon M, Bowers RG, Schauber EM (2003) Parasite establishment in host communities. Ecol Lett 6:837–842

Hudson PJ, Dobson AP, Newborn D (1998) Prevention of population cycles by parasite removal. Science 282(5397):2256–2258

Huyse T, Vanhove MPM, Mombaerts M, Volckaert FAM, Verreycken H (2015) Parasite introduction with an invasive goby in Belgium: double trouble? Parasitol Res 114(7):2789–2793

Kalantan AMN, Arfin M, Nizami WA (1987) Seasonal incidence and pathogenicity of the metacercariae of *Clinostomum complanatum* in *Aphanius dispar*. Jap J Parasitol 36:17–23

Kalfa-Papaioannou AM, Sinis AI (1985) Metacercariae of *Diplostomurn spathaceum* in freshwater fish of the Lake System Koronia and Volvi (Macedonia, Greece). Zbl Vet Med B 32:65–70

Kalogianni E, Giakoumi S, Zogaris S, Chatzinikolaou Y, Zimmerman B, Economou AN (2010a) Current distribution and ecology of the critically endangered *Valencia letourneuxi* in Greece. Biologia 65:128–139

Kalogianni E, Giakoumi S, Andriopoulou A, Chatzinikolaou Y (2010b) Feeding ecology of the critically endangered *Valencia letourneuxi* (Valenciidae). Aquat Ecol 44(1):289–299

Kennedy CR (1993) Introductions, spread and colonization of new localities by fish helminth and crustacean parasites in the British Isles: a perspective and appraisal. J Fish Biol 43:287–301

Kondo Y, Ohtsuka S, Hirabayashi T, Okada S, Ogawa NO, Ohkouchi N, Shimazu T, Nishikawa J (2016) Seasonal changes in infection with trematode species utilizing jellyfish as hosts: evidence of transmission to definitive host fish via medusivory. Parasite 23:16

Kottelat M, Freyhof J (2007) Handbook of European freshwater fishes. Kottelat, Cornol and Freyhof, Berlin

Kvach Y, Jurajda P, Bryjová A, Trichkova T, Ribeiro F, Přikrylová I, Ondračková M (2017) Distribution of North American digenean, *Posthodiplostomum* cf. *minimum centrarchi* (Strigeiformes: Diplostomidae) metacercariae in Europe. Parasitol Int. https://doi.org/10.1016/j.parint.2017.06.003

Locke SA, McLaughlin JD, Dayanandan S, Marcogliese DJ (2010a) Diversity and specificity in *Diplostomum* spp. metacercariae in freshwater fishes revealed by cytochrome *c* oxidase I and internal transcribed spacer sequences. Int J Parasitol 40:333–343

Locke SA, McLaughlin JD, Marcogliese DJ (2010b) DNA barcodes show cryptic diversity and a potential physiological basis for host specificity among Diplostomoidea (Platyhelminthes: Digenea) parasitizing freshwater fishes in the St. Lawrence River, Canada. Mol Ecol 19:2813–2827

Lucký Z (1970) Pathological changes with posthodiplostomosis of fish fry. Acta Vet Brno 1:51–66

Machado PM, Takemoto RM, Pavanelli GC (2005) Diplostomum (Austrodiplostomum) compactum (Lutz, 1928) (Platyhelminthes, Digenea) metacercariae in fish from the floodplain of the Upper Paraná River, Brazil. Parasitol Res 97(6):436–444

Marcogliese DJ (2004) Parasites: small players with crucial roles in the ecological theatre. EcoHealth 1:151–164

Marcogliese DJ (2005) Parasites of the superorganism: are they indicators of ecosystem health? Int J Parasitol 35:705–716

Mbokane EM, Theron J, Luus-Powell WJ (2015) Seasonal occurrence of some larval stages of endoparasites in three cyprinids from the Nwanedi-Luphephe dams, the Limpopo River System, South Africa. Helminthologia 52(3):229–235

McCallum H, Dobson A (1995) Detecting disease and parasite threats to endangered species and ecosystems. Trends Ecol Evol 10:190–194

Miller JH (1954) Studies on the life history of *Posthodiplostomum minimum* (MacCallum 1921). J Parasitol 3(40):255–270

Milne I, Lindner D, Bayer M, Husmeier D, McGuire G, Marshall DF, Wright F (2009) TOPALi v2: a rich graphical interface for evolutionary analyses of multiple alignments on HPC clusters and multi-core desktops. Bioinformatics 25:126–127

Moszczynska A, Locke SA, McLaughlin JD, Marcogliese DJ, Crease TJ (2009) Development of primers for the mitochondrial cytochrome *c* oxidase I gene in digenetic trematodes (Platyhelminthes) illustrates the challenge of barcoding parasitic helminths. Mol Ecol Resour 9: 75–82

Nezhybová V, Reichard M, Blažek R, Ondračková M (2017) Metazoan parasites of African annual killifish (Nothobranchiidae): abundance, diversity, and their environmental correlates. Biotropica 49(2):229–238

Nguyen TC, Li Y-C, Makouloutou P, Jimenez LA, Sato H (2012) *Posthodiplostomum* sp. metacercariae in the trunk muscle of northern snakeheads (*Channa argus*) from the Fushinogawa River, Yamaguchi, Japan. J Vet Med Sci 74(10):1367–1372

Niewiadomska K (2002) Superfamily Diplostomoidea Poirier, 1886. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, vol 1. The Natural History Museum, London, pp 159–166

Ondráčková M, Reichard M, Jurajda P, Gelnar M (2004a) Seasonal dynamics of *Posthodiplostomum cuticola* (Digenea, Diplostomatidae) metacercariae and parasite-enhanced growth of juvenile host fish. Parasitol Res 93:131–136

Ondráčková M, Šimková A, Gelnar M, Jurajda P (2004b) *Posthodiplostomum cuticola* (Digenea: Diplostomatidae) in intermediate fish hosts: factors contributing to the parasite infection and prey selection by the definitive bird host. Parasitology 129: 761–770

Ondráčková M, Dávidová M, Gelnar M, Jurajda P (2006) Susceptibility of Prussian carp infected by metacercariae of *Posthodiplostomum cuticola* (v. Nordmann, 1832) to fish predation. Ecol Res 21:526–529

Paes JVK, Carvalho ED, da Silva RJ (2010) Infection levels of *Austrodiplostomum compactum* (Digenea, Diplostomidae) metacercariae in *Plagioscion squamosissimus* (Teleostei, Sciaenidae) from the Nova Avanhandava reservoir, Sao Paulo State, Brazil. J Helminthol 84:284–291

Poulin R (1993) Age-dependent effects of parasites on anti-predator responses in two New Zealand freshwater fish. Oecologia 96:431–438

Prenter J, MacNeil C, Dick JTA, Dunn AM (2004) Roles of parasites in animal invasions. Trends Ecol Evol 19:385–390

Rambaut A, Drummond A (2009) FigTree: molecular evolution, phylogenetics and epidemiology. Available at http://tree.bio.ed.ac.uk/

Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer v1.6. Available at http://beast.bio.ed.ac.uk/Tracer

Rodríguez F, Olivier JL, Marín A, Medina JR (1990) The general stochastic model of nucleotide substitution. J Theor Biol 142:485–501

Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2011) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61(3):539–542

Saad-Fares A, Combes C (1992) Abundance/host size relationship in a fish trematode community. J Helminthol 66:187–192

Seppälä O, Karvonen A, Valtonen ET (2005) Manipulation of fish host by eye flukes in relation to cataract formation and parasite infectivity. Anim Behav 70:89–894

Shareef PAA, Abidi SMA (2015) Studies on the epidemiology and histopathology of *Euclinostomum heterostomum* (Trematoda; Digenea) infection in *Channa punctata* from North India. Arch Pol Fish 23(3): 133–140

Sharriff M, Richards RH, Sommerville C (1980) The histopathology of acute and chronic infections of rainbow trout *Salmo gairdneri* Richardson with eye flukes, *Diplostomum* spp. J Fish Dis 3:455–465

Silva-Souza AT, Ludwig G (2005) Parasitism of *Cichlasoma paranaense* Kullander, 1983 and *Gymnotus carapo* Linnaeus, 1814 by *Clinostomum complanatum* (Rudolphi, 1814) metacercariae in the Taquari river. Braz J Biol 65(3):513–519

Sonin MD (1986) Key to the trematode parasites of fish-eating birds in Palearctic region. Nauka, Moscow

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9):1312–1313

Stoyanov B, Georgieva S, Pankov P, Kudlai O, Kostadinova A, Georgiev BB (2017) Morphology and molecules reveal the alien *Posthodiplostomum centrarchi* Hoffman, 1958 as the third species of *Posthodiplostomum* Dubois, 1936 (Digenea: Diplostomidae) in Europe. Syst Parasitol 94:1–20

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30:2725–2729

Vanhove MPM, Tessens B, Schoelinck C, Jondelius U, Littlewood DTJ, Artois T, Huyse T (2013) Problematic barcoding in flatworms: a case-study on monogeneans and rhabdocoels (Platyhelminthes). ZooKeys 365:355–379

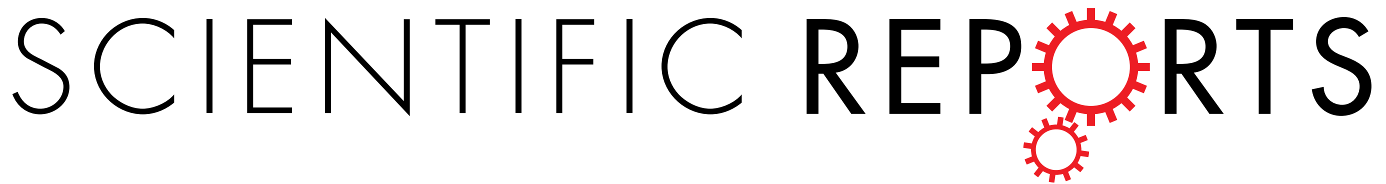
Vianna RT, Pereira Júnior J, Brandão DA (2005) *Clinostomum complanatum* (Digenea, Clinostomidae) density in *Rhamdia quelen* (Siluriformes, Pimelodidae) from South Brazil. Braz Arch Biol Technol 48(4):635–642

Vidal-Martinez VM, Pech D, Sures B, Purucker ST, Poulin R (2010) Can parasites really reveal environmental impact? Trends Parasitol 26: 44–51

Whiteman NK, Kimball RT, Parker PG (2007) Co-phylogeography and comparative population genetics of the threatened Galápagos hawk and three ectoparasite species: ecology shapes population histories within parasite communities. Mol Ecol 16:4759–4773

Kmentová N., Gelnar M., Mendlová M., Van Steenberge M., Koblmüller S. & Vanhove M. P. M. 2016. Reduced host-specificity in a parasite infecting non-littoral Lake Tanganyika cichlids evidenced by intraspecific morphological and genetic diversity. *Scientific Reports* 6:39605. DOI: 10.1038/srep39605 [Q1, IF (2016) = 4.259].

Publication available at: https://www.nature.com/articles/srep39605



OPEN

Received: 24 May 2016
Accepted: 24 November 2016
Published: 22 December 2016

Reduced host-specificity in a parasite infecting non-littoral Lake Tanganyika cichlids evidenced by intraspecific morphological and genetic diversity

Nikol Kmentová[1], Milan Gelnar[1], Monika Mendlová[1], Maarten Van Steenberge[2,3,4], Stephan Koblmüller[3,5] & Maarten P. M. Vanhove[1,2,4,6,†]

Lake Tanganyika is well-known for its high species-richness and rapid radiation processes. Its assemblage of cichlid fishes recently gained momentum as a framework to study parasite ecology and evolution. It offers a rare chance to investigate the influence of a deepwater lifestyle in a freshwater fish-parasite system. Our study represents the first investigation of parasite intraspecific genetic structure related to host specificity in the lake. It focused on the monogenean flatworm *Cichlidogyrus casuarinus* infecting deepwater cichlids belonging to *Bathybates* and *Hemibates*. Morphological examination of *C. casuarinus* had previously suggested a broad host range, while the lake's other *Cichlidogyrus* species are usually host specific. However, ongoing speciation or cryptic diversity could not be excluded. To distinguish between these hypotheses, we analysed intraspecific diversity of *C. casuarinus*. Monogeneans from nearly all representatives of the host genera were examined using morphometrics, geomorphometrics and genetics. We confirmed the low host-specificity of *C. casuarinus* based on morphology and nuclear DNA. Yet, intraspecific variation of sclerotized structures was observed. Nevertheless, the highly variable mitochondrial DNA indicated recent population expansion, but no ongoing parasite speciation, confirming, for the first time in freshwater, reduced parasite host specificity in the deepwater realm, probably an adaptation to low host availability.

Host specificity is one of the basic biological factors influencing the life cycle and diversity of parasitic organisms[1]. It is highly variable among groups and within taxa, ranging from strict specialist to generalist species, but always limited by the occurrence of potential hosts[2]. Host specificity is characterised by a trade-off of costs and benefits. While specialists have evolved specific adaptations to their host and therefore maximise profits, generalist species infecting a broad range of hosts are less affected by possible host extinction.

But to what extent is this important aspect of parasite biodiversity dependent on host ecology? The capability of a parasite to infect a host is determined by their co-evolutionary history and also ecological determinants such as host species longevity, stability and seasonality of a particular ecosystem. However, it seems that host species' ecological similarity is more important than host phylogeny[3].

[1]Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic. [2]Biology Department, Royal Museum for Central Africa, Leuvensesteenweg 13, B-3080 Tervuren, Belgium. [3]Institute of Zoology, University of Graz, Universitätsplatz 2, A-8010 Graz, Austria. [4]Laboratory of Biodiversity and Evolutionary Genomics, Department of Biology, University of Leuven, Ch. Deberiotstraat 32, B-3000 Leuven, Belgium. [5]Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, 603 65 Brno, Czech Republic. [6]Hasselt University, Centre for Environmental Sciences, Research Group Zoology: Biodiversity & Toxicology, Agoralaan Gebouw D, B-3590 Diepenbeek, Belgium. [†]Present address: Capacities for Biodiversity and Sustainable Development, Operational Directorate Natural Environment, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium. Correspondence and requests for materials should be addressed to N.K. (email: kmentovan@mail.muni.cz)

Lower host-specificity affected by decreasing host population density in deepwater habitats has been documented in marine environments[4–6] but never in freshwater systems. Decreased host-specificity in marine pelagic deepwater habitats was proposed to increase the chance of finding a host if host species exhibit low population densities, which is characteristic for most deepwater taxa[4–6]. In the present study we focus on yet unexplored parasite host choice patterns in the non-littoral habitat (i.e. the pelagic and deepwater zone) of one of the biggest and in terms of biodiversity most exceptional freshwater ecosystems in the world.

Lake Tanganyika, situated in the East African Rift Valley, is the second deepest and second oldest lake in the world. It is known for its remarkable species diversity characterised by rapid radiation processes in many vertebrate and invertebrate taxa[7], including parasitic flatworms that infect cichlids[8]. Therefore, it has been intensively studied for many decades. Although the first record of parasitic flatworms in Lake Tanganyika stems from a study on cestodes from 1914[9], the knowledge about the diversity and role of parasitic organisms in this unique environment is still poor and fragmentary. In the last years, parasitological research in the lake has mainly focused on the monogenean fauna of its cichlids and the number of described species is increasing[10–18]. Monogenea van Beneden, 1858 is one of the most species-rich groups of Platyhelminthes[19,20] with more than 3,500 already described species[21]. Most monogeneans are ectoparasites that infect the body or gills of freshwater and marine fishes. One species has a mammalian host and some have also colonised invertebrates or adopted an endoparasitic lifestyle inside fishes, turtles or amphibians[22].

The most important attachment organ of monogeneans is the opisthaptor, which is located posteriorly and which contains sclerotized structures such as hooks, clamps or suckers[23]. The evolutionary expansion of this parasitic group is related to the opisthaptor diversity and its adaptability to different hosts and infection sites[24]. Whereas the haptoral region is characteristic of species groups or lineages, the morphology of the male copulatory organ (MCO) is important for species-level diagnosis in many groups of Monogenea[22]. The ecological, behavioural and phylogenetic diversity of cichlid fishes, especially in Lake Tanganyika[25,26], make them ideal models for investigating parasite speciation mechanisms such as the influence of host ecology on parasite diversity[27–29]. Cichlids (Teleostei, Cichlidae) form one of the most diverse vertebrate families with around 2,200 known species[30]. In each of the African Great Lakes, hundreds of endemic species evolved within a short period of time[31–35]. Currently, 13 monogenean genera are known to infect cichlid species and six of these have been observed on African representatives[36]. *Cichlidogyrus* (Monopisthocotylea, Dactylogyridae) is the most species rich, with 102 representatives recorded from 88 different host species[10–15,18,37–42]. This genus displays variation in host-specificity and contains generalist but also strictly specialist species[39,43]. In Lake Tanganyika, most species of *Cichlidogyrus* described to date are strict or intermediate specialists[8,10,12,13,15] following the terminology used in Mendlová & Šimková[43]. While strict specialists infect only a single host species, intermediate specialists parasitise on two or more congeneric host species and intermediate generalist infect heterogeneric host species from the same tribe. The host range of generalists includes two or more hosts from different tribes. A complete list of *Cichlidogyrus* species from Lake Tanganyika with their host species is provided in Table 1. It was suggested that the relatively high degree of monogenean host-specificity is the result of adaptive processes related to their direct life cycle and to the tight co-evolutionary interactions with their hosts[24,44,45], depending on the species-specific response to both mechanical structures and the chemical composition of fish tissue[46,47]. To date, 24 species of *Cichlidogyrus* have been described from 20 different cichlid host species from Lake Tanganyika[10–15,18]. Only three of these have been reported from the benthopelagic and truly pelagic deepwater environment: *Cichlidogyrus brunnensis, C. attenboroughi* Kmentová, Gelnar, Koblmüller & Vanhove, 2016 and *C. casuarinus* Pariselle, Muterezi Bukinga & Vanhove, 2015. The present study focuses on exploring the intraspecific diversity of *C. casuarinus* infecting two deepwater cichlid genera, *Bathybates* Boulenger, 1898 and *Hemibates* Regan, 1920. These genera constitute the endemic tribe Bathybatini Poll, 1986. Until recently, also the genus *Trematocara* was included in the tribe[48], but genome-wide data[49–51] suggest that Poll's[52] original classification into Bathybatini, comprising the genera *Bathybates* and *Hemibates*, and Trematocarini, consisting of *Trematocara*, is more reasonable than alternative classifications[34,48,51,53]. The tribe Bathybatini contains eight currently recognised benthopelagic and truly pelagic species in two deeply divergent genera[48,49,53] (see Fig. 1). Whereas *Bathybates* species are chiefly piscivorous, *Hemibates* has a broader diet that also includes shrimps. With the exceptions of *B. ferox*, which has not been recorded below 70 meters and *B. horni*, of which no information is available, all species within these two genera have maximal recorded depth ranges ranging from 160 down to 210 meters. Hence, some of these species occur just above the lake's anoxic zone[54]. Morphological and genetic data were collected to test the hypothesis of Pariselle *et al.*[15], who suggested that *Cichlidogyrus casuarinus* has a broader host range than its congeners in Lake Tanganyika because it infects pelagic deepwater hosts. Here, the *Cichlidogyrus* host-specificity in the deepwater habitat in Lake Tanganyika is tested for all fish hosts within the presumed host range of *C. casuarinus*, potentially the first intermediate generalist of *Cichlidogyrus* reported for Lake Tanganyika (see Table 1), on a lake-wide geographical scale. However, in other monogeneans there are reports of cryptic speciation, with allegedly generalist monogeneans representing a complex of more host-specific cryptic species[28] or incipient speciation, with haplotypes or morphotypes of the same generalist species preferring a certain host species[55]. These scenarios can only be verified by studying *C. casuarinus* at the intraspecific level. There are only few studies about African monogeneans focusing on intraspecific aspects[56]. Here, the *Cichlidogyrus* host-specificity in the non-littoral habitat in Lake Tanganyika is tested for all fish species within the presumed host genera of *C. casuarinus* and on a lake-wide geographical scale. Multivariate statistic approaches of morphological characters and genetic characterisation using markers with different rates of molecular evolution were used to answer the following questions:

(1) How broad is the host range of this parasite species among members of the Bathybatini?
(2) Is there any morphological intraspecific variation?
(3) Does the apparently broad host range of *Cichlidogyrus casuarinus* infecting *Bathybates* and *Hemibates* reflect cryptic speciation or a lack of host preference?
(4) What is the population structure and recent demographic history of this deepwater species of *Cichlidogyrus*?

Monogenean species	Host species	Host-specificity[43]
Cichlidogyrus. attenboroughi Kmentová, Gelnar, Koblmüller & Vanhove, 2016	*Benthochromis horii* Poll, 1948	strict specialist
C. banyankimbonai Pariselle & Vanhove, 2015	*Simochromis diagramma* (Günther, 1894)	strict specialist
C. brunnensis Kmentová, Gelnar, Koblmüller & Vanhove, 2016	*Trematocara unimaculatum* Boulenger, 1901	strict specialist
C. buescheri Pariselle & Vanhove, 2015	*Interochromis loocki* (Poll, 1949)	strict specialist
C. casuarinus Pariselle, Muterezi Bukinga & Vanhove, 2015	*Bathybates minor* Boulenger, 1906; *B. fasciatus* Boulenger, 1901; *B. vittatus* Boulenger, 1914 Potentially also on *B. leo* Poll, 1956 and *Hemibates stenosoma* (Boulenger, 1901)	**intermediate generalist?**
C. centesimus Vanhove, Volckaert & Pariselle, 2011	*Ophthalmotilapia ventralis* (Boulenger, 1898); *O. nasuta* (Poll & Matthes, 1962); *O. boops* (Boulenger, 1901)	intermediate specialist
C. frankwillemsi Pariselle & Vanhove, 2015	*Pseudosimochromis curvifrons* (Poll, 1942)	strict specialist
C. franswittei Pariselle & Vanhove, 2015	*P. marginatus* (Poll, 1956); *P. curvifrons*	intermediate specialist
C. georgesmertensi Pariselle & Vanhove, 2015	*P. babaulti* (Pellegrin, 1927)	strict specialist
C. gillardinae Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle, 2012	*Astatotilapia burtoni* (Günther, 1894)	strict specialist
C. gistelincki Gillardin, Vanhove, Pariselle, Huyse & Volckaert, 2012	*Ctenochromis horei* (Günther, 1894)	strict specialist
C. irenae Gillardin, Vanhove, Pariselle, Huyse & Volckaert, 2012	*Gnathochromis pfefferi* (Boulenger, 1898)	strict specialist
C. makasai Vanhove, Volckaert & Pariselle, 2011	*Opthalmotilapia ventralis* (Boulenger, 1898); *O. nasuta* (Poll Matthes, 1932); *O. boops* (Boulenger, 1901)	intermediate specialist
C. mbirizei Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle, 2012	*Oreochromis tanganicae* (Günther, 1894), *O. niloticus, O. mossambicus*	intermediate specialist
C. mulimbwai Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle, 2012	*Tylochromis polylepis* (Boulenger, 1900)	strict speciliast
C. muterezii Pariselle & Vanhove, 2015	*S. diagramma*	strict specialist
C. muzumanii Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle, 2012	*T. polylepis*	strict specialist
C. nshomboi Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle, 2012	*Boulengerochromis microlepis* (Boulenger, 1899)	strict specialist
C. raeymaekersi Pariselle & Vanhove, 2015	*S. diagramma*	strict specialist
C. schreyenbrichardorum Pariselle & Vanhove, 2015	*I. loocki*	strict specialist
C. steenbergei Gillardin, Vanhove, Pariselle, Huyse & Volckaert, 2012	*Limnotilapia dardennii* (Boulenger, 1899)	strict specialist
C. sturmbaueri Vanhove, Volckaert & Pariselle, 2011	*O. ventralis; O. nasuta*	intermediate specialist
C. vandekerkhovei Vanhove, Volckaert & Pariselle, 2011	*O. ventralis; O. nasuta; O. boops*	intermediate specialist
C. vealli Pariselle & Vanhove, 2015	*I. loocki*	strict specialist

Table 1. List of the 24 monogenean species of *Cichlidogyrus* reported in Lake Tanganyika with host specification[10,12,14,15,42,112,113]. Terminology: Strict specialist – infecting only a single host species, intermediate specialist – infecting two or more congeneric host species, intermediate generalist – infecting non-congeneric host species from the same tribe, generalist – infecting two or more hosts from different tribes.

Results

Morphological species identification. In total, 764 *Cichlidogyrus* specimens were retrieved and identified from 24 fish specimens belonging to six host species, namely *B. leo* Poll, 1956, *B. minor* Boulenger, 1906, *B. horni* Steindachner, 1911, *B. vittatus* Boulenger, 1914, *B. fasciatus* Boulenger, 1901 and *H. stenosoma* (Boulenger, 1901), making use of fresh material from an expedition in 2013 and of the historical ichthyological collections of the Royal Museum for Central Africa (Tervuren, Belgium) (Fig. 1; Table 2). All these *Cichlidogyrus* specimens were collected from the hosts' gills. No monogeneans were found on *B. graueri* Steindachner, 1911 and *B. ferox* Boulenger, 1898. The overall prevalence on most of the species was 100%. The lowest prevalence was recorded on *B. leo* (25%). Infection intensity ranged from 1 to 263 individuals per gill chamber. This parameter was counted only for one gill chamber as one side needed to remain undamaged in museum specimens. Infection parameters are detailed in Table 2. They are only indicative because of the small sample size. All *Cichlidogyrus* specimens were identified as *C. casuarinus* based on the original description[15] according to the corresponding shape and measurements of haptoral and male genital hardparts. The often slightly wider range of measurements is interpreted as a logical consequence of a larger sample size and the wider host and geographical range of the measured individuals (Table 3).

Morphometric and geomorphometric assessment of intraspecific variation. Principal component analysis (PCA) was performed on measurements taken on the haptoral hardparts of 182 individuals of *C. casuarinus* to assess intraspecific variation. The first PC explained 60% and the second 11% of the variation in our dataset. The shape of the bars had the highest contribution to PC1 whereas the size of one component of the

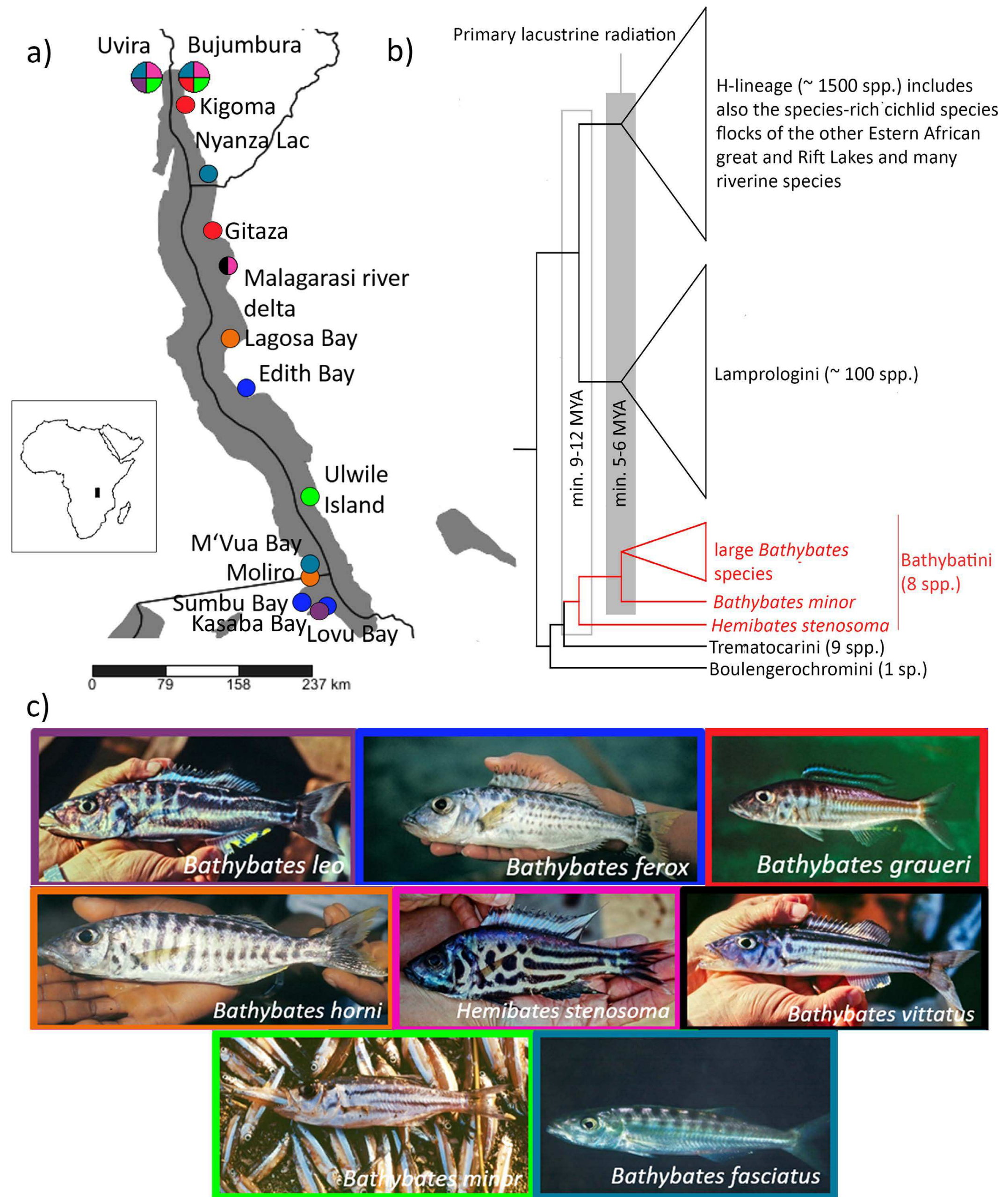


Figure 1. Host species information. (**a**) Geographical positions of sampling localities in Lake Tanganyika with indication of host species (pictures by Ad Konings). (**b**) Schematic phylogenetic tree of the Lake Tanganyika cichlid radiation, showing the phylogenetic position and relative divergence of the tribe Bathybatini and its major lineages[48,50,52]. (**c**) Host species pictures (Ad Konings). The map was created using SimpleMappr software v7.0.0. (available at http://www.simplemappr.net. Accessed February 20, 2016).

dorsal anchor (outer root) was the main contributor to PC2. The resultant biplot graph, in which samples were grouped according to host species as well as to the three lake subbasins following Danley *et al.*[57] (Table 2), showed some differentiation according to host species (Fig. 2). Individuals collected from *H. stenosoma* and *B. minor* clustered mostly along the positive side of the first axis. Specimens collected from *B. fasciatus* and *B. leo* had low values for this axis, with the exception of the three specimens coming from M'Vua Bay (southern basin). Another group was formed by parasites retrieved from *B. horni* and *B. vittatus*. These worms displayed lower values for the second axis. Most of the specimens coming from the central and the southern part of the lake displayed low values for the first axis while values for parasites collected in the northern part were widespread across the graph (Fig. 2). However, the fact that data from the central and southern parts of the lake are comprised only by specimens collected from *B. horni* could have influenced the result. Analyses of variance (ANOVA) provided information about intraspecific variation of *C. casuarinus* in copulatory tube and heel length (see Supplementary Tables S2 and 5). Box-plot graphs with the length of male copulatory organ structures are showed in Supplementary Figs S1 and S2.

Host species (host maximum size[68], cm)	Locality (geographic coordinates)	Locality – basins[57] (date of sampling)	Number of fish specimens (accession number in RMCA)	Number of *Cichlidogyrus* individuals (accession numbers in RMCA)	Prevalence (%)	Infection intensity/one gill chamber	Abundance (range)
Bathybates fasciatus (39, 7)	Uvira (3°22′S 29°08′ E)	The northern basin (9/9/2013)	1 (MRAC 2016-22-P)	12 (MRAC 37926-8)	100	6	6
	Bujumbura (3°23′S 29°22′E)	The northern basin (4/9/2013)	3 (MRAC 2016-22-P)	42 (MRAC 37921-5)	100	7.7	7.7 (1–19)
	M'Vua Bay (08°05′ S-30°34′E)	The southern basin (23/3/1947)	2 (MRAC 112235-242 A, 115)	3 (MRAC 37898-9)	50	1.5	1.5 (0–3)
	Nyanza Lac (04°20′ S-29°35′E)	The northern basin (1/1/1937)	3 (MRAC 54746-60 A, B, C)	7 (MRAC 37900-3)	66.6	3.5	2.3 (0–4)
Bathybates horni (27, 2)	Moliro (08°13′ S-30°35′E)	The southern basin (12/3/1947)	1 (MRAC 112481)	263 (MRAC 37847, 49-73)	10	263	263
	Lagosa Bay (05°57′ S-29°51′E)	The central basin (11/4/1947)	1 (MRAC 112484)	162 (MRAC 37827-46, 48)	100	162	162
Bathybates leo (26, 0)	Uvira	The northern basin (9/9/2013)	4 (—)	2 (MRAC 37758-9)	25	1	162
	Kasaba Bay (-08°31′ S-30°39′E)	The southern basin (23/11/1995)	2 (MRAC 99-31P-896-904)	0	0	0	0
	near Malagarasi River delta (05°14′S-29°45′E and 05°13′S-29°43′E)	The northern basin (26/2/1947)	1 (112492-496)	0	0	0	0
Bathybates minor (20, 5)	Bujumbura	The northern basin (4/9/2013)	7 (MRAC 2016-22-P)	50 (MRAC 37904-8)	71.4	5	3.55 (0–9.5)
	Ulwile Island (07°25′ S-30°34′ E)	The central basin (4/9/2013)	1 (2016-22-P)	8 (MRAC 37909-14)	100	4	4
Bathybates ferox (38, 5)	Sumbu Bay (08°31′ S-30°29′E)	The southern basin (31/3/1947)	3 (MRAC 112187-97 A, B, F)	0	0	0	0
	Lovu Bay (08°34′ S-30°44′E)	The southern basin (26/3/1947)	1 (MRAC 112175-80)	0	0	0	0
	Edith Bay (06°30′ S-29°55′E)	The central basin (14/2/1947)	3 (MRAC 112152-62 A, B, C)	0	0	0	0
Hemibates stenosoma (26, 0)	Bujumbura	The northern basin (25/9/2013)	4 (MRAC 2016-22-P)	28 (MRAC 37915-6)	75	4.7	3.5 (0–8)
	Uvira	The northern basin (9/9/2013)	4 (MRAC 2016-22-P)	36 (MRAC 37917-20)	100	4.5	4.5 (2.5–7.5)
	near Malagarasi River delta	The northern basin (22/5/1947)	1 (MRAC 112136)	27 (MRAC 37891-7)	100	27	27
Bathybates viitatus (42, 0)	near Malagarasi River delta	The northern basin (26/2/1947)	1 (MRAC 112489)	124 (MRAC 37874-90)	100	124	124
Bathybates graueri (30, 0)	Bujumbura	The northern basin (25/9/2013)	7 (MRAC 2016-22-P)	0	0	0	0
	Uvira	The northern basin (9/9/2013)	6 (MRAC 2016-22-P)	0	0	0	0
	Kigoma (04°52′S-29°38′E)	The northern basin (10/1/1947)	3 (MRAC 112430-452)	0	0	0	0
	Gitaza (03°37′ S-29°20′E)	The northern basin (20/10/1995)	1 (MRAC 95-98-P-253-62)	0	0	0	0

Table 2. An overview of host spe cies examined for *Cichlidogyrus* parasites with localities and infection parameters.

The analyses of copulatory tube length were based on 157 individuals while analyses of heel length were based on 149 individuals. Significant differences in copulatory tube length were observed between *C. casuarinus* from all of the host species except for *B. fasciatus* and *B. vittatus*. Comparisons of heel lengths showed a significant difference between *C. casuarinus* collected from *B. fasciatus* and the other host species, except for *B. vittatus*. There was no significant difference among individuals collected from *H. stenosoma*, *B. minor* and *B. horni.* No influence of geographical range was observed on the intraspecific variation in copulatory tube length. Individuals collected from the south of the lake differed significantly in heel length from specimens coming from the north.

Intraspecific shape plasticity of *C. casuarinus* was analysed using landmarks and semilandmarks placed on one of the dorsal and ventral anchors. Specimens collected from *B. leo* were not included in the geomorphometric analysis because only two individuals were available. Scatterplots of relative warps showed some clustering according to host species, mainly along the second axis. Sample distribution along the first axis was caused by allometric effects, which were due to differences in the total size of the structures (individuals collected from *B. fasciatus* with the smallest ventral/dorsal anchor contrary to *C. casuarinus* from *B. horni* with the largest measured structure). Differences in the second axis were caused by variation in the shape of the anchors, for which no size effect could be found. For both analyses, specimens collected from *H. stenosoma*, *B. minor* and, to a lesser extent, *B. fasciatus* had relatively high values for the second axis. Values on the second axis were highly variable for *B. horni* and *B. vittatus* for the dorsal anchors, whereas they had low values on this axis for the ventral anchor.

Based on the values of the bending energy (see Supplementary Table S6), individuals found on *H. stenosoma* seemed to have anchors that correspond most with the mean shape of both anchors in the dataset. The most divergent shape was displayed by specimens of *C. casuarinus* recorded from *B. vittatus* hosts (Fig. 3). The values for specimens collected in the northern part of Lake Tanganyika clustered mainly in the area with high values for the second relative warp. Specimens coming from southern and central localities tended to have lower values for this axis. This result was most evident in the shape of the ventral anchor but less so for the dorsal anchor (Fig. 3).

Based on the results of scatterplots, we defined two groups. The first was formed by the specimens collected from *H. stenosoma*, *B. minor* and *B. fasciatus*. The specimens recorded from *B. horni* and *B. vittatus* hosts were placed in a second group. Non-parametric Mann-Whitney U tests showed significant differences between these groups for both the dorsal ($Z_{1,117} = -3.14122$, $P < 0.01$) and the ventral anchor ($Z = -3.59488$, $P < 0.001$). Another test was performed to check for significant geographic differences in anchor shapes, comparing specimens collected in the north of the lake with those collected elsewhere. Whereas a significant difference was found between these groups in the shape of the ventral anchor ($Z = -2.3227$, $P < 0.05$), this was not the case for the shape of the dorsal anchor ($Z = -1.77484$, $P > 0.05$).

Genetic species identification. All host specimens of which parasites were available for genetic analyses (*H. stenosoma, B. minor, B. fasciatus*) came from the very northern end of the lake. Genetic species identification of parasites was performed using three nuclear markers (28 S rDNA, 18 S rDNA, ITS-1) generally considered as suitable for monogenean species level determination[58,59]. The length of the successfully sequenced 28 S rDNA fragment ranged from 641 to 747 base pairs (bp). The 18 S rDNA fragment was 195–482 bp long, while the length of ITS-1 sequences ranged between 141 and 474 bp. In total, 27 sequences of 28 S rDNA and 25 sequences of 18 S + ITS1 rDNA were acquired. The *Cichlidogyrus* parasites shared an identical haplotype for all three rDNA regions and are hence confirmed to be conspecific. Formaldehyde fixation prevented the use of samples from the historical RMCA collections for the genetic part of this study.

Population structure and past population size trajectories. Population structure was assessed using the mitochondrial marker COI. This region was used because of its fast rate of molecular evolution as compared to the nuclear sequences[60]. Since specimens suitable for DNA extraction were only available from the northern basin, a geographical effect could not be taken into account in the genetic analyses. The length of the COI sequences ranged from 466 to 1120 bp. Sequences for COI mtDNA were obtained from 42 individuals of *C. casuarinus* collected from three host species (*H. stenosoma, B. minor, B. fasciatus*), comprising 35 different haplotypes and containing 50 polymorphic sites. Analyses were based on a 402 bp fragment of COI. Haplotype and nucleotide diversity were estimated to be 0.987 and 0.02045, respectively. Genetic distance among haplotypes ranged from 0.2% to 4.7%. The haplotype network representing the relationships among *C. casuarinus* COI haplotypes is depicted in Fig. 4. There was no evident clustering according to host species and therefore no indication for cryptic diversity or incipient speciation. Moreover, a non-significant F_{ST} indicates no barriers between the groups defined by host species (see Supplementary Table S1), at least in northern Lake Tanganyika. The unimodal mismatch distribution with non-significant SSD ($SSD = 0.00340$, $P = 0.476$) and rg ($rg = 0.01168$, $P = 0.355$) indicated past population expansion of the *C. casuarinus* population (Fig. 5a). In addition, this result is well supported by the negative and significant value of Fu's F_S (-24.01572, $P < 0.001$). Tajima's D was negative, as expected for recent population growth, but not significantly different from zero (-1.03465, $P = 0.142$), which is probably due to the reduced power of Tajima's D for detecting population expansion as compared to Fu's F_S[61]. Also the Bayesian Skyline Plot (BSP; Fig. 5b) indicated that *C. casuarinus* experienced population expansion in the recent past. The time to the most recent common ancestor (TMRCA) was dated to 144.4 KYA (95% HPD: 92.1–204.1 KYA). The onset of population expansion was dated to 87.0 KYA (95% CI: 51.4–167.0 KYA) based on the parameter $\tau = 6.994$ (95% CI: 4.129–13.428) from the mismatch distribution ($\tau = 2\mu t$; where μ is the mutation rate per locus and t is the time since the onset of population growth).

Discussion

The main aim of this study was to test for host-specificity of monogeneans in the non-littoral habitat of Lake Tanganyika. Genetic and morphological methods were used to answer questions about the diversity of the monogenean fauna occurring on deepwater fishes. Using multivariate statistical approaches, we investigated the host range and intraspecific variation of *Cichlidogyrus casuarinus*. Population-level analyses using mtDNA were performed to check whether host preference is driving speciation in *C. casuarinus*. This also allowed us to infer the species' demographic history.

The previously proposed low host-specificity of *C. casuarinus* in the deepwater habitat[15], which contrasts with the high host-specificity of many of its congeners in the littoral zone[8], was supported by the fact that no variation was observed at the three rDNA regions in *C. casuarinus* sampled across different host species. Nuclear rDNA regions are considered to be suitable markers for species-level identification of monogeneans[59,62–64]. They have been used to show that allegedly generalist *Cichlidogyrus* species might actually comprise a complex of cryptic species, which are more host-specific than previously assumed[28]. However, our rDNA data confirm that *Cichlidogyrus* specimens infecting various bathybatine species are truly conspecific. Hence, we are dealing with an intermediate generalist species, parasitizing on a range of host-species within the same tribe[43]. Such weak host preference is probably an adaptation to the lower host availability in the deepwater realm, as has been suggested in previous studies on marine systems[4–6]. Therefore, *C. casuarinus* has evolved a different strategy compared to its congeners in the littoral zone (it is the only generalist species of *Cichlidogyrus* collected from the lake so far[15]), by broadening its host range, probably increasing the chance of contact and reducing that of extinction. The host range of *C. casuarinus* spans *Hemibates stenosoma* and the whole phylogenetic range of *Bathybates*. However, there are also species of *Bathybates* where monogenean infection has not been recorded

Parameters (µm)	*C. casuarinus* (n = 182)	*C. casuarinus* Pariselle *et al.*[15] (n = 35)
Total length	628.7 ± 93.2[a] (n = 83); (379.1–1003.4)[b]	915 (n = 19); (766–1105)
Ventral anchor		
Total length	51 ± 4.7 (n = 158); (38.3–62.5)	51 ± 2.5 (n = 35); (47–59)
Length to notch	41.7 ± 3.8 (n = 157); (32.1–49.9)	43 ± 1.7 (n = 35); (39–47)
Inner root length	16.4 ± 2.6 (n = 156); (10.1–21.6)	17 ± 1.6 (n = 35); (12–19)
Outer root length	9.5 ± 2 (n = 154); (5.1–17.3)	8 ± 1.5 (n = 35); (5–11)
Point length	16.3 ± 2.1 (n = 157); (10.9–22.8)	16 ± 1.5 (n = 35); (12–20)
Dorsal anchor		
Total length	56.7 ± 5.5 (n = 156); (40–73.8)	58 ± 2.8 (n = 32); (52–64)
Length to notch	39.6 ± 3.6 (n = 156); (29.7–46.8)	40 ± 2.0 (n = 32); (35–44)
Inner root length	22.1 ± 3.1 (n = 156); (15.5–31.3)	24 ± 1.9 (n = 32); (20–27)
Outer root length	8.4 ± 2 (n = 154); (2.5–14.1)	8 ± 1.3 (n = 32); (6–11)
Point length	14.2 ± 1.6 (n = 154); (8.9–18.2)	15 ± 0.8 (n = 32); (13–17)
Ventral bar		
Branch length	64.2 ± 8.3 (n = 157); (43.4–90.3)	59 ± 3.2 (n = 39); (54–67)
Branch maximum width	9.5 ± 1.8 (n = 164); (5.1–14.5)	9 (n = 20); (7–12)
Dorsal bar		
Maximum straight width	79.6 ± 13.1 (n = 138); (54.7–115.2)	71 (n = 20); (64–85)
Thickness at midlength	15.4 ± 3.7 (n = 174); (9.3–39.2)	15 (n = 15); (12–20)
Distance between auricles	33.7 ± 7 (n = 169); (21.8–55.4)	30 (n = 20); (23–40)
Auricle length	18.7 ± 3.2 (n = 154); (7.8–28.6)	17 ± 1.8 (n = 40); (13–23)
Hooks		
Pair I	33.8 ± 4.3 (n = 157); (22–49.4)	30 ± 1.2 (n = 30); (27–33)
Pair II	22.3 ± 2.6 (n = 129); (11.8–33.5)	—
Pair III	23.7 ± 2.8 (n = 121); (10.6–29.9)	—
Pair IV	25.8 ± 2.7 (n = 111); (13.8–31.2)	—
Pair V	10.8 ± 0.9 (n = 109); (6.2–13.8)	11 (n = 17); (10–12)
Pair VI	26.3 ± 3.8 (n = 69); (15.9–33.1)	—
Pair VII	27.4 ± 3.6 (n = 64); (14.2–32.5)	—
Pair II, III, IV, VI, VII average size	24.6 ± 3.5 (n = 494); (10.6–33.5)	23 ± 1.9 (n = 120); (19–28)
Copulatory tube straight length	37 ± 3.1 (n = 163); (29.7–43.2)	37 (n = 20); (34–44)
Accessory piece curved length	32.8 ± 5.8 (n = 27); (33.1–103.2)	31 (n = 20); (26–38)
Heel straight length	60 ± 15.7 (n = 157); (25.7–46.9)	47 (n = 20); (40–59)
Vagina curved length	56.3 ± 12.7 (n = 34); (38.1–83.1)	46 (36–59)
Vagina maximum width	12 ± 2.2 (n = 49); (7.7–16.5)	7 (5–8)

Table 3. Comparison of measurements performed on *C. casuarinus* haptoral and genital hardparts between the present study and the original description[15] (a – mean value ± standard deviation, b – range).

yet (*B. graueri* and *B. ferox*). In Lake Tanganyika, 24 *Cichlidogyrus* species have already been described from 20 different host species[10–15,18]. Intermediate specialists were recorded in a previous study, namely *C. vandekerkhovei*, *C. makasai*, *C. centesimus* and *C. sturmbaueri* Vanhove, Volckaert & Pariselle, 2011 recorded from two to three *Ophthalmotilapia* species[13] as well as *C. franswittei* Pariselle & Vanhove, 2015 infecting two species of *Pseudosimochromis*[14]. However, whereas past and/or ongoing hybridisation between *Ophthalmotilapia* species might explain their shared parasite species[65], this does not seem to have been the case in the bathybatines. The divergence between them is ancient (Fig. 1b), and there is no evidence for any past or ongoing interspecific geneflow[49,53]. Hence, the lower host-specificity of *C. casuarinus* cannot be attributed to a shallow host phylogeny, confirming its more generalist lifestyle. Close morphological similarity of *C. casuarinus* with *C. nshomboi* and *C. centesimus*, which infect species from other Lake Tanganyika cichlid tribes (Boulengerochromini and Ectodini, respectively), was recorded[15]. These three monogenean species are the only known representatives of their genus exhibiting a spirally coiled thickening of the wall of the copulatory tube (see also Fannes *et al.*[66]). Together, they infect a variety of Tanganyika cichlids, with different feeding and reproductive strategies, occurring in different habitats and belonging to different tribes. This indicates that this morphotype of *Cichlidogyrus* is characterised by a rather broad niche.

Considering COI is the fastest evolving marker currently available for these monogeneans[59], it was used to investigate the intraspecific variability of *C. casuarinus* in our study. Although the characterisation of *C. casuarinus* as an intermediate generalist species is well supported by ribosomal DNA, morphometric analyses of haptoral elements showed intraspecific variation, which was linked to host species. Based on the available knowledge about the member species of the Bathybatini, we could not discern a clear link between the morphological differentiation of *C. casuarinus* and host ecology (e.g., prey and habitat)[49,67,68]. Host body size and phylogenetic history

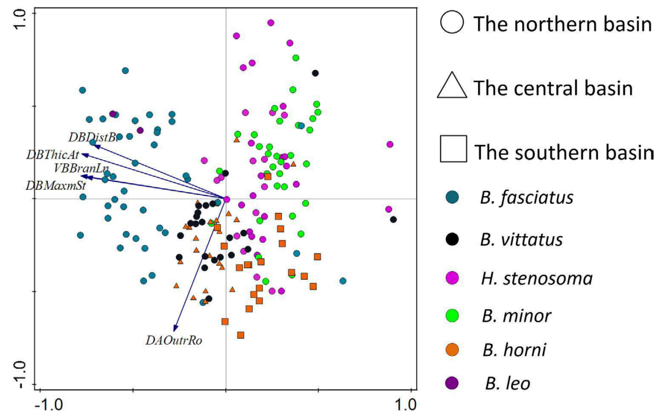


Figure 2. A biplot of PCA (first two axes) based on measurements of haptoral sclerotized structures only showing the five best fitting morphological characters selected by CANOCO. Symbols denote host species and their origin in each of the three subbasins of Lake Tanganyika. DALENGTO – Dorsal anchor, Length to notch, DATotlLn- Dorsal anchor, Total length, DBMaxmSt – Dorsal bar, Maximum straight width, VATotlLn – Ventral anchor, Total length, VBBranLn – Ventral bar, Branch length.

could explain some of the groupings observed in the analyses of haptoral structures. Individuals collected from *H. stenosoma* and *B. minor*, which are the smallest species of the clade[68] and which represent more basal lineages[49,53], clustered together. Parasites originating from *B. horni*, *B. vittatus, B. leo* and *B. fasciatus* also clustered in our analysis. These host species stem from a phylogenetic lineage that also includes *B. ferox* and *B. graueri*[49]. Our data therefore suggest a correlation between morphological variation in *C. casuarinus* and the size and phylogenetic position of the host.

The most important morphometric features showing intraspecific variability in our dataset were maximum straight width, thickness at the middle and distance between auricles of the dorsal bar, branch length of the ventral bar and length of the outer root of the dorsal anchor. Although various sclerotized structures (heel length, length of copulatory tube, length of dorsal bar auricle) in other previously described *Cichlidogyrus* species exhibit a considerable size range[10,12,13,15], the range observed in this study is wider. This difference could be explained by the increased geographical range and host range included in the present study compared to the original descriptions of parasites in Lake Tanganyika. Only one previous study[13] also looked at some aspects on intraspecific morphometric variability of monogenean species in Lake Tanganyika, demonstrating intraspecific morphological variation of the MCO heel. The greatest infection intensity was observed on two relatively large cichlid species (*B. horni* and *B. vittatus*). This confirms the correlation between infection intensity and host body size[69]. However, individuals from another large host species included in our study, *B. fasciatus*, were not affected so severely by monogeneans. This discrepancy could be caused by limited sample size (random choice) or by massive infection in certain areas or times (the *B. horni* and *B. vittatus* specimens originated from 1949 and were collected at three different localities, see Table 2). In theory, prevalence and infection intensity could help us to identify the original and the more recent hosts of *C. casuarinus*[70,71]. Yet, it is hard to reliably quantify such parameters in view of the rarity of several of the host fishes[54]. Another way to establish which hosts have been colonized earlier would be through (co-)phylogenetic analyses. Although the observed groups based on parasite morphology correspond with the separate position of *H. stenosoma* and *B. minor* relative to the other species in the published host phylogeny[49,53] unfortunately, our taxon coverage of Bathybatini was only exhaustive for morphological analysis, as museum specimens were unsuitable for molecular work. Moreover, the sequence data generated in this study did not consistently differ between parasites from different host species.

The geomorphometric approach suggested the existence of intraspecific shape variation in both dorsal and ventral anchors. Clustering along the relative warps axes shows almost the same sample distribution according to host species as the PCA of our set of linear haptoral measurements. Phenotypic changes of haptoral sclerites have already been described in many previous studies and are supposed to be influenced by a combination of host characteristics[72,73] geographical origin[74–76] and other environmental factors[77,78]. While some researchers prefer the haptoral region for reconstructing evolutionary history[28,58], other investigations devote more attention to the reproductive organs[79,80]. We found significant intraspecific differences in certain parts of the male copulatory organ between parasites collected from different host species. Although this could be a sign of a possible reproductive barrier, it is known from this morphotype of Lake Tanganyika *Cichlidogyrus* that heel length can vary substantially within a species[13]. Moreover, geographic variation of reproductive and haptoral sclerotized structures was found by both morphometric techniques. However, unequal sampling of host species across different basins might have influenced our results. *Bathybates minor*, *B. fasciatus*, *B. leo*, *B. vittatus* and *H. stenosoma* were mostly or exclusively collected from the northern part of the lake, whereas the sample of hosts from the central and southern basins was dominated by *B. horni*.

The observed high haplotype diversity is consistent with a large population size of *C. casuarinus*[81]. Non-significant F_{ST} estimates suggested a lack of population genetic structure with respect to host species. Equally, there was no indication of ongoing speciation influenced by host preference apparent in the haplotype

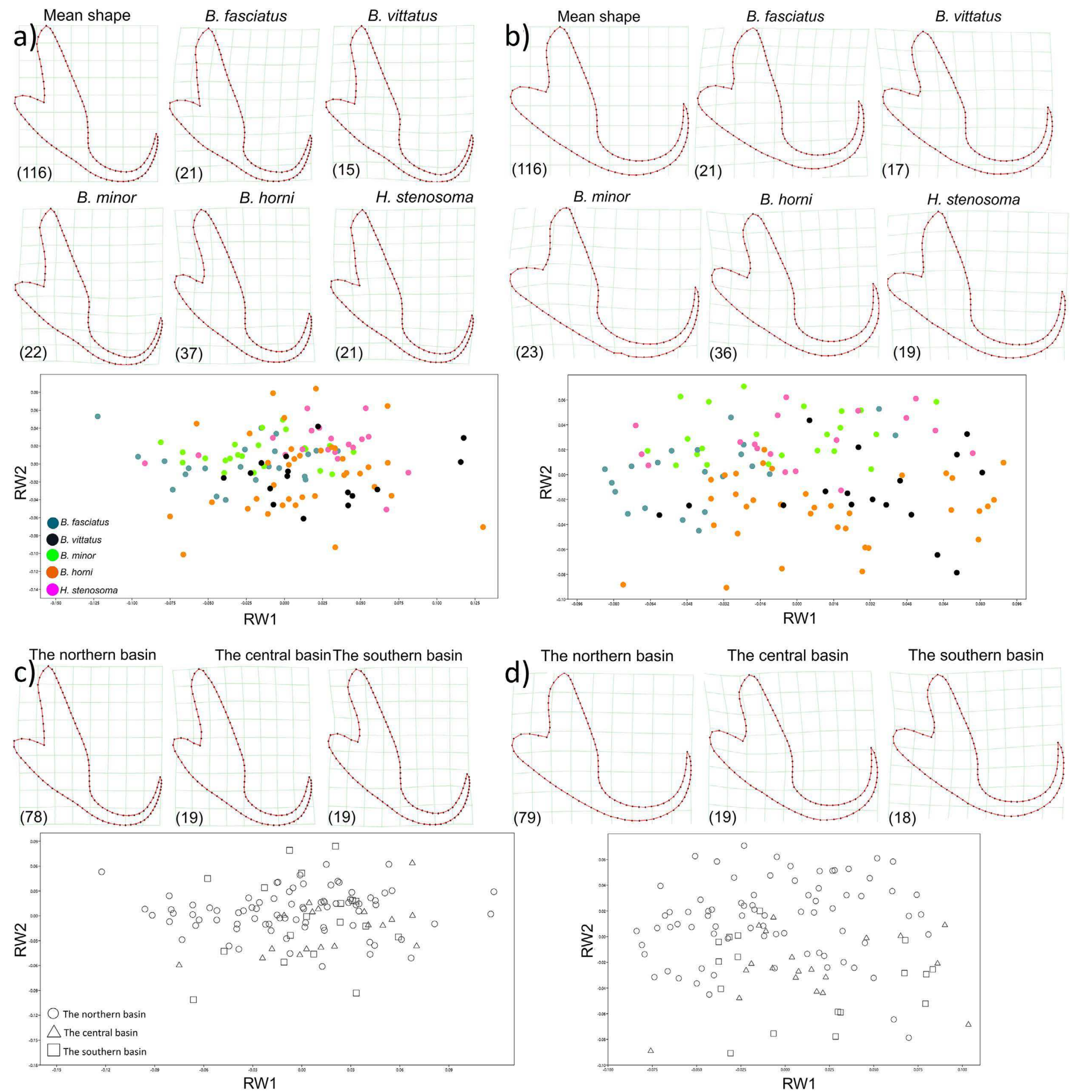


Figure 3. Scatterplots of the first two relative warps showing shape variation of the dorsal and ventral anchor with deformation grids (thin-plate) depicting mean anchor differences among groups. Symbols denote host species and sampling localities: (**a**) dorsal anchor, separation according to the host species; (**b**) ventral anchor, separation according to the host species; (**c**) dorsal anchor, separation according to the sampling localities; (**d**) ventral anchor, separation according to the sampling localities. The number of specimens investigated is indicated in brackets.

network. Its non-hierarchical topology indicated the absence of host related population structure. It has been suggested that a broad host range of morphometrically similar monogeneans can result from cryptic speciation processes[28,82–84]. However, in that case we should be able to recognise different haplotype variants corresponding with host preference: "choice matters"[55]. Since the intraspecific genetic variation was independent of host species, no cryptic or incipient speciation was evident in this system. Rather, the observed pattern of morphological variation seemed to be caused by phenotypic changes during ontogenetic development as an adaptation to the host or to the environment. Regarding the differences in MCO morphology, it is unclear how this may be influenced by the host. Poor correlation between genetic and morphometric variation may also be caused by limited sample size, only including COI sequences of *C. casuarinus* individuals from three host species or by a higher rate of genital morphological changes as compared to mutations in COI[55]. Even though there is no evidence for population structure according to host species or geographic origin based on COI fragments, future studies employing a large number of unlinked nuclear loci (i.e. generated by next-generation sequencing approaches) might reveal some population structure[85,86]. However, even if that was the case, it would indicate recent population splitting postdating the ancient divergence of the hosts, since the markers available for this study are generally used to distinguish closely related monogenean populations or species[8,55].

Mismatch distribution, BSP and neutrality test all suggested past population expansion of *C. casuarinus*. These analyses were based on COI sequences and all indicated a recent increase in effective population size. However, only one of the neutrality tests (Fu's F_S) was significant. While Fu's F_S compares expected and observed haplotype

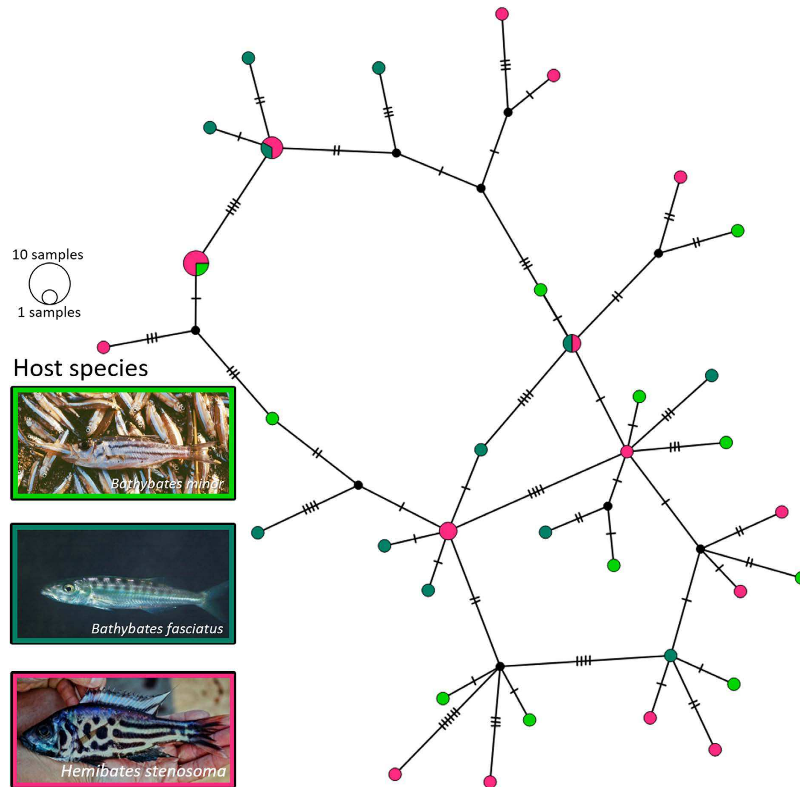


Figure 4. Haplotype network of *C. casuarinus* COI sequences (n = 42). The circles represent different haplotypes with size proportional to the number of individuals represented. Haplotypes are connected with lines, indicating number of mutations. Colours correspond with the host species (pictures by Ad Konings).

diversity and while it is sensitive to demographic expansion[87], significantly negative values of Tajima's D can result from positive selection, a bottleneck, or population expansion[88]. Compared to other neutrality test statistics, Tajima's D has considerably lower power to detect population expansion[61]. Hence the data (non-significant negative D) were still compatible with population expansion. The reported lake level lowstand during the mega-draught period ~100 KYA, when the water level dropped by up to 435 m below the present level (which was not enough to separate the lake into its three sub-basins[89]), reduced the inhabitable lake area considerably, even for pelagic and benthopelagic deepwater fish species. The subsequent lake level rise resulted in an expansion of the available habitat and might have triggered population expansion, a pattern reported for other pelagic and benthopelagic cichlid species from lakes Malawi and Tanganyika[90,91]. Moreover, recent work suggests congruent population expansion in some of the Bathybatini species (S. Koblmüller, unpublished data). Alternatively, recent host colonization or a bottleneck event might be responsible for the observed pattern[55].

The present study is one of the most detailed investigations about intraspecific structure in monogeneans, and the first in an ancient lake. It confirmed the previously suggested decrease of host-specificity in *Cichlidogyrus* in the non-littoral habitat, with *C. casuarinus* as the first generalist species of the genus described from the lake. Therefore, it corroborates a pattern also observed in marine systems. There is a trophic relationship between bathybatine cichlids and economically much more important endemic clupeids of Lake Tanganyika's open water. The predator-prey relationship was already suggested in previous studies to play an important role in host range expansion by transmission of parasites with a direct life cycle[92]. Therefore, it is recommended to also scrutinise these fisheries target species to assess the diversity and dynamics of parasites in the pelagic zone of this unique freshwater ecosystem. The lack of evidence for genetic population structure related to host preference in *C. casuarinus*, the significant intraspecific phenotypic plasticity influenced by the host and the reported population expansion of *C. casuarinus* suggest a high ability of (morphological) adaptation in this monogenean.

Material and Methods

Study area and sampling.

Fish specimens (*Bathybates leo*, *B. minor*, *B. fasciatus, B. graueri* and *H. stenosoma*) were bought at several fish markets in the northern part of Lake Tanganyika, more specifically in the cities of Bujumbura and Uvira. Fishes were identified to the species level *in situ*. Gills were removed according to the standard protocol of Ergens and Lom[93] and immediately preserved in pure ethanol. Some fresh gills were also inspected *in situ* for monogenean parasites under a stereomicroscope. Protocols were approved by the competent local authorities (mission statement 022/MINEURS/CRH-U/2013) and the Animal Care and Use Committee of Masaryk University, and carried out in accordance with permit CZ01308. To complete the taxon coverage and include geographical variation, fishes from the collection of the Royal Museum for Central Africa (Tervuren, Belgium) were also dissected (*B. ferox, B. horni, B. vittatus, B. fasciatus* and *H. stenosoma*) (Table 2,

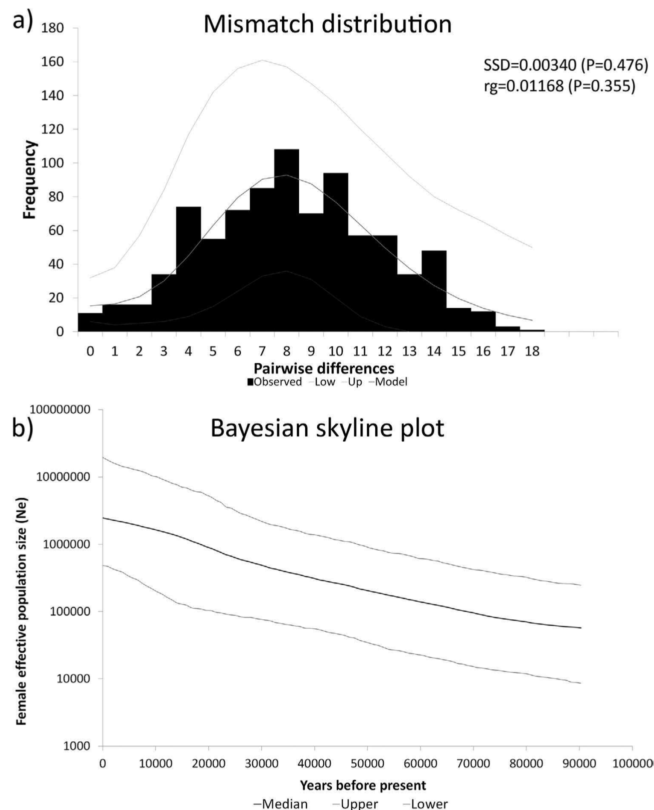


Figure 5. Demographic history of *Cichlidogyrus casuarinus*. (**a**) Mismatch distribution. The black bars show the observed frequency of pairwise differences. The grey lines refer to the expected distribution based on parameter estimates (plus 95% confidence limits) under a model of population growth. The sum of squared differences (SSD) and raggedness index (rg) and their respective P-values are given to describe the fit of the observed distribution to the expectations based on growth parameter estimates, as well as τ, the modal value of the mismatch distribution. (**b**) Bayesian skyline plot (BSP) based on 402 base pairs of COI sequences of *Cichlidogyrus casuarinus* showing the effective populations size through time, assuming a substitution rate of 10% per site per million years. The thick line represents the median values; the thin lines denote 95% highest posterior density (HPD) intervals.

Fig. 1). Monogeneans that were to be used for morphometric analyses were mounted on a slide under a coverslip in Hoyer's medium[94]. These specimens were deposited in the invertebrate collection of the RMCA (Table 2). Worms to be used for genetic work were mounted on a slide with a little drop of water under a cover slip after which pictures of the sclerotized structures were taken under phase contrast using an Olympus BX51 microscope and MicroImage analysis software 4.0. This procedure allowed for *post hoc* species-level identification of specimens of which the entire body was used for DNA extraction. Afterwards, these monogeneans were stored in 1.2 ml eppendorf tubes filled with 99.8% ethanol for subsequent DNA isolation.

Morphometrics and geomorphometrics. Morphological characterization was based on the sclerotized structures of the parasite body; i.e. the opisthaptor and the genital parts. Measurements and photos were taken using an Olympus BX51 microscope with incorporated phase contrast at a magnification of 1000x (objective x100 immersion, ocular x10) with MicroImage 3.1. In total, 26 different features were measured on each individual. The terminology combined Řehulková *et al.*[38] and Pariselle *et al.*[15]. To check for within-species variation in haptor morphology, a principal component analysis was performed on linear haptoral measurements of *C. casuarinus* monogenean parasites from different hosts and localities. For this, the length of pairs of hooks VI and VII was excluded because of the small sample size. This analysis was conducted in CANOCO 5.01[95] on the basis of measurements of 21 selected morphological characters of the haptoral region. Missing data were replaced by the average value of each morphological character. ANOVA tests of MCO structures (only when data was available for more than 15 specimens collected per host species/locality) were performed in STATISTICA 12. To take possible geographical intraspecific variation into consideration, samples were also grouped into three basins according to Danley *et al.*[57] (Table 2). The assumption of homogeneous variance within our sample groups was verified by Levene's test. This prerequisite was only met in the case of the copulatory tube length and for groups defined by host species where Bonferroni's post-hoc test was performed. Other analyses were therefore conducted using the non-parametric variant, namely Kruskal-Wallis ANOVA (see Supplementary Tables 2 and 5).

Geomorphometric analyses focuses on the visualisation of complex shape variation and provides an additional view to the classical morphometric study (linear measurements)[96,97]. Since a significant phylogenetic signal in anchor shape was detected in previous studies[98], the ventral and dorsal anchors of *C. casuarinus* were digitalized using landmarks and semi-landmarks with TPSDIG2 software (Rohlf, 2006, TPS package, Stony Brook University). Positions and number of landmarks (5) and semi-landmarks (95) follow Vignon *et al.*[56]. Semi-landmarks were distributed in equal intervals. Generalized least square superpositions of landmark and semi-landmark coordinates was computed in TPSRELW (Rohlf, 2006, TPS package, Stony Brook University). The degree of shape deformation was quantified by estimating the minimal shape parameters (relative warps) needed to deform the consensus configuration to each specimen computed from partial warps[96,99,100]. As above, groups were defined in two ways: according to the host species and to the geographical origin of the specimen. To visualize mean shape anchor differences, thin-plate spline deformation grids were depicted in TPSSpline (Rohlf, 2006, TPS package, Stony Brook University). Parasites collected from *B. leo* were excluded from the analysis because of the small number of parasite specimens.

Genetic species identification and intraspecific structure. To study the genetic diversity within *Cichlidogyrus casuarinus*, markers with varying rates of molecular evolution were used. These are: the 18 S ribosomal DNA (rDNA) gene, the 28 S rDNA gene, the first internal transcribed spacer region (ITS-1), and the mitochondrial cytochrome *c* oxidase subunit I gene (COI) (GenBank accession numbers: 28 S: KX007796-822, 18 S + ITS1: KX007775-95, COI: KX007823-64). Unfortunately, samples from the RMCA collections could not be used in the genetic part of this study because of fixation in formaldehyde. PCR conditions are mentioned in Supplementary Methods.

The analyses of population structure and demographic history within *C. casuarinus* were based on the COI sequences. COI was used because of its fast rate of molecular evolution as compared to the nuclear markers[59,60]. This allows for the detection of recent evolutionary events, such as possible incipient speciation as a result of host preference[55]. The number of haplotypes and polymorphic sites, haplotype diversity and nucleotide diversity[101] were calculated using DnaSP 5.1[102]. Phylogenetic relationships among COI haplotypes were inferred by means of a Median Joining network[103] in PopART 1.7[104]. Differentiation among pre-defined populations (according to host species) was estimated by F_{ST} in Arlequin 3.5.1.2[105]. To test for signals of past population expansion, a mismatch distribution and two different neutrality test statistics, Tajima's D[88] and Fu's F_S[106] were calculated in Arlequin. Then the fit of the observed mismatch distribution to the expectations based on growth parameter estimates was evaluated by the sum of squared differences (SSD) and the raggedness index (rg). Significance was assessed with 10,000 permutations. Past population size trajectories were inferred employing a Bayesian coalescent approach (Bayesian skyline tree prior[107]) as implemented in BEAST 1.8.1[108]. We employed the model of evolution selected under the Bayesian information criterion in MEGA 6.06, assuming a strict molecular clock and a substitution rate of 10% per million years. Among monogeneans, a substitution rate estimate for COI is only available for *Gyrodactylus* (13.7–20.0% per million years;[109]). In view of the short generation time of *Gyrodactylus* compared to many other monogeneans[110], it can be assumed that mutation rates of other monogeneans are somewhat lower[111], and that the employed 10% per million years represents a reasonable approximation[8]. Two independent MCMC runs of 10 million generations each at a sampling frequency of 1,000 were conducted, with a burn-in of the first 10% of sampled generations. The number of grouped intervals was set to 5. Verification of effective sample sizes ($ESS > 200$ for all parameters), trace of MCMC runs and visualisation of past population size changes were done in Tracer 1.6 (Rambaut A, Suchard MA, Drummond AJ. 2014. Tracer v1.6, available from http://tree.bio.ed.ac.uk/software/tracer/).

References

1. Vanhove, M. P. M. & Huyse, T. In *Parasite diversity and diversification: evolutionary ecology meets phylogenetics*. (eds Morand, S., Krasnov, B. & Littlewood, D. T. J.) 401–419 (Cambridge University Press, 2015).
2. Dunn, R. R., Harris, N. C., Colwell, R. K., Koh, L. P. & Sodhi, N. S. The sixth mass coextinction: are most endangered species parasites and mutualists? *Proc. R. Soc. B* **276,** 3037–45 (2009).
3. Poulin, R. Relative infection levels and taxonomic distances among the host species used by a parasite: insights into parasite specialization. *Parasitology* **130,** 109–115 (2005).
4. Schoelinck, C., Cruaud, C. & Justine, J. L. Are all species of *Pseudorhabdosynochus* strictly host specific? - A molecular study. *Parasitol. Int.* **61,** 356–359 (2012).
5. Justine, J.-L. *et al.* An annotated list of fish parasites (Isopoda, Copepoda, Monogenea, Digenea, Cestoda, Nematoda) collected from Snappers and Bream (Lutjanidae, Nemipteridae, Caesionidae) in New Caledonia confirms high parasite biodiversity on coral reef fish. *Aquat. Biosyst*. **8,** 22, 10-9063–8–22 (2012).
6. Rohde, K. Diversity gradients of marine Monogenea in the Atlantic and Pacific Oceans. *Experientia* **36,** 1368–1369 (1980).
7. Salzburger, W., Van Bocxlaer, B. & Cohen, A. S. Ecology and Evolution of the African Great Lakes and Their Faunas. *Annu. Rev. Ecol. Evol. Syst.* **45,** 519–545 (2014).
8. Vanhove, M. P. M. *et al.* Hidden biodiversity in an ancient lake: phylogenetic congruence between Lake Tanganyika tropheine cichlids and their monogenean flatworm parasites. *Sci. Rep*. **5,** 13669, 10.1038/srep13669 (2015).
9. De Beauchamp, P. M. Sur quelques parasites provenant du Congo belge. *Revue de Zoologie et de Botanique Africaines* **4,** 109–116 (1914).
10. Muterezi Bukinga, F., Vanhove, M. P. M., Van Steenberge, M. & Pariselle, A. Ancyrocephalidae (Monogenea) of Lake Tanganyika: III: *Cichlidogyrus* infecting the world's biggest cichlid and the non-endemic tribes Haplochromini, Oreochromini and Tylochromini (Teleostei, Cichlidae). *Parasitol. Res.* **111,** 2049–2061 (2012).
11. Pariselle, A. *et al.* Ancyrocephalidae (Monogenea) of Lake Tanganyika: Does the *Cichlidogyrus* parasite fauna of *Interochromis loocki* (Teleostei, Cichlidae) reflect its host's phylogenetic affinities? *Contrib. Zool.* **84,** 25–38 (2015).
12. Gillardin, C., Vanhove, M. P. M., Pariselle, A., Huyse, T. & Volckaert, F. A. M. Ancyrocephalidae (Monogenea) of Lake Tanganyika: II: description of the first *Cichlidogyrus* spp. parasites from Tropheini fish hosts (Teleostei, Cichlidae). *Parasitol. Res.* **110,** 305–313 (2012).

13. Vanhove, M. P. M., Volckaert, F. A. M. & Pariselle, A. Ancyrocephalidae (Monogenea) of Lake Tanganyika: I: Four new species of *Cichlidogyrus* from *Ophthalmotilapia ventralis* (Teleostei: Cichlidae), the first record of this parasite family in the basin. *Zoologia*. **28,** 253–263 (2011).
14. Van Steenberge, M. *et al.* Morphology, molecules, and monogenean parasites: an example of an integrative approach to cichlid biodiversity. *PLoS One* **10,** e0124474 (2015).
15. Pariselle, A., Muterezi Bukinga, F., Van Steenberge, M. & Vanhove, M. P. M. Ancyrocephalidae (Monogenea) of Lake Tanganyika: IV: *Cichlidogyrus* parasitizing species of Bathybatini (Teleostei, Cichlidae): reduced host-specificity in the deepwater realm? *Hydrobiologia* **748,** 99–119 (2015).
16. Grégoir, A. F. *et al.* A link between host dispersal and parasite diversity in two sympatric cichlids of Lake Tanganyika. *Freshw. Biol*. **60,** 323–335 (2015).
17. Vanhove, M. P. M., Snoeks, J., Volckaert, F. A. M. & Huyse, T. First description of monogenean parasites in Lake Tanganyika: the cichlid *Simochromis diagramma* (Teleostei, Cichlidae) harbours a high diversity of *Gyrodactylus* species (Platyhelminthes, Monogenea). *Parasitology* **138,** 364–80 (2011).
18. Kmentová, N., Gelnar, M., Koblmüller, S. & Vanhove, M. P. M. Deep-water parasite diversity in Lake Tanganyika: description of two new monogenean species from benthopelagic cichlid fishes. *Parasit. Vectors* **9,** 426, 10.1186/s13071–016–1696–x (2016).
19. Dušek, L., Gelnar, M. & Šebelová, Š. Biodiversity of parasites in a freshwater environment with respect to pollution: metazoan parasites of chub (*Leuciscus cephalus* L.) as a model for statistical evaluation. *Int. J. Parasitol.* **32,** 1555–1571 (1998).
20. Gelnar, M., Koubková, B., Plánková, P. & Jurajda, P. Preliminary report of helminths parasitizing fishes of the river Morava with remarks on the effect of water pollution. *Helminthologia* **31,** 47–56 (1994).
21. Buchmann, K. & Bresciani, J. In *Fish Diseases and Disorders, Volume 1: Protozoan and Metazoan Infections*, *Second Edition*. (eds Woo, P. T. K. & Leatherland, J. F.) 297–344 (CAB International, 2006).
22. Pugachev, O. N., Gerasev, P. I., Gussev, A. V., Ergens, R. & Khotenowsky, I. *Guide to Monogenoidea of freshwater fish of Palaeartic and Amur regions*. (Ledizione-LediPublishing, 2009).
23. Paperna, I. *Parasites, infections and diseases of fishes in Africa: An update*. (Food and Agriculture Organization of the United Nations, 1996).
24. Kearn, G. C. Evolutionary expansion of the Monogenea. *Int. J. Parasitol.* **24,** 1227–1271 (1994).
25. Kocher, T. D. Adaptive evolution and explosive speciation: the cichlid fish model. *Nat. Rev. Genet.* **5,** 288–298 (2004).
26. Koblmüller, S., Sefc, K. M. & Sturmbauer, C. The Lake Tanganyika cichlid species assemblage: Recent advances in molecular phylogenetics. *Hydrobiologia* **615,** 5–20 (2008).
27. Pariselle, A., Morand, S., Deveney, M. & Pouyaud, L. In Taxonomy, ecology and evolution of metazoan parasites. (eds Combes, C. & Jourdan, J.) 147–166 (Universitaires de Perpignan, 2003).
28. Pouyaud, L., Desmarais, E., Deveney, M. & Pariselle, A. Phylogenetic relationships among monogenean gill parasites (Dactylogyridea, Ancyrocephalidae) infesting tilapiine hosts (Cichlidae): Systematic and evolutionary implications. *Mol. Phylogenet. Evol.* **38,** 241–249 (2006).
29. Vanhove, M. P. M. *et al.* Cichlids: A Host of Opportunities for Evolutionary Parasitology. *Trends Parasitol.* **32,** 820–832 (2016).
30. Koblmüller, S., Albertson, R. C., Genner, M. J., Sefc, K. M. & Takahashi, T. Preface: Advances in cichlid research: behavior, ecology, and evolutionary biology. *Hydrobiologia* **748,** 1–5 (2015).
31. Salzburger, W. The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes. *Mol. Ecol*. **18,** 169–185 (2009).
32. Turner, G. F. Adaptive radiation of cichlid fish. *Curr. Biol.* **17,** 827–831 (2007).
33. Sturmbauer, C., Husemann, M. & Danley, P. In *Biodiversity* Hotspots (eds Zachos, F. E. & Habel, J. C.) 333–362 (Springer Berlin Heidelberg, 2011).
34. Muschick, M., Indermaur, A. & Salzburger, W. Convergent evolution within an adaptive radiation of cichlid fishes. *Curr. Biol.* **22,** 2362–2368 (2012).
35. Takahashi, T. & Koblmüller, S. The adaptive radiation of cichlid fish in Lake Tanganyika: a morphological perspective. *Int. J. Evol. Biol*. **2011,** 620754, 10.4061/2011/620754 (2011).
36. Pariselle, A. *et al.* The monogenean parasite fauna of cichlids: a potential tool for host biogeography. *Int. J. Evol. Biol*. **2011,** 471480, 10.4061/2011/471480 (2011).
37. Pariselle, A. & Euzet, L. Systematic revision of dactylogyridean parasites (Monogenea) from cichlid fishes in Africa, the Levant and Madagascar. *Zoosystema* **31,** 849–898 (2009).
38. Řehulková, E., Mendlová, M. & Šimková, A. Two new species of *Cichlidogyrus* (Monogenea: Dactylogyridae) parasitizing the gills of African cichlid fishes (Perciformes) from Senegal: Morphometric and molecular characterization. *Parasitol. Res.* **112,** 1399–1410 (2013).
39. Pariselle, A., Bitja Nyom, A. R. & Bilong Bilong, C. F. Checklist of the ancyrocephalids (Monogenea) parasitizing Tilapia species in Cameroon, with the description of three new species. *Zootaxa* **3599,** 78–86 (2013).
40. Pariselle, A., Bitja Nyom, A. R. & Bilong Bilong, C. F. Four new species of *Cichlidogyrus* (Monogenea, Ancyrocephalidae) from *Sarotherodon mvogoi* and *Tylochromis sudanensis* (Teleostei, Cichlidae) in Cameroon. *Zootaxa* **3881,** 258–66 (2014).
41. Messu Mandeng, F. D. *et al.* A phylogeny of *Cichlidogyrus* spp. (Monogenea. Dactylogyridea) clarifies a host-switch between fish families and reveals an adaptive component to attachment organ morphology of this parasite genus. *Parasit. Vectors* **8,** 582 (2015).
42. Kmentová, N., Gelnar, M., Koblmüller, S. & Vanhove, M. P. M. First insights into the diversity of gill monogeneans of '*Gnathochromis*' and *Limnochromis* (Teleostei, Cichlidae) in Burundi: do the parasites mirror host ecology and phylogenetic history? *PeerJ* **4,** e1629, 10.7717/peerj.1629 (2016).
43. Mendlová, M. & Šimková, A. Evolution of host specificity in monogeneans parasitizing African cichlid fish. *Parasit. Vectors* **7,** 69 (2014).
44. Poulin, R. Determinants of host-specificity in parasites of freshwater fishes. *Int. J. Parasitol.* **22,** 753–758 (1992).
45. Barker, S. C. Evolution of host-parasite associations among species of lice and rock-wallabies: Coevolution? *Int. J. Parasitol.* **21,** 497–501 (1991).
46. Whittington, I. D., Cribb, B. W., Hamwood, T. E. & Halliday, J. a. Host-specificity of monogenean (platyhelminth) parasites: A role for anterior adhesive areas? *Int. J. Parasitol*. **30,** 305–320 (2000).
47. Buchmann, K. Interactions between monogenean parasites and their fish hosts. *Int. J. Parasitol*. **32,** 309–319 (2002).
48. Takahashi, T. Systematics of Tanganyikan cichlid fishes (Teleostei: Perciformes). *Ichthyol. Res.* **50,** 367–382 (2003).
49. Kirchberger, P. C., Sefc, K. M., Sturmbauer, C. & Koblmüller, S. Evolutionary History of Lake Tanganyika's Predatory Deepwater Cichlids. *Int. J. Evol. Biol*. **2012,** 716209, 10.1155/2012/716209 (2012).
50. Takahashi, T. & Sota, T. A robust phylogeny among major lineages of the East African cichlids. *Mol. Phylogenet. Evol.* **100,** 234–242 (2016).
51. Meyer, B. S., Matschiner, M. & Salzburger, W. A tribal level phylogeny of Lake Tanganyika cichlid fishes based on a genomic multi-marker approach. *Mol. Phylogenet. Evol.* **83,** 56–71 (2015).
52. Poll, M. Classification des cichlidae du lac Tanganika. *Académie R. Belgique Membres la Cl. des Sci*. **45,** 1–163 (1986).
53. Koblmüller, S., Duftner, N., Katongo, C., Phiri, H. & Sturmbauer, C. Ancient divergence in bathypelagic Lake Tanganyika deepwater cichlids: Mitochondrial phylogeny of the tribe Bathybatini. *J. Mol. Evol.* **60,** 297–314 (2005).

54. Coulter, G. W. *Lake Tanganyika and Its Life. Lake Tanganyika and Its Life* (Oxford University Press, 1991).
55. Bueno-Silva, M., Boeger, W. A. & Pie, M. R. Choice matters: Incipient speciation in *Gyrodactylus corydori* (Monogenoidea: Gyrodactylidae). *Int. J. Parasitol.* **41,** 657–667 (2011).
56. Vignon, M., Pariselle, A. & Vanhove, M. P. M. Modularity in attachment organs of African *Cichlidogyrus* (Platyhelminthes: Monogenea: Ancyrocephalidae) reflects phylogeny rather than host specificity or geographic distribution. *Biol. J. Linn. Soc.* **102,** 694–706 (2011).
57. Danley, P. D. *et al.* The impact of the geologic history and paleoclimate on the diversification of East african cichlids. *Int. J. Evol. Biol.* **2012,** 574851, 10.1155/2012/574851 (2012).
58. Šimková, A., Morand, S., Jobet, E., Gelnar, M. & Verneau, O. Molecular phylogeny of congeneric monogenean parasites (*Dactylogyrus*): a case of intrahost speciation. *Evolution* **58,** 1001–1018 (2004).
59. Vanhove, M. P. M. *et al.* Problematic barcoding in flatworms: A case-study on monogeneans and rhabdocoels (Platyhelminthes). *ZooKeys* **365,** 355–379 (2013).
60. Hillis, D. M. & Dixon, N. T. Ribosomal DNA: Molecular Evolution and Phylogenetic Inference. *Q. Rev. Biol.* **44,** 411–451 (1991).
61. Ramos-Onsins, S. E. & Rozas, J. Statistical properties of new neutrality tests against population growth. *Mol. Biol. Evol.* **19,** 2092–2100 (2002).
62. Matějusová, I., Gelnar, M., McBeath, A. J. A., Collins, C. M. & Cunningham, C. O. Molecular markers for gyrodactylids (Gyrodactylidae: Monogenea) from five fish families (Teleostei). *Int. J. Parasitol.* **31,** 738–745 (2001).
63. Cunningham, C. O. Species Variation within the Internal Transcribed Spacer (ITS) Region of *Gyrodactylus* (Monogenea: Gyrodactylidae) Ribosomal RNA Genes. *J. Parasitol.* **83,** 215–219 (1997).
64. Cable, J. & Harris, P. D. Gyrodactylid developmental biology: historical review, current status and future trends. *Int. J. Parasitol.* **32,** 255–80 (2002).
65. Nevado, B., Fazalova, V., Backeljau, T., Hanssens, M. & Verheyen, E. Repeated unidirectional introgression of nuclear and mitochondrial DNA between four congeneric Tanganyikan cichlids. *Mol. Biol. Evol.* **28,** 2253–67 (2011).
66. Fannes, W., Vanhove, M. P. M., Huyse, T. & Paladini, G. A scanning electron microscope technique for studying the sclerites of *Cichlidogyrus*. *Parasitol. Res.* **114,** 2031–4 (2015).
67. Konings, A. *Guide to Tanganyika Cichlids*. (Cichlid Press, 1998).
68. Maréchal, C. & Poll, M. In *Checklist of the Freshwater Fishes of* Africa (C*LOFFA*) (eds Daget, J., Gosse, J.-P., Teugels, G. G. & Thys van den Audenaerde, D. F. E.) 21–24, 186 (Royal Museum for Central Africa, 1991).
69. Buchmann, K. Relationship between host size of *Anguilla anguilla* and the infection level of the monogeneans *Pseudodactylogyrus* spp. *J. Fish Biol.* **35,** 599–601 (1989).
70. Šimková, A., Ondráčková, M., Gelnar, M. & Morand, S. Morphology and coexistence of congeneric ectoparasite species: reinforcement of reproductive isolation? *Biol. J. Linn. Soc.* **76,** 125–135 (2002).
71. Jiménez-García, M. I., Vidal-Martínez, V. M. & López-Jiménez, S. Monogeneans in introduced and native cichlids in México: evidence for transfer. *J. Parasitol.* **87,** 907–9 (2001).
72. Šimková, A., Desdevise, Y., Gelnar, M. & Morand, S. Morphometric correlates of host specificity in *Dactylogyrus* species (Monogenea) parasites of European Cyprinid fish. *Parasitology* **123,** 169–77 (2001).
73. Jarkovský, J., Morand, S., Šimková, A. & Gelnar, M. Reproductive barriers between congeneric monogenean parasites (*Dactylogyrus*: Monogenea): Attachment apparatus morphology or copulatory organ incompatibility? *Parasitol. Res.* **92,** 95–105 (2004).
74. Rohde, K. & Watson, N. Morphology, microhabitats and geographical variation of *Kuhnia* spp. (Monogenea: Polyopisthocotylea). *Int. J. Parasitol.* **15,** 569–586 (1985).
75. Rohde, K. Size differences in hamuli of *Kuhnia scombri* (Monogenea: Polyopisthocotylea) from different geographical areas not due to differences in host size. *Int. J. Parasitol.* **21,** 113–4 (1991).
76. Ergens, R. Variability of hard parts of opisthaptor of two species of *Gyrodactylus* Nordmann, 1832 (Monogenoidea) from *Phoxinus phoxinus* (L.). *Folia Parasitol.* (*Praha*). **23,** 111–126 (1976).
77. Ergens, R. & Gelnar, M. Experimental verification of the effect of temperature on the size of the hard parts of haptor of *Gyrodactylus katharineri* Malberg 1964. *Folia Parasitol.* (*Praha*). **32,** 377–380 (1985).
78. Beaumont, M. Book review: Sublethal and chronic effects of pollutants on freshwater fish. edited by R. Müller & R. Lloyd. *Regul. Rivers Res. Manag.* **13**, 95–96 (1997).
79. Kritsky, D. C. & Boeger, W. A. Neotropical Monogenoidea: 41. New and previously described species of Dactylogyridae (Platyhelminthes) from the gills of marine and freshwater perciform fishes (Teleostei) with proposal of a new genus and a hypothesis on phylogeny. *J. Parasitol.* **76,** 1–21 (1990).
80. Kritsky, D. C. & Boeger, W. A. Neotropical Monogenoidea: 41. New and previously described species of Dactylogyridae (Platyhelminthes) from the gills of marine and freshwater perciform fishes (Teleostei) with proposal of a new genus and a hypothesis on phylogeny. *Zoosystema* **24,** 7–40 (2002).
81. Donnelly, P. & Tavare, S. The ages of alleles and a coalescent. *Adv. Appl. Probab.* **18,** 1–19 (1986).
82. Ziętara, M. S. & Lumme, J. Speciation by host switch and adaptive radiation in a fish parasite genus *Gyrodactylus* (Monogenea, Gyrodactylidae). *Evolution* **56,** 2445–58 (2002).
83. Huyse, T. & Malmberg, G. Molecular and morphological comparisons between *Gyrodactylus ostendicus* n. sp. (Monogenea: Gyrodactylidae) on *Pomatoschistus microps* (Krøyer) and *G. harengi* Malmberg, 1957 on *Clupea harengus membras*. *Syst. Parasitol.* **58,** 105–13 (2004).
84. Kuusela, J., Ziętara, M. & Lumme, J. Description of three new European cryptic species of *Gyrodactylus* Nordmann, 1832 supported by nuclear and mitochondrial phylogenetic characterization. *Acta Parasitol.* **53,** 120–126 (2008).
85. Falush, D., Stephens, M. & Pritchard, J. K. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Mol. Ecol. Notes* **7,** 574–578 (2007).
86. Spinks, P. Q., Thomson, R. C. & Shaffer, H. B. The advantages of going large: genome-wide SNPs clarify the complex population history and systematics of the threatened western pond turtle. *Mol. Ecol.* **23,** 2228–2241 (2014).
87. Fu, Y. X. & Li, W. H. Statistical test of neutrality of mutations. *Genetics* **133,** 693–709 (1993).
88. Tajima, F. Statistical methods to test for nucleotide mutation hypothesis by DNA polymorphism. *Genetics* **123,** 585–595 (1989).
89. McGlue, M. M. *et al.* Seismic records of late Pleistocene aridity in Lake Tanganyika, tropical East Africa. *J. Paleolimnol.* **40,** 635–653 (2007).
90. Koblmüller, S., Odhiambo, E. A. & Sinyinza, D. Big fish, little divergence: phylogeography of Lake Tanganyika's giant cichlid, *Boulengerochromis microlepis*. **748,** 29–38 (2015).
91. Genner, M. J. & Turner, G. F. Age of populations in the Lake Malawi cichlid radiation. *Hydrobiologia* **748,** 121–132 (2015).
92. Strona, G. The underrated importance of predation in transmission ecology of direct lifecycle parasites. *Oikos* **124,** 685–690 (2015).
93. Ergens, R. & Lom, J. *Causative agents of fish diseases*. (Academia, 1970).
94. Humason, G. L. *Animal tissue techniques:4th*. (Freeman and Company, 1979).
95. Ter Braak, C. J. F. & Šmilauer, P. J. *CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Ordination (Version 4)*. (Wageningen: Centre for Biometry, 1998).
96. Rohlf, F. J. & Marcus, L. F. A revolution morphometrics. *Trends Ecol. Evol.* **8,** 129–32 (1993).
97. Mitteroecker, P. & Gunz, P. Advances in Geometric Morphometrics. *Evol. Biol.* **36,** 235–247 (2009).

98. Khang, T. F., Soo, O. Y. M., Tan, W. B. & Lim, L. H. S. Monogenean anchor morphometry: systematic value, phylogenetic signal, and evolution. *PeerJ* **4,** e1668, 10.7717/peerj.1668 (2016).
99. Rohlf, F. *Tpsdig, digitize landmarks and outlines. Version 2.10*. (Stony Brook, NY: State University, 2006).
100. Bookstein, F. L. *Morphometric Tools for Landmark Data: Geometry and Biology*. (Cambridge University Press, 1997).
101. Nei, M. *Molecular evolutionary genetics*. (Columbia University Press, 1987).
102. Librado, P. & Rozas, J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25,** 1451–2 (2009).
103. Bandelt, H. J., Forster, P. & Rohl, A. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **16,** 37–48 (1999).
104. Leigh, J. W. & Bryant, D. PopART: full-feature software for haplotype network construction. *Methods Ecol. Evol.* **6,** 1110–1116 (2015).
105. Excoffier, L. & Lischer, H. E. L. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour*. **10,** 564–7 (2010).
106. Fu, Y. X. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147,** 915–925 (1997).
107. Drummond, A. J., Rambaut, A., Shapiro, B. & Pybus, O. G. Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol. Biol. Evol.* **22,** 1185–1192 (2005).
108. Drummond, A. J., Suchard, M. A., Xie, D. & Rambaut, A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **29,** 1969–73 (2012).
109. Meinilä, M., Kuusela, J., Ziętara, M. S. & Lumme, J. Initial steps of speciation by geographic isolation and host switch in salmonid pathogen *Gyrodactylus salaris* (Monogenea: Gyrodactylidae). *Int. J. Parasitol.* **34,** 515–526 (2004).
110. Bakke, T. A., Harris, P. D. & Cable, J. Host specificity dynamics: Observations on gyrodactylid monogeneans. *Int. J. Parasitol*. **32,** 281–308 (2002).
111. Li, M., Shi, S. F., Brown, C. L. & Yang, T. B. Phylogeographical pattern of *Mazocraeoides gonialosae* (Monogenea, Mazocraeidae) on the dotted gizzard shad, *Konosirus punctatus*, along the coast of China. *Int. J. Parasitol.* **41,** 1263–1272 (2011).
112. Kmentová, N. *Monogenean parasites from deepwater Lake Tanganyika cichlids: reduced specificity or hidden diversity?* (MSc thesis, Masaryk University, 2015).
113. Lerssutthichawal, T., Maneepitaksanti, W. & Purivirojkul, W. Gill Monogeneans of Potentially Cultured Tilapias and First Record of *Cichlidogyrus mbirizei* Bukinga *et al.*, 2012. in Thailand. **13,** 543–553 (2015).

Acknowledgements

The authors would like to thank the following people for their contribution to the field work: Andrea Vetešníková-Šimková, Eva Řehulková, Šárka Mašová, Iva Přikrylová, Veronika Nezhybová, Radim Blažek, Gaspard Banyankimbona, Fidel Muterezi Bukinga, Constantin Amundala Shekani, Donatien Muzumani Risasi, Théophile Mulimbwa N'sibula, Joseph Mbirize Ndalozibwa, Axel Meyer, Christian Sturmbauer, Martin Reichard, the Schreyen-Brichard family and the technical staff of Fishes of Burundi. We are grateful to Ad Konings (Cichlid Press) for providing us with fish pictures. Daniel R. Brooks and Walter A. Boeger are acknowledged for useful comments on this study. We also thank Tine Huyse, Jos Snoeks, Miguël Parrent (RMCA) and people in the parasitological group, Masaryk University, Brno for their hospitality and the RMCA for providing access to the collections. Research was supported by the Czech Science Foundation (GBP505/12/G112 – ECIP).

Author Contributions

M.P.M.V. designed the study, planned the expedition, contributed to data analyses and interpretation and revised the manuscript. N.K. performed molecular work, analysed sequences, obtained morphometric and geomorphometric data, contributed to data analyses and interpretation and wrote the manuscript. M.G. provided financial support and necessary experience of monogenean taxonomy. M.M. performed molecular work and contributed to alignment of sequences. M.V.S. contributed to geomorphometric analyses and manuscript writing. S.K. identified the hosts, provided background on the host system, performed demographic reconstruction and contributed to expedition planning, data interpretation and manuscript writing. All authors contributed to the final version of the manuscript.

Additional Information

Accession Codes: The sequences obtained were deposited in NCBI GenBank under accession numbers KX007775-864.

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Kmentová, N. *et al.* Reduced host-specificity in a parasite infecting non-littoral Lake Tanganyika cichlids evidenced by intraspecific morphological and genetic diversity. *Sci. Rep.* **6**, 39605; doi: 10.1038/srep39605 (2016).

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

© The Author(s) 2016

Kmentová N., Gelnar M., Koblmüller S. & Vanhove M. P. M. 2016. Deep-water parasite diversity in Lake Tanganyika: description of two new monogenean species from benthopelagic cichlid fishes. *Parasites & Vectors* 9:426. DOI: 10.1186/s13071-016-1696-x [Q1, IF (2016) = 3.035].

Publication available at:

https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-016-1696-x

DOI 10.1186/s13071-016-1696-x

Parasites & Vectors

RESEARCH Open Access

Deep-water parasite diversity in Lake Tanganyika: description of two new monogenean species from benthopelagic cichlid fishes

Nikol Kmentová[1*], Milan Gelnar[1], Stephan Koblmüller[2,3] and Maarten P. M. Vanhove[1,4,5,6]

Abstract

Background: Lake Tanganyika is the world's second deepest lake. Its diverse cichlid assemblage offers a unique opportunity for studying a deep-water host-parasite model in freshwater. Low host specificity and a broad host range including representatives of the Bathybatini tribe in the only monogenean parasite described from this habitat, *Cichlidogyrus casuarinus* Pariselle, Muterezi Bukinga & Vanhove, 2015 suggest a link between lower specificity and lower host density. Conversely, high host specificity and species richness are reported for monogeneans of the lake's littoral cichlids. We further investigated whether the deep-water environment in Lake Tanganyika is really monogenean species-depauperate by investigating the monogenean fauna of *Trematocara unimaculatum* (a representative of the tribe Trematocarini, the sister lineage of the Bathybatini) and *Benthochromis horii*, a member of the tribe Benthochromini, found in the same deep-water habitat as the already known hosts of *C. casuarinus*.

Methods: Sclerotised structures of the collected monogenean individuals were characterised morphologically using light microscopy and morphometrics.

Results: Both examined cichlid species are infected by a single monogenean species each, which are new to science. They are described as *Cichlidogyrus brunnensis* n. sp., infecting *T. unimaculatum*, and *Cichlidogyrus attenboroughi* n. sp., parasitising on *B. horii*. Diagnostic characteristics include the distal bifurcation of the accessory piece in *C. brunnensis* n. sp. and the combination of long auricles and no heel in *C. attenboroughi* n. sp. In addition *C. brunnensis* n. sp. does not resemble *C. casuarinus*, the only species of *Cichlidogyrus* thus far reported from the Bathybatini. Also *Cichlidogyrus attenboroughi* n. sp. does not resemble any of the monogenean species documented from the pelagic zone of the lake and is among the few described species of *Cichlidogyrus* without heel.

Conclusions: As two new and non-resembling *Cichlidogyrus* species are described from *T. unimaculatum* and *B. horii*, colonisation of the deep-water habitat by more than one morphotype of *Cichlidogyrus* is evident. Based on morphological comparisons with previously described monogenean species, parasite transfers with the littoral zone are possible. Therefore, parasites of pelagic cichlids in the lake do not seem to only mirror host phylogeny and the evolutionary history of this host-parasite system merits further attention.

Keywords: *Benthochromis horii*, Cichlidae, *Cichlidogyrus*, Monogenea, *Trematocara unimaculatum*

* Correspondence: kmentovan@mail.muni.cz
[1]Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic
Full list of author information is available at the end of the article

BioMed Central

© 2016 The Author(s). **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

Background

Considering the high number of vertebrate [1–4] and invertebrate [5–11] radiations described from Lake Tanganyika, it is surprising that its parasite fauna has been mostly overlooked for many years. Parasitological research in Lake Tanganyika has increased since about five years, improving our understanding of mainly its monogenean fauna [12–17]. The Monogenea van Beneden, 1858 is a group of mostly ectoparasitic flatworms described mainly from freshwater fishes and phylogenetically closely related to Cestoda van Beneden, 1849 [18, 19]. Due to their direct life-cycles and high degree of structural adaptations influenced by host preferences, they are considered as useful targets for investigations focusing on evolutionary processes in parasites [20–23] as well as for research on the taxonomy [16], biogeography [24–26] or phylogeny of their host species [27]. This is nicely exemplified when focusing on the cichlid fishes (family Cichlidae), one of the most diverse host fish families with a remarkable evolutionary history featuring rapid radiation processes [1, 28, 29]. Cichlids display huge species richness and are usually classified into tribes [30].

Lake Tanganyika in the African Rift Valley is the second deepest lake in the world and figures as a natural experiment displaying the greatest diversity of speciation mechanisms in cichlids compared to the other major African lakes [28, 31]. Lake Tanganyika is inhabited by more than 200 cichlid species [31], classified into 15 different tribes [32–34]. Six monogenean genera have been reported to infect African cichlid fishes. *Cichlidogyrus* Paperna, 1960 is the most species-rich one [15, 24, 35]. To date, 22 species of *Cichlidogyrus* have been described in Lake Tanganyika from 18 cichlid hosts representing seven different tribes [12–17]. Most records originated from the littoral zone where these parasites were shown to display quite strong host specificity [27]. Cichlid species richness in Lake Tanganyika decreases with water depth [31], with most of the diversity found in shallow littoral habitats. This situation is caused by three main factors: reduction of niche diversity in the deep-water habitat, the fact that the short-wave length blue light spectrum in the depths does not promote diversification mechanisms, and the absence of strong geographic barriers [36–39]. The same pattern of lower species richness, along with lower host specificity, was documented in monogeneans and suggested to be a consequence of lower host availability. For example, the generalist *C. casuarinus* Pariselle, Muterezi Bukinga & Vanhove, 2015 has been reported from six bathybatine cichlid species belonging to the genera *Bathybates* and *Hemibates* [13, Kmentová et al., unpublished observation]. To further our knowledge of the monogenean diversity in deep-water Tanganyika cichlids, we examined *Trematocara unimaculatum* Boulenger, 1901, a representative of the tribe Trematocarini, the sister group of the Bathybatini [34, 40], and *Benthochromis horii* Takahashi, 2008, a cichlid species belonging to another deep-water tribe, Benthochromini, which is only distantly related to the Bathybatini [31, 34], but found in the same habitat as the previously reported hosts of *C. casuarinus*.

Methods

Fishes were bought on fish markets in the capital city of Burundi, Bujumbura (3°23'S, 29°22'E) in September 2013 and identified in situ. Eight specimens of *Trematocara unimaculatum* and ten of *Benthochromis horii* were dissected according to the standard protocol of Ergens & Lom [41]. Gills were preserved in ethanol until subsequent inspection for monogeneans under an Olympus SYX7 stereomicroscope. Parasites were mounted on slides under coverslips using Hoyer's medium, enabling visualisation of sclerotised structures [42]. Measurements of sclerotised structures were taken at a magnification of 1000× (objective × 100 immersion, ocular × 10) using an Olympus BX51 microscope with incorporated phase contrast and the software MicroImage version 4.0. In total, 26 different metrical features were measured and are presented in micrometres. The terminology follows [17, 43] while "straight length" and "straight width" mean the linear length of the measured structure. Drawings were made using an Olympus BX51 microscope equipped with a drawing tube and OLYMPUS KL 1500 LED illumination and edited with a graphics tablet compatible with Adobe Illustrator 16.0.0 and Adobe Photoshop 13.0. The type-material was deposited in the invertebrate collection of the Royal Museum for Central Africa (RMCA), Tervuren, Belgium; the Iziko South African Museum (SAMC), Cape Town, Republic of South Africa; the Muséum national d'Histoire naturelle (MNHN), Paris, France; and the Natural History Museum (NHMUK), London, United Kingdom. Tissue samples of the hosts are available in the ichthyology collection of the RMCA.

Results

Trematocara unimaculatum and *B. horii* were each infected by a single different monogenean species belonging to *Cichlidogyrus*. Following Paperna [44] and Pariselle et al. [45], the genus is characterised by a haptor consisting of two pairs of medium-sized anchors, seven pairs of marginal hooks, two transversal bars (the dorsal one with two auricles; the ventral one curved and articulated), a male copulatory organ (MCO) with copulatory tube and most often a heel and (see [15]) an accessory piece; and a vagina which is not always sclerotised. Both collected *Cichlidogyrus* spp. are new to science and their descriptions are presented below. Since the species description in dactylogyrid monogenean taxonomy is more than anything else based on the

morphology of their sclerotised structures [19, 46], the depiction of soft parts and internal organs is omitted and a differential diagnosis focused on details of the parasites' hard parts is provided.

Family Dactylogyridae Yamaguti, 1963
Genus *Cichlidogyrus* Paperna, 1960

***Cichlidogyrus brunnensis* n. sp.**

Type-host: *Trematocara unimaculatum* Boulenger, 1901 (Cichlidae).
Type-locality: Bujumbura, Lake Tanganyika, Burundi (3° 23'S, 29°22'E), coll. 4.ix.2013.
Type-material: Holotype: MRAC MT.37812. Paratypes: MRAC MT.37812-4 (16 specimens); MNHN HEL549-550 (4 specimens); NHMUK 2015.12.10.1-2 (3 specimens); SAMC-A082649-50 (3 specimens).
Site in host: Gills.
Infection parameters: Five of eight fish infected with 2–23 specimens.
ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN) [47], details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:F7E8CC4E-8B91-48A9-9131-3BBBC80F798F. The LSID for the new name *Cichlidogyrus brunnensis* is urn:lsid:zoobank.org:act:270003FD-6002-404B-BD38-37F5ED161EEA.
Etymology: The species epithet was chosen after the biggest Moravian city, Brno, Czech Republic, where Masaryk University was founded, in gratitude for the education and support provided.

Description

[Based on 30 specimens; Figs. 1, 3a, c; see measurements in Table 1.] Dorsal anchor with poorly incised roots and well-developed, regularly curved short point. Ventral anchors larger than dorsal anchors, with longer distance between base and point of hook, poorly incised roots, short point. Dorsal bar large, wide, straight, with relatively short, wide auricles. Ventral bar thick, short, branches straight with constant width. Hooks 7 pairs, pairs 1–4, 6 and 7 relatively short (*sensu* [35]) compared to pair 5, considering ontogenetic development as pair 5 retains its larval size); pair 7 largest. MCO small, with narrow, thin-walled tubular copulatory tube; accessory piece of same length as copulatory tube with distal bifurcation starting in distal quarter, and short heel. Sclerotised vagina not observed.

Differential diagnosis

The anchors of this species resemble those of its non-Tanganyika congeners *Cichlidogyrus sclerosus* Paperna & Thurston, 1969 [48], *C. amphoratus* Pariselle & Euzet, 1996

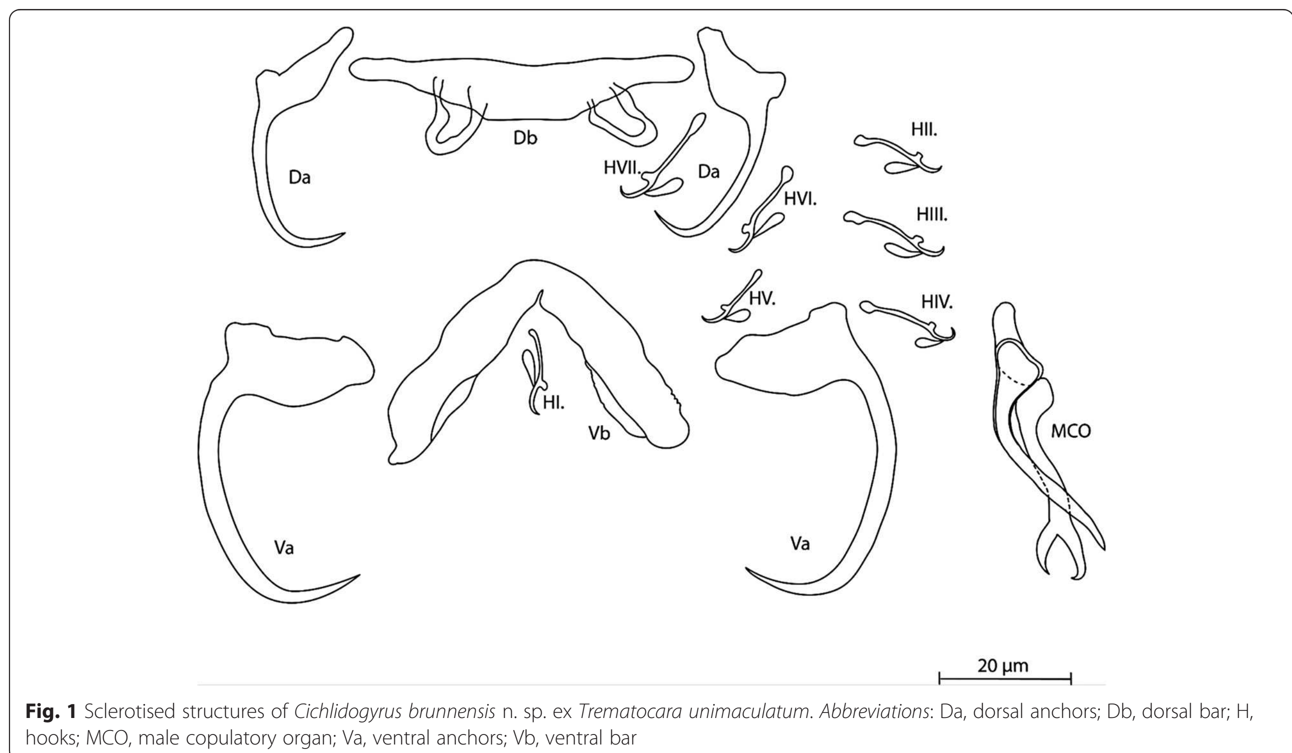


Fig. 1 Sclerotised structures of *Cichlidogyrus brunnensis* n. sp. ex *Trematocara unimaculatum*. *Abbreviations*: Da, dorsal anchors; Db, dorsal bar; H, hooks; MCO, male copulatory organ; Va, ventral anchors; Vb, ventral bar

Table 1 Measurements (in micrometres) for the two new species of *Cichlidogyrus* described in this study

Character	*C. brunnensis* n. sp. (*n* = 30)			*C. attenboroughi* n. sp. (*n* = 30)		
	Range	Mean	*n*	Range	Mean	*n*
Total body length	493.9–772.5	657.1	3	436–344.7	378.9	10
Ventral anchor						
Total length	38.1–42.7	41.6	25	30.2–34.5	32.8	21
Length to notch	36.6–45.9	40.0	24	25.2–30.4	27.4	18
Inner root length	3.3–15.2	11.8	19	5.9–10.4	8.0	18
Outer root length	2.4–7.6	5.4	11		4.6	12
Point length	10.6–16.0	13.5	23	3.3–5.3	8.2	
Dorsal anchor						
Total length	30.2–36.6	33.1	20	25.8–33.6	29.9	23
Length to notch	25.0–29.4	27.1	19	18.3–26.5	23.0	20
Inner root length	5.6–12.7	10.5	19	7.3–11.2	9.2	21
Outer root length	0.9–5.0	4.0	16	3.4–8.0	5.1	18
Point length	7.3–10.0	8.7	20	6.0–8.8	7.4	17
Ventral bar						
Branch length	32.3–48.2	38.5	19	30.4–41.0	34.7	26
Branch maximum width	8.2–10.4	9.2	22	4.0–5.8	5.1	28
Dorsal bar						
Maximum straight width	35.8–51.1	43.5	15	31.8–44.6	38.1	24
Thickness at midlength	6.8–10.6	8.2	23	4.1–6.7	5.6	28
Distance between auricles	14.6–22.2	17.4	21	9.3–15.5	13.0	23
Auricle length	5.7–14.8	11.1	15	13.6–23.1	18.4	14
Hooks						
First pair	13.7–19.2	16.2	11	11.1–16.8	11.9	28
Second pair	14.8–18.7	16.1	14	12.3–18.1	16.3	25
Third pair	15.5–20.9	17.6	18	13.4–21.9	17.9	22
Fourth pair	14.1–18.4	16.5	12	19.9–23.4	21.5	15
Fifth pair	11.2–15.4	13.3	11	10.2–14.1	11.6	11
Sixth pair	15.1–27.6	18.0	9	18.4–22.9	20.3	11
Seventh pair	17.0–23.0	18.7	9	15.0–22.5	17.2	10
MCO straight length	40.1–69.4	48.6	17		–	
Copulatory tube curved length	24.5–45.3	37.8	16	47.4–64.5	52.8	27
Accessory piece curved length	29.6–44.8	37.2	14	35.9–53.2	43.4	22
Heel straight length	10.0–4.8	7.6	28		–	

[49] and *C. giostrai* Pariselle, Bilong Bilong & Euzet, 2003 [45] described from *Oreochromis mossambicus* (Peters, 1852), *Tilapia louka* Thys van den Audenaerde, 1969 and *Sarotherodon caudomarginatus* (Boulenger, 1916), respectively, in their broad base and almost non-incised roots of the anchors. However, the exceptional shape of its accessory piece, with a forked ending, as well as the large ventral anchor in comparison to the dorsal one, make *C. brunnensis* n. sp. clearly distinguishable among all species of *Cichlidogyrus* described so far. Moreover, the shape of the anchors, specifically their poorly incised roots and the proportionally large hook, is unique among all other known congeners in Lake Tanganyika: *Cichlidogyrus vandekerkhovei* Vanhove, Volckaert & Pariselle, 2011; *C. makasai* Vanhove, Volckaert & Pariselle, 2011; *C. sturmbaueri* Vanhove, Volckaert & Pariselle, 2011; *C. centesimus* Vanhove, Volckaert & Pariselle, 2011; *C. gillardinae* Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle, 2012; *C. mbirizei* Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle, 2012; *C. nshomboi* Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle, 2012; *C. mulimbwai* Muterezi Bukinga, Vanhove, Van

Steenberge & Pariselle, 2012; *C. muzumanii* Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle, 2012; *C. steenbergei* Gillardin, Vanhove, Pariselle, Huyse & Volckaert, 2012; *C. gistelincki* Gillardin, Vanhove, Pariselle, Huyse & Volckaert, 2012; *C. irenae* Gillardin, Vanhove, Pariselle, Huyse & Volckaert, 2012; *C. buescheri* Pariselle & Vanhove, 2015; *C. schreyenbrichardorum* Pariselle & Vanhove, 2015; *C. vealli* Pariselle & Vanhove, 2015; *C. banyankimbonai* Pariselle & Vanhove, 2015; *C. muterezii* Pariselle & Vanhove, 2015; *C. raeymaekersi* Pariselle & Vanhove, 2015; *C. georgesmertensi* Pariselle & Vanhove, 2015; *C. franswittei* Pariselle & Vanhove, 2015; *C. frankwillemsi* Pariselle & Vanhove, 2015 and *C. casuarinus*. The latter species infects bathybatine cichlids, the sister group of the Trematocarini, which among others also comprises *T. unimaculatum*, the type-host of *C. brunnensis* n. sp., but *C. casuarinus* is morphologically very different; it is easily distinguished by the spirally-coiled thickening in the wall of the copulatory tube. Although the forked shape of the accessory piece of *C. brunnensis* n. sp. is similar to an undescribed species collected from *Limnochromis auritus* [50], a member of Lake Tanganyika's benthic deep water cichlid tribe Limnochomini, differences in haptoral sclerotised structures, namely the length of the dorsal bar auricles (on average 11.1 μm in *C. brunnensis vs c.*45 μm in the undescribed species) and the shape of the anchors allows clear distinction between them.

Cichlidogyrus attenboroughi n. sp.

Type-host: *Benthochromis horii* Takahashi, 2008 (Cichlidae).
Type-locality: Bujumbura, Lake Tanganyika, Burundi (3° 23'S, 29°22'E).
Type-material: Holotype: MRAC MT.37815. Paratypes: MRAC: MT.37815-7 (10 specimens); MNHN HEL551-552 (5 specimens); NHMUK 2015.12.10. 3–4 (5 specimens); SAMC-A082651-2 (4 specimens).
Site in host: Gills.
Infection parameters: Three of ten fish infected with 4–27 specimens.
ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN) [47], details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:F7E8CC4E-8B91-48A9-9131-3BBBC80F798F. The LSID for the new name *Cichlidogyrus attenboroughi* is urn:lsid:zoobank.org:act:AC051EA5-FCAC-49A8-9048-02E44E80654D.
Etymology: The species epithet honours the English scientist and broadcaster Sir David Frederick Attenborough, in gratitude for the insights and inspiration he gave to so many people to study and protect nature and biodiversity.

Description

[Based on 30 specimens; Figs. 2, 3b, d; see measurements in Table 1.] Dorsal anchors with different root size and regularly curved points. Ventral anchors larger in total size and more similar root size than dorsal anchors. Dorsal bar thin, with relatively long narrow auricles. Ventral bar thin, long, with constant width. Hooks 7 pairs, pair 4 relatively long; pairs 1 and 5 of equal length. MCO with slender copulatory tube with relatively thick wall; accessory piece broader than copulatory tube. No heel or sclerotised vagina observed.

Differential diagnosis

There are congeners in Lake Tanganyika that share with *C. attenboroughi* n. sp. the small size of the first pair of hooks and the presence of long dorsal bar auricles, namely *C. makasai* and *C. vandekerkhovei* recorded from *Ophthalmotilapia ventralis* (Boulenger, 1898),*O. nasuta* (Poll & Matthes, 1962) and *O. boops* (Boulenger, 1901). *Cichlidogyrus attenboroughi* n. sp. differs from these species by the shorter length of the auricles (18.4 μm in *C. attenboroughi vs* 20 μm in *C. makasai* and 30 μm in *C. vandekerkhovei*) and in possessing a MCO with a simple accessory piece and without a heel. Because of the shape of the ventral anchor and the equal size among the first and the second pairs of hooks this species could be mistaken with *C. gistelincki* infecting *Ctenochromis horei* (Günther, 1894), *C. irenae* described from *Gnathochromis pfefferi* (Boulenger, 1898) and *C. steenbergei* parasitising *Limnotilapia dardennii* (Boulenger, 1899) all of which are also present in Lake Tanganyika. However, in contrast to these species, there is no developed heel in the MCO of *C. attenboroughi* n. sp. Three other species have been described lacking a heel: *C. haplochromii* Paperna & Thurston, [48] described from *Pharyngochromis darlingi* (Boulenger, 1911); *C. tilapiae* Paperna, 1960 recorded from, among others, *Oreochromis leucostictu*s (Trewavas, 1933) and *Sarotherodon galilaeus* (Linnaeus, 1758) and *C. arfii* Pariselle & Euzet, 1995 described from *Pelmatochromis buettikoferi* (Steindachner, 1894). However, the haptoral region of the latter species cannot be confused with *C. attenboroughi* n. sp. because of the different edge of the dorsal anchor roots, the relative length of the first pairs of hooks (the smallest pair in *C. attenboroughi* n. sp. and the biggest pair in *C. arfii*) or the length of the auricles (18.4 μm in *C. attenboroughi* n. sp. *vs* 9 μm in *C. arfii*) [51]. The most evident difference between *C. attenboroughi* n. sp. and *C. tilapiae* is the size of the dorsal anchor as well as the maximal straight width of the dorsal bar and the length

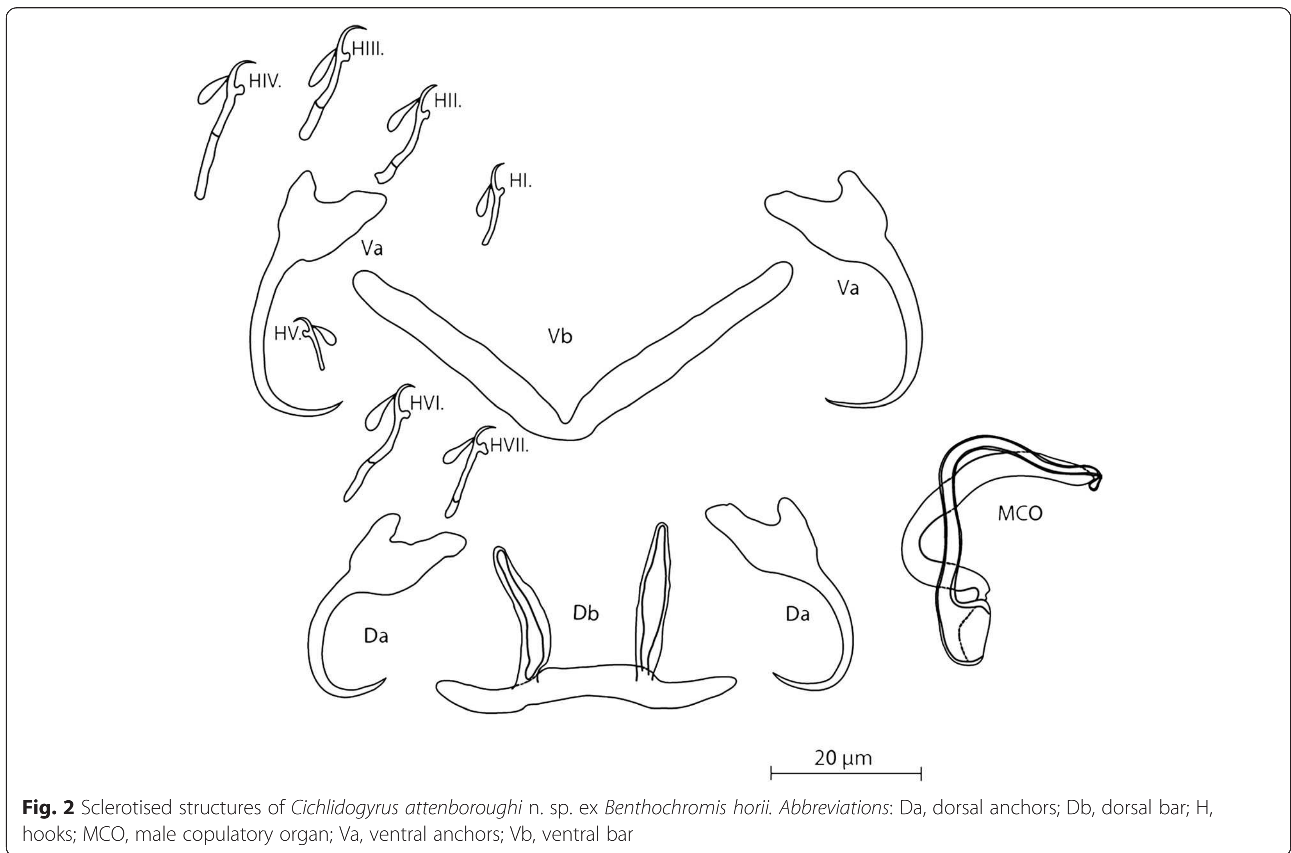


Fig. 2 Sclerotised structures of *Cichlidogyrus attenboroughi* n. sp. ex *Benthochromis horii. Abbreviations*: Da, dorsal anchors; Db, dorsal bar; H, hooks; MCO, male copulatory organ; Va, ventral anchors; Vb, ventral bar

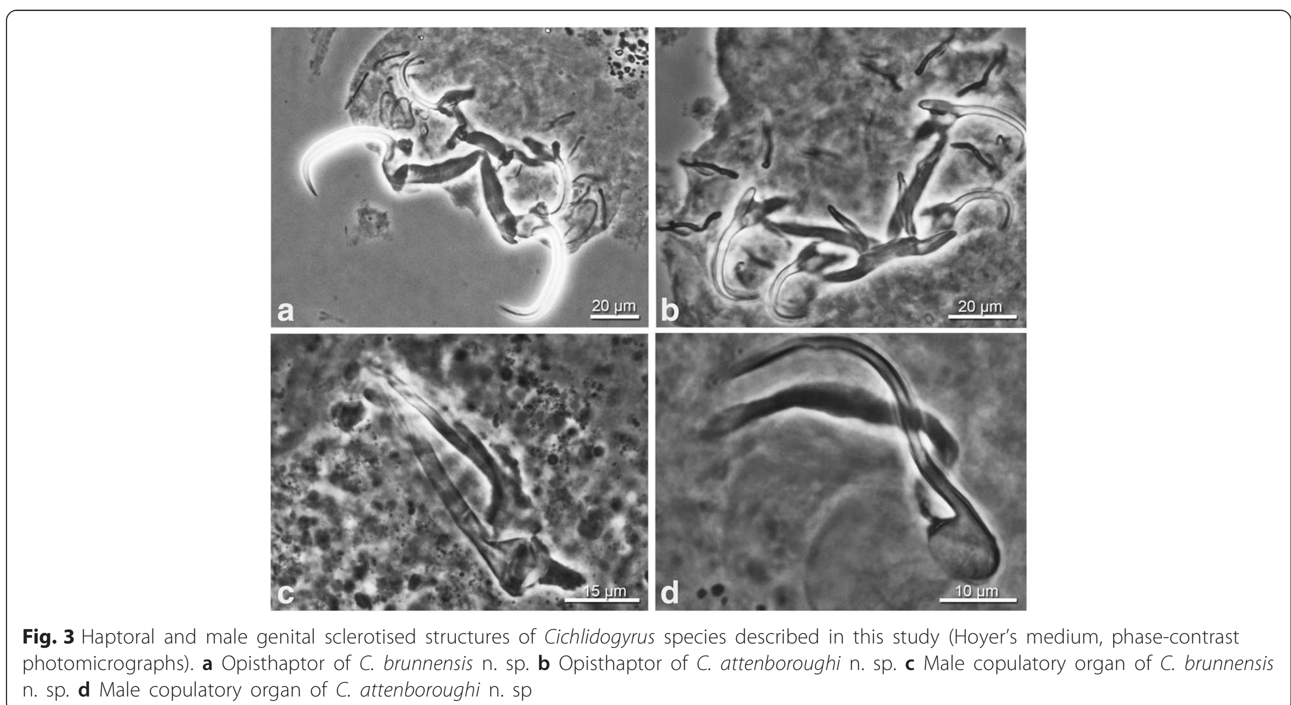


Fig. 3 Haptoral and male genital sclerotised structures of *Cichlidogyrus* species described in this study (Hoyer's medium, phase-contrast photomicrographs). **a** Opisthaptor of *C. brunnensis* n. sp. **b** Opisthaptor of *C. attenboroughi* n. sp. **c** Male copulatory organ of *C. brunnensis* n. sp. **d** Male copulatory organ of *C. attenboroughi* n. sp

of its auricles [45]. Differences with *C. haplochromii* are visible in the dorsal bar, which has shorter and less slender auricles in comparison to *C. attenboroughi* n. sp. [48].

Discussion

The presence of a single monogenean species, phenotypically substantially different from *C. casuarinus*, on *T. unimaculatum*, indicates that closely related deep-water cichlid lineages have been colonised by several *Cichlidogyrus* lineages. Moreover, comparison with other species reported from Lake Tanganyika so far points to multiple origins of the deep-water representatives of *Cichlidogyrus*. Indeed, this is suggested by the phenotypic similarity of *C. brunnensis* n. sp. to an undescribed species collected from the benthic cichlid *Limnochromis auritus* (Boulenger, 1901) [50] and its quite different morphology of sclerotised structures as compared to *C. attenboroughi* n. sp., which infects another deep-water cichlid species, *Benthochromis horii*. Furthermore, *C. attenboroughi* n. sp. shares morphological characteristics of its haptoral region with two species of *Cichlidogyrus* (*C. vandekerkhovei* and *C. makasai*) recorded from three species of *Ophthalmotilapia* as well as with species described from tropheine cichlids [14, 15]. Interestingly, *C. casuarinus* infecting Bathybatini and *C. nshomboi* infecting *Boulengerochromis microlepis* (Boulenger, 1899), a cichlid species phylogenetically closely related to the Trematocarini and Bathybatini [52–54], are similar to *C. centesimus*. The latter infects three species of the genus *Ophthalmotilapia*, which belongs to the tribe Ectodini. *Cichlidogyrus casuarinus*, *C. nshomboi* and *C. centesimus* share the unique spirally coiled thickening of the wall of the copulatory tube [12, 13, 15]. Both ectodine and tropheine cichlids occur in shallow water and are only distantly related to Bathybatini, Trematocarini, Benthochromini and *Boulengerochromis*. Therefore, other scenarios such as host habitat preferences influencing the chance of transmission [55] or shared morphological characters of the host affecting monogenean phenotypes might have played a role in the evolutionary history of this monogenean assemblage [56, 57].

Conclusions

The inventory of monogeneans from Lake Tanganyika has been supplemented by the description of *C. brunnensis* n. sp. and *C. attenboroughi* n. sp. collected from *T. unimaculatum* and *B. horii*, respectively. These are the first monogeneans reported from the respective cichlid species and tribes. The known host range of *C. casuarinus* still remains limited to the genera *Bathybates* and *Hemibates*, although further investigations are needed to confirm this observation. Our result is consistent with taxonomic hypotheses that include the Trematocarini as a separate tribe [34, 58], which, together with the Bathybatini, constitute the sister group of the Boulengerochromini [53]. *Cichlidogyrus casuarinus*, parasitising bathybatine cichlids, morphologically resembles *C. nshomboi* (collected from *B. microlepis*, see [12, 13]) more than *C. brunnensis* n. sp. which infects *T. unimaculatum*, a representative of the Bathybatini's sister group Trematocarini. Hence, probably other speciation mechanisms rather than co-speciation have occurred in the evolutionary history of this deep-water parasite-host system. An exhaustive list of *Cichlidogyrus* species occurring on deep-water cichlid species in Lake Tanganyika, together with genetic analyses and a cophylogenetic approach, are needed to verify these alternative scenarios. The reported lower monogenean host specificity is probably correlated with small diversity and population densities of hosts [58–60] influenced by lower temperature and reduction of light as communication of cichlids is mainly based on visual signals [61]. Decline of parasite diversity is probably also related to the distance from the shore as well as to specific host behavioural characteristics [60]. Although the deep-water monogenean fauna in Lake Tanganyika seems to be less species-rich than the littoral one [27] it is premature to exactly quantify differences in monogenean species richness per host, or to conclude whether the deep-water monogenean fauna is indeed depauperate.

Abbreviations

MCO, male copulatory organ

Acknowledgements

The authors appreciate the help of the people involved in the field work and sampling procedure (Andrea Šimková, Eva Řehulková, Šárka Mašová, Iva Přikrylová, Radim Blažek, Veronika Nezhybová, Gaspard Banyankimbona and the Schreyen-Brichard family and the technical staff of Fishes of Burundi). Tine Huyse and Maarten Van Steenberge are thanked for their valuable comments, Tetsumi Takahashi for confirming our *Benthochromis* species identification and the parasitological lab of Masaryk University in Brno for its hospitality.

Funding

This study was financially supported by the Czech Science Foundation (GBP505/12/G112-ECIP).

Availability of data and materials

The data supporting the conclusions of this article are included within the article. The type-material of the new species described in this study is deposited in the invertebrate collection of the Royal Museum for Central Africa (RMCA), Tervuren, Belgium; the Iziko South African Museum (SAMC), Cape Town, Republic of South Africa; the Muséum national d'Histoire naturelle (MNHN), Paris, France; and the Natural History Museum (NHMUK), London, United Kingdom (see "Type-material" for accession numbers). Tissue samples of the hosts are available in the ichthyology collection of the RMCA.

Authors' contributions

MPMV designed and supervised this study. MG provided scientific background in the field of monogenean research. SK identified fish species, contributed to sampling and provided scientific background on Lake Tanganyika and its ichthyofauna. NK performed the morphological characterisation and described the species. MPMV and NK analysed the data and wrote the paper. SK revised the manuscript. All authors read and approved the final version of the manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
The research was approved by the Ethics Committee of Masaryk University. The approval number which allows us to work with vertebrate animals is CZ01308.

Author details
[1]Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic. [2]Institute of Zoology, University of Graz, Universitätsplatz 2, A-8010 Graz, Austria. [3]Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, 603 65 Brno, Czech Republic. [4]Biology Department, Royal Museum for Central Africa, Leuvensesteenweg 13, B-3080 Tervuren, Belgium. [5]Laboratory of Biodiversity and Evolutionary Genomics, Department of Biology, University of Leuven, Ch. Deberiotstraat 32, B-3000 Leuven, Belgium. [6]Present address: Capacities for Biodiversity and Sustainable Development, Operational Directorate Natural Environment, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium.

Received: 25 January 2016 Accepted: 13 July 2016
Published online: 03 August 2016

References
1. Sturmbauer C. Explosive speciation in cichlid of the African Great Lakes: a dynamic model of adaptive radiation. J Fish Biol. 1998;53:18–36.
2. Day JJ, Peart CR, Brown KJ, Friel JP, Bills R, Moritz T. Continental diversification of an African catfish radiation (Mochokidae: *Synodontis*). Syst Biol. 2013;62:351–65.
3. Brown KJ, Rüber L, Bills R, Day JJ. Mastacembelid eels support Lake Tanganyika as an evolutionary hotspot of diversification. BMC Evol Biol. 2010;10:188.
4. Peart CR, Bills R, Wilkinson M, Day JJ. Nocturnal claroteine catfishes reveal dual colonisation but a single radiation in Lake Tanganyika. Mol Phylogenet Evol. 2014;73:119–28.
5. Michel E. Evolutionary diversification of rift lake gastropods: Morphology, anatomy, genetics and biogeography of *Lavigeria* (Mollusca: Thiaridae) in Lake Tanganyika. Tucson: The University of Arizona; 1995.
6. Glaubrecht M. Adaptive radiation of thalassoid gastropods in Lake Tanganyika, East Africa: morphology and systematization of a paludomid species flock in an ancient lake. Zoosystematics Evol. 2008;84:71–122.
7. Fryer G. Comparative aspects of adaptive radiation and speciation in Lake Baikal and the great rift lakes of Africa. Hydrobiologia. 1991;211:137–46.
8. Schön I, Martens K. Molecular analyses of ostracod flocks from Lake Baikal and Lake Tanganyika. Hydrobiologia. 2011;682:91–110.
9. Marijnissen SAE, Michel E, Daniels SR, Erpenbeck D, Menken SBJ, Schram FR. Molecular evidence for recent divergence of Lake Tanganyika endemic crabs (Decapoda: Platythelphusidae). Mol Phylogenet Evol. 2006;40:628–34.
10. Erpenbeck D, Weier T, de Voogd NJ, Wörheide G, Sutcliffe P, Todd JA, Michel E. Insights into the evolution of freshwater sponges (Porifera: Demospongiae: Spongillina): Barcoding and phylogenetic data from Lake Tanganyika endemics indicate multiple invasions and unsettle existing taxonomy. Mol Phylogenet Evol. 2011;61:231–6.
11. Meixner MJ, Lüter C, Eckert C, Itskovich V, Janussen D, von Rintelen T, et al. Phylogenetic analysis of freshwater sponges provide evidence for endemism and radiation in ancient lakes. Mol Phylogenet Evol. 2007;45:875–86.
12. Muterezi Bukinga F, Vanhove MPM, Van Steenberge M, Pariselle A. Ancyrocephalidae (Monogenea) of Lake Tanganyika: III: *Cichlidogyrus* infecting the world's biggest cichlid and the non-endemic tribes Haplochromini, Oreochromini and Tylochromini (Teleostei, Cichlidae). Parasitol Res. 2012;111:2049–61.
13. Pariselle A, Muterezi Bukinga F, Van Steenberge M, Vanhove MPM. Ancyrocephalidae (Monogenea) of Lake Tanganyika: IV: *Cichlidogyrus* parasitizing species of Bathybatini (Teleostei, Cichlidae): reduced host-specificity in the deepwater realm? Hydrobiologia. 2015;748:99–119.
14. Gillardin C, Vanhove MPM, Pariselle A, Huyse T, Volckaert FAM. Ancyrocephalidae (Monogenea) of Lake Tanganyika: II: description of the first *Cichlidogyrus* spp. parasites from Tropheini fish hosts (Teleostei, Cichlidae). Parasitol Res. 2012;110:305–13.
15. Vanhove MPM, Volckaert FAM, Pariselle A. Ancyrocephalidae (Monogenea) of Lake Tanganyika: I: Four new species of *Cichlidogyrus* from *Ophthalmotilapia ventralis* (Teleostei: Cichlidae), the first record of this parasite family in the basin. Zoologia. 2011;28:253–63.
16. Van Steenberge M, Pariselle A, Huyse T, Volckaert FAM, Snoeks J, Vanhove MPM. Morphology, molecules, and monogenean parasites: an example of an integrative approach to cichlid biodiversity. PLoS One. 2015;10:e0124474.
17. Pariselle A, Van Steenberge M, Snoeks J, Volckaert FAM, Huyse T, Vanhove MPM. Ancyrocephalidae (Monogenea) of Lake Tanganyika: Does the *Cichlidogyrus* parasite fauna of *Interochromis loocki* (Teleostei, Cichlidae) reflect its host's phylogenetic affinities? Contrib Zool. 2015;84:25–38.
18. Boeger WA, Kritsky DC. Coevolution of the Monogenoidea (Platyhelminthes) based on a revised hypothesis of parasite phylogeny. Int J Parasitol. 1997;27:1495–511.
19. Pugachev ON, Gerasev PI, Gussev AV, Ergens R, Khotenowsky I. Guide to Monogenoidea of freshwater fish of Palaeartic and Amur Regions. Milan: Ledizione-LediPublishing; 2009.
20. Šimková A, Ondráčková M, Gelnar M, Morand S. Morphology and coexistence of congeneric ectoparasite species: reinforcement of reproductive isolation? Biol J Linn Soc. 2002;76:125–35.
21. Šimková A, Morand S, Jobet E, Gelnar M, Verneau O. Molecular phylogeny of congeneric monogenean parasites (*Dactylogyrus*): a case of intrahost speciation. Evolution. 2004;58:1001–18.
22. Mendlová M, Desdevises Y, Civáňová K, Pariselle A, Šimková A. Monogeneans of west African cichlid fish: Evolution and cophylogenetic interactions. PLoS One. 2012;7:e37268.
23. Vanhove MPM, Huyse T. Host-specificity and species-jumps in fish-parasite systems. In: Morand S, Krasnov B, Littlewood DTJ, editors. Parasite diversity and diversification: evolutionary ecology meets phylogenetics. Cambridge: Cambridge University Press; 2015. p. 401–19.
24. Pariselle A, Boeger WA, Snoeks J, Bilong Bilong CF, Morand S, Vanhove MPM. The monogenean parasite fauna of cichlids: a potential tool for host biogeography. Int J Evol Biol. 2011;2011:ID 471480.
25. Vanhove MPM, Van Steenberge M, Dessein S, Volckaert FAM, Snoeks J, Huyse T, Pariselle A. Biogeographical implications of Zambezian *Cichlidogyrus* species (Platyhelminthes: Monogenea: Ancyrocephalidae) parasitizing Congolian cichlids. Zootaxa. 2013;3608:398–400.
26. Barson M, Přikrylová I, Vanhove MPM, Huyse T. Parasite hybridization in African *Macrogyrodactylus* spp. (Monogenea, Platyhelminthes) signals historical host distribution. Parasitology. 2010;137:1585–95.
27. Vanhove MPM, Pariselle A, Van Steenberge M, Raeymaekers JAM, Hablützel PI, Gillardin C, et al. Hidden biodiversity in an ancient lake: phylogenetic congruence between Lake Tanganyika tropheine cichlids and their monogenean flatworm parasites. Sci Rep. 2015;5:13669.
28. Sturmbauer C, Husemann M, Danley P. Explosive speciation and adaptive radiation of East African cichlid fishes. In: Frank EH, Habel JC, editors. Biodiversity hotspots. Distribution and protection of conservation priority areas. London: Springer; 2011. p. 333–62.
29. Santos ME, Salzburger W. Evolution. How cichlids diversify. Science. 2012;338:619–21.
30. Poll M. Classification des cichlidae du lac Tanganika. Académie R Belgique Membres la Cl des Sci. 1986;45:1–163.
31. Koblmüller S, Sefc KM, Sturmbauer C. The Lake Tanganyika cichlid species assemblage: Recent advances in molecular phylogenetics. Hydrobiologia. 2008;615:5–20.
32. Takahashi T. Greenwoodochromini Takahashi from Lake Tanganyika is a junior synonym of Limnochromini Poll (Perciformes: Cichlidae). J Fish Biol. 2014;84:929–36.
33. Takahashi T. Systematics of Tanganyikan cichlid fishes (Teleostei: Perciformes). Ichthyol Res. 2003;50:367–82.
34. Takahashi T, Sota T. A robust phylogeny among major lineages of the East African cichlids. Mol Phylogenet Evol. 2016;100:234–42.
35. Pariselle A, Euzet L. Systematic revision of dactylogyridean parasites (Monogenea) from cichlid fishes in Africa, the Levant and Madagascar. Zoosystema. 2009;31:849–98.
36. Seehausen O. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. Science. 1997;277:1808–11.
37. Seehausen O, Van Alphen JJM, Lande R. Color polymorphism and sex ratio distortion in a cichlid fish as an incipient stage in sympatric speciation by sexual selection. Ecol Lett. 1999;2:367–78.
38. Knight ME, Turner GF. Laboratory mating trials indicate incipient speciation by sexual selection among populations of the cichlid fish *Pseudotropheus zebra* from Lake Malawi. Proc R Soc B. 2004;271:675–80.

39. Maan ME, Seehausen O, Söderberg L, Johnson L, Ripmeester EAP, Mrosso HDJ, et al. Intraspecific sexual selection on a speciation trait, male coloration, in the Lake Victoria cichlid *Pundamilia nyererei*. Proc R Soc B. 2004;271:2445–52.
40. Kirchberger PC, Sefc KM, Sturmbauer C, Koblmüller S. Evolutionary history of Lake Tanganyika's predatory deepwater cichlids. Int J Evol Biol. 2012;2012:716209.
41. Ergens R, Lom J. Causative Agents of Fish Diseases. Prague: Academia; 1970.
42. Humason GL. Animal tissue techniques. 4th ed. San Francisco: Freeman and Company; 1979.
43. Řehulková E, Mendlová M, Šimková A. Two new species of *Cichlidogyrus* (Monogenea: Dactylogyridae) parasitizing the gills of African cichlid fishes (Perciformes) from Senegal: Morphometric and molecular characterization. Parasitol Res. 2013;112:1399–410.
44. Paperna I. Studies on monogenetic trematodes in Israel, 2: Monogenetic trematodes of cichlids. Bamidgeh. 1960;12:20–33.
45. Pariselle A, Bilong Bilong CF, Euzet L. Four new species of *Cichlidogyrus* Paperna, 1960 (Monogenea, Ancyrocephalidae), all gill parasites from African mouthbreeder tilapias of the genera *Sarotherodon* and *Oreochromis* (Pisces, Cichlidae), with a redescription of *C. thurstonae* Ergens, 1981. Syst Parasitol. 2003;56:201–10.
46. Vignon M, Pariselle A, Vanhove MPM. Modularity in attachment organs of African *Cichlidogyrus* (Platyhelminthes: Monogenea: Ancyrocephalidae) reflects phylogeny rather than host specificity or geographic distribution. Biol J Linn Soc. 2011;102:694–706.
47. International Commission on Zoological Nomenclature. Amendment of articles 8, 9, 10, 21 and 78 of the International Code of Zoological Nomenclature to expand and refine methods of publication. Zootaxa. 2012;3450:1–7.
48. Paperna I, Thurston JP. Monogenetic trematodes collected from cichlid fish in Uganda;including the description of five new species of *Cichlidogyrus*. Rev Zool Bot Afr. 1969;79:15–33.
49. Pariselle A, Euzet L. *Cichlidogyrus* Paperna, 1960 (Monogenea, Ancyrocephalidae): gill parasites from West African Cichlidae of the subgenus *Coptodon* Regan, 1920 (Pisces), with descriptions of six new species. Syst Parasitol. 1996;34:109–24.
50. Kmentová N, Gelnar M, Koblmüller S, Vanhove MPM. First insights into the diversity of gill monogeneans of'*Gnathochromis*' and *Limnochromis* (Teleostei, Cichlidae) in Burundi: do the parasites mirror host ecology and phylogenetic history? PeerJ. 2016;4:e1629.
51. Pariselle A, Euzet L. Trois Monogènes nouveaux parasites branchiaux de *Pelmatochromis buettikoferi* (Steindachner, 1895)(Cichlidae) en Guinée. Parasite. 1995;2:203–9.
52. Meyer BS, Matschiner M, Salzburger W. A tribal level phylogeny of Lake Tanganyika cichlid fishes based on a genomic multi-marker approach. Mol Phylogenet Evol. 2015;83:56–71.
53. Muschick M, Indermaur A, Salzburger W. Convergent evolution within an adaptive radiation of cichlid fishes. Curr Biol. 2012;22:2362–8.
54. Weiss JD, Cotterill FPD, Schliewen UK. Lake Tanganyika - A “melting pot” of ancient and young cichlid lineages (Teleostei: Cichlidae)? PLoS One. 2015;10:e0125043.
55. Desdevises Y, Morand S, Legendre P. Evolution and determinants of host specificity in the genus *Lamellodiscus* (Monogenea). Biol J Linn Soc. 2002;77:431–43.
56. Vignon M, Sasal P. The use of geometric morphometrics in understanding shape variability of sclerotized haptoral structures of monogeneans (Platyhelminthes) with insights into biogeographic variability. Parasitol Int. 2010;59:183–91.
57. Morand S, Šimková A, Matějusová I, Plaisance L, Verneau O, Desdevises Y. Investigating patterns may reveal processes: evolutionary ecology of ectoparasitic monogeneans. Int J Parasitol. 2002;32:111–9.
58. Schoelinck C, Cruaud C, Justine JL. Are all species of *Pseudorhabdosynochus* strictly host specific? - A molecular study. Parasitol Int. 2012;61:356–9.
59. Justine J-L, Beveridge I, Boxshall G, Bray RA, Miller TL, Moravec F, et al. An annotated list of fish parasites (Isopoda, Copepoda, Monogenea, Digenea, Cestoda, Nematoda) collected from Snappers and Bream (Lutjanidae, Nemipteridae, Caesionidae) in New Caledonia confirms high parasite biodiversity on coral reef fish. Aquat Biosyst. 2012;8:22.
60. Campbell RA, Headrich RL, Munroe TA. Parasitism and ecological relationships among deep-see benthic fishes. Mar Biol. 1980;57:301–13.
61. Barlow G. The Cichlid Fishes. Nature’s Grand Experiment In Evolution. Cambridge: Perseus Publishing; 2000.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at www.biomedcentral.com/submit

Kmentová N., Gelnar M., Koblmüller S. & Vanhove M. P. M. 2016. Gill monogeneans of *'Gnathochromis'* and *Limnochromis* (Teleostei, Cichlidae) in Burundi: do the parasites mirror host ecology and phylogenetic history? *PeerJ* 4(1): e1629. https://doi.org/10.7717/peerj.1629 [Q1, IF (2016) = 2.177].

Publication available at: https://peerj.com/articles/1629/

PeerJ

First insights into the diversity of gill monogeneans of '*Gnathochromis*' and *Limnochromis* (Teleostei, Cichlidae) in Burundi: do the parasites mirror host ecology and phylogenetic history?

Nikol Kmentová[1], Milan Gelnar[1], Stephan Koblmüller[2,3] and Maarten P.M. Vanhove[1,4,5,6]

[1] Department of Botany and Zoology, Masaryk University, Brno, Czech Republic
[2] Institute of Zoology, University of Graz, Graz, Austria
[3] Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic
[4] Biology Department, Royal Museum for Central Africa, Tervuren, Belgium
[5] Laboratory of Biodiversity and Evolutionary Genomics, Deparment of Biology, University of Leuven, Leuven, Belgium
[6] Capacities for Biodiversity and Sustainable Development, Royal Belgian Institute of Natural Sciences, Brussels, Belgium

ABSTRACT

Monogenea is one of the most species-rich groups of parasitic flatworms worldwide, with many species described only recently, which is particularly true for African monogeneans. For example, *Cichlidogyrus*, a genus mostly occurring on African cichlids, comprises more than 100 nominal species. Twenty-two of these have been described from Lake Tanganyika, a famous biodiversity hotspot in which many vertebrate and invertebrate taxa, including monogeneans, underwent unique and spectacular radiations. Given their often high degrees of host specificity, parasitic monogeneans were also used as a potential tool to uncover host species relationships. This study presents the first investigation of the monogenean fauna occurring on the gills of endemic '*Gnathochromis*' species along the Burundese coastline of Lake Tanganyika. We test whether their monogenean fauna reflects the different phylogenetic position and ecological niche of '*Gnathochromis*' *pfefferi* and *Gnathochromis permaxillaris*. Worms collected from specimens of *Limnochromis auritus*, a cichlid belonging to the same cichlid tribe as *G. permaxillaris*, were used for comparison. Morphological as well as genetic characterisation was used for parasite identification. In total, all 73 *Cichlidogyrus* individuals collected from '*G.*' *pfefferi* were identified as *C. irenae*. This is the only representative of *Cichlidogyrus* previously described from '*G.*' *pfefferi*, its type host. *Gnathochromis permaxillaris* is infected by a species of *Cichlidogyrus* morphologically very similar to *C. gillardinae*. The monogenean species collected from *L. auritus* is considered as new for science, but sample size was insufficient for a formal description. Our results confirm previous suggestions that '*G.*' *pfefferi* as a good disperser is infected by a single monogenean species across the entire Lake Tanganyika. Although *G. permaxillaris* and *L. auritus* are placed in the same tribe, *Cichlidogyrus* sp. occurring on *G. permaxillaris* is morphologically more similar to *C. irenae* from '*G.*' *pfefferi*, than to the *Cichlidogyrus* species found on *L. auritus*. Various evolutionary processes, such as host-switching or duplication events, might underlie the pattern observed in

Submitted 2 November 2015
Accepted 5 January 2016
Published 25 January 2016

Corresponding author
Nikol Kmentová,
kmentovan@mail.muni.cz

Academic editor
María Ángeles Esteban

Additional Information and Declarations can be found on page 11

DOI 10.7717/peerj.1629

Copyright
2016 Kmentová et al.

Distributed under
Creative Commons CC-BY 4.0

OPEN ACCESS

this particular parasite-host system. Additional samples for the *Cichlidogyrus* species occuring on *G. permaxillaris* and *L. auritus* are needed to unravel their evolutionary history by means of (co-)phylogenetic analyses.

Subjects Fisheries and Fish Science, Biodiversity, Parasitology
Keywords *Cichlidogyrus*, Lake Tanganyika, Ectoparasites, Limnochromini, Tropheini

INTRODUCTION

Cichlid fishes (Cichlidae) are considered an ideal study system for evolutionary biologists because of their remarkable species richness, high rates of speciation and often high levels of endemicity, derived from diverse speciation and adaptive radiation processes (*Salzburger et al., 2005*; *Turner, 2007*; *Muschick, Indermaur & Salzburger, 2012*). Studies about cichlid adaptation mechanisms provided important information, generally applicable in evolutionary biology (*Kocher, 2004*; *Koblmüller, Sefc & Sturmbauer, 2008*). Cichlids range from Central and South America, across Africa, Iran, the Middle East and Madagascar to India and Sri Lanka, but most species are concentrated in the Neotropics and in Africa (*Chakrabarty, 2004*). A place famous for its extraordinary cichlid diversity is Lake Tanganyika in East Africa (*Koblmüller, Sefc & Sturmbauer, 2008*). It is considered a prime study area for evolutionary research as its cichlids show the greatest diversity in speciation mechanisms of all the African Great Lakes' cichlid fishes (*Salzburger et al., 2002*; *Salzburger, 2009*). In Lake Tanganyika, there are more than 200 described cichlid species belonging to 53 genera (*Snoeks, 2000*; *Takahashi, 2003*; *Koblmüller, Sefc & Sturmbauer, 2008*), usually classified into 15 tribes (*Takahashi, 2003*; *Takahashi, 2014*).

Although cichlids have been subjects of interest for many decades, there are still gaps in the understanding of their phylogenetic history and taxonomy (*Koblmüller, Sefc & Sturmbauer, 2008*). According to recent molecular findings, the two species of '*Gnathochromis*', *G. permaxillaris* (LR David, 1936) and '*G.*' *pfefferi* (GA Boulenger, 1898) belong to different cichlid tribes (Limnochromini and Tropheini, respectively) and their classification therefore needs revision (*Salzburger et al., 2002*; *Duftner, Koblmüller & Sturmbauer, 2005*; *Koblmüller et al., 2010*; *Muschick, Indermaur & Salzburger, 2012*; *Kirchberger et al., 2014*). A possible source for a better understanding of cichlid taxonomy and phylogeny, and a particularly diverse group of organisms in Lake Tanganyika, are monogenean parasites (*Mendlová et al., 2012*; *Vanhove et al., 2015*; *Van Steenberge et al., 2015*). Monogenea P-J Van Beneden, 1858 is a group of parasitic flatworms mainly occurring on fish gills, skin and fins (*Pugachev et al., 2009*). These often tiny animals have a direct life cycle, and relatively strong host specificity was reported on cichlid hosts (*Pariselle & Euzet, 2009*; *Gillardin et al., 2012*; *Muterezi Bukinga et al., 2012*; *Řehulková, Mendlová & Šimková, 2013*), which makes them an ideal model for investigating co-evolutionary processes in host-parasite systems (*Pouyaud et al., 2006*). While there is no published data available for the monogenean fauna on any of the tribe members of Limochromini, there is a pretty good record regarding the *Cichlidogyrus* diversity on the

various species within Tropheini, with a high degree of host specificity and phylogenetic congruence (Vanhove et al., 2015). '*Gnathochromis*' *pfefferi, Limnotilapia dardennii* (GA Boulenger, 1899) and '*Ctenochromis*' *horei* (A Günther, 1894) are infected by a single dactylogyridean monogenean species each: *Cichlidogyrus irenae, C. steenbergei* and *C. gistelincki* C Gillardin, MPM. Vanhove, A Pariselle et al., 2012, respectively (Gillardin et al., 2012). *Astatotilapia burtoni* (A Günther 1894), a haplochromine cichlid closely related to the Tropheini (Koblmüller et al., 2008; Meyer, Matschiner & Salzburger, 2015), is infected by *C. gillardinae* F Muterezi Bukinga, MPM Vanhove, M Van Steenberge et al., 2012 (Muterezi Bukinga et al., 2012). These observations are hitherto only based on reports from several localities along the Congolese, Tanzanian and Zambian coasts of the lake (Gillardin et al., 2012; Muterezi Bukinga et al., 2012; Vanhove et al., 2015). Thorough sampling covering as many host localities as possible is, however, needed to conclude about the full extent of a species' parasite fauna (Price & Clancy, 1983; Brooks et al., 2006; Caro, Combes & Euzet, 1997).

As mentioned above, '*Gnathochromis*' is a polyphyletic genus and no comparison of the parasite fauna of its two species has been performed to date. Do the parasites reflect the phylogenetic position and ecological characteristics of their hosts? We investigated the monogenean fauna of both '*Gnathochromis*' species to answer the following questions:

(1) Does the Burundese population of '*G.*' *pfefferi* confirm that this host is only infected by a single species of *Cichlidogyrus*?
(2) Since '*Gnathochromis*' is considered polyphyletic, is the phylogenetic distinctness of its two representatives also reflected in their parasite fauna?

MATERIAL & METHODS

Sampling

Fish specimens were obtained from commercial fishermen along the Burundese coastline of Lake Tanganyika. Two '*G.*' *pfefferi* individuals from Mvugo (4°15′S, 29°34′E) and four from Mukuruka (4′14′S, 29°33′E) were examined, as well as seven *G. permaxillaris* and six *Limnochromis auritus* (GA Boulenger, 1901) individuals from Bujumbura (3°23′S 29°22′E) (Fig. 1). Maps were created using SimpleMappr software (Shorthouse, 2010). The latter species was included to allow a comparison between the monogeneans of *G. permaxillaris* and another member of the Limnochromini, a tribe from which no monogeneans have been described previously. Fish were sacrificed by severing the spinal cord and dissected immediately. Gills were removed according to the standard protocol of Ergens & Lom (1970) and immediately preserved in pure ethanol in plastic tubes until further inspection in the lab. Some fresh gills were also inspected *in situ* for monogenean parasites using dissecting needles and a stereomicroscope. Slides prepared *in situ* were fixed in glycerine ammonium picrate (GAP) (Malmberg, 1957) or in Hoyer's solution (Humason, 1979). Monogeneans were isolated in the lab using a dissecting needle and an Olympus SZX7 stereomicroscope. They were mounted on a slide under a cover slip. Parasite individuals used for genetic characterisation were identified using an Olympus BX51 microscope with incorporated phase contrast at a magnification of

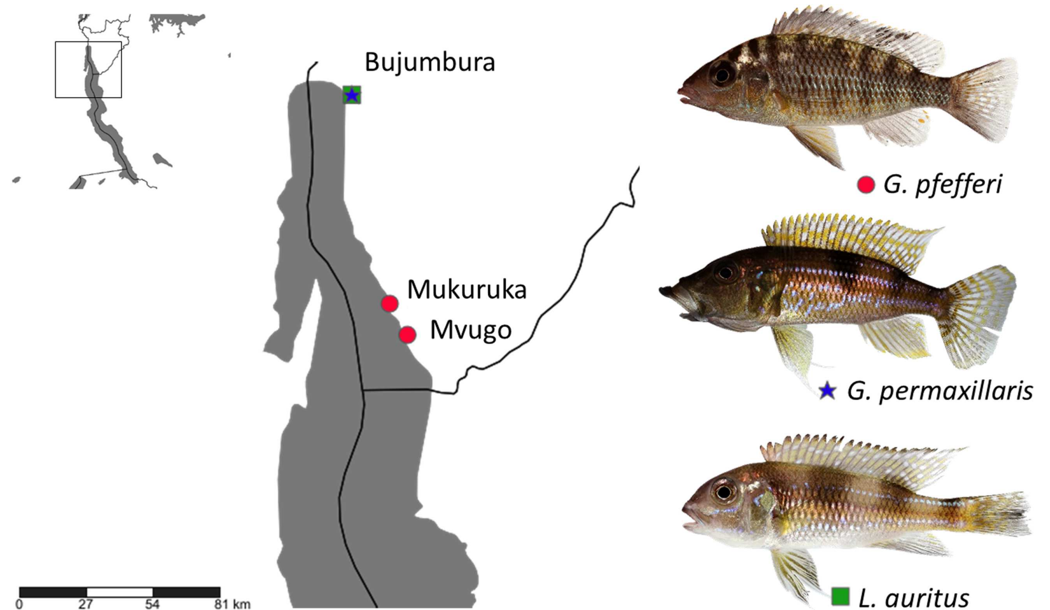


Figure 1 **Sampling localities in Lake Tanganyika with indication of host species (photos by Wolfgang Gessl).**

100× (oil immersion, 10× ocular) with Micro Image software and photographed for *post hoc* confirmation of species identity. They were stored in 1.2 ml Eppendorf tubes with 99.8% ethanol for subsequent DNA isolation. The research was approved by the Ethics Committee of Masaryk University. The approval number which allows us to work with vertebrate animals is CZ01308.

Morphometrics

The morphometric characterisation was based on 26 different metrics measured according to *Řehulková, Mendlová & Šimková (2013)* and *Gillardin et al. (2012)*. Measurements and photos were taken using the same configuration as above. In some cases an extra magnification of 2× had to be used. Voucher specimens were deposited in the invertebrate collection of the Royal Museum for Central Africa (Tervuren, Belgium) under accession numbers MRAC 37792-802.

DNA extraction and genetic characterisation

Ethanol was removed by evaporation in a vacuum centrifuge. DNA was extracted using the Qiagen Blood and Tissue Isolation Kit according to the manufacturer's instructions with some modifications (samples in ATL buffer (180 µl) with protein kinase (20 µl) were kept in 1.5 ml Eppendorf tubes overnight at room temperature). The DNA extract was then concentrated to a volume of 80 µl in 1.5 ml Eppendorf tubes using a vacuum centrifuge and stored at a temperature of −20 °C until polymerase chain reaction amplification. Part of the 18S nuclear ribosomal DNA gene, together with the first Internal Transcribed Spacer (ITS-1) region was amplified for 5 individuals using the S1 (5′-ATTCCGATAACGAACGAGACT-3′) (*Sinnappah et al., 2001*) and IR8

(5′-GCAGCTGCGTTCTTCATCGA-3′) (*Šimková et al., 2003*) primers. Each amplification reaction contained 1.5 unit of *Taq* Polymerase, 1X buffer containing 0.1 mg/ml BSA, 1.5 mM $MgCl_2$, 200 mM dNTPs, 0.5 mM of each primer and 30 ng of genomic DNA in a total reaction volume of 30 µl under the following conditions: 2 min at 94 °C, 39 cycles of 1 min at 94 °C, 1 min at 53 °C and 1 min and 30 s at 72 °C, and finally 10 min at 72 °C . The obtained nucleic acid sequences were aligned using MUSCLE (*Edgar, 2004*) under default distance measures and sequence weighting schemes as implemented in MEGA 6.06 (*Tamura et al., 2013*), together with previously published sequences of *Cichlidogyrus* from '*G.*' *pfefferi* (GenBank accession numbers KT037169, KT037170, KT037171, KT037172, KT037173; *Vanhove et al., 2015*). Sequences and their alignment were visually inspected and corrected using the same software. Uncorrected pairwise distances were calculated in MEGA. The newly obtained haplotype sequence was deposited in NCBI GenBank under accession number KT692939.

RESULTS

All 73 adult monogeneans collected from '*G.*' *pfefferi* specimens were identified as *C. irenae* following the original description of *Gillardin et al. (2012)*. The prevalence was 83.3%, mean infection intensity 18.2 and mean abundance 15.1 (calculated using adult monogeneans only). Although there are slight differences visible, mainly in the dorsal anchors and the attachment of the accessory piece to the base of the copulatory tube, our set of measurements matches with the original description of *C. irenae* (*Gillardin et al., 2012*) (Table 1). Differences in heel length are caused by different metrics (measuring up to the base of the heel *versus* to the base of the copulatory tube).

Only one specimen of *G. permaxillaris* was infected by monogeneans. It carried a single representative of a species of *Cichlidogyrus* similar in morphology to *C. gillardinae* parasitizing on *Astatotilapia burtoni*. Unfortunately, we cannot confidently confirm conspecificity based on only one specimen and therefore we refer to it as *C*. cf. *gillardinae*. Its pairs of anchors are asymmetrical: the dorsal anchor has a much longer guard than shaft while in the ventral anchor, guard and shaft are equal in size. The auricles and ventral bar branches are relatively short. Its male copulatory organ is characterised by a short heel, a simple copulatory tube with constant diameter and an accessory piece with easily overlooked distal bulb. No sclerotized vagina was observed. Despite these similarities with *C. gillardinae*, some differences compared to the original description were noted, e.g., *Cichlidogyrus* cf. *gillardinae* from *G. permaxillaris* has a more slender heel and shorter ventral anchor roots (Table 2).

Two monogenean specimens of an undescribed species of *Cichlidogyrus* were collected from one individual of *L. auritus*. One of the most noticeable structures within this parasite's haptor are the extremely long auricles of the dorsal transverse bar. There is no visible difference between the length of guard and shaft in any of the anchors. The copulatory tube is thin with a constant diameter; a heel was not recognized. The accessory piece is robust and thick with a fork-shaped ending. No sclerotized vagina was observed. In view of the remarkably long auricles, this species morphologically resembles *C. vandekerkhovei* and *C. makasai* MPM Vanhove, F Volckaert and A Pariselle, 2011 described

Table 1 **Comparison of measurements (in µm) on Burundese *Cichlidogyrus irenae* with the original description.**

	C. irenae from Burundi ($n = 30$[a])	*C. irenae* (Gillardin et al., 2012)
Ventral anchor		
Total length	30.3 ± 2.3[b] ($n = 28$)[c]; (26.9–36.4)[d]	31.4 ± 1,6 ($n = 14$); (29.3–34.6)
Length to notch	25.7 ± 0.9 ($n = 25$); (22.6–29.8)	28.5 ± 1.4 ($n = 14$); (26.1–30.2)
Inner root length	8.7 ± 1.7 ($n = 24$); (5.6–10.8)	8.1 ± 1.3 ($n = 14$); (5.9–10.1)
Outer root length	5.5 ± 0.7 ($n = 18$); (4.9–6.8)	5.4 ± 1.2 ($n = 14$); (3.2–7.8)
Point length	8.5 ± 1.1 ($n = 25$); (6.9–10.4)	10.0 ± 1.5 ($n = 14$); (7.9–12.8)
Dorsal anchor		
Total length	30.5 ± 2.6 ($n = 22$); (27–37.5)	35.0 ± 2.8 ($n = 15$); (30.0–38.5)
Length to notch	21.8 ± 1.1 ($n = 16$); (19.8–23.9)	25.8 ± 1.6 ($n = 15$); (22.4–28.8)
Inner root length	10.6 ± 1.3 ($n = 16$); (7.9–13.4)	12.3 ± 1.5 ($n = 15$); (9.6–14.7)
Outer root length	5.3 ± 0.9 ($n = 16$); (4,1–7,2)	4.6 ± 0.7 ($n = 15$); (3.6–5.9)
Point length	7.1 ± 1 ($n = 12$); (5.7–8.7)	9.1 ± 1.0 ($n = 15$); (6.9–11.1)
Ventral bar		
Branch length	38.4 ± 4.4 ($n = 22$); (32–49.5)	31.6 ± 4.6 ($n = 15$); (24.8–39.5)
Branch maximum width	6 ± 0.9 ($n = 28$); (3.6–8.1)	4.8 ± 0.9 ($n = 15$); (3.2–6.5)
Dorsal bar		
Maximum straight width	40.1 ± 4.1 ($n = 14$); (35–48.6)	32.7 ± 7.0 ($n = 15$); (17.9–45.8)
Thickness at middle length	7.5 ± 1.2 ($n = 28$); (5.7–10.3)	6.1 ± 1.1 ($n = 15$); (4.2–8.2)
Distance between auricles	15.2 ± 1.9 ($n = 28$); (12.1–18.4)	11.5 ± 1.8 ($n = 15$); (8.3–15.2)
Auricle length	15.3 ± 2.3 ($n = 15$); (12.2–19.9)	14.2 ± 2.4 ($n = 15$); (9.6–19.0)
Hooks		
Pair I	12.3 ± 0.6 ($n = 26$); (11.5–13.2)	11.6 ± 0.4 ($n = 15$); (10.8–12.1)
Pair II	18.5 ± 2.1 ($n = 28$); (14.8–22.8)	–
Pair II	20.6 ± 1.2 ($n = 25$); (18.4–22.2)	–
Pair IV	21.1 ± 1.5 ($n = 25$); (19.4–25)	–
Pair V	10.1 ± 0.9 ($n = 10$); (9.4–12.2)	11.4 ± 0.9 ($n = 15$); (9.2–12.6)
Pair VI	21.4 ± 2.4 ($n = 10$); (16.1–22.8)	–
Pair VII	20.6 ± 3.3 ($n = 18$); (17.5–25.7)	–
Average size of pairs II, III, IV, VI, VII	20.2 ± 2.5 ($n = 105$); (13.3–27.3)	16.3 ± 2.1 ($n = 15$); (11.9–19.3)
Copulatory tube curved length	69.9 ± 5.3 ($n = 30$); (59.3–81.4)	69.5 ± 5.7 ($n = 20$); (48.0–73.3)
Accessory piece curved length	68.8 ± 8.2 ($n = 30$); (54–91)	59.5 ± 5.8 ($n = 20$); (37.8–64.8)
Heel straight length	11.1 ± 3.9 ($n = 30$); (6–12.6)	4.1 ± 0.2 ($n = 20$); (3.6–4.4)

Notes.
[a] Number of specimens.
[b] Standard deviation.
[c] Number of specimens.
[d] Range.

from *Opthalmotilapia* J Pellegrin, 1904 species. However, there are clear differences in MCO structure. For example, the copulatory tube tapers distally in *C. vandekerkhovei* and *C. makasai*, whereas it is of constant diameter in the undescribed parasite of *L. auritus*.

Micrographs of the collected monogenean species are presented in Fig. 2.

The rDNA dataset included four successfully amplified sequences of parasites collected from '*G.*' *pfefferi*. Only one haplotype (1,060 base pairs) was recognised. The maximum

Table 2 **Comparison of measurements (in μm) on Burundese *Cichlidogyrus* cf. *gillardinae* with the original description.**

	C. cf. *gillardinae* from Burundi (*n* = 1)[a]	*C. gillardinae* (Muterezi Bukinga et al., 2012) (*n* = 30)[a]
Ventral anchor		
Total length	29.5	32 (27–37)
Length to notch	26	28 (23–32)
Inner root length	6.5	10 (8–13)
Outer root length	3.8	6 (4–9)
Point length	10.8	8 (6–11)
Dorsal anchor		
Total length	31	33 (29–38)
Length to notch	22.5	23 (19–29)
Inner root length	10.5	12 (9–16)
Outer root length	4.6	5 (4–7)
Point length	7.75	7 (5–8)
Ventral bar		
Branch length	29	31 (27–35)
Branch maximum width	3.7	5 (3–6)
Dorsal bar		
Maximum straight width	33	33 (27–39)
Thickness at middle length	6.5	6 (4–8)
Distance between auricles	11.8	12 (9–15)
Auricle length	9.3	11 (8–14)
Hooks		
Pair I	14.5	11 (9–13)
Pair II	13.5	14 (11–17)
Pair III	15.1	21 (18–26)
Pair IV	21.5	22 (19–24)
Pair V	9.5	10 (8–12)
Pair VI	21.5	15 (13–17)
Pair VII	14.1	17 (15–21)
Copulatory tube curved length	51	47 (42–55)
Accessory piece curved length	30	35 (29–42)
Heel straight length	6.5	5 (4–7)

Notes.
[a]Number of specimens.

overlap with sequences of more southern parasites of '*G.*' *pfefferi* obtained from GenBank was 571 base pairs, situated within ITS-1. The uncorrected pairwise genetic distance reached a maximum of 0.8%, which is below the species-level cut-off of 1%, suggested for this region for the best-studied monogenean, *Gyrodactylus* A von Nordmann, 1832 (*Ziętara & Lumme, 2002*). This result confirms the identification, based on morphology and morphometrics, of a single monogenean species infecting '*G.*' *pfefferi*, namely *C. irenae*.

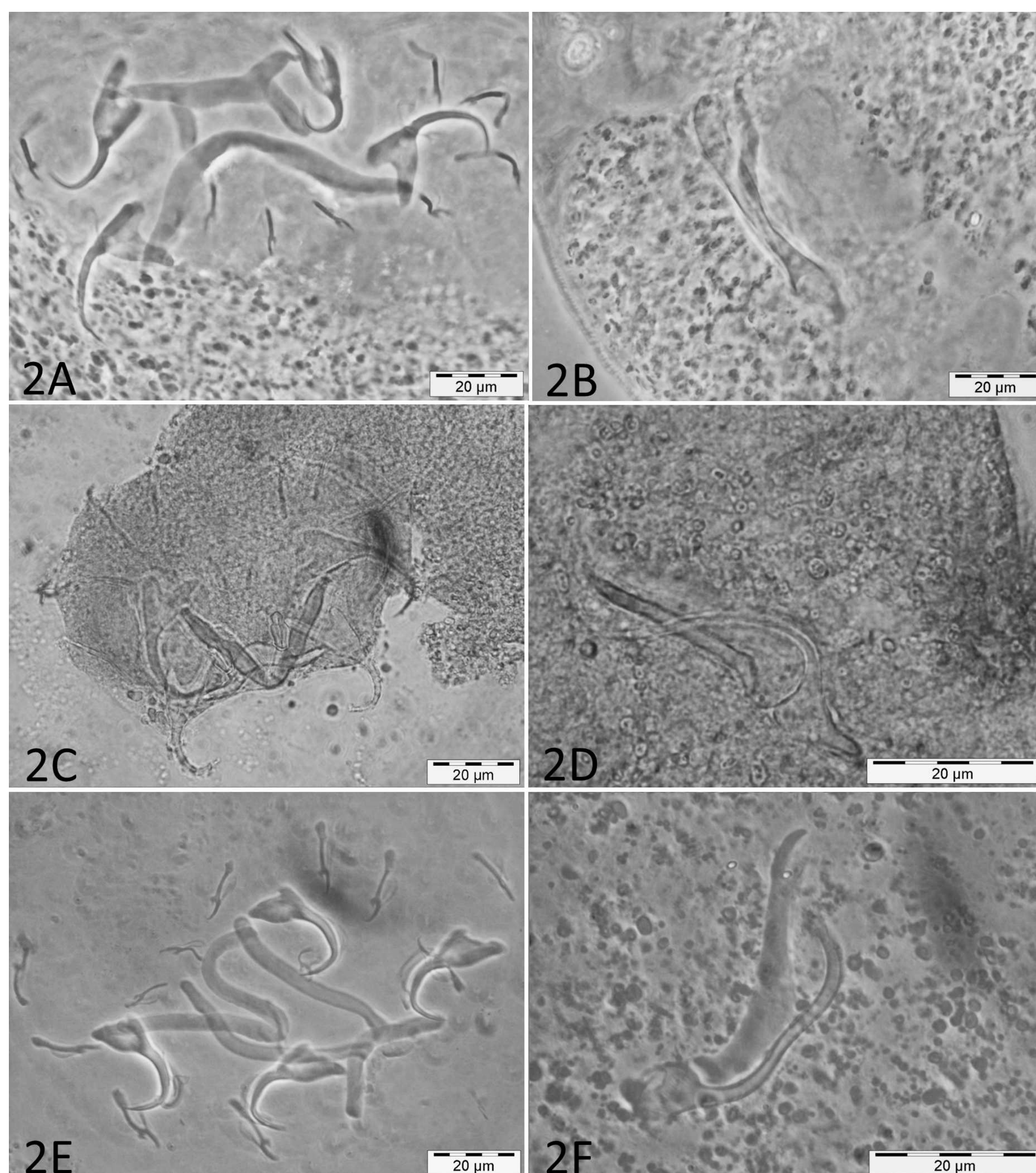


Figure 2 Micrographs of haptoral and male genital sclerotized structures from monogenean species belonging to *Cichlidogyrus*. Host species: (A) '*G.*' *pfefferi* (opisthaptor, Hoyer's medium, phasecontrast); (B) '*G.*' *pfefferi* (MCO, Hoyer's medium, phasecontrast); (C) *G. permaxillaris* (opisthaptor, GAP); (D) *G. permaxillaris* (MCO, GAP); (E) *L. auritus* (opisthaptor, Hoyer's medium, phasecontrast); (F) *L. auritus* (MCO, Hoyer's medium, phasecontrast).

DISCUSSION

The monogenean fauna of the cichlid '*G.*' *pfefferi* in Burundi was characterised morphologically and genetically. We confirmed the occurrence of *C. irenae*, representing the first record of this species in Burundi. According to previous results, the species richness of *Cichlidogyrus* on Tanganyika cichlids is influenced by the dispersal ability or isolation of the host species (Pariselle et al., 2015a; Grégoir et al., 2015). Although some differences

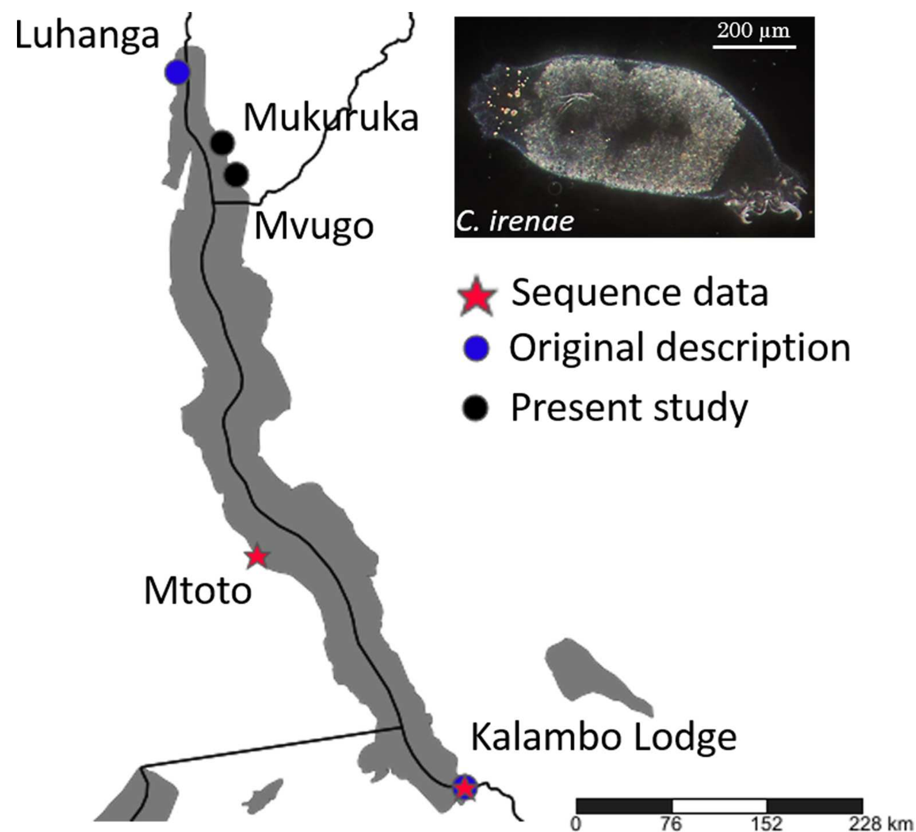


Figure 3 **Geographical position of records of *C. irenae*, monogeneans infecting *'G.' pfefferi*.**

in the size of parasite sclerotized structures were recorded (Table 1), these are only minor and likely reflect phenotypic intraspecific variability across entire Lake Tanganyika. Our results therefore support previous suggestions that '*G.*' *pfefferi*, as a cichlid with good dispersal ability, hosts only a single representative of *Cichlidogyrus*, now recorded from several localities in the northern as well as the southern part of the Lake (*Vanhove et al., 2015*) (see Fig. 3).

Monogenean parasites belonging to *Cichlidogyrus* were also used as an additional way to look at species interrelationships within '*Gnathochromis*.' The parasite from *G. permaxillaris* was identified as *C*. cf. *gillardinae*. Since *C. gillardinae* was originally described from the haplochromine *A. burtoni*, a fish occurring in aquatic systems along Lake Tanganyika's shores, it is most likely a generalist parasite infecting representatives of two unrelated cichlid genera with different habitat preferences (*Konings, 1998*; *Muterezi Bukinga et al., 2012*). Although the limnochromine *G. permaxillaris* is hence infected by a monogenean species different from *C. irenae* described from '*G.*' *pfefferi*, its parasite seems more similar to its congeners infecting tropheine hosts like '*G.*' *pfefferi* (*Gillardin et al., 2012*; *Pariselle et al., 2015b*). *Cichlidogyrus* can be divided into different lineages based on the configuration of their haptoral hard parts, in particular the relative length of the pairs of hooks (also termed uncinuli) (*Pariselle & Euzet, 2003*; *Vignon, Pariselle & Vanhove, 2011*). Indeed, both parasites' haptor shares important characteristics: asymmetry between anchors, small

(*sensu* Pariselle & Euzet, 2009) hooks. *Cichlidogyrus* cf. *gillardinae* differs substantially from the *Cichlidogyrus* species collected from the closely related host *L. auritus*, another limnochromine cichlid. In the latter flatworm, the extremely long dorsal bar auricles represent an evident similarity with *C. vandekerkhovei* and *C. makasai* (Vanhove, Volckaert & Pariselle, 2011) collected from species of *Ophthalmotilapia*, belonging to the Ectodini, another cichlid tribe endemic to Lake Tanganyika. This feature was hitherto never found in other monogenean congeners. The gill monogenean retrieved from *Limnochromis* hence seems to belong to an endemic Tanganyika lineage. The discussion about the evolution of the haptoral sclerotized structures is still ongoing. Morand et al. (2002) assume that haptoral structures do not reflect a phylogenetic pattern as a result of adaptation to microhabitat within the host. Moreover, Messu Mandeng et al. (2015) point out an adaptive component in the attachment organ morphology of *Cichlidogyrus*. However, other studies suggest the existence of a phylogenetic signal in sclerite morphology and shape within dactylogyridean monogeneans (Šimková et al., 2002; Šimková et al., 2006) and specifically within *Cichlidogyrus* (Vignon, Pariselle & Vanhove, 2011).

According to Mendlová & Šimková (2014) the host specificity of *Cichlidogyrus* parasitising African cichlid fishes is significantly influenced by fish phylogeny and by form of parental care. No *Cichlidogyrus* species was hitherto observed to infect cichlid species with different parental care systems (i.e., substrate brooders as well as mouthbrooders) (Pouyaud et al., 2006). However, the form of parental care in cichlids is directly influenced by phylogenetic history and relationships (Goodwin, Balshine-Earn & Reynolds, 1998). Possible explanations for the affinities of monogenean species on '*Gnathochromis*' are therefore host evolutionary history as well as habitat characteristics. While '*G.*' *pfefferi* is a typical rock dwelling littoral cichlid occurring at depths between 1 and 15 m, *G. permaxillaris* occurs over muddy bottoms and is rarely seen in water shallower than 30 m (Maréchal & Poll, 1991; Konings, 1998). *Limnochromis auritus* is placed together with *G. permaxillaris* in the Limnochromini and prefers similar habitats with muddy bottoms at depths ranging from 5 to 125 m (Maréchal & Poll, 1991; Konings, 1998). Given that the haplochromine *A. burtoni* occurs in wetlands adjacent to the lake, in river mouths and in vegetated areas in the lake proper, it is unclear how it came to share a species with *G. permaxillaris* from which it differs ecologically and phylogenetically. On the other hand, the deepwater limnochromines *G. permaxillaris* and *L. auritus* seem to host entirely different monogeneans. However, these findings are based on a limited number of specimens (only one specimen of *Cichlidogyrus* collected from *G. permaxillaris*). Due to the lack of genetic data, we cannot perform (co-)phylogenetic analyses. According to Mendlová et al. (2012) duplication and host-switching events have played the most important role in the evolutionary history of African cichlid dactylogyridean species. Vanhove et al. (2015), however, found evidence for an important role of co-speciation in the evolution of *Cichlidogyrus* infecting Lake Tanganyika's tropheine cichlids. Although representatives of *Cichlidogyrus* occuring on littoral cichlid assemblages including Tropheini display strong host specificity (Gillardin et al., 2012; Muterezi Bukinga et al., 2012; Vanhove et al., 2015), a lower specificity was observed within the Bathybatini, a deepwater cichlid tribe from Lake Tanganyika (Pariselle et al., 2015a). Hence, some lineages of *Cichlidogyrus* in Lake Tanganyika were already

shown to have a wide host range. The observed low host specificity and the apparent low infestation rate most likely correlate with low host density in the deepwater habitat (*Justine et al., 2012*; *Schoelinck, Cruaud & Justine, 2012*). Given the low prevalence and infection intensities observed in this study, and the deepwater habitat of the limnochromine hosts, it is a challenge to retrieve additional material for species identification and molecular analyses. These, together with a broadened geographical coverage, are needed to uncover the whole co-phylogenetic history of '*Gnathochromis*' and its monogenean fauna.

ACKNOWLEDGEMENTS

We would like to thank Maarten Van Steenberge, Tine Huyse and the parasitology group at Masaryk University, Brno for their hospitality and cooperation. Eva Řehulková, Šárka Mašová, Iva Přikrylová, Radim Blažek, Veronika Nezhybová, Gaspard Banyankimbona and the Schreyen-Brichard family and the technical staff of Fishes of Burundi are thanked for their help in collecting samples, and Wolfgang Gessl for providing fish pictures. Antoine Pariselle, Miguel Rubio-Godoy and an anonymous referee are acknowledged for their constructive comments.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This study was financially supported by the Czech Science Foundation (GBP505/12/G112-ECIP). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
Czech Science Foundation: GBP505/12/G112-ECIP.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Nikol Kmentová conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables.
- Milan Gelnar contributed reagents/materials/analysis tools, reviewed drafts of the paper.
- Stephan Koblmüller analyzed the data, reviewed drafts of the paper.
- Maarten P.M. Vanhove conceived and designed the experiments, analyzed the data, wrote the paper.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

The research was approved by Ethics Committee of Masaryk University, approval number CZ01308.

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

GenBank accession number KT692939.

Data Availability

The following information was supplied regarding data availability:

The research in this article did not generate any raw data.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.1629#supplemental-information.

REFERENCES

Brooks DR, León-Règagnon V, McLennan DA, Zelmer D. 2006. Ecological fitting as a determinant of the community structure of platyhelminth parasites of anurans. *Ecology* **87**:76–85 DOI 10.1890/05-0023.

Caro A, Combes C, Euzet L. 1997. What makes a fish a suitable host for Monogenea in the Mediterranean? *Journal of Helminthology* **71**:203–210 DOI 10.1017/S0022149X00015923.

Chakrabarty P. 2004. Cichlid biogeography: comment and review. *Fish and Fisheries* **5**:97–119 DOI 10.1111/j.1467-2979.2004.00148.x.

Duftner N, Koblmüller S, Sturmbauer C. 2005. Evolutionary relationships of the Limnochromini, a tribe of benthic deepwater cichlid fish endemic to Lake Tanganyika, East Africa. *Journal of Molecular Evolution* **60**:277–289 DOI 10.1007/s00239-004-0017-8.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**:1792–1797 DOI 10.1093/nar/gkh340.

Ergens R, Lom J. 1970. *Causative agents of fish diseases.* Prague: Academia.

Gillardin C, Vanhove MPM, Pariselle A, Huyse T, Volckaert FAM. 2012. Ancyrocephalidae (Monogenea) of Lake Tanganyika: II: description of the first *Cichlidogyrus* spp. parasites from Tropheini fish hosts (Teleostei, Cichlidae). *Parasitology Research* **110**:305–313 DOI 10.1007/s00436-011-2490-5.

Goodwin NB, Balshine-Earn S, Reynolds JD. 1998. Evolutionary transitions in parental care in cichlid fish. *Proceedings of the Royal Society B: Biological Sciences* **265**:2265–2272 DOI 10.1098/rspb.1998.0569.

Grégoir AF, Hablützel PI, Vanhove MPM, Pariselle A, Bamps J, Volckaert FAM, Raeymaekers JAM. 2015. A link between host dispersal and parasite diversity in two sympatric cichlids of Lake Tanganyika. *Freshwater Biology* **60**:323–335 DOI 10.1111/fwb.12492.

Humason GL. 1979. *Animal tissue techniques:4th.* San Francisco: Freeman and Company.

Justine J-L, Beveridge I, Boxshall GA, Bray RA, Miller TL, Moravec F, Trilles J-P, Whittington ID. 2012. An annotated list of fish parasites (Isopoda, Copepoda, Monogenea, Digenea, Cestoda, Nematoda) collected from Snappers and

Bream (Lutjanidae, Nemipteridae, Caesionidae) in New Caledonia confirms high parasite biodiversity on coral reef fish. *Aquatic Biosystems* **8**: Article 22 DOI 10.1186/2046-9063-8-22.

Kirchberger PC, Sefc KM, Sturmbauer C, Koblmüller S. 2014. Outgroup effects on root position and tree topology in the AFLP phylogeny of a rapidly radiating lineage of cichlid fish. *Molecular Phylogenetics and Evolution* **70**:57–62 DOI 10.1016/j.ympev.2013.09.005.

Koblmüller S, Egger B, Sturmbauer C, Sefc KM. 2010. Rapid radiation, ancient incomplete lineage sorting and ancient hybridization in the endemic Lake Tanganyika cichlid tribe Tropheini. *Molecular Phylogenetics and Evolution* **55**:318–334 DOI 10.1016/j.ympev.2009.09.032.

Koblmüller S, Schliewen UK, Duftner N, Sefc KM, Katongo C, Sturmbauer C. 2008. Age and spread of the haplochromine cichlid fishes in Africa. *Molecular Phylogenetics and Evolution* **49**:153–169 DOI 10.1016/j.ympev.2008.05.045.

Koblmüller S, Sefc KM, Sturmbauer C. 2008. The Lake Tanganyika cichlid species assemblage: recent advances in molecular phylogenetics. *Hydrobiologia* **615**:5–20 DOI 10.1007/s10750-008-9552-4.

Kocher TD. 2004. Adaptive evolution and explosive speciation: the cichlid fish model. *Nature Reviews Genetics* **5**:288–298 DOI 10.1038/nrg1316.

Konings A. 1998. *Guide to Tanganyika cichlids.* El Paso: Cichlid Press.

Malmberg G. 1957. On the occurrence of *Gyrodactylus* on Swedish fishes. In: *Skrifter utgivna av Södra Sveriges Fiskeriföreningen Årsskrift*, 19–56.

Maréchal C, Poll M. 1991. *Gnathochromis, Limnochromis*. In: Daget J, Gosse J-P, Teugels GG, Thys van den Audenaerde DFE, eds. *Checklist of the freshwater fishes of Africa (CLOFFA).* Tervuren: Royal Museum for Central Africa, 94–95, 243–245.

Mendlová M, Desdevises Y, Civáňová K, Pariselle A, Šimková A. 2012. Monogeneans of west African cichlid fish: evolution and cophylogenetic interactions. *PLoS ONE* **7**:e37268 DOI 10.1371/journal.pone.0037268.

Mendlová M, Šimková A. 2014. Evolution of host specificity in monogeneans parasitizing African cichlid fish. *Parasites & Vectors* **7**: Article 69 DOI 10.1186/1756-3305-7-69.

Messu Mandeng FD, Bilong Bilong CF, Pariselle A, Vanhove MPM, Bitja Nyom AR, Agnèse J-F. 2015. A phylogeny of *Cichlidogyrus* species (Monogenea, Dactylogyridea) clarifies a host switch between fish families and reveals an adaptive component to attachment organ morphology of this parasite genus. *Parasites & Vectors* **8**: Article 582 DOI 10.1186/s13071-015-1181-y.

Meyer BS, Matschiner M, Salzburger W. 2015. A tribal level phylogeny of Lake Tanganyika cichlid fishes based on a genomic multi-marker approach. *Molecular Phylogenetics and Evolution* **83**:56–71 DOI 10.1016/j.ympev.2014.10.009.

Morand S, Šimková A, Matějusová I, Plaisance L, Verneau O, Desdevises Y. 2002. Investigating patterns may reveal processes: evolutionary ecology of ectoparasitic monogeneans. *International Journal for Parasitology* **32**:111–119 DOI 10.1016/S0020-7519(01)00347-2.

Muschick M, Indermaur A, Salzburger W. 2012. Convergent evolution within an adaptive radiation of cichlid fishes. *Current Biology* **22**:2362–2368 DOI 10.1016/j.cub.2012.10.048.

Muterezi Bukinga F, Vanhove MPM, Van Steenberge M, Pariselle A. 2012. Ancyrocephalidae (Monogenea) of Lake Tanganyika: III: *Cichlidogyrus* infecting the world's biggest cichlid and the non-endemic tribes Haplochromini, Oreochromini and Tylochromini (Teleostei, Cichlidae). *Parasitology Research* **111**:2049–2061 DOI 10.1007/s00436-012-3052-1.

Pariselle A, Euzet L. 2003. Four new species of *Cichlidogyrus* (Monogenea: Ancyrocephalidae), gill parasites of *Tilapia cabrae* (Teleostei: Cichlidae), with discussion on relative length of haptoral sclerites. *Folia Parasitologica* **50**:195–201 DOI 10.14411/fp.2003.035.

Pariselle A, Euzet L. 2009. Systematic revision of dactylogyridean parasites (Monogenea) from cichlid fishes in Africa, the Levant and Madagascar. *Zoosystema* **31**:849–898 DOI 10.5252/z2009n4a6.

Pariselle A, Muterezi Bukinga F, Van Steenberge M, Vanhove MPM. 2015a. Ancyrocephalidae (Monogenea) of Lake Tanganyika: IV: *Cichlidogyrus* parasitizing species of Bathybatini (Teleostei, Cichlidae): reduced host-specificity in the deepwater realm? *Hydrobiologia* **748**:99–119 DOI 10.1007/s10750-014-1975-5.

Pariselle A, Van Steenberge M, Snoeks J, Volckaert FAM, Huyse T, Vanhove MPM. 2015b. Ancyrocephalidae (Monogenea) of Lake Tanganyika: does the *Cichlidogyrus* parasite fauna of *Interochromis loocki* (Teleostei, Cichlidae) reflect its host's phylogenetic affinities? *Contributions to Zoology* **84**:25–38.

Pouyaud L, Desmarais E, Deveney M, Pariselle A. 2006. Phylogenetic relationships among monogenean gill parasites (Dactylogyridea, Ancyrocephalidae) infesting tilapiine hosts (Cichlidae): systematic and evolutionary implications. *Molecular Phylogenetics and Evolution* **38**:241–249 DOI 10.1016/j.ympev.2005.08.013.

Price PW, Clancy KM. 1983. Patterns in number of helminth parasite species in freshwater fishes. *The Journal of Parasitology* **69**:449–454 DOI 10.2307/3281352.

Pugachev ON, Gerasev PI, Gussev AV, Ergens R, Khotenowsky I. 2009. *Guide to Monogenoidea of freshwater fish of Palaeartic and Amur regions.* Milan: Ledizione-LediPublishing.

Řehulková E, Mendlová M, Šimková A. 2013. Two new species of *Cichlidogyrus* (Monogenea: Dactylogyridae) parasitizing the gills of African cichlid fishes (Perciformes) from Senegal: morphometric and molecular characterization. *Parasitology Research* **112**:1399–1410 DOI 10.1007/s00436-013-3291-9.

Salzburger W. 2009. The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes. *Molecular Ecology* **18**:169–185 DOI 10.1111/j.1365-294X.2008.03981.x.

Salzburger W, Mack T, Verheyen E, Meyer A. 2005. Out of Tanganyika: genesis, explosive speciation, key-innovations and phylogeography of the haplochromine cichlid fishes. *BMC Evolutionary Biology* **5**:17 DOI 10.1186/1471-2148-5-17.

Salzburger W, Meyer A, Baric S, Verheyen E, Sturmbauer C. 2002. Phylogeny of the Lake Tanganyika cichlid species flock and its relationship to the Central and East African haplochromine cichlid fish faunas. *Systematic Biology* **51**:113–135 DOI 10.1080/106351502753475907.

Schoelinck C, Cruaud C, Justine J-L. 2012. Are all species of *Pseudorhabdosynochus* strictly host specific?—A molecular study. *Parasitology International* **61**:356–359 DOI 10.1016/j.parint.2012.01.009.

Shorthouse D. 2010. SimpleMappr. *Available at http:// www.simplemappr.net*.

Šimková A, Ondráčková M, Gelnar M, Morand S. 2002. Morphology and coexistence of congeneric ectoparasite species: reinforcement of reproductive isolation? *Biological Journal of the Linnean Society* **76**:125–135.

Šimková A, Plaisance L, Matějusová I, Morand S, Verneau O. 2003. Phylogenetic relationships of the Dactylogyridae Bychowsky, 1933 (Monogenea: Dactylogyridea): the need for the systematic revision of the Ancyrocephalinae Bychowsky, 1937. *Systematic Parasitology* **54**:1–11 DOI 10.1023/A:1022133608662.

Šimková A, Verneau O, Gelnar M, Morand S. 2006. Specificity and specialization of congeneric monogeneans parasitizing cyprinid fish. *Evolution* **60**:1023–1037 DOI 10.1111/j.0014-3820.2006.tb01180.x.

Sinnappah ND, Lim LHS, Rohde K, Tinsley RC, Combes C, Verneau O. 2001. A paedormorphic parasite associated with a neotenic Amphibian host: phylogenetic evidence suggests a revised systematic position for Sphyranuridae with Anuran and Turtle Polytomatoineans. *Molecular Phylogenetics and Evolution* **18**:189–201 DOI 10.1006/mpev.2000.0877.

Snoeks J. 2000. How well known is the ichthyodiversity of the large East African lakes? *Advances in Ecological Research* **31**:17–38 DOI 10.1016/S0065-2504(00)31005-4.

Takahashi T. 2003. Systematics of Tanganyikan cichlid fishes (Teleostei: Perciformes). *Ichthyological Research* **50**:367–382 DOI 10.1007/s10228-003-0181-7.

Takahashi T. 2014. Greenwoodochromini Takahashi from Lake Tanganyika is a junior synonym of Limnochromini Poll (Perciformes: Cichlidae). *Journal of Fish Biology* **84**:929–936 DOI 10.1111/jfb.12309.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**:2725–2729 DOI 10.1093/molbev/mst197.

Turner GF. 2007. Adaptive radiation of cichlid fish. *Current Biology* **17**:827–831 DOI 10.1016/j.cub.2007.07.026.

Vanhove MPM, Pariselle A, Van Steenberge M, Raeymaekers JAM, Hablützel PI, Gillardin C, Hellemans B, Breman FC, Koblmüller S, Sturmbauer C, Snoeks J, Volckaert FAM, Huyse T. 2015. Hidden biodiversity in an ancient lake: phylogenetic congruence between Lake Tanganyika tropheine cichlids and their monogenean flatworm parasites. *Scientific Reports* **5**:13669 DOI 10.1038/srep13669.

Vanhove MPM, Volckaert FAM, Pariselle A. 2011. Ancyrocephalidae (Monogenea) of Lake Tanganyika: I: four new species of *Cichlidogyrus* from *Ophthalmotilapia*

ventralis (Teleostei: Cichlidae), the first record of this parasite family in the basin. *Zoologia* **28**:253–263 DOI 10.1590/S1984-46702011000200016.

Van Steenberge M, Pariselle A, Huyse T, Volckaert FAM, Snoeks J, Vanhove MPM. 2015. Morphology, molecules, and monogenean parasites: an example of an integrative approach to cichlid biodiversity. *PLoS ONE* **10**:e0124474 DOI 10.1371/journal.pone.0124474.

Vignon M, Pariselle A, Vanhove MPM. 2011. Modularity in attachment organs of African *Cichlidogyrus* (Platyhelminthes: Monogenea: Ancyrocephalidae) reflects phylogeny rather than host specificity or geographic distribution. *Biological Journal of the Linnean Society* **102**:694–706 DOI 10.1111/j.1095-8312.2010.01607.x.

Ziętara MS, Lumme J. 2002. Speciation by host switch and adaptive radiation in a fish parasite genus *Gyrodactylus* (Monogenea, Gyrodactylidae). *Evolution* **56**:2445–2458 DOI 10.1111/j.0014-3820.2002.tb00170.x.