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

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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.

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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 Shirakhara, 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 (Raemyaekers 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; Schoelink 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 (Huys 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
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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

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

<table>
<thead>
<tr>
<th>Host species</th>
<th>Locality (geographic coordinates, year)</th>
<th>Locality – basins (Danley et al., 2012)</th>
<th>Number of fish specimens (accession number in RMCA)</th>
<th>Number of monogenean individuals</th>
<th>Prevalence (%)</th>
<th>Infection intensity/one gill chamber</th>
<th>Abundance/one gill chamber (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Limnothrissa miodon</em></td>
<td>Bujumbura (3°23’S-29°22’E, 1.7.-31.7.1928)</td>
<td>The northern basin</td>
<td>2 (MRAC 23567-68)</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td></td>
<td>Bujumbura (1.2.-28.2.1935)</td>
<td>The northern basin</td>
<td>11 (MRAC 43554-64)</td>
<td>1/0</td>
<td>9/0</td>
<td>1/0</td>
<td>0.09 (0-1)/0</td>
</tr>
<tr>
<td></td>
<td>Kalemie (5°56’S-29°12’E, 22.10.1946)</td>
<td>The central basin</td>
<td>8 (MRAC 88891-89098)</td>
<td>4/0</td>
<td>25/0</td>
<td>2/0</td>
<td>0.5 (0-3)/0</td>
</tr>
<tr>
<td></td>
<td>Kalemie (1.1.-31.1.1946)</td>
<td>The central basin</td>
<td>1 (MRAC 89151)</td>
<td>2/0</td>
<td>100/0</td>
<td>2/0</td>
<td>2/0</td>
</tr>
<tr>
<td></td>
<td>Kalemie (20.11.1946)</td>
<td>The central basin</td>
<td>8 (MRAC 89137-144)</td>
<td>22/0</td>
<td>25/0</td>
<td>5.5/0</td>
<td>2.75 (0-9)/0</td>
</tr>
<tr>
<td></td>
<td>Kalemie (11.8.2016)</td>
<td>The central basin</td>
<td>10 (-)</td>
<td>55/5</td>
<td>80/33</td>
<td>6.9/1.7</td>
<td>5.5 (0-15)/0.5(0-2)</td>
</tr>
<tr>
<td></td>
<td>Kasaba Bay (8°31’S-30°42’E, 1.1.1967)</td>
<td>The southern basin</td>
<td>2 (MRAC 190150-151)</td>
<td>0/49</td>
<td>0/100</td>
<td>0/29.5</td>
<td>0/29.5 (12-37)</td>
</tr>
<tr>
<td></td>
<td>Kigoma Bay (4°88’S-29°61’E, 12.4.-13.4.1947)</td>
<td>The northern basin</td>
<td>4 (MRAC 89367-70)</td>
<td>2/0</td>
<td>25/0</td>
<td>2/0</td>
<td>0.5 (0-4)/0</td>
</tr>
<tr>
<td></td>
<td>Kiranda (07°25’S-30°36’E, 11.3.1947)</td>
<td>The southern basin</td>
<td>2 (MRAC 89311-12)</td>
<td>9/0</td>
<td>50/0</td>
<td>9/0</td>
<td>4.5 (0-9)/0</td>
</tr>
<tr>
<td></td>
<td>Kivugwe (3°80’S-29°34’E, 22.2.1994)</td>
<td>The northern basin</td>
<td>7 (MRAC 94069.0369-70)</td>
<td>2/0</td>
<td>28.6/0</td>
<td>1/0</td>
<td>0.28 (0-1)/0</td>
</tr>
<tr>
<td></td>
<td>Kasaba Bay (5.3.1947)</td>
<td>The southern basin</td>
<td>1 (MRAC 89353)</td>
<td>1/2</td>
<td>100/100</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td></td>
<td>Luhanga (3°52’S-29°15’E, 26.4.1994)</td>
<td>The northern basin</td>
<td>2 (MRAC 94069.2375-76)</td>
<td>0/1</td>
<td>0/50</td>
<td>0/1</td>
<td>0/0.5 (0-1)</td>
</tr>
<tr>
<td></td>
<td>Moba Bay (7°03’S-29°47’E, 21.3.1947)</td>
<td>The central basin</td>
<td>2 (MRAC 89335-36)</td>
<td>9/0</td>
<td>50/0</td>
<td>11/0</td>
<td>5.5 (0-11)/0</td>
</tr>
<tr>
<td></td>
<td>Mpulungu (8°46’S-31°07’E, 14.3.1966)</td>
<td>The southern basin</td>
<td>3 (MRAC 189612-14)</td>
<td>1/0</td>
<td>33.3/0</td>
<td>1/0</td>
<td>0.33 (0-1)/0</td>
</tr>
<tr>
<td></td>
<td>Mpulungu (19.8.2016)</td>
<td>The southern basin</td>
<td>2 (-)</td>
<td>4/0</td>
<td>50/0</td>
<td>4/0</td>
<td>2 (0-4)/0</td>
</tr>
<tr>
<td></td>
<td>Mvugo (4°18’S-29°34’E, 4.8.2016)</td>
<td>The northern basin</td>
<td>6 (-)</td>
<td>9/25</td>
<td>50/100</td>
<td>3/4.2</td>
<td>1.5/4.2(1-10)</td>
</tr>
<tr>
<td>Host species</td>
<td>Locality (geographic coordinates, year)</td>
<td>Locality – basins (Danley et al., 2012)</td>
<td>Number of fish specimens (accession number in RMCA)</td>
<td>Number of monogenean individuals</td>
<td>Prevalence (%)</td>
<td>Infection intensity/one gill chamber</td>
<td>Abundance/one gill chamber (range)</td>
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</tr>
<tr>
<td><em>Stolothrissa tanganicae</em></td>
<td>Mvuna Island (7°20’S 30°32’E, 18.8.2015)</td>
<td>The southern basin</td>
<td>6 (-)</td>
<td>11/5</td>
<td>50/50</td>
<td>3/1.7</td>
<td>1.8 (0-8)/0.83(0-3)</td>
</tr>
<tr>
<td></td>
<td>Near Ruzizi (2°50’S-29°02’E, 2.12.1954)</td>
<td>The northern basin</td>
<td>2 (MRAC 99633-34)</td>
<td>6/0</td>
<td>50/0</td>
<td>6/0</td>
<td>3 (0-6)/0</td>
</tr>
<tr>
<td></td>
<td>Near Ruzizi (26.10.1954)</td>
<td>The northern basin</td>
<td>4 (MRAC 99623-32)</td>
<td>7/0</td>
<td>50/0</td>
<td>7/0</td>
<td>3.5 (0-7)/0</td>
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<td></td>
<td>Rumonge (3°58’S-29°25’E, 1.2. - 28.2.1935)</td>
<td>The northern basin</td>
<td>12 (MRAC 43763-72)</td>
<td>10/4</td>
<td>25/16.7</td>
<td>3.3/2</td>
<td>0.8 (0-6)/0.2(0-2)</td>
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<tr>
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<td>Ilagala (05°14’S-29°47’E, 24.2.1947)</td>
<td>The northern basin</td>
<td>8 (MRAC 89211-18.41)</td>
<td>14/0</td>
<td>62.5/0</td>
<td>2.8/0</td>
<td>1.75 (0-6)/0</td>
</tr>
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<td>Uvira (3°22’ S 29°09’E, 12.8.2016)</td>
<td>The northern basin</td>
<td>41 (-)</td>
<td>12/28</td>
<td>62.5/0</td>
<td>2.8/0</td>
<td>0.6 (0-3)/1.4(0-9)</td>
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<td></td>
<td>Bujumbura (4.8.2016)</td>
<td>The northern basin</td>
<td>29 (-)</td>
<td>7</td>
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<td>1.5</td>
<td>0.14 (0-2)</td>
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<td></td>
<td>Kalambo Lodge (8°59’S-31°18’E, 20.8.2016)</td>
<td>The southern basin</td>
<td>48 (-)</td>
<td>6</td>
<td>4.2</td>
<td>1.5</td>
<td>0.06 (0-2)</td>
</tr>
<tr>
<td></td>
<td>Kalemie (9.2.-10.2.1947)</td>
<td>The central basin</td>
<td>6 (MRAC 89428-33)</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>Kalemie (12.8.2016)</td>
<td>The central basin</td>
<td>33 (-)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Kigoma Bay (12.4.-13.4.1947)</td>
<td>The northern basin</td>
<td>4 (MRAC 89494-98)</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td></td>
<td>Kigoma Bay (13.5.1947)</td>
<td>The northern basin</td>
<td>6 (MRAC 89462-65)</td>
<td>5</td>
<td>33.3</td>
<td>2.5</td>
<td>2.5 (1-4)</td>
</tr>
<tr>
<td></td>
<td>Mpulungu (4.7-5.7.1965)</td>
<td>The southern basin</td>
<td>7 (MRAC 189618-19)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mpulungu (3.10.1966)</td>
<td>The southern basin</td>
<td>2 (MRAC 189595-601)</td>
<td>3</td>
<td>28.6</td>
<td>1.5</td>
<td>0.4 (0-2)</td>
</tr>
<tr>
<td></td>
<td>Mpulungu (19.8.2016)</td>
<td>The southern basin</td>
<td>18 (-)</td>
<td>2</td>
<td>11.1</td>
<td>1</td>
<td>0.11 (0-1)</td>
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<tr>
<td></td>
<td>Musende Bay (8°46’S-31°06’E, 7.4.1967)</td>
<td>The southern basin</td>
<td>5 (MRAC 190171-74)</td>
<td>4</td>
<td>20</td>
<td>3.5</td>
<td>0.8 (0-4)</td>
</tr>
<tr>
<td></td>
<td>Mvugo (15.8.2015)</td>
<td>The northern basin</td>
<td>6 (-)</td>
<td>7</td>
<td>33.3</td>
<td>4</td>
<td>1.2 (0-6)</td>
</tr>
<tr>
<td></td>
<td>Rumonge (1.1.-31.12.1935)</td>
<td>The northern basin</td>
<td>18 (MRAC 43763-72)</td>
<td>28</td>
<td>61</td>
<td>3.5</td>
<td>1.55 (0-7)</td>
</tr>
<tr>
<td></td>
<td>Utinta Bay (7°10’S-30°53’E, 17.2.1947)</td>
<td>The southern basin</td>
<td>1 (MRAC 89442)</td>
<td>1</td>
<td>100</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Uvira (1.1.1935)</td>
<td>The northern basin</td>
<td>4 (MRAC 43787-88.90,98)</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td></td>
<td>Uvira (1.1.1954)</td>
<td>The northern basin</td>
<td>2 (MRAC 99603-4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Uvira (12.8.2016)</td>
<td>The northern basin</td>
<td>27 (-)</td>
<td>31</td>
<td>44</td>
<td>2.6</td>
<td>1.1 (0-6)</td>
</tr>
<tr>
<td></td>
<td>Uvira (12.8.2016)</td>
<td>The northern basin</td>
<td>25 (-)</td>
<td>12</td>
<td>28</td>
<td>1.7</td>
<td>0.5 (0-3)</td>
</tr>
</tbody>
</table>
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 1000x 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](image_url). 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 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’-ATTCGGATAAAGCAACGAGACT-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 MgCl₂, 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 94 °C, 39 cycles of 20 seconds at 94 °C, 30 seconds at 58 °C and 1 min and 30 s at 72 °C, and finally 10 min at 72 °C. 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 °C and 15 min at 80 °C. 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,
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 (10⁷ 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

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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subbasin</th>
<th>Season</th>
<th>Host body size</th>
<th>Subbasin</th>
<th>Season</th>
<th>Season</th>
<th>Host body size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>K. limnotrissae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorsal bar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Branch length</td>
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<td>F₁,₉₀=18.19; p&lt;0.001</td>
<td>F₁,₉₀=10.10; p&lt;0.01</td>
<td>F₁,₉₀=6.2; p&lt;0.05</td>
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<td>F₁,₉₀=6.54; p&lt;0.05</td>
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</tr>
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<td>-</td>
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</tr>
<tr>
<td>Branch length</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>F₁,₉₀=12.50; p&lt;0.01</td>
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<td>Branch maximum width</td>
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<td>-</td>
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<td>F₁,₉₀=21.14; p&lt;0.001</td>
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<td><strong>Hooks</strong></td>
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<td>Pair I</td>
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<td>F₁,₉₀=3.97; p&lt;0.05</td>
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<td>F₁,₉₀=6.36; p&lt;0.05</td>
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<td>F₁,₉₀=4.03; p&lt;0.05</td>
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<td>-</td>
<td>-</td>
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<td>F₁,₉₀=5.41; p&lt;0.05</td>
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<td>Pair VII</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Inner root length</td>
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<td>-</td>
<td>-</td>
<td>F₁,₉₀=10.10; p&lt;0.01</td>
<td>F₁,₉₀=10.5; p&lt;0.01</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Length to notch</td>
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<td>-</td>
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<td></td>
</tr>
<tr>
<td>Inner root length</td>
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<td>F₁,₉₀=9.98; p&lt;0.01</td>
<td>F₁,₉₀=10.1; p&lt;0.01</td>
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<tr>
<td>Outer root length</td>
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<td>-</td>
<td>F₁,₉₀=6.00; p&lt;0.05</td>
<td>F₁,₉₀=5.2; p&lt;0.05</td>
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</tr>
<tr>
<td>Length to notch</td>
<td>F₁,₉₀=7.24; p&lt;0.01 (S, C)</td>
<td>F₁,₉₀=7.24; p&lt;0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

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.
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. brunensis Kmentová, Gelnar, Koblmüller and Vanhove, 2016 and C. casuarinus Pariselle, Muterezi Buki nga 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_{1,20}=-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).

<table>
<thead>
<tr>
<th>Parameters (µm)</th>
<th><em>K. limnotrissae</em> (original description)</th>
<th><em>K. limnotrissae</em> (present study)</th>
<th><em>K. tanganicanus</em> (<em>L. miodon</em>)</th>
<th><em>K. tanganicanus</em> (<em>S. tanganicae</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total length</td>
<td>440-610</td>
<td>458.1±128.5 (n=21); (286.2-748.3)</td>
<td>214.2±39.6 (n=23); (121.7-285.8)</td>
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<td>Total width</td>
<td>90-130</td>
<td>143.9±26.2 (n=20); (97.8-220.3)</td>
<td>779.5±170.1 (n=25); (474.4-1171.3)</td>
</tr>
<tr>
<td>Ventral anchor</td>
<td>Total length</td>
<td>20-27</td>
<td>26.2±1.5 (n=99); (22.3-31.8)</td>
<td>31.6±2.4 (n=77); (25.3-36.7)</td>
</tr>
<tr>
<td></td>
<td>Length to notch</td>
<td>19-20</td>
<td>18.8±1.6 (n=98); (15.5-28.3)</td>
<td>21.3±1.8 (n=72); (16.5-26.7)</td>
</tr>
<tr>
<td></td>
<td>Inner root length</td>
<td>7-13</td>
<td>15.6±1.4 (n=99); (11.4-18.6)</td>
<td>18.6±2.5 (n=77); (10.0-22.9)</td>
</tr>
<tr>
<td></td>
<td>Outer root length</td>
<td>5-8</td>
<td>5.0±0.8 (n=94); (3.6-7.9)</td>
<td>8.7±1.5 (n=75); (4.9-11.4)</td>
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<td>Point length</td>
<td>5-7</td>
<td>8.1±1.1 (n=90); (5.3-11.0)</td>
<td>8.7±1.1 (n=69); (6.7-12.3)</td>
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<tr>
<td>Dorsal anchor</td>
<td>Total length</td>
<td>23-26</td>
<td>21.48±1.55 (n=88); (18.7-26.1)</td>
<td>27.7±2.0 (n=73); (20.832.6)</td>
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<tr>
<td></td>
<td>Length to notch</td>
<td>15-19</td>
<td>16.5±1.3 (n=87); (13.8-20.5)</td>
<td>19.9±2.1 (n=74); (13.4-28.2)</td>
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<td></td>
<td>Inner root length</td>
<td>10-13</td>
<td>11.2±1.3 (n=87); (7.8-14.6)</td>
<td>14.4±2.3 (n=72); (6.6-20.6)</td>
</tr>
<tr>
<td></td>
<td>Outer root length</td>
<td>5-7</td>
<td>4.5±1.0 (n=84); (2.5-8.0)</td>
<td>8.1±1.6 (n=70); (4.9-16.1)</td>
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<td>Point length</td>
<td>5-7</td>
<td>7.8±1.1 (n=84); (5.1-10.9)</td>
<td>8.2±1.2 (n=67); (5.7-11.4)</td>
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<td>Ventral bar</td>
<td>Branch length</td>
<td>27-35</td>
<td>16.7±3.0 (n=84); (12.5-33.3)</td>
<td>21.4±3.7 (n=66); (14.5-32.7)</td>
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<td>Branch maximum width</td>
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<td>4.4±0.7 (n=86); (3.0-7.0)</td>
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<td>Dorsal bar</td>
<td>Branch length</td>
<td>22-35</td>
<td>17.4±3 (n=69); (12.0-27.1)</td>
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<td>Thickness at midlength</td>
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<td>6.8±1.3 (n=75); (4-10)</td>
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<tr>
<td>Hooks</td>
<td>Pair I</td>
<td>-</td>
<td>14.4±1.4 (n=67); (11.4-17.9)</td>
<td>13.3±1.3 (n=63); (10.0-17.8)</td>
</tr>
<tr>
<td></td>
<td>Pair II</td>
<td>-</td>
<td>15.4±1.3 (n=63); (12.1-18.2)</td>
<td>14.6±1.6 (n=47); (11.8-17.9)</td>
</tr>
<tr>
<td></td>
<td>Pair III</td>
<td>-</td>
<td>15.9±1.2 (n=68); (13.3-19.3)</td>
<td>14.8±1.5 (n=53); (11.2-17.7)</td>
</tr>
<tr>
<td></td>
<td>Pair IV</td>
<td>-</td>
<td>16.2±1.1 (n=59); (13.0-19.3)</td>
<td>15.3±1.6 (n=42); (10.8-19.4)</td>
</tr>
<tr>
<td></td>
<td>Pair V</td>
<td>-</td>
<td>14.2±1.6 (n=35); (9.3-17)</td>
<td>13.5±1.4 (n=32); (10.4-16.2)</td>
</tr>
<tr>
<td></td>
<td>Pair VI</td>
<td>-</td>
<td>16.3±1.1 (n=34); (13.0-18.8)</td>
<td>15.3±1.4 (n=32); (11.9-18.1)</td>
</tr>
<tr>
<td></td>
<td>Pair VII</td>
<td>-</td>
<td>16.7±1.4 (n=24); (14.3-20.8)</td>
<td>15±1.3 (n=22); (12.9-17.5)</td>
</tr>
<tr>
<td></td>
<td>Pair I, II, III, IV, VI, VII average size</td>
<td>13-15</td>
<td>15.5±1.5 (n=350); (9.3-20.8)</td>
<td>14.6±1.6 (n=259); (10.0-19.4)</td>
</tr>
<tr>
<td>Copulatory tube</td>
<td>curved length</td>
<td>21-23</td>
<td>30.4±2.5 (n=75); (25.1-38.3)</td>
<td>39.7±4.6 (n=63); (30.4-49.9)</td>
</tr>
<tr>
<td>Accessory piece</td>
<td>curved length</td>
<td>-</td>
<td>36.1±3.6 (n=69); (28.0-47.1)</td>
<td>52.5±6.0 (n=62); (33.9-62.6)</td>
</tr>
</tbody>
</table>
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 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
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.
Monogeneans of 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 luxophthalmus (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 haptorial 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 geographical 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 *Limnothrisa miodon*, *Kapentagyrus tanganicanus* infects both clupeid species in Lake Tanganyika. Although large phenotypic variation in *K. tanganicanus* related to host species was documented, the conspicuity 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).

**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 haptor 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.

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**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), 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 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 Annullotrema 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
lato include the genera *Haploleidus* Mueller, 1936, *Urocleidus* Mueller, 1936 and *Actinoleidus* 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 *Halitrematidae* Johnston and Tiegs, 1922 and *Euryhalitrematidae* 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 *Lethrinotrema* 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); MZM 10076-79 (4 specimens).

Additional localities. Bujumbura (3°23'S-29°22'E), Kalemie (5°56'S-29°12'E), Kiloma 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 Kapentagyrus limnotrissae was 35% with a mean infection intensity of 1.7.

Diagnosis. Kapentagyrus limnotrissae is the type species of the genus, infecting gills of Limnothrissa 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 Kapentagyrus limnotrissae were identified based on comparison with the holotype material. Importantly, as mentioned in the original description, it differs from Ancyrocephalus sensu stricto Crepin, 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](image.png)
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°57'E), Mpulungu (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), Luwanga (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 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)
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.