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**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**

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## **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, *Kapentagyris 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 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

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## 1 **Introduction**

2

3 Helminths are the most commonly detected parasite group co-introduced with non-native species, with fish as the  
4 most common alien hosts (Gozlan 2008; Lymbery et al. 2014). Parasite co-introduction and its possible impact on  
5 ecosystems is usually underestimated (Peeler et al. 2004; Lymbery et al. 2014). The success of parasite  
6 establishment in a non-native environment is affected by many factors such as the size of the founder host  
7 population (Anderson and May 1991; Sakai et al. 2001; Dlugosch and Parker 2008), the parasite's life cycle (direct  
8 versus indirect) and environmental biotic and abiotic conditions (Taraschewski 2006; Lymbery et al. 2014).  
9 Moreover, the success of parasite co-introduction is influenced by host transportation, whereby factors such as  
10 salinity, the life stage of the introduced population or antiparasitic treatment might hamper parasite introduction  
11 (Mombaerts et al. 2014; Kvach et al. 2014). While the potential use of parasites as tags for host population,  
12 introduction pathway and historical distribution has been discussed for decades, there are only a small number of  
13 studies demonstrating this concept (Jiménez-García et al. 2001; Oliva and Gonzalez 2004; Huyse et al. 2015;  
14 Kmentová et al. in press).

15 A taxonomically diverse range of fish has been anthropogenically introduced or translocated in Africa. Fish  
16 introductions out of their native range mainly occur with fishery target species like the Nile perch *Lates niloticus*  
17 (Linnaeus 1758) (Latidae), “tilapias” (*Oreochromis* spp., *Tilapia* spp.) (Cichlidae) and the clupeid *Limnothrissa*  
18 *miodon* (Boulenger, 1906) (Ogutu-Ohwayo and Hecky 1991). Other species acting as potential agents for disease  
19 control, such as the poeciliid *Gambusia affinis* (Baird & Girard, 1853), which feeds on the mosquito vectors of  
20 malaria, were translocated to non-native areas (Welcomme 1981).

21 Clupeids (Clupeiformes; Actinopterygii) form highly productive commercial stocks of worldwide importance  
22 (Naylor et al. 2000). Although they are primarily a marine family, more than half of the clupeid species can be  
23 found in brackish waters or freshwater. Some of them have adopted a continental lifestyle without any link to the  
24 marine realm. In African freshwaters, clupeids are represented by 27 species belonging to the Dorosomatinae  
25 (Lavoué et al. 2014). In this study, we focused on *Limnothrissa miodon*, a clupeid species endemic to Lake  
26 Tanganyika, and particularly on its non-native populations from lakes Kivu and Kariba.

27 Lake Tanganyika is the oldest and deepest of the African Great Lakes. It is famous for its explosive and adaptive  
28 evolution of many fish and other taxa (Salzburger et al. 2014). In contrast to the high species richness of fish in  
29 the littoral zone, the pelagic realm is mainly inhabited by two endemic clupeid species, *Limnothrissa miodon*  
30 (Boulenger, 1906) and *Stolothrissa tanganyicae* Regan, 1917, both belonging to monotypic genera (Coulter 1991).

31 Lake Tanganyika sprat (*S. tanganicae*) and sardines (*L. miodon*), together with their main predator, *Lates stappersii*  
32 (Boulenger, 1914), comprise up to 95% of commercial catches in the lake, with an estimated annual production in  
33 the range of 165,000 to 200,000 tons (Mölsä et al. 1999). Both clupeid species are short-lived and numerous. They  
34 show schooling behaviour, seasonal fluctuations in abundance, and form the main link between the planktonic and  
35 piscivorous trophic levels in the pelagic realm (Mulimbwa and Shirakihara 1994).

36 The Lake Tanganyika sardine, *L. miodon*, was introduced into several water bodies in Africa, including Lake Kivu  
37 (Spliethoff et al. 1983) and the man-made reservoir Lake Kariba (Balon and Cache 1974). In addition, starting  
38 from Lake Kariba, this species further invaded the Cahora Bassa reservoir via the Zambezi River (Cross et al.  
39 2011).

40 Lake Kivu is one of the Great African Lakes and is known for the vast amounts of carbon dioxide and methane in  
41 its anoxic waters, which cause unusual biochemical and limnological conditions. It has a species-poor fish fauna  
42 compared to the other Great Lakes (Beadle, 1981) because of historical volcanic activity, periods of drought and  
43 the higher salinity and recent origin of the present-day lake (Snoeks et al. 1997). Lake Kariba is a man-made lake  
44 that was constructed by damming the Zambezi River in 1958.

45 *Limnothrissa miodon* was introduced to both non-native areas to fill in the empty pelagic niche. However, initially,  
46 *S. tanganicae* was planned to be introduced into Lake Kivu as a fishery target (Collart 1960; Dumont 1986). The  
47 introduction of both clupeid species, rather than just *Stolothrissa tanganicae*, was unintentional. The success of *L.*  
48 *miodon* is probably due to its greater habitat and diet plasticity (Mulimbwa and Shirakihara 1994).

49 As the introduction of *S. tanganicae* to Lake Kivu in 1959 was unsuccessful, *L. miodon* was targeted for  
50 introduction into Lake Kariba in 1967. Transport of adult sardines was not recommended because of their fragile  
51 skin that becomes severely damaged upon contact and becomes susceptible to infection. Therefore, fry occurring  
52 in shallow water at night were chosen for transport because they were easier to handle. The fry were scooped with  
53 large containers to avoid high fry density, and a tranquilizer was added. As the species migrates to deeper water  
54 during the day, the containers were transported by plane at night and emptied into Lake Kivu and Lake Kariba  
55 (Collart, 1960; Bell-Cross & Bell-Cross, 1971). Based on available reports, the origin of the population currently  
56 inhabiting Lake Kivu was the northern end of Lake Tanganyika near Bujumbura in Kabezi (Collart 1960). Fish  
57 for Lake Kariba, however, were caught near Mpulungu and Kasaba Bay at the south-western coast of Lake  
58 Tanganyika (Bell-Cross and Bell-Cross 1971). In contrast to Lake Kivu, where only fry were introduced, some  
59 somewhat larger specimens were also present in the transports to Lake Kariba (Bell-Cross and Bell-Cross 1971).

60 In Lake Tanganyika, *L. miodon* is infected with two species of dactylogyrid monogeneans, *Kapentagyris*  
61 *limnotrissae* (Paperna, 1973) and *K. tanganicanus* Kmentová, Gelnar & Vanhove, 2018 (Paperna 1973; Kmentová  
62 et al. in press). Monogeneans (Platyhelminthes) are parasitic flatworms with a direct life cycle (they infect a single  
63 host species). They occur worldwide and are mainly ectoparasites on the gills, skin and fins of fish (Pugachev et  
64 al. 2009). Parasites with a direct life cycle have an increased chance of establishment after translocation compared  
65 to parasites where more than one host is involved in the life cycle (Bauer 1991). Importantly, monogeneans and  
66 other parasites are considered to be potential tags for the characterisation of host stock structure (Oliva and  
67 Gonzalez 2004; Criscione et al. 2006). Monogeneans have already been used to reconstruct their host's historical  
68 distribution (Lumme et al. 2016) but also their introduction route (Huyse et al. 2015). Although co-introductions  
69 can be viewed as natural experiments to test the potential of parasites in detecting their host's origin, very few  
70 introductions are sufficiently documented to allow testing this. The introductions of *L. miodon*, however, do  
71 provide us with such a case, as the procedures were described in detail for Lake Kivu (Collart 1960) and Lake  
72 Kariba (Bell-Cross and Bell-Cross 1971). Based on historical reports, solely sardine fry was introduced to Lake  
73 Kivu (Collart 1960), while some somewhat larger sardine specimens are thought to have been potentially  
74 introduced to Lake Kariba (Bell-Cross and Bell-Cross 1971). Infection of dactylogyrid monogeneans on fry was  
75 reported as a result of high fish population densities and stress in farmed/artificial conditions (Paperna 1963;  
76 Thoney and Hargis 1991; Jalali and Barzegar 2005) and the gills of fry populations in natural environments are  
77 usually less affected or not affected (Pugachev et al. 2009). Therefore, we hypothesize a higher co-introduction  
78 success of monogenean parasites in the latter case, as sardine fry are not known to be infected by monogeneans  
79 (11 specimens from Uvira (northern basin of Lake Tanganyika) and 9 specimens from Bujumbura (MRAC MT.  
80 43554-64) of *L. miodon* below 5.0 cm standard length were not infected by monogeneans, suggesting that sardine  
81 fry are not infected by monogeneans: unpublished results).

82 This study is designed as a natural experiment in two different ways: 1) Since some larger specimens of *L. miodon*  
83 were transported to Lake Kariba whereas only fry was introduced to Lake Kivu, we can test the effect of host life  
84 stage on the co-introduction of monogeneans. In this study, the potential presence of monogenean species in their  
85 non-native range was verified by using a combination of morphological and molecular identification. 2)  
86 Additionally, intraspecific geographical variation in morphology was reported in dactylogyrids, including in  
87 species of *Kapentagyris* Kmentová, Gelnar & Vanhove 2018 which infect *L. miodon* in Lake Tanganyika  
88 (Kmentová et al. in press). We investigated whether the morphology of introduced parasites might indicate the  
89 geographic origin of the host population which was used in the introduction. To date, few parasitological surveys

90 have been conducted in Lake Kivu (Baer and Fain 1958; Vercammen-Grandjean 1960) and only a small portion  
91 of host species have been investigated in Lake Kariba (Douëllou 1993; Barson et al. 2010). Although a previous  
92 study reported on the presence of a species of *Kapentagyris* on *L. miodon* in Lake Kariba (Douëllou 1991), only  
93 one species was known from *L. miodon* at that time (*Kapentagyris limnotrissae* (Paperna, 1973)) with the second  
94 species, *K. tanganicanus* discovered at a later date. As such, the presence of the latter species remained to be  
95 checked.

## 96 **Material and Methods**

### 97 **Sampling**

98 Individuals of *Limnothrissa miodon* from two non-native areas, Lake Kivu and Lake Kariba, were examined. Fish  
99 specimens from the Rwandese side of Lake Kivu originated from the ichthyology collection of the Royal Museum  
100 for Central Africa (RMCA) (Tervuren, Belgium). Fresh specimens were obtained by scientists from the Unité  
101 d'Enseignement et de Recherche en Hydrobiologie Appliquée (UERHA) of the department of Biology of the  
102 Institut Supérieur Pédagogique (ISP) of Bukavu located at the Congolese side of the lake (see Table 1). Fish from  
103 Lake Kariba were caught by gillnets during several months in 2016 and the beginning of 2017 in Sanyati East  
104 Basin (see Table 1). In total, gills and fins of 251 fish specimens were examined following the standard protocol  
105 of Ergens & Lom (1970). Monogeneans were mounted on a slide with a drop of water, which was later replaced  
106 by Hoyer's medium, and covered with a cover slip that was fixed with nail polish. With the exception of museum  
107 specimens, at least two monogenean individuals from each infected fish were cut in two, followed by the transfer  
108 of the anterior body part into an Eppendorf tube (1.5 ml) containing 99% ethanol. In addition, 80 specimens of *L.*  
109 *miodon* fry under 3.1 cm of standard length, originating from Chituta Bay, the southern basin of Lake Tanganyika,  
110 were examined (8°43'25"S, 31°9'0"E). Parasite identification and measurements were carried out using an  
111 Olympus BX51 microscope. Specimens were compared with type material of *K. limnotrissae* and *K. tanganicanus*,  
112 respectively, deposited in the RMCA (MRAC MT.35572 and MT.38201). Fish tissue samples from Lake Kariba  
113 were deposited in the collection of the research group Zoology: Biodiversity and Toxicology of Hasselt University  
114 under accession number HU hostvouchers xxxx. Fish tissue samples from Lake Kivu were deposited in the  
115 ichthyology collection of the RMCA under collection number MRAC P. 2016.20 and parasite voucher specimens  
116 are available in the invertebrate collection of the RMCA (MRAC MT. 38237-8 and 38450-60).

### 117 **Morphometrics**



118 Since monogenean taxonomy is mainly based on the parasites' sclerotized structures, 25 different variables of the  
119 hard parts of the haptor and male copulatory organ (MCO) were measured for species identification (see Table 2).  
120 Measurements were taken using an Olympus BX51 microscope with incorporated phase contrast and the Olympus  
121 Stream Motion software at a magnification x1000 (objective × 100 immersion, ocular × 10). Terminology was  
122 based on Řehulková et al. (2013). To check for intraspecific phenotypic diversity (in haptor morphology),  
123 measurements were analysed using multivariate statistical techniques in the R (R development core team, 2011)  
124 adegenet package (Jombart, 2008), where a principal component analysis (PCA) was conducted on a covariance  
125 matrix with 19 measured and standardised variables. Outliers were identified and removed using Mahalanobis  
126 distances in the mvoutlier package (Filzmoser and Gschwandtner 2017). Morphometric data generated in this study  
127 were compared with previously published data on *Kapentagyryus limnotrissae* from Lake Tanganyika (Kmentová  
128 et al. in press) which stemmed from specimens from all three subbasins (Danley et al. 2012). Since significant  
129 intraspecific variation of *K. limnotrissae* among subbasins was documented (Kmentová et al. in press),  
130 comparisons for the different subbasins were made separately. The assumption of normality was tested by Shapiro-  
131 Wilk's W test implemented in the stats package (R Core Team, 2013). Morphological differences between  
132 monogeneans from the native and introduced range were also tested using multiple one-way MANOVA in the  
133 package stats as a set of independent tests, with Pillai's test of significance and Bonferroni's correction ( $\alpha$  value  
134 of 0.05/number of variables). To test the significance of intraspecific differences in haptor and MCO structures,  
135 Mann-Whitney U tests were performed in STATISTICA 12. The assumption of homogeneous variance within  
136 sample groups was verified by Levene's test.

### 137 **Molecular characterisation**

138 Total genomic DNA was extracted following Zavodna et al. (2008): ethanol evaporation took place in a vacuum  
139 centrifuge and the tissue was homogenized in 200  $\mu$ l of extraction buffer (100 mM Tris-HCl, 10 mM EDTA, 100  
140 mM NaCl, 1% SDS, 0.06 mg Proteinase K, 1.5 mM dithiothreitol) and incubated at 56 °C overnight. After  
141 incubation, proteins were precipitated using 10 M ammonium acetate (1/3 of the lysate volume). The lysate was  
142 then vortexed, centrifuged at the highest speed (13,800 rpm) and the supernatant containing DNA was precipitated  
143 using a double volume of ice-cold 100% ethanol. Following centrifugation, the DNA pellet was washed using 70%  
144 ethanol. Finally, the DNA pellet was air-dried and dissolved in 60  $\mu$ l of sterile Millipore water. To confirm parasite  
145 species identification genetically, we used three nuclear fragments: from the small and large ribosomal subunit  
146 gene (18 and 28 rDNA) and internal transcribed spacer 1 (ITS-1). Partial 18S rDNA together with ITS-1 were  
147 amplified using the S1 (5'-ATTCCGATAACGAACGAGACT-3') (Sinnappah et al. 2001) and Lig5.8R (5'-

148 GATACTCGAGCCGAGTGATCC-3') (Blasco-Costa et al. 2012) primers. Each reaction mix contained 1.5 units  
149 of *Taq* Polymerase, 1X buffer containing 0.1 mg/ml BSA, 1.5 mM MgCl<sub>2</sub>, 200 mM dNTPs, 0.8 mM of each primer  
150 and 3 µl of isolated DNA (concentration was not measured) in a total reaction volume of 30 µl under the following  
151 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  
152 min at 72 °C. Primers C1 (5'-ACCCGCTGAATTTAAGCAT-3') and D2 (5'-TGGTCCGTGTTTCAAGAC-3')  
153 (Hassouna et al. 1984) were used for amplification of the partial 28S rDNA gene. Each PCR reaction contained  
154 1.5 unit of *Taq* Polymerase, 1X buffer containing 0.1 mg/ml BSA, 1.5 mM MgCl<sub>2</sub>, 200 mM dNTPs, 0.5 mM of  
155 each primer and 50 ng of genomic DNA in a total reaction volume of 30 µl under the following conditions: 2 min  
156 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  
157 72 °C. The PCR products were visualized using horizontal gel electrophoresis using a GoldView stained agarose  
158 gel (1%) followed by enzymatic cleaning of the positive samples using 1 µl of ExoSAP-IT reagent and 2,5 µl of  
159 PCR product under the following conditions: 15 min at 37 °C and 15 min at 80 °C. Identical primers as in the  
160 amplification reactions were used for sequencing with a Big Dye Chemistry Cycle Sequencing Kit 3.1, following  
161 the manufacturer's recommendations. Fragments were cleaned using the BigDye XTerminator® Purification Kit  
162 and visualized on an ABI3130 capillary sequencer. Sequences were visually inspected and corrected using MEGA  
163 v7 (Kumar et al. 2016) and aligned using MUSCLE (Edgar 2004) under default distance measures as implemented  
164 in MEGA v7. Previously published sequences of *Kapentagyris limnotrissae* (GenBank accession numbers  
165 MH071808 and MH071782) were added to the dataset. Sequences obtained in the present study were deposited in  
166 the NCBI GenBank under the accession numbers MH620705 and MH623076.

167

## 168 **Results**

169

### 170 **Morphological and molecular characterisation**

171 Based on 136 fish individuals, no monogenean parasites were recorded from Lake Kivu. In total, 58 monogenean  
172 individuals were collected from 115 individuals of *L. miodon* from Lake Kariba (Table 1). Morphological  
173 identification combined with genetic characterisation revealed the presence of only one parasite species:  
174 *Kapentagyris limnotrissae*. The observed prevalence in Lake Kariba ranged from 0 to 55.5%. An average infection  
175 intensity of 1.5 individuals was documented in both positive samplings in Lake Kariba. No monogenean parasites  
176 were found in 80 specimens of sardine fry.

177 The amplified fragments of 18S, ITS-1 and 28S rDNA from 9 individuals were 451, 328 and 650 base pairs long,  
178 respectively. No intraspecific differences either among individuals collected from Lake Kariba and Lake  
179 Tanganyika or between the lakes were found.

180

#### 181 **Taxonomic account**

182 New record

183 Family: Dactylogyridae Yamaguti, 1963

184 Genus: *Kapentagyryus* Kmentová, Gelnar & Vanhove, 2018

185 Species: *K. limnotrissae*

186 Type-host: *Limnothrissa miodon* (Boulenger, 1906) (Clupeidae)

187 Type-locality: Lake Tanganyika, Tanzania

188 Vouchers: MRAC MT. 38237-8 and 38450-60.

189 Additional locality: Sanyati East Basin, Lake Kariba (-16°59'S-28°82'E; -16°60'S-28°87'E)

190 Site of infection: Gills.

191 Infection parameters: 16 of 115 *L. miodon* infected with 1 – 10 specimens (Table 1).

192 Species identification was based on morphology (Fig. 2) and morphometrics (Table 2) of sclerotized structures.  
193 The presence of two pairs of anchors with well-incised roots and a regularly curved point, slightly larger ventral  
194 compared to dorsal anchors with more developed inner roots and V-shaped bars with similar branch lengths and  
195 constant width, enabled identification to the genus level. The proportion of the inner/outer root length of both  
196 ventral and dorsal anchors of around 3, combined with a straight copulatory tube and a coiled accessory piece,  
197 correspond to the original description of *K. limnotrissae* and the redescription provided by Kmentová et al. (in  
198 press).

#### 199 **Morphometrics**

200 Intraspecific phenotypic variability was analysed by PCA using 19 haptoral variables (the length of the sixth and  
201 seventh pair of hooks were omitted given the small number of replicates) of 127 individuals of *K. limnotrissae*, 95  
202 of which were from Lake Tanganyika and stem from a previous study (Kmentová et al. in press) (Fig. 3). The first  
203 PCA axis, which explained 16.6% of the variation, failed to clearly separate specimens originating from Lake

204 Kariba and Lake Tanganyika. The five variables with the highest contribution were the branch length and thickness  
205 of both bars and the outer root length of the ventral anchor. Other PCs did not show a clearer separation.  
206 Morphometric results were then compared with the samples from Lake Tanganyika divided in groups based on  
207 their subbasin origin. Multiple one-way MANOVA, after applying strict Bonferroni's correction, revealed that  
208 specimens from the southern basin turned out to be more similar to the population from Lake Kariba compared to  
209 the other two subbasins. In contrast to the significant difference in length of the dorsal and ventral bar branches  
210 and of the fourth pair of marginal hooks between the central and the northern subbasin of Lake Tanganyika and  
211 Lake Kariba, respectively, no difference in these parameters was reported between specimens from Lake Kariba  
212 and the southern subbasin of Lake Tanganyika (see Table 3).

213 Copulatory organ variables from a total of 88 individuals of *K. limnotrissae* originating from Lake Kariba (21) and  
214 Lake Tanganyika stem from a previous study (Kmentová et al. in press) (67) were compared. Mann-Whitney U  
215 tests showed no difference in MCO structures between *K. limnotrissae* from Lake Kariba and Lake Tanganyika  
216 (copulatory tube -  $Z_{1,86}=-1.10$ ;  $p>0.05$ ; accessory piece -  $Z_{1,79}=-1.89$ ;  $p>0.05$ ) (Fig. 4).

217

## 218 **Discussion**

219

220 Co-introduction of the monogenean *K. limnotrissae* from Lake Tanganyika with *L. miodon* to Lake Kariba was  
221 documented by combining morphological and genetic results. On the other hand, *L. miodon* was seen to be free of  
222 monogenean infection in Lake Kivu, where this sardine was also introduced. Intraspecific diversity of *K.*  
223 *limnotrissae* was analysed to evaluate morphological differences between native and introduced populations. This  
224 has potential for the identification of host origins. The effect of host life stage on parasite co-introduction is  
225 discussed. Co-introduction of *K. tanganicanus* was not detected.

226

### 227 ***Kapentagyus limnotrissae* in Lake Kariba**

228 The higher observed abundance (1.5 versus 0.6 individuals/gill chamber) and prevalence (56% versus 35% and  
229 70% reported by Douëllou (1991)) of *K. limnotrissae* in Lake Kariba compared to its native Lake Tanganyika is  
230 in contrast to previously studied monogenean introductions finding the opposite pattern (Ondračková et al. 2010;  
231 Sheath et al. 2015; Gabagambi and Skorpung 2017; Sarabeev et al. 2017a). Interestingly, the relatively faster  
232 growth but smaller size of *L. miodon* was reported in Lake Kariba compared to natural lakes (Lake Tanganyika  
233 and Lake Kivu), probably as a result of unstable conditions and high predation pressure (Marshall 1987, 1993).

234 Therefore, we can suggest that a higher parasite prevalence of *K. limnotrissae* in Lake Kariba could be caused by  
235 different environmental conditions such as predation pressure or host life history (Dunn 2009; Gabagambi and  
236 Skorping 2017; Sarabeev et al. 2017b). However, the observed differences in prevalence could also be the  
237 consequence of different abiotic factors in Lake Kariba compared to Lake Tanganyika, such as temperature or  
238 water chemical composition (Coche 1974; Edmond et al. 1993), as these factors are known to influence  
239 monogenean population dynamics (Buchmann 1988; Šimková et al. 2001; Marchiori et al. 2015).

240 Seasonal differences in the prevalence and abundance of *K. limnotrissae* were documented in Lake Kariba (see  
241 Table 1). The pattern seems to follow changes in water temperature, which reaches its maximum of 30 °C in  
242 January and its minimum of 17 °C in July (Balon and Cache 1974). This absence of monogeneans in the colder  
243 period of the year corresponds to previous studies on dactylogyrids in temporal climates (Šimková et al. 2001;  
244 Marchiori et al. 2015). As the hatching of monogenean eggs is temperature-dependent (Whittington and Kearn  
245 2011), the lack of seasonal temperature differences in Lake Tanganyika explains the year-round abundance of *K.*  
246 *limnotrissae* in the latter lake. However, differences in *K. limnotrissae* prevalence between native and non-native  
247 localities as well as within Lake Kariba need to be further tested over several years to reveal the general pattern of  
248 the parasites' population dynamics (Hudson et al. 2002).

249 Although *L. miodon* does not seem to have been infected by monogeneans native to Lake Kariba, Douëllou (1991)  
250 mentioned the presence of eight endoparasite species infecting this sardine in Lake Kariba which have not yet been  
251 reported in the population from Lake Tanganyika (Kmentová et al. in press). This result indicates parasite spill-  
252 back of native fauna to the introduced *L. miodon* and is explained by the generally lower host specificity in fish of  
253 endoparasites' larval stages compared to monogeneans (Cribb et al. 2001; Jensen and Bullard 2010). Finally, it  
254 would be interesting to investigate the effect of the combined stressors, predators and increased parasite loads on  
255 the introduced sardines, as interactions between parasitism and predation are known to exist (Hudson et al. 1992;  
256 Rohlenová et al. 2011).

257 Another question regarding the infection of *K. limnotrissae* in Lake Kariba is why only one of the species of  
258 *Kapentagyryus* infecting *L. miodon* (Kmentová et al. in press) was co-introduced. There are two possible scenarios:  
259 either *K. tanganicanus* was not co-introduced, as it possibly might not have been present in the source population  
260 of *L. miodon*, or it may not have survived the environmental conditions in Lake Kariba. However, based on present  
261 knowledge and available data, we cannot determine which of these two possibilities are the cause.

262 Co-introduced flatworm parasites have been observed to cause population decline (Tanum 1983; Johnsen and  
263 Jensen 1988; Britton et al. 2011) and extinction of native fish fauna (Zholdasova 1997). However, considering the

264 generally high host-specificity of dactylogyrid monogeneans and the fact that *K. limnotrissae* is strictly host-  
265 specific in Lake Tanganyika, the potential for spill-over to native fish seems low. Co-introduction of monogenean  
266 species without any known impact on the native fauna has been documented (Truter et al. 2017). However,  
267 monitoring the potential presence of non-native monogenean species on the local fish fauna in Lake Kariba is  
268 recommended as parasite spill-over does not always occur in a predictable manner (Jiménez-García et al. 2001).

269

#### 270 **Testing the possibility of inferring host origin using the morphometrics of *K. limnotrissae***

271 To check for possible differences between native and introduced parasite populations, measurements of the  
272 parasites' sclerotized structures were analysed. Knowledge of stock structure and the degree of mixing among  
273 populations is crucial for the management of *L. miodon* not only in Lake Kariba but also in other areas of its  
274 distribution. Parasites are considered potential biological tags revealing host structure (Poulin and Kamiya 2015).  
275 PCA showed only a slight differentiation between the specimens from Lake Kariba and Lake Tanganyika as well  
276 as among Lake Tanganyika's subbasins. Morphometric variables were tested using MANOVA and sub-tests were  
277 discussed, as possibly one or a few variables, rather than the full set of haptor variables, could indicate the host's  
278 subbasin fidelity. As rather continuous morphometric variability in *K. limnotrissae* found in PCA was reported,  
279 tests on haptor morphometrics indicated a greater level of similarity of the specimens from Lake Kariba with the  
280 individuals from the southern compared to the northern and central subbasin of Lake Tanganyika. This result  
281 corresponds with the documented origin of the introduced *L. miodon*, namely Mpulungu and Kasaba Bay, both  
282 located in the southern part of Lake Tanganyika. The low percentage of explained variation in the PCA indicates  
283 that there is either continuous phenotypic plasticity among the specimens from different subbasins/lakes, or that  
284 the potential geographic segregation of parasite populations does not affect all haptor variables to the same extent  
285 (Vignon et al. 2011). While significant differences in haptor morphometrics were revealed by sub-tests of  
286 MANOVA, no significant differences in MCO characteristics between Lake Tanganyika and Lake Kariba were  
287 observed. Phenotypic variation without a genetic basis has already been observed in various parasite taxa (Stunkard  
288 1957; de Leon 1995; Mariniello et al. 2004; Steinauer et al. 2007; Ondračková et al. 2012; Kmentová et al. 2016;  
289 Truter et al. 2017). Even if the intraspecific variability was not mirrored in the three rDNA gene portions amplified  
290 in this study, highly variable markers with a faster rate of molecular evolution, such as mitochondrial genes, may  
291 identify potential divergence as a consequence of founder effects, adaptation or geographical isolation (Steinauer  
292 et al. 2007; Dlugosch and Parker 2008).

293

## 294 **Release from monogenean infection in Lake Kivu**

295 In contrast to Lake Kariba, the introduced population of *L. miodon* in Lake Kivu consisted only of small fry. Since  
296 monogenean infection has been reported to depend on the size of fish fry (Bagge and Valtonen 1999), introducing  
297 only fry will have decreased the possibility of monogenean co-introduction with *L. miodon*. Therefore, the absence  
298 of monogenean parasites of *L. miodon* in Lake Kivu can be explained by the host's life stage. Moreover, no  
299 monogenean parasite was found in 80 specimens of sardine fry examined as a part of this study. This hypothesis  
300 is also supported by the fact that there is no report documenting any type of antiparasitic treatment before or after  
301 the respective translocations of *L. miodon* (Collart 1960; Bell-Cross and Bell-Cross 1971). In previous studies,  
302 characterisation of the monogenean parasite fauna matched the suggested method of transport of invasive goby  
303 species, with differences between arrival with ballast water and active dispersal (Mombaerts et al. 2014; Huysse et  
304 al. 2015). However, different biochemical and limnological conditions (Degens et al. 1973; Schmid and Wüest  
305 2012) together with a different surface temperature compared to Lake Tanganyika (26°C and 24°C) (Katsev et al.  
306 2014) may have also precluded the establishment of sardine-infecting monogeneans in Lake Kivu. Moreover,  
307 founder effects related to the small population size of introduced parasites could have influenced the parasites'  
308 ability to adapt (Gavrilets and Hastings 1996). However, distinguishing between the effect on the establishment  
309 success of a particular parasite of the translocation procedure or of different environmental conditions is impossible  
310 without an experimental study. The enemy release hypothesis suggests that a lack of parasite infections can  
311 increase the invasion success of alien species (Colautti et al. 2004). According to Guillard et al. (2012) *L. miodon*  
312 is well established in Lake Kivu, showing the same schooling behaviour, seasonal fluctuations and cannibalistic  
313 behaviour as in Lake Tanganyika (de Iongh et al. 1983; Spliethoff et al. 1983; Hauser et al. 1995). The species  
314 impacts the community composition of zooplankton (de Iongh et al. 1995). The invasion success of the sardine is  
315 probably also correlated with the absence of planktivorous competitors and predators (Snoeks 2000).

316

## 317 **Conclusion**

318 The main question of our study was whether we could use parasites to investigate host introductions. While parasite  
319 co-introduction with the fishery target *L. miodon* to Lake Kariba was documented, a release of monogenean  
320 infection into Lake Kivu is suggested. Two possible scenarios to explain the current situation in Lake Kivu were  
321 proposed: monogenean parasites not having been translocated, as the founder population consisted only of fry,  
322 would support previous studies highlighting introduction conditions as crucial for parasite survival. Therefore, the  
323 absence of monogenean parasites in some introduced areas could be the result of circumstances surrounding host

324 translocation and host life stage. This should be considered as a parameter when considering fish introductions.  
325 The other possibility is that the parasites were unsuccessfully established because of differences in biotic and  
326 abiotic conditions in their native area compared to Lake Kivu. Experimental studies are needed to discern between  
327 these two scenarios. In contrast, the increased prevalence of *K. limnotrissae* in Lake Kariba compared to Lake  
328 Tanganyika was reported. This pattern was suggested to be due to different environmental conditions such as  
329 different predation pressure, differences in host life history or in abiotic conditions, as these factors are known to  
330 influence monogenean population dynamics (Buchmann 1988; Šimková et al. 2001; Marchiori et al. 2015).  
331 Despite only slight phenotypic differences in morphology of *K. limnotrissae* between populations from its native  
332 range and from Lake Kariba, our results revealed a greater similarity to the specimens from the southern part of  
333 Lake Tanganyika using morphometric results. This finding corresponds with historical reports about the  
334 introduction events. Therefore, the potential of *K. limnotrissae* as a tag for its host's origins was supported and  
335 should be further scrutinised by detailed genetic characterisation, including fast evolving markers.

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558 **Figure captions**

559 Figure 1: Geographical positions of sampling localities in a) Lake Kariba and b) Lake Kivu. Map created using  
560 SimpleMappr software v7.0.0. (available at <http://www.simplemappr.net>. Accessed July 25, 2017).

561 Figure 2: Sclerotised haptor and male genital structures of *Kapentagyris limnotrissae* from Lake Kariba (Hoyer's  
562 medium, phase-contrast photomicrographs). A) Opisthaptor B) Male copulatory organ.

563 Figure 3: A biplot of PCA (first two axes) based on measurements of haptor and sclerotized structures of *K.*  
564 *limnotrissae* from Lake Tanganyika and Lake Kariba. Symbols denote the lake origin of specimens (dot – Lake  
565 Kariba, triangle – Lake Tanganyika), colour is used to specify the subbasins of Lake Tanganyika.



566 Figure 4: Box-plot graph with male copulatory organ structures of *K. limnotrissae* defined by study area: a)  
567 copulatory tube length; b) accessory piece length. The number of specimens is indicated in brackets.

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