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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, *Kapentagyrus limnotrissae* and *K. tanganicanus*. One of these species, *K. limnotrissae,* was found on *L. miodon* from Lake Kariba. In contrast, not a single monogenean individual was found in specimens from Lake Kivu. Morphometric results suggested that the origin of *K. limnotrissae* introduced into Lake Kariba may be 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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Introduction

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

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

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

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

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

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

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

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

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

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

This study is designed as a natural experiment in two different ways: 1) Since some larger specimens of *L. miodon* were transported to Lake Kariba whereas only fry was introduced to Lake Kivu, we can test the effect of host life stage on the co-introduction of monogeneans. In this study, the potential presence of monogenean species in their non-native range was verified by using a combination of morphological and molecular identification. 2) Additionally, intraspecific geographical variation in morphology was reported in dactylogyrids, including in species of *Kapentagyrus* Kmentová, Gelnar & Vanhove 2018 which infect *L. miodon* in Lake Tanganyika (Kmentová et al. in press). We investigated whether the morphology of introduced parasites might indicate the geographic origin of the host population which was used in the introduction. To date, few parasitological surveys have been conducted in Lake Kivu (Baer and Fain 1958; Vercammen-Grandjean 1960) and only a small portion of host species have been investigated in Lake Kariba (Douëllou 1993; Barson et al. 2010). Although a previous study reported on the presence of a species of *Kapentagyrus* on *L. miodon* in Lake Kariba (Douëllou 1991), only one species was known from *L. miodon* at that time (*Kapentagyrus limnotrissae* (Paperna, 1973)) with the second species, *K. tanganicanus* discovered at a later date. As such, the presence of the latter species remained to be checked.

Material and Methods

Sampling

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

Morphometrics

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

Molecular characterisation

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

GATACTCGAGCCGAGTGATCC-3′) (Blasco-Costa et al. 2012) primers. Each reaction mix contained 1.5 units of *Taq* Polymerase, 1X buffer containing 0.1 mg/ml BSA, 1.5 mM $MgCl_2$, 200 mM dNTPs, 0.8 mM of each primer and 3 µl of isolated DNA (concentration was not measured) in a total reaction volume of 30 µl under the following conditions: 2 min at 95 ºC, 39 cycles of 1 min at 95 ºC, 1 min at 55 ºC and 1 min and 30 s at 72 ºC, and finally 10 min at 72 ºC. Primers C1 (5′-ACCCGCTGAATTTAAGCAT-3′) and D2 (5′-TGGTCCGTGTTTCAAGAC-3′) (Hassouna et al. 1984) were used for amplification of the partial 28S rDNA gene. Each PCR reaction contained 1.5 unit of *Taq* Polymerase, 1X buffer containing 0.1 mg/ml BSA, 1.5 mM $MgCl_2$, 200 mM dNTPs, 0.5 mM of each primer and 50 ng of genomic DNA in a total reaction volume of 30 µl under the following conditions: 2 min at 94 ºC, 39 cycles of 20 seconds at 94 ºC, 30 seconds at 58 ºC and 1 min and 30 s at 72 ºC, and finally 10 min at 72 ºC. The PCR products were visualized using horizontal gel electrophoresis using a GoldView stained agarose gel (1%) followed by enzymatic cleaning of the positive samples using 1 µl of ExoSAP-IT reagent and 2,5 µl of PCR product under the following conditions: 15 min at 37 ºC and 15 min at 80 ºC. Identical primers as in the amplification reactions were used for sequencing with a Big Dye Chemistry Cycle Sequencing Kit 3.1, following the manufacturer’s recommendations. Fragments were cleaned using the BigDye XTerminator® Purification Kit and visualized on an ABI3130 capillary sequencer. Sequences were visually inspected and corrected using MEGA v7 (Kumar et al. 2016) and aligned using MUSCLE (Edgar 2004) under default distance measures as implemented in MEGA v7. Previously published sequences of *Kapentagyrus limnotrissae* (GenBank accession numbers MH071808 and MH071782) were added to the dataset. Sequences obtained in the present study were deposited in the NCBI GenBank under the accession numbers MH620705 and MH623076.

Results

Morphological and molecular characterisation

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

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

Taxonomic account

New record

Family: Dactylogyridae Yamaguti, 1963

Genus: *Kapentagyrus* Kmentová, Gelnar & Vanhove, 2018

Species: *K. limnotrissae*

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

Type-locality: Lake Tanganyika, Tanzania

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

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

Site of infection: Gills.

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

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

Morphometrics

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

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

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

Discussion

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

Kapentagyrus limnotrissae **in Lake Kariba**

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

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

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

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

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

Co-introduced flatworm parasites have been observed to cause population decline (Tanum 1983; Johnsen and Jensen 1988; Britton et al. 2011) and extinction of native fish fauna (Zholdasova 1997). However, considering the generally high host-specificity of dactylogyrid monogeneans and the fact that *K. limnotrissae* is strictly host-specific in Lake Tanganyika, the potential for spill-over to native fish seems low. Co-introduction of monogenean species without any known impact on the native fauna has been documented (Truter et al. 2017). However, monitoring the potential presence of non-native monogenean species on the local fish fauna in Lake Kariba is recommended as parasite spill-over does not always occur in a predictable manner (Jiménez-García et al. 2001).

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

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

Release from monogenean infection in Lake Kivu

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

Conclusion

The main question of our study was whether we could use parasites to investigate host introductions. While parasite co-introduction with the fishery target *L. miodon* to Lake Kariba was documented, a release of monogenean infection into Lake Kivu is suggested. Two possible scenarios to explain the current situation in Lake Kivu were proposed: monogenean parasites not having been translocated, as the founder population consisted only of fry, would support previous studies highlighting introduction conditions as crucial for parasite survival. Therefore, the absence of monogenean parasites in some introduced areas could be the result of circumstances surrounding host translocation and host life stage. This should be considered as a parameter when considering fish introductions. The other possibility is that the parasites were unsuccessfully established because of differences in biotic and abiotic conditions in their native area compared to Lake Kivu. Experimental studies are needed to discern between these two scenarios. In contrast, the increased prevalence of *K. limnotrissae* in Lake Kariba compared to Lake Tanganyika was reported. This pattern was suggested to be due to different environmental conditions such as different predation pressure, differences in host life history or in abiotic conditions, as these factors are known to influence monogenean population dynamics (Buchmann 1988; Šimková et al. 2001; Marchiori et al. 2015). Despite only slight phenotypic differences in morphology of *K. limnotrissae* between populations from its native range and from Lake Kariba, our results revealed a greater similarity to the specimens from the southern part of Lake Tanganyika using morphometric results. This finding corresponds with historical reports about the introduction events. Therefore, the potential of *K. limnotrissae* as a tag for its host's origins was supported and should be further scrutinised by detailed genetic characterisation, including fast evolving markers.

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Figure captions

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

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

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

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