Weak population structure and expansive demographic history of the monogenean parasite *Kapentagyrus* spp. infecting clupeid fishes of Lake Tanganyika

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Abstract

Lake Tanganyika is the oldest and deepest African Great Lake and harbours one of the most diverse fish assemblages on earth. Two clupeid fishes, *Limnothrissa miodon* and *Stolothrissa tanganicae*, constitute a major part of the total fish catch, making them indispensable for local food security. Parasites have been proposed as indicators of stock structure in highly mobile pelagic hosts. We examined the monogeneans *Kapentagyrus limnotrissae* and *K. tanganicanus* (Dactylogyridae) infecting these clupeids to explore the parasites' lake-wide population structure and patterns of demographic history.

Samples were collected at seven sites distributed across three subbasins of the lake. Intraspecific morphological variation of the monogeneans (N = 380) was analysed using morphometrics and geomorphometrics of sclerotised structures. Genetic population structure of both parasite species (N = 246) was assessed based on a 415 bp fragment of the mitochondrial COI gene.

Overall, we observed a lack of clear geographical morphological differentiation in both parasites along a north-south axis. This lack of geographical population structure was also reflected by a large proportion of shared haplotypes, and a pattern of seemingly unrestricted gene flow between populations. Significant morphological and genetic differentiation between some populations might reflect temporal differentiation rather than pure geographical isolation. Overall, the shallow population structure of both species of *Kapentagyrus* reflects the near-panmictic population structure of both host species reported in previous studies. Morphological differences related to host species identity of *K. tanganicanus* were consistent with incipient speciation observed at the genetic level. Both parasite species experienced a recent demographic expansion, which might be linked to paleohydrological events. Finally, hybridisation between species of *Kapentagyrus* was found, representing the first case in dactylogyrid monogeneans.

Keywords: Clupeidae, Dactylogyridae, Fisheries target species, *Kapentagyrus limnotrissae,* *Kapentagyrus tanganicanus*, Phenotypic plasticity, Population genetics

1. Introduction

The pelagic realm of the African Great Lakes harbours a lower species diversity than the littoral habitat. This might be attributed to the lower number of niches and a lack of barriers to gene flow (Kirchberger et al., 2012; Shaw et al., 2000). Lake Tanganyika's pelagic zone is dominated by two clupeid species (*Limnothrissa miodon* (Boulenger, 1906) and *Stolothrissa tanganicae* Regan, 1917) and their latid fish predators. The two clupeids make up 65% (in mass) of the total catch in Lake Tanganyika, making them a key component of the local fishery andan important factor for the food security in the countries bordering the lake (Mölsä et al., 1999). Clupeids play an important role in the food chain, because they are a link between the plankton and the piscivores (Coulter, 1991). Lake Tanganyika clupeids are parasitized by two species of *Kapentagyrus* Kmentová, Gelnar & Vanhove, 2018 (Monogenea, Dactylogyridae), *Kapentagyrus limnotrissae* (Paperna, 1973) and *Kapentagyrus tanganicanus* Kmentová, Gelnar & Vanhove, 2018. Both parasite species have a lake-wide distribution throughout the year (Kmentová et al., 2018). While *K. limnotrissae* is host specific to *L. miodon*, *K. tanganicanus* has a more generalist lifestyle and infects both *L. miodon* and *S. tanganicae*. In *K. tanganicanus*, two distinct morphotypes related to sardine species identity have been observed (Kmentová et al., 2018).

Clupeids in Lake Tanganyika are short-lived species with a lifespan of usually one year and maximally three years. Other biological characteristics include schooling behaviour and a diurnal vertical migration that follows that of zooplankton (Coulter, 1991; Mulimbwa and Shirakihara, 1994). Migration and population connectivity of clupeids in the lake are poorly understood, but are thought to be linked to seasonal changes in the plankton distribution (Kurki et al., 1999; Plisnier et al., 2009). Generally, the delineation of pelagic fish stocks is crucial for fisheries management (Emmett et al., 2005). Classical methods to track the movement of fish populations, such as data storage tags (DST) and passive physical tags are no option for clupeids because of

their fragility (James et al., 1988). Hence, a combination of biological markers such as morphometry, parasites, otolith elemental profiles, and molecular markers appears to be a more promising approach (Svedäng et al., 2010). Lake-wide genome screening of both clupeids in Lake Tanganyika using SNPs did not identify a clear population structure, suggesting near-panmictic populations (De Keyzer et al., 2019; Junker et al., 2019). However, differences in chemical composition of otoliths in both species (Sako et al., 2005) and a pattern of isolation by distance along a north-south gradient in *S. tanganicae* (De Keyzer et al., 2019) pointed to restricted long-distance migration.

A combination of host- and parasite genetics has been proposed as an integrative approach to reconstruct host population structure (Catalano et al., 2014) or stock structure over small geographical and temporal scales (Baldwin et al., 2012). Monogenean parasites are excellent targets for such research for several reasons. Foremost, the direct life cycle and often high host specificity of monogeneans prevents their life history from being influenced by any other than the targeted host taxon (Catalano et al., 2014; Pariselle et al., 2011). Secondly, due to their short generation time, monogeneans may accumulate genetic changes more rapidly than their hosts (Poulin, 2007). Thirdly, their high mutation rate in comparison to that of their hosts may reflect historical events that are too recent to be inferred from host genetics (Nieberding et al., 2004; Nieberding and Olivieri, 2007), and therefore parasites have been proposed to act as a "magnifying glass" for their hosts. To date, few studies have used monogeneans in such an approach. E.g., Pettersen et al. (2015) used a portion of the cytochrome c oxidase of *Gyrodactylus thymalli* Žitňan, 1960 combined with dehydrogenase subunit 5 to indirectly infer barriers to gene flow in grayling (*Thymallus thymallus* L.). Monogenean genetics was also used to track the historical distribution of clariid catfishes in Africa (Barson et al., 2010) as well as to reconstruct introduction pathways in *Perccottus glenii* Dybowski, 1877 (Ondračková et al., 2012).

Several steps have to be considered before using parasites as tags for host population structure, including parasite species identification, the availability of more than one genetic marker to verify cryptic species, and temporal stability in the presence of the parasite species across the host's geographic range (Mattiucci et al., 2004; Vilas et al., 2005). All above-mentioned criteria are fulfilled in the system studied here. Since the morphology of their sclerotised structures was shown to vary along a north-south gradient (Kmentová et al., 2018), the species of *Kapentagyrus* are proposed as candidates for unravelling the clupeids' population structure in Lake Tanganyika. Moreover, several periods of draught in the past led to low lake levels and at times even separation into up to four paleolakes corresponding with the current subbasins (Danley et al., 2012; Sturmbauer et al., 2017). Such events repeatedly caused periods of population separation followed by periods of secondary admixture across the north-south gradient. These left their signature in the genetics of various animal taxa (Sturmbauer et al., 2001) and influenced their current population structure (Nevado et al., 2013; Sefc et al., 2017; Sturmbauer et al., 2017), or their demographic history, even in the barrier-free pelagic realm (Koblmüller et al., 2019). We assume that the demographic history of *Kapentagyrus* spp. is connected with past population trajectories of clupeid hosts, because historical lake level fluctuations influenced the demographic history of cichlid fishes and their respective monogenean species in a similar way (Kmentová et al., 2016; Koblmüller et al., 2019).

In this study, we test two species of *Kapentagyrus* as potential markers for spatio-temporal population structure of both clupeid species. We hypothesise that there is more differentiation among parasite populations than among host populations. We also compare the degree of morphological and genetic differentiation among a host-specific versus a more generalist species of *Kapentagyrus*. Finally, we test the relation between the hydrological history of Lake Tanganyika and the recent demographic history of *Kapentagyrus* spp.

2. Material and methods

2.1. Sampling design

In total, 380 monogenean individuals collected from 497 host specimens were morphologically analysed in this study. We used samples listed in Kmentová et al. (2018) as well as new specimens collected in April 2018 (see Table 1). Monogeneans were collected from ethanol-preserved fish samples collected along the lake's shoreline within two days in April 2018 (off Bujumbura, Kalemie, Mpulungu and Uvira). As clupeids are highly mobile pelagic fish (De Keyzer et al., 2019; Marshall, 1993; Mulimbwa and Mannini, 1993), this short time window enabled us to analyse the spatial population structure of the parasites without the potential effect of school migration. Additionally, fresh specimens collected within two days in August 2016 (off Kalemie and Uvira) and within two weeks in Mpulungu 2018 were included in this study to analyse spatio-temporal patterns in the parasites' morphology. We also included fresh specimens collected at Baraka in 2017, Mpulungu in 2016, Mvugo in 2016 and Mvuna Island in 2015 to increase spatial resolution of population genetic analyses. In total, 246 individuals of *Kapentagyrus* spp. were characterised genetically (see Table 1).

All host specimens were either bought at fish markets in the above-mentioned cities or caught with gills nets during experimental fishing. Fishes were identified to species level *in situ*. Voucher specimens of the two clupeid species are part of the ichthyology collection of the Royal Museum for Central Africa in Tervuren (RMCA 2016.20). Monogenean individuals collected from fresh fish specimens were placed on a slide in a drop of glycerine ammonium picrate solution (GAP) in 1:1 ratio. Ethanol-preserved samples were cleaned of host tissue in a drop of water followed by adding Hoyer's solution. In both cases, the individuals were fixed under a cover slip. All collected monogenean species were identified as either *K. limnotrissae* or *K. tanganicanus*. Infection parameters are listed in Table 1. Voucher specimens of *Kapentagyrus* spp. are deposited in the

168 collection of the Research Group Zoology: Biodiversity and Toxicology at Hasselt University in
169 Diepenbeek, Belgium (HU) (see Table 1 for accession numbers).

170 2.2. Morphometrics and geomorphometrics

171 Morphological variation on a lake wide geographical scale was inferred via both morphometric
172 and geomorphometric approaches. Haptoral and male copulatory hardparts of the two species of
173 *Kapentagyrus* were measured and photographed using an Olympus BX51 microscope with
174 incorporated phase contrast at a magnification of 1000x (objective x100 immersion, ocular x10)
175 with *MicroImage* v3.1. In total, we obtained 23 different morphometric parameters following the
176 terminology of Řehulková et al. (2013).

177 Geomorphometric data were obtained by digitising the shape of the dorsal and ventral anchor,
178 respectively. For this we used *tps Dig* v2.30 from the thin-plate spline (TPS) packages (Rohlf, 2006).
179 We chose the anchors for geomorphometric analyses as their shape had been successfully used in
180 intraspecific studies on members of *Ligophorus* Euzet & Suriano, 1977 (Monogenea,
181 Dactylogyridae) (Rodríguez-González et al., 2015). The shape of other monogeneans' sclerotised
182 structures, such as the shape of bars and marginal hooks, was shown to be highly related to the
183 method of sample preparation (Vignon et al., 2011). Eight fixed landmarks were selected on each
184 of the anchors. Additionally, semi-landmarks were placed in equal intervals on each anchor,
185 resulting in 98 of them in the case of *K. limnotrissae* and 102 in *K. tanganicanus* (see Fig S1).

186 2.3. DNA extraction and genetic characterisation

187 Monogeneans were stored in 99% ethanol prior to DNA isolation. Subsequently, ethanol was
188 evaporated using a vacuum centrifuge and lysis buffer was poured onto the specimens. Whole
189 genomic DNA was extracted using either the Qiagen DNeasy Blood & Tissue Kit or Nucleospin

Tissue Genomic DNA kit following the manufacturer's instructions. The extracted DNA was eluted in a volume of 50 µl.

Part of the monogenean mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene was amplified using a nested PCR reaction, in view of the low content of genomic DNA extracted from in most cases 1/3 of the worm. The first PCR reaction was performed with ASmit1 (5'-TTTTTTGGGCATCCTGAGGTTTAT-3') (Littlewood et al., 1997) and Schisto3 (5'-TAATGCATMGGAAAAAAACA-3') (Lockyer et al., 2003) primers in 24 µl of PCR mix (one unit of *Taq* Polymerase, 1X buffer containing 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.8 mM of each primer) for a total reaction volume of 25 µl. It was carried out under the following conditions: initial denaturation at 95°C for 5 min, then 40 cycles of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C, and final elongation for 7 min at 72°C. The nested PCR with ASmit1 and ASmit2 (5'-TAAAGAAAGAACATAATGAAAATG-3') (Littlewood et al., 1997) primers followed the same protocol as the first one with 1:100 dilution of template DNA. The final PCR products were enzymatically purified 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. The same primers as in the amplification reactions were used for sequencing with a BigDye Terminator® Cycle Sequencing Kit v3.1 (ThermoFisher Scientific), following the manufacturer's recommendations. The fragments were cleaned up using the BigDye XTerminator® Purification Kit (ThermoFisher Scientific) and visualized on an ABI 3130 capillary sequencer (Applied Biosystems).

For *S. tanganicae*, sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene were obtained from De Keyzer et al. (2019) (GenBank accession numbers MH290064-159). For *L. miodon*, DNA was extracted from finclips using the NucleoSpin Tissue kit (Macherey-Nagel GmBH) according to the manufacturer's instructions. Subsequently, the COI gene was amplified using the universal primer combination HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') (Folmer et al., 1994). PCR reaction was performed in 24

µl of PCR mix (12.5µl MyTaq HS mix (2x) (Bioline, London, UK), 10.5µl H2O and 1µl primer mix (20µM of each primer) to which 1 µl of purified DNA was added for a total reaction volume of 25 µl (Handy et al., 2011). It was carried out under the following conditions: initial denaturation at 94°C for 3 min, then 35 cycles of 45 sec at 94°C, 40 sec min at 52°C and 90 sec at 72°C, and final elongation for 10 min at 72°C. PCR products were purified using CleanPCR beads (CleanNA, GC Biotech). The same primers as in the amplification reactions were used for sequencing with a BigDye Terminator® Cycle Sequencing Kit v3.1 (ThermoFisher Scientific), following the manufacturer's recommendations. The fragments were purified CleanDTR beads (CleanNA, GC Biotech) and visualized on an ABI 3500XL capillary sequencer (Applied Biosystems).

DNA 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. COI sequences of *Kapentagyrus* spp. are deposited in NCBI GenBank under the accession numbers MK598125-323. Corresponding nuclear data generated by Kmentová et al. (2018) are available on NCBI GenBank under the accession numbers MH071782 and MH071808 (28S, 18S and ITS-1 region of *K. limnotrissae*), MH071783 and MH071807 (28S, 18S and ITS-1 region of *K. tanganicanus* collected from *L. miodon*), MK522517-520 (28S, 18S and ITS-1 region of *K. tanganicanus* collected from *Stolothrissa tanganicae*), MK521656-MK521659 (28S rDNA portion of *K. limnotrissae* individuals identified as hybrids) and MK521661-MK521664 (18S and ITS-1 rDNA portion of *K. limnotrissae* individuals identified as hybrids). COI sequences of *L. miodon* are deposited in NCBI GenBank under the accession numbers MT040511-78. Individuals of both host species originate from different localities covering all three major subbasins of Lake Tanganyika (see Table S1).

2.4. Data analysis

2.4.1. Morphological differentiation

To avoid any possible influence of ethanol fixation on the size and shape of sclerotised structures, the samples were subdivided into spatial (ethanol-preserved) and spatio-temporal (fresh) data sets. To evaluate intraspecific and intrahost variation, data sets were further subdivided into six different sample sets according to parasite species and host species. These sample sets were always analysed separately by a) morphometric analyses of haptoral structures, and geomorphometric analyses of b) dorsal and c) ventral anchors. Samples in all sample sets and subsequent analyses were grouped by sampling site to check for possible geographical structure in both species of *Kapentagyrus.* As preliminary analyses indicated a significant influence of host size on morphological characters, individuals of *K. tanganicanus* ex *L. miodon* from Kalemie 2018 were analysed as two groups using 12 cm of host standard length (SL) as a cut off value (referred to as Kalemie 2018 Big and Kalemie 2018 Small, respectively). As the same pattern was discovered with the fresh samples from Mpulungu, a threshold in SL of host specimens was set to 10 cm (referred to as Mpulungu 2018 Big and Mpulungu 2018 Small, respectively).

Morphometrics - Principal component analysis (PCA) of haptoral morphometric parameters standardised to unit scale was performed in the R package *adegenet* (Jombart, 2008). Missing data were replaced by the average value for each morphological character. To increase the resolution of the resulting pattern, morphological characters with more than 50% missing data were excluded prior to the analysis. Then, linear or generalised linear models were calculated in the R package *stats* (R Core Team, 2013) to evaluate the effect of sampling site, host size and their interaction on each of the morphological characters followed by F-statistic and Chi Square statistics, respectively. In case of an overall significant effect of sampling site, *post hoc* Welsh`s t test and Tukey test, respectively, were performed to assess pairwise significance between sampling sites. Sampling sites with insufficient number of specimens (< 10) were excluded from the analyses.

Geomorphometrics - Configurations of fixed landmarks were superimposed using Generalized Full Procrustes Analyses (Cox and Cox, 1989; Zelditch et al., 2012), under the Least Squares criterion to minimize bending energy with respect to a mean reference shape. Canonical variate analyses (CVA) (Klingenberg and Monteiro, 2005) and PCA using only the fixed landmarks were performed in *MorphoJ* v2.0 (Klingenberg, 2011). A permutation test with 10,000 iterations was used to statistically validate pairwise differences between the pre-defined groups. Additionally, the overall shape of both anchors, captured using fixed landmarks and semi-landmarks, was analysed using *tpsRelw* v1.49. A Relative Warp Analysis (RWA) (Rohlf, 1993) was performed with the Procrustes coordinates. The scaling option was set to $\alpha = 0$ to give all landmarks equal weight. Sampling sites with insufficient number of specimens (<6 as in the case of *K. tanganicanus* ex *L. miodon* from Bujumbura 2018 and *K. tanganicanus* ex *S. tanganicae* from Bujumbura 2018 and Mpulungu 2018 in spatial sample sets) were excluded from the analyses.

Relationships between the individual scores inferred with PCA and RWA analyses, respectively, and the host size were checked via linear regression analyses in the R package *stats* (R Core Team, 2013). This was done for each sample set and within the respective groups. All sample sets were visually inspected for outliers, which were excluded from the analyses. Normality of the data was checked by Shapiro-Wilks tests in the R package *onewaytests* (Dag et al., 2018). The homogeneity of variance among groups within each sample set was assessed by Levene's tests in the R package *car* (Fox and Weisberg, 2011). Biplots of PC and RW scores were visualised with the packages *ggplot2* (Wickham, 2009) and *factoextra* (Kassambara and Mundt, 2017).

2.4.2. Genetic structure

The genetic diversity of the two monogenean species and the two host species was studied based on 415 bp (*Kapentagyrus* spp.), 646 bp (*L. miodon*) and 643 bp (*S. tanganicae*), respectively, of the COI gene. Genetic diversity was assessed as the number of haplotypes and polymorphic sites,

haplotype diversity and nucleotide diversity, all calculated using *Arlequin* v3.5 (Excoffier and Lischer, 2010).

The genealogy of the COI haplotypes for the parasites were inferred by means of a Median Joining network in *PopART* v1.71 (Leigh and Bryant, 2015). Differentiation among pre-defined populations was estimated by F_{ST} in *Arlequin* v3.5 (Excoffier and Lischer, 2010): for *K. tanganicanus* collected from *L. miodon* and *S. tanganicae*, respectively, in Uvira 2016, as well as for populations of *K. tanganicanus* ex *L. miodon* with at least 17 individuals available. Analysis of molecular variance (AMOVA) based on F-statistics was used to test for significant population structure of *K. tanganicanus* at the level of subbasins within Lake Tanganyika. Sample size for *K. limnotrissae* was generally too low to allow for any meaningful population genetic analyses.

2.4.3. Demographic history

To test for signals of past population expansion in both species of monogeneans and their host species, two different neutrality test statistics, Tajima's D (Tajima, 1989) and Fu's F_s (Fu, 1997), were calculated in *Arlequin* v3.5 (Excoffier and Lischer, 2010).

The demographic history of *Kapentagyrus* spp. was further assessed by mismatch distribution analyses in *Arlequin* v3.5 (Excoffier and Lischer, 2010). The sum of square deviations (SSD) and raggedness index (rg) were used to assess the fit of the observed mismatch distributions to the expectations based on estimates of the growth parameter. Past population size trajectories of monogenean species were further investigated with a Bayesian skyline plot (Drummond et al., 2005), as implemented in *BEAST* v1.8.2. (Suchard et al., 2018). The substitution rate was set to 10% per million years, which is lower than the rates previously used for viviparous gyrodactylid monogenean species characterised by asexual multiplication (Meinilä et al., 2004), and should take into account the assumed comparatively longer generation time and lower reproductive capacity of *Kapentagyrus* as oviparous dactylogyrid monogeneans (Tinsley, 2004). Two

312 independent MCMC runs of 300 million generations each and a sampling frequency of 30,000
313 were conducted, with a burn-in of the first 10% of sampled generations. The number of grouped
314 intervals was set to 5. Verification of effective sample sizes (ESS > 200 for all parameters), tracing
315 MCMC runs and visualisation of past population size changes were done in *Tracer* v1.7 (Rambaut
316 et al., 2018).

317 **3. Results**

318 3.1. Morphological variation

319 3.1.1. *Kapentagyrus limnotrissae* ex *Limnothrissa miodon*

320 Overall, the intraspecific morphological variation of *K. limnotrissae* was primarily affected by
321 several parameters of the dorsal anchor, ventral anchor and branch length of the ventral bar (Fig.
322 1B, Table S2). An overview of measurements from haptoral as well as from the male copulatory
323 organ region is listed in Table S4.

324 *Haptoral structures* - PCA did not reveal any clear geographical separation based on haptoral
325 morphometric parameters in *K. limnotrissae* along any of the PC axes in any of the two sample sets
326 (Fig. 2A&B). However, differentiation was visible between the specimens from Mpulungu 2018
327 and Uvira 2016 along the first and the second axis (Fig. 2B). In half of the comparisons between
328 sampling sites tested, at least one of the morphological parameters was found to differ
329 significantly. The length of the outer root of the ventral anchor was the only morphological
330 character that differed between sampling sites in both sample sets (see Fig. 1 and Table S2).

331 *Dorsal and ventral anchor* - In *K. limnotrissae*, the PCA biplot based on fixed landmarks revealed a
332 clearer differentiation between Uvira and Bujumbura 2018 for the dorsal than for the ventral
333 anchor (Fig. 2C and Fig. S2A). This differentiation was further reflected in the CVA results (Table
334 S3). The shape of the ventral anchor was significantly different between the specimens from Uvira

and Kalemie 2016, and between Kalemie 2016 and Mpulungu 2018 (detailed results presented in Table S2). The results of RWA (including sliding landmarks) confirmed the pattern obtained via PCA (Fig. S2).

Effect of host size - No effect of host size on the position of specimens in neither of the presented biplots was detected (not shown). Linear models for the spatial sample set revealed that the total length of the dorsal anchor decreased with host size ($F_{1,58} = 5.32$, $P < 0.05$). This was also the case for the point length of the dorsal anchor ($X^2_{2,57} = 9.98$, $P = 0.002$) in the spatio-temporal sample set. Linear models revealed an increasing effect of host size on the branch length of the dorsal bar ($F_{2,60} = 9.17$, $P < 0.05$) and the inner root length of the ventral anchor ($X^2_{2,61} = 5.34$, $P = 0.02$) in the spatio-temporal sample set.

3.1.2. *Kapentagyrus tanganicanus* ex *Limnothrissa miodon*

Overall, the intraspecific morphological variation in *K. tanganicanus* ex *L. miodon* was affected by several parameters of both the dorsal and the ventral anchor, the dorsal and the ventral bar as well as some of the pairs of marginal hooks (Fig. 1C, Table S2). An overview of measurements from the haptoral as well as the male copulatory organ region are given in Table S5.

Haptoral structures - The first PC axis of haptoral morphometric parameters revealed that specimens from Mpulungu 2018 were intermediate between those from Kalemie 2018 and Uvira 2018 (Fig 3A). The separation was further reflected in the number of significantly different characters between the sampling sites (see Table S2). A separation was also visible along the first PC axis of haptoral morphometric parameters between specimens collected in Mpulungu 2018 from those from Kalemie 2016 and Uvira 2016 (Fig. 3B). Only two morphological parameters differed significantly between Mpulungu 2018 and Uvira 2016 (see Table S2). A single one differed significantly between the two host-size categories from Kalemie 2018 and Mpulungu 2018.

Dorsal and ventral anchor - In *K. tanganicanus* ex *L. miodon*, the PCAs of the shape of both anchors, based on a fixed landmark geomorphometric approach, reflected the gradient visible in the biplot of the haptoral morphometric approach in the both spatial and the spatio-temporal sample sets (Fig. 3C-F). This differentiation was further supported by the CVA results. Here, a significant difference was observed in at least one of the anchors in comparisons between each of the sampling sites. The only exception was the comparison between Mpulungu 2018 and Uvira 2018, where no difference was found (see also Table S3). Moreover, the shape of the ventral anchor and of both anchors differed significantly between the two host-size categories from Kalemie 2018 and Mpulungu 2018, respectively. The results of RWA (including sliding landmarks) confirmed the pattern obtained via PCA (Fig. S3).

Effect of host size - A significant effect of host size was detected only on the individual RW scores of the first axis for the ventral anchor of the spatio-temporal sample set ($F_{1,64} = 7.08$, $P = 0.010$). Linear models revealed an increasing effect of host size on the total length of the ventral anchor ($F_{3,77} = 34.31$, $P < 0.0010$) and the length to notch of the ventral anchor ($x^2_{3,77} = 10.81$, $P = 0.001$) in the case of spatio-temporal sample set.

3.1.3 *Kapentagyrus tanganicanus* ex *Stolothrissa tanganicae*

Overall, the intraspecific morphological variation of *K. tanganicanus* ex *S. tanganicae* was affected by the length to notch of the dorsal anchor and the second pair of marginal hooks (Fig. 1D, Table S2). An overview of measurements from the haptoral as well as from the male copulatory organ region is listed in Table S6.

Haptoral structures - In total, two sample sets, both containing specimens of *K. tanganicanus* ex *S. tanganicae* from four and two different groups, respectively, were analysed. Clear differentiation was visible in the second PC axis of haptoral morphometric parameters between the specimens from Kalemie 2018 and Uvira 2018 (Fig. 4A). A single morphological character, the size of the first

pair of marginal hooks, differed significantly between sampling sites. Additionally, a separation in the spatio-temporal sample set was found between specimens from Mpulungu 2018 and Uvira 2016 along the first and second PC axes (Fig. 4B). Similar to the previous sample set, only one morphological character, the length to the notch of the dorsal anchor, differed between sampling sites.

Dorsal and ventral anchor - The position of specimens along the first PC axis of the anchor shape based on a fixed landmark geomorphometric approach mirrored the pattern observed in haptoral morphometric characters in both sample sets (Fig. 4C-F). In the spatial sample set, the shape of ventral anchor was found to be related to the sampling site in the comparison between Kalemie 2018 and Uvira 2018. In the spatio-temporal sample set, the shape of botch anchors was different between Mpulungu 2018 and Uvira 2016 (Table S3). The results of RWA (including sliding landmarks) followed the pattern (Fig. S4).

Effect of host size - No effect of the host size on the position of specimens was detected in neither of the presented biplots nor in the linear models for the morphological parameters.

In total, just a single morphological character, the length to notch of the dorsal anchor, differed between the sampling sites in all sample sets. Two additional characters, the branch length of the ventral bar and the total length of the ventral anchor, differed between the sampling sites in specimens of *Kapentagyrus* spp. collected from *L. miodon*. Finally, the length of the first pair of marginal hooks differed between the sampling sites for the specimens of *K. tanganicanus* collected from different host species (see 3.1.2.). For further details see Table S2.

3.2. Genetic diversity

The number of polymorphic sites in COI found per monogenean species was 18 (N = 51) for *K. limnotrissae* and 68 (N = 140) for *K. tanganicanus*. Both clupeids had a similar number of

polymorphic sites, 45 (N = 69) in *L. miodon* and 48 (N = 96) in *S. tanganicae,* in the COI gene. Similar levels of nucleotide and haplotype diversity were observed between the two parasite species and one of the host species: *S. tanganicae*. Lower genetic diversity was observed for *K. tanganicanus* when only individuals collected from *S. tanganicae* were included. The other host species, *L. miodon*, had higher genetic diversity than both species of *Kapentagyrus* (Table 2).

3.3. Parasite population genetics

First, there was no evident clustering of *K. tanganicanus* according to host species (see Fig. 5A). However, significant F_{ST} value were found between *K. tanganicanus* infecting different host species collected in Uvira 2016 (F_{ST} = 0.0668; P = 0.0112). Haplotype networks indicated neither geographic, nor school-related structure in either of the monogenean species. All networks showed a star-like topology with a single dominant haplotype (see Fig. 5B-D). Satellite haplotypes were mostly separated by a single mutation from the central haplotypes. Significant F_{ST} values were also recorded in *K. tanganicanus* ex *L. miodon* between several sampling sites on a temporal scale (see enclosed table in Fig. 5). AMOVA calculated for *K. tanganicanus* ex *L. miodon* showed that most of the variation was present within populations (96.85%) in comparison to 1.67% among populations within subbasins and 1.47% among subbasins.

3.4. Demographic history

Signatures of population expansion were detected in both monogenean species and their host species. Recent population growth was suggested by significantly negative values of Fu's F_s in *K. limnotrissae* (-20.98; P < 0.001), in *K. tanganicanus* (-27.90; P < 0.001), in *L. miodon* (24.09; P < 0.001) and in *S. tanganicae* (-61.64; P < 0.001) as well as Tajima's D in *K. limnotrissae* (-2.48; P < 0.001), in *K. tanganicanus* (-2.41; P < 0.001) and in *S. tanganicae* (-2.41; P < 0.001). In *L. miodon*, the value of Tajima's D was negative (-1.31) but not significant (P = 0.06).

The unimodal mismatch distribution was well supported by a non-significant SSD and rg, indicating recent population expansion in both *Kapentagyrus* species (see Fig. 6A, B). Mismatch analyses dated the onset of population expansion to 11.8 KYA in *K. limnotrissae* (95% CI: 6.5–16.8 KYA) and to 17.6 KYA in *K. tanganicanus* (95% CI: 3.3–30.1 KYA).

Based on Bayesian Skyline Plot analysis, the start of population growth for *K. tanganicanus* was estimated around 12 KYA (see Fig. 6D) and the time to the most recent common ancestor (TMRCA) around 70.9 KYA (95% HDP: 15.6–143.1 KYA). Due to the insufficient number of haplotypes, BSP could not track past changes of effective population size back to more than 7 KYA in the case of *K. limnotrissae* (see Fig. 6C). No sign of population growth was observed and the TMRCA was estimated at 14.4 KYA (95% HDP: 6.5–24.1 KYA).

3.5. Nuclear-mitochondrial discordance

Based on the comparison of rDNA markers published in Kmentová et al. (2018) and the obtained COI sequences of the same specimens, nuclear–mitochondrial discordance was documented for four individuals of *Kapentagyrus* collected from *L. miodon* (see Fig. 5A). For two of these four cases, morphological vouchers are available (specimens on slides deposited under X.4.04 and XI.1.20 in HU). Their morphology and haplotype of 28S and/or 18S and ITS-1 rDNA (MK521656–59 and MK521661–64) are characteristic for *K. limnotrissae,* whereas the mitochondrial COI haplotype is that of *K. tanganicanus.*

Discussion

The geographic and temporal population structure, and demographic history of two monogenean species of *Kapentagyrus* infecting clupeid hosts in Lake Tanganyika were investigated. Although, morphological comparison of the parasites' sclerotised structures did not show clear patterns of differentiation along a north-south axis, significant differences between some of them indicate

spatio-temporal differentiation. Moreover, molecular analyses suggest a weak geographic population structure with some temporal differentiation. Finally, both species of *Kapentagyrus* showed a similar pattern of recent population expansion, presumably correlated with Pleistocene climate change and subsequent lake-level fluctuations.

Monogeneans as tags for the geographical population structure of clupeids

The pelagic environment, promoting dispersal, and the large effective population size constrain genetic drift and differentiation in pelagic fishes (Gonzalez and Zardoya, 2007; Koblmüller et al., 2019; Martínez et al., 2006). Moreover, the patchy production of phytoplankton in Lake Tanganyika may promote seasonal migration following the prey and population mixing in pelagic fishes (Phiri and Shirakihara, 1999; Plisnier et al., 2009; van Zwieten et al., 2002). On the other hand, population differentiation in the pelagic realm might be facilitated by the presence of physical barriers such as currents (Podsetchine and Huttula, 2000) or geographical distance (Gonzalez and Zardoya, 2007). Although the migration patterns of clupeids have not been resolved yet, some isolation by distance along a north-south gradient was detected, suggesting limits to lake-wide migration in *S. tanganicae* (De Keyzer et al., 2019). This was also seen in the chemical composition of otoliths in both species (Sako et al., 2005).

Morphological variation - Based on our comprehensive study, morphometrics of monogenean haptoral and male copulatory organ structures showed in some cases significant intraspecific shape variation with respect to sampling site. However, none of the approaches used identified a morphological character that was unambiguously specific to a particular sampling site in neither of the various sample sets. Interestingly, even though the shape of both anchors mirrored the pattern of the overall haptoral morphology, the detailed morphology of neither of these structures provided sufficient resolution to resolve the geographic origin of a monogenean individual. Significantly different morphological characters between the sampling sites in 2016

were not found between the specimens from the same localities in 2018. This suggests dependency of phenotypic differentiation on environmental conditions rather than fidelity to a geographic location in *Kapentagyrus* spp. and consequently of their clupeid host species. Temperature (Brazenor et al., 2018; Dávidová et al., 2005; Ergens and Gelnar, 1985; Mo, 1993), pollutants (Beaumont, 1997) or other environmental factors (Cable and Harris, 2002; Olstad et al., 2009) influence the morphology of monogeneans. The morphological differences of *Kapentagyrus* spp. observed between some of the sampling sites might hence be attributed to environmental factors directly influencing the parasites' morphology or indirectly through host morphology. These may induce geographical patterns via restricted host - parasite migration, or via similar environmental conditions in geographically isolated locations. This might explain the clustering of geographically isolated specimens of *K. tanganicanus* from Mpulungu and Kalemie (Fig. 3A). Interestingly, spatio-temporal variation in sample sets of fresh supported the hypothesis of environmentally-dependent variation, specific to site and time. In Lake Tanganyika, geographical and seasonal variation in thermal stratification, the level of oxygen (Hecky et al., 1978; Langenberg et al., 2002), pH (Plisnier et al., 1999), chemical (Degens et al., 1971), phytoplankton composition (Descy et al., 2005) and algal succession (Agawin et al., 2000) have been reported. They are driven mostly by wind conditions (Hecky et al., 1978; Langenberg et al., 2003). However, no experimental data for representatives of *Kapentagyrus* are currently available to attribute observed morphological differentiation to specific environmental factor.

Different host, different story - Interestingly, the number of morphometric characters related to sampling site differed between the sample sets. While a maximum of two characters was informative in the case of *K. limnotrissae*, a species specific to *L. miodon*, this number was considerably higher in *K. tanganicanus* collected from the same host species. Clupeids form size-dependent schools (Misund, 1993). Host-size dependent intensity of infection between

Kapentagyrus spp. collected from *L. miodon* was observed (*K. limnotrissae* being more prevalent on smaller *L. miodon* and vice versa, own unpublished results). Therefore, observed discrepancy in differentiation between species of *Kapentagyrus* might be explained by a difference in migration capacity between fish schools (Nøttestad et al., 1999). Moreover, the number of significantly different morphological characters between the groups of *K. tanganicanus* ex *S. tanganicae* was lower compared to the individuals collected from *L. miodon*. Such a pattern might be related to the difference in ecology between the host species as *S. tanganicae* displays more pelagic life style compared to *L. miodon* (Mannini et al., 1996; Mulimbwa and Mannini, 1993). However, this result might also be influenced by the small number of specimens collected from *S. tanganicae*.

Genetic population structure - Generally, the genetic structure of parasites is strongly connected with the dispersal capacity of their hosts (Miura et al., 2006) and their reproductive mode, generation time and population size. The COI-based median joining networks of both monogenean species exhibited comparable core-satellite topologies with similar levels of variation in haplotype and nucleotide diversity. Given that no clear geographical structure appeared from the haplotype network of none of the three monogenean/host species combinations, we suggest geographical panmixia with temporal effects in both species of monogeneans infecting Lake Tanganyika clupeids. This result corresponds with the general biology of clupeids, with assumed lake-wide migration patterns (De Keyzer et al., 2019; Hauser et al., 1998; Junker et al., 2019; Mulimbwa and Shirakihara, 1994). In general, studies conducted on marine clupeids do not show strong population structure, neither using genetic markers (García-Rodríguez et al., 2011; Gonzalez and Zardoya, 2007; Kinsey et al., 1994) nor fish tags (Clark, 1945). Nevertheless, and despite the shared COI haplotypes, significant genetic divergence among some of the pre-defined populations of *K. tanganicanus* from *L. miodon* was detected based on F_{ST}. Such temporal genetic structure without clear evidence for a geographical pattern could be explained by restricted migration of clupeid

hosts, random genetic drift across generations or cohorts related to overfishing of declining clupeid populations in the lake (Mölsä et al., 1999) or recruitement-dependent population fluctuations in r-strategic fish stocks (Watanabe et al., 1995). Interestingly, a recent study by Junker et al. (2019) suggested that population structure in *L. miodon* is linked to chromosomal inversions. Notably, genetic diversity of *K. tanganicanus* in COI is comparable to its host: *S. tanganicae*. However, when only parasite individuals collected from this host species were included, the value was lower. Nucleotide diversity in *L. miodon* is much higher than in both species of *Kapentagyrus* (see Table 2). The level of genetic variation and impact of genetic drift depends on effective population size (Nei and Tajima, 1981). Given the observed variability in prevalence and infection intensity of *Kapentagyrus* spp. (see Table 1), it is hard to estimate population size relative to their clupeid hosts and subsequently evaluate the effect of host population fluctuations. However, no temporal differentiation was observed in neither of the clupeid hosts so far (De Keyzer et al., 2019; Junker et al., 2019). The reported genetic divergence of *K. tanganicanus* among pre-defined geographical populations can be influenced by stochasticity related to the small sample size and short fragment length rather than persisting gene flow barriers. Moreover, the generally reported short generation time of less than a month in dactylogyrid monogeneans (Harris, 1983; Scott and Nokes, 1984; Xiaoqin et al., 2000). Indeed, multiple spawning events per year were reported for both species of Tanganyika clupeids (Mulimbwa and Shirakihara, 1994). Together with their short life span, this may erase the expected effect of a faster molecular evolution in parasites. Alternatively, monogenean reproduction in the pelagic habitat connected with planktonic larval dispersal might cause differences in local genetic diversity of parasites. This is known as fluctuating genetic patchiness (Hellberg et al., 2002). A similar mechanism was suggested for populations of several monogenean species infecting pelagic fish hosts along the coast of China (Li et al., 2011; Shi et al., 2014; Wang et al., 2016; Yan et al., 2016).

548 We need to know more about the population dynamics of the hosts and parasites to identify the
549 cause of the mosaic population structure revealed in this study. In order to further evaluate the
550 magnifying potential of *Kapentagyrus* spp., genome-wide markers need to be applied and
551 compared with similar data on the host species as this study is limited by the single genetic marker
552 being used. A promising approach to clarify the true nature of the interaction between
553 environment, host and parasite are waterscape genomic and transcriptomic studies (Grummer et
554 al., 2019). Here, the peculiarities of the aquatic environment are taken in consideration in the
555 analysis of populations. Promising examples are the highly resolved population structure of well-
556 dispersing taxa (Clucas et al., 2018) and dual transcriptomic studies in host and parasite (Feis et al.,
557 2018).

558 *Parasite diversification in the pelagic zone of Lake Tanganyika*

559 The core-satellite structure of the haplotype networks and the lower haplotype and nucleotide
560 diversity in comparison to *Cichlidogyrus casuarinus* Pariselle, Muterezi Bukinga & Vanhove, 2015, a
561 monogenean species infecting bathybatine benthopelagic cichlids in Lake Tanganyika (Kmentová
562 et al., 2016; Pariselle et al., 2015) points to more recent diversification in both species of
563 *Kapentagyrus.* This might be attributed to limited allopatric divergence in view of a higher
564 dispersal capacity and larger population densities of clupeids in Lake Tanganyika compared to
565 pelagic cichlid species (Coulter, 1991; Koblmüller et al., 2019, 2015).

566 Interestingly, morphological differentiation of *K. tanganicanus,* influenced by the host species and
567 detected in a previous study (Kmentová et al., 2018), was supported by genetic differentiation of
568 the specimens sampled off Uvira in 2016. Our results indicate genetic differentiation of *K.*
569 *tanganicanus* with respect to the clupeid host species. Most probably it happened after a recent
570 host switch. However, given the uniformity in three nuclear gene fragments, the low F_{ST} value and
571 the many shared COI haplotypes of *K. tanganicanus* collected from different host species, we

hypothesize that speciation is prevented as hosts occupy the same environment and have a prey-predator relationship between them (Coulter, 1991; Mulimbwa and Shirakihara, 1994), which has been proposed to be linked to host sharing in monogeneans (Strona, 2015). This should be further verified by genetic characterisation of more individuals combined with genome wide data. The results fit the scenario of a relatively low rate of intraspecific divergence in barrier-free pelagic compared to littoral fish species (Kmentová et al., 2016; Koblmüller et al., 2019, 2015).

Demographic history

Haplotype structure pointed to a recent population expansion for both species of *Kapentagyrus* and their clupeid hosts. The time of the onset of population growth inferred for *K. tanganicanus* corresponded with global climate changes and subsequent lake level rise. Indeed, 10 KYA is the estimated end of the last Little Ice Age, which corresponds with the end of a dry period in East Africa (McGlue et al., 2008). Sea level changes and climate oscillations have measurably influenced the demographic history of monogeneans (Wang et al., 2016; Yan et al., 2016). We suggest that expansion and population growth of *Kapentagyrus* spp. are linked to rising lake levels. We assume that such patterns might be also found in the clupeid host species as climate induced lake level fluctuations have also influenced the demographic history of eupelagic bathybatine cichlids (Koblmüller et al., 2019) and their monogenean parasite *C. casuarinus* (Kmentová et al., 2016). The onset of population expansion and the time to the most recent common ancestor was estimated for both species of *Kapentagyrus* to have been more recent than for *C. casuarinus* (using the same substitution rate). Possible explanations for this difference could be the different life-style and population size of the hosts, host range of the parasites and difference in substitution rates.

Nuclear–mitochondrial discordance

The mitochondrial haplotype of *K. tanganicanus* was detected in four specimens identified as *K. limnotrissae*. We interpret this as evidence for mitochondrial introgression of *K. tanganicanus* into

K. limnotrissae. All four individuals were homozygous at all three nuclear loci analysed and identical to other individuals of *K. limnotrissae*, which excludes that they are F1 hybrids of *Kapentagyrus* spp. Given the broader host range of *K. tanganicanus* compared to its congener, the introgression might result from a recent host switch and a demographic expansion of *K. tanganicanus* (Barson et al., 2010; Rieseberg et al., 2007; Seixas et al., 2018). However, our data does not allow unambiguous differentiation among incomplete lineage sorting, introgression, or contemporary hybridisation. Nevertheless, as no intermediate nuclear haplotype was captured, the presence of a mitochondrial genome of one species in the nuclear environment of another species suggests mitochondrial introgression followed by recurrent backcrossing into the paternal species. Eventually, the introgression resulted in dilution and loss of alleles inherited from the maternal species (Okamoto et al., 2010). A hybridisation event would support the above-mentioned scenario of a recent host switch of *K. tanganicanus* followed by temporal differentiation of infection related to host size (own unpublished data). Moreover, the apparent morphological similarity in the male copulatory organ of the two parasite species contradicts the scenario of intrahost speciation (Jarkovský et al., 2004). Although hybridisation has been reported in gyrodactylid monogeneans (Barson et al., 2010; Schelkle et al., 2012), this is the first case for dactylogyrid monogeneans. The poor documentation of hybridisation in monogeneans might be related to the lack of studies combining morphology, nuclear and mitochondrial markers. In general, hybridisation is considered a major driver of evolution (Franssen et al., 2015; Hedrick, 2013; Huyse et al., 2013; King et al., 2015) which also impacts the host range of parasites (Henrich et al., 2013; Huyse et al., 2009).

Conclusion

In conclusion, no consistent geographical structure along a north-south axis in neither *Kapentagyrus* spp. was found (distance between the two most extreme sampling sites is > 600

620 km), suggesting ongoing gene flow throughout Lake Tanganyika. Therefore, our results correspond
621 with a pattern of weak to no lake-wide population structure of both host species (De Keyzer et al.,
622 2019; Junker et al., 2019). Temporal structure in some morphological characters might be
623 attributed to similar environmental conditions in geographically isolated sampling sites, in
624 combination with restricted host migration. Moreover, significant genetic differentiation was
625 found between some of the parasite populations. Serial sampling and genomic data should
626 increase spatio-temporal resolution to track host migration. Some evidence for incipient
627 speciation in *K. tanganicanus* according to host species was found based on mitochondrial data,
628 despite uniformity in nuclear gene portions. Our findings provide additional support for the impact
629 of historical lake level changes also on organisms inhabiting the lake's pelagic zone. Finally,
630 mitonuclear discordance suggests past hybridisation between the two species of *Kapentagyrus*,
631 which is the first documented case of hybridisation in dactylogyrid monogeneans.

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653 **References**

654 Agawin, N.S.R., Duarte, C.M., Agustí, S., 2000. Nutrient and temperature control of the
655 contribution of picoplankton to phytoplankton biomass and production. Limnol. Oceanogr.
656 45, 591–600. https://doi.org/10.4319/lo.2000.45.3.0591

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

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

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

666 Brazenor, A.K., Saunders, R.J., Miller, T.L., Hutson, K.S., 2018. Morphological variation in the

667 cosmopolitan fish parasite *Neobenedenia girellae* (Capsalidae: Monogenea). Int. J. Parasitol.
668 48, 125–134. https://doi.org/10.1016/J.IJPARA.2017.07.009

669 Cable, J., Harris, P.D., 2002. Gyrodactylid developmental biology: historical review, current status
670 and future trends. Int. J. Parasitol. 32, 255–80.

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

675 Clark, F.N., 1945. Results of tagging experiments in California on Sardine (*Sardinops caerulea*). Fish.
676 Bull. 61, 93.

677 Clucas, G. V., Younger, J.L., Kao, D., Emmerson, L., Southwell, C., Wienecke, B., Rogers, A.D., Bost,
678 C.A., Miller, G.D., Polito, M.J., Lelliott, P., Handley, J., Crofts, S., Phillips, R.A., Dunn, M.J.,
679 Miller, K.J., Hart, T., 2018. Comparative population genomics reveals key barriers to dispersal
680 in Southern Ocean penguins. Mol. Ecol. 27, 4680–4697. https://doi.org/10.1111/mec.14896

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

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

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

688 Danley, P.D., Husemann, M., Ding, B., Dipietro, L.M., Beverly, E.J., Peppe, D.J., 2012. The impact of

689 the geologic history and paleoclimate on the diversification of East African cichlids. Int. J. Evol.
690 Biol. 574851. https://doi.org/10.1155/2012/574851

691 Dávidová, M., Jarkovský, J., Matějusová, I., Gelnar, M., 2005. Seasonal occurrence and metrical
692 variability of *Gyrodactylus rhodei* Žitňan 1964 (Monogenea, Gyrodactylidae). Parasitol. Res.
693 95, 398–405. https://doi.org/10.1007/s00436-005-1311-0

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

699 Degens, E.T., Von Herzen, R.P., Wong, H.-K., 1971. Lake Tanganyika: Water chemistry, sediments,
700 geological structure. Naturwissenschaften 58, 229–241. https://doi.org/10.1007/BF00602986

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

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

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

709 Emmett, R.L., Brodeur, R.D., Miller, T.D., Pool, S.S., Krutzikowsky, G.K., Bentley, P.J., Mccrae, J.,
710 2005. Pacific sardine (*Sardinops sagax*) abundance, distribution, and ecological relationships
711 in the Pacific Northwest. Calif. Coop. Ocean. Fish. Investig. Rep. 46, 122–143.

712 Ergens, R., Gelnar, M., 1985. Experimental verification of the effect of temperature on the size of
713 the hard parts of haptor of *Gyrodactylus kathariner*i Malberg 1964. Folia Parasitol. (Praha).
714 32, 377–380.

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

718 Feis, M.E., John, U., Lokmer, A., Luttikhuizen, P.C., Wegner, K.M., 2018. Dual transcriptomics
719 reveals co-evolutionary mechanisms of intestinal parasite infections in blue mussels *Mytilus*
720 *edulis*. Mol. Ecol. 27, 1505–1519. https://doi.org/10.1111/mec.14541

721 Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of
722 mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol.
723 Mar. Biol. Biotechnol. 3, 294–299. https://doi.org/10.1071/ZO9660275

724 Fox, J., Weisberg, S., 2011. An {R} companion to applied regression, Second. ed. Sage, Thousand
725 Oaks {CA}.

726 Franssen, F., Bilska-Zając, E., Deksne, G., Sprong, H., Pozio, E., Rosenthal, B., Rozycki, M., van der
727 Giessen, J., 2015. Genetic evidence of interspecies introgression of mitochondrial genomes
728 between *Trichinella spiralis* and *Trichinella britovi* under natural conditions. Infect. Genet.
729 Evol. 36, 323–332. https://doi.org/10.1016/J.MEEGID.2015.10.005

730 Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking
731 and background selection. Genetics 147, 915–25.

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

https://doi.org/10.1016/J.FISHRES.2010.11.002

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

Grummer, J.A., Beheregaray, L.B., Bernatchez, L., Hand, B.K., Luikart, G., Narum, S.R., Taylor, E.B., 2019. Aquatic landscape genomics and environmental effects on genetic variation. Trends Ecol. Evol. https://doi.org/10.1016/j.tree.2019.02.013

Handy, S.M., Deeds, J.R., Ivanova, N. V., Hebert, P.D.N., Hanner, R.H., Ormos, A., Weigt, L.A., Moore, M.M., Yancy, H.F., 2011. A single-laboratory validated method for the generation of DNA barcodes for the identification of fish for regulatory compliance. J. AOAC Int. 94, 201–210.

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

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

Hecky, R.E., Fee, E.J., Kling, H., Rudd, J.W.M., 1978. Studies on the planktonic ecology of Lake Tanganyika. Winnipeg, Man.:Fisheries and Marine Service.

Hedrick, P.W., 2013. Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. Mol. Ecol. 22, 4606–4618. https://doi.org/10.1111/mec.12415

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

759 Henrich, T., Benesh, D.P., Kalbe, M., 2013. Hybridization between two cestode species and its
760 consequences for intermediate host range. Parasit. Vectors 6, 33.
761 https://doi.org/10.1186/1756-3305-6-33

762 Huyse, T., Van den Broeck, F., Hellemans, B., Volckaert, F.A.M., Polman, K., 2013. Hybridisation
763 between the two major African schistosome species of humans. Int. J. Parasitol. 43, 687–689.
764 https://doi.org/10.1016/J.IJPARA.2013.04.001

765 Huyse, T., Webster, B.L., Geldof, S., Stothard, J.R., Diaw, O.T., Polman, K., Rollinson, D., 2009.
766 Bidirectional introgressive hybridization between a cattle and human schistosome species.
767 PLoS Pathog. 5, e1000571. https://doi.org/10.1371/journal.ppat.1000571

768 James, A.G., Hutchings, L., Brownell, C.L., Horstman, D.A., 1988. Methods of capture and transfer
769 to the laboratory of wild pelagic fish. South African J. Mar. Sci. 6, 17–21.
770 https://doi.org/10.2989/025776188784480519

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

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

777 Junker, J., Rick, J.A., McIntyre, P.B., Kimirei, I., Sweke, E.A., Mosille, J.B., Werli, B., Dinkel, C.,
778 Mwaiko, S., Seehausen, O., Wagner, C.E., 2019. Sex differentiation and a chromosomal
779 inversion lead to cryptic diversity in Lake Tanganyika sardines. bioRxiv 800904.

https://doi.org/10.1101/800904

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

King, K.C., Stelkens, R.B., Webster, J.P., Smith, D.F., Brockhurst, M.A., 2015. Hybridization in parasites: consequences for adaptive evolution, pathogenesis, and public health in a changing w. PLOS Pathog. 11, e1005098. https://doi.org/10.1371/journal.ppat.1005098

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

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

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

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

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

Kmentová, N., Van Steenberge, M., Raeymaekers, J.A.R., Koblmüller, S., Hablützel, P.I., Muterezi

802 Bukinga, F., Mulimbwa N'sibula, T., Masilya Mulungula, P., Nzigidahera, B., Ntakimazi, G.,
803 Gelnar, M., Vanhove, M.P.M., 2018. Monogenean parasites of sardines in Lake Tanganyika:
804 diversity, origin and intra-specific variability. Contrib. to Zool. 87, 105–132.

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

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

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

814 Kurki, H., Vuorinen, I., Bosma, E., Bwebwa, D., 1999. Spatial and temporal changes in copepod
815 zooplankton communities of Lake Tanganyika, in: Lindqvist, O. V., Molsd, H., Salonen, K.,
816 Sarvala, J. (Eds.), From Limnology to Fisheries: Lake Tanganyika and Other Large Lakes. Kluwer
817 Academic Publishers., Dordrecht, pp. 105–114. https://doi.org/10.1007/978-94-017-1622-
818 2_10

819 Langenberg, V.T., Mwape, L.M., Tshibangu, K., Tumba, J.-M., Koelmans, A.A., Roijackers, R.,
820 Salonen, K., Sarvala, J., Mölsä, H., 2002. Comparison of thermal stratification, light
821 attenuation, and chlorophyll- a dynamics between the ends of Lake Tanganyika. Aquat.
822 Ecosyst. Health Manag. 5, 255–265. https://doi.org/10.1080/14634980290031956

823 Langenberg, V.T., Sarvala, J., Roijackers, R., 2003. Effect of wind induced water movements on
824 nutrients, chlorophyll- a, and primary production in Lake Tanganyika. Aquat. Ecosyst. Health

825 Manag. 6, 279–288. https://doi.org/10.1080/14634980301488

826 Leigh, J.W., Bryant, D., 2015. PopART: full-feature software for haplotype network construction.
827 Methods Ecol. Evol. 6, 1110–1116.

828 Li, M., Shi, S.F., Brown, C.L., Yang, T.B., 2011. Phylogeographical pattern of *Mazocraeoides*
829 *gonialosae* (Monogenea, Mazocraeidae) on the dotted gizzard shad, *Konosirus punctatus*,
830 along the coast of China. Int. J. Parasitol. 41, 1263–1272.
831 https://doi.org/10.1016/j.ijpara.2011.07.012

832 Littlewood, D.T.J., Rohde, K., Clough, K.A., 1997. Parasite speciation within or between host
833 species? - Phylogenetic evidence from site-specific polystome monogeneans. Int. J. Parasitol.
834 27, 1289–1297. https://doi.org/10.1016/S0020-7519(97)00086-6

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

840 Mannini, P., Aro, E., Katonda, K.I., Kassaka, B., Mambona, C., Milindi, G., Paffen, P., Verburg, P.,
841 1996. Pelagic fish stocks of Lake Tanganyika: biology and exploitation. FAO/FINNIDA research
842 for the management of the fisheries of Lake Tanganyika., GCP/RAF/271/FIN—TD/53 (En).
843 Bujumbura.

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

846 Martínez, P., González, E.G., Castilho, R., Zardoya, R., 2006. Genetic diversity and historical
847 demography of Atlantic bigeye tuna (*Thunnus obesus*). Mol. Phylogenet. Evol. 39, 404–416.

https://doi.org/10.1016/j.ympev.2005.07.022

Mattiucci, S., Abaunza, P., Ramadori, L., Nascetti, G., 2004. Genetic identification of *Anisakis* larvae in European hake from Atlantic and Mediterranean waters for stock recognition. J. Fish Biol. 65, 495–510. https://doi.org/10.1111/j.0022-1112.2004.00465.x

McGlue, M.M., Lezzar, K.E., Cohen, A.S., Russell, J.M., Tiercelin, J.-J., Felton, A.A., Mbede, E., Nkotagu, H.H., 2008. Seismic records of late Pleistocene aridity in Lake Tanganyika, tropical East Africa. J. Paleolimnol. 40, 635–653. https://doi.org/10.1007/s10933-007-9187-x

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

Misund, O., 1993. Dynamics of moving masses: variability in packing density, shape, and size among herring, sprat, and saithe schools. ICES J. Mar. Sci. 50, 145–160. https://doi.org/10.1006/jmsc.1993.1016

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

Mo, A., 1993. Seasonal variation of haptoral hard parts of *Gyrodactylus derjavini* Mikailov 1975 (Monogenea: Gyrodactylidae) on brown trout *Salmo trutta* L. parr and Atlantic salmon *Salmo salar* L. parr in the River Sandviksela, N. Syst. Parasitol. 26, 225–231.

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

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

873 Mulimbwa, N., Shirakihara, K., 1994. Growth, recruitment and reproduction of sardines
874 (*Stolothrissa tanganicae* and *Limnothrissa miodon*) in northwestern Lake Tanganyika. Tropics
875 4, 57–67. https://doi.org/10.3759/tropics.4.57

876 Nei, M., Tajima, F., 1981. Genetic drift and estimation of effective population size. Genetics 98,
877 625–640.

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

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

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

886 Nøttestad, L., Giske, J., Holst, J.C., Huse, G., 1999. A length-based hypothesis for feeding
887 migrations in pelagic fish. Can. J. Fish. Aquat. Sci. 56, 26–34. https://doi.org/10.1139/f99-222

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

891 Olstad, K., Bachmann, L., Bakke, T. a, 2009. Phenotypic plasticity of taxonomic and diagnostic

892 structures in gyrodactylosis-causing flatworms (Monogenea, Platyhelminthes). Parasitology
893 136, 1305–1315. https://doi.org/10.1017/S0031182009990680

894 Ondračková, M., Matějusová, I., Grabowska, J., 2012. Introduction of *Gyrodactylus perccotti*
895 (Monogenea) into Europe on its invasive fish host, Amur sleeper (*Perccottus glenii*, Dybowski
896 1877). Helminthologia 49, 21–26. https://doi.org/10.2478/s11687-012-0004-3

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

900 Pariselle, A., Muterezi Bukinga, F., Van Steenberge, M., Vanhove, M.P.M., 2015. Ancyrocephalidae
901 (Monogenea) of Lake Tanganyika: IV: *Cichlidogyrus* parasitizing species of Bathybatini
902 (Teleostei, Cichlidae): reduced host-specificity in the deepwater realm? Hydrobiologia 748,
903 99–119. https://doi.org/10.1007/s10750-014-1975-5

904 Pettersen, R.A., Mo, T.A., Hansen, H., Vøllestad, L.A., 2015. Genetic population structure of
905 *Gyrodactylus thymalli* (Monogenea) in a large Norwegian river system. Parasitology 142,
906 1693–1702. https://doi.org/10.1017/S003118201500133X

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

909 Plisnier, P.-D., Chitamwebwa, D., Mwape, L., Tshibangu, K., Langenberg, V., Coenen, E., 1999.
910 Limnological annual cycle inferred from physical-chemical fluctuations at three stations of
911 Lake Tanganyika, in: Lindqvist, O.V., Mölsä, H., Salonen, K., Sarvala, J. (Eds.), From Limnology
912 to Fisheries: Lake Tanganyika and Other Large Lakes. Springer Netherlands, Dordrecht, pp.
913 45–58. https://doi.org/10.1007/978-94-017-1622-2_4

914 Plisnier, P.D., Mgana, H., Kimirei, I., Chande, A., Makasa, L., Chimanga, J., Zulu, F., Cocquyt, C.,

915 Horion, S., Bergamino, N., Naithani, J., Deleersnijder, E., André, L., Descy, J.P., Cornet, Y.,
916 2009. Limnological variability and pelagic fish abundance (*Stolothrissa tanganicae* and *Lates*
917 *stappersii*) in Lake Tanganyika. Hydrobiologia 625, 117–134. https://doi.org/10.1007/s10750-
918 009-9701-4

919 Podsetchine, V., Huttula, T., 2000. Numerical simulation of wind-driven circulation in lake
920 tanganyika. Aquat. Ecosyst. Heal. Manag. 3, 55–64.
921 https://doi.org/10.1080/14634980008656991

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

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

926 Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior summarization in
927 Bayesian phylogenetics using Tracer 1.7. Syst. Biol 67, 2.
928 https://doi.org/10.1093/sysbio/syy032

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

933 Rieseberg, L.H., Kim, S.-C., Randell, R.A., Whitney, K.D., Gross, B.L., Lexer, C., Clay, K., 2007.
934 Hybridization and the colonization of novel habitats by annual sunflowers. Genetica 129,
935 149–165. https://doi.org/10.1007/s10709-006-9011-y

936 Rodríguez-González, A., Míguez-Lozano, R., Llopis-Belenguer, C., Balbuena, J.A., 2015. Phenotypic
937 plasticity in haptoral structures of *Ligophorus cephali* (Monogenea: Dactylogyridae) on the

938 flathead mullet (*Mugil cephalus*): a geometric morphometric approach. Int. J. Parasitol. 45,
939 295–303. https://doi.org/10.1016/j.ijpara.2015.01.005

940 Rohlf, F., 2006. Tpsdig, digitize landmarks and outlines. Version 2.10. Stony Brook, NY: State
941 University.

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

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

949 Schelkle, B., Faria, P.J., Johnson, M.B., van Oosterhout, C., Cable, J., 2012. Mixed infections and
950 hybridisation in monogenean parasites. PLoS One 7, e39506.
951 https://doi.org/10.1371/journal.pone.0039506

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

955 Sefc, K., Mattersdorfe, K., Ziegelbecker, A., Neuhüttler, N., Steiner, O., Goessler, W., Koblmüller, S.,
956 2017. Shifting barriers and phenotypic diversification by hybridization. Ecol. Lett. 20, 651–
957 662.

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

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

964 Shi, S.-F., Li, M., Yan, S., Wang, M., Yang, C.-P., Lun, Z.-R., Brown, C.L., Yang, T.-B., 2014.
965 Phylogeography and demographic history of *Gotocotyla sawara* (Monogenea: Gotocotylidae)
966 on Japanese Spanish mackerel (*Scomberomorus niphonius*) along the coast of China. J.
967 Parasitol. 100, 85–92. https://doi.org/10.1645/13-235.1

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

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

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

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

978 Svedäng, H., André, C., Jonsson, P., Elfman, M., Limburg, K.E., 2010. Migratory behaviour and
979 otolith chemistry suggest fine-scale sub-population structure within a genetically
980 homogenous Atlantic Cod population. Environ. Biol. Fishes 89, 383–397.
981 https://doi.org/10.1007/s10641-010-9669-y

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

Tinsley, R.C., 2004. Platyhelminth parasite reproduction: some general principles derived from monogeneans. Can. J. Zool. 82, 270–291. https://doi.org/10.1139/z03-218

van Zwieten, P.A.M., Roest, F.C., Machiels, M.A.M., van Densen, W.L.T., 2002. Effects of inter-annual variability, seasonality and persistence on the perception of long-term trends in catch rates of the industrial pelagic purse-seine fishery of northern Lake Tanganyika (Burundi). Fish. Res. 54, 329–348. https://doi.org/10.1016/S0165-7836(01)00267-3

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

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

Wang, M., Yan, S., Brown, C.L., Shaharom-harrison, F., Shi, S., Yang, T., 2016. Phylogeography of *Tetrancistrum nebulosi* (Monogenea, Dactylogyridae) on the host of mottled spinefoot (*Siganus fuscescens*) in the South China Sea, inferred from mitochondrial COI and ND2 genes. Mitochondrial DNA 27, 3865–3875. https://doi.org/10.3109/19401736.2014.971240

Watanabe, Y., Zenitani, H., Kimura, R., 1995. Population decline off the Japanese sardine *Sardinops melanostictus* owing to recruitment failures. Can. J. Fish. Aquat. Sci. 52, 1609–1616. https://doi.org/10.1139/f95-154

Wickham, H., 2009. Introduction, in: Ggplot2. Springer New York, New York, NY, pp. 1–7. https://doi.org/10.1007/978-0-387-98141-3_1

Xiaoqin, X., Weijun, W., Weijian, Y., 2000. The life span of *Dactylogyrus vaginulatus* on

Hypophthalmichthys molitrix. Jounrnal Nanjing Agric. Univ. 22, 95–98.

Yan, S., Wang, M., Yang, C.-P., Zhi, T.-T., Brown, C.L., Yang, T.-B., 2016. Comparative phylogeography of two monogenean species (Mazocraeidae) on the host of chub mackerel, *Scomber japonicus*, along the coast of China. Parasitology 143, 594–605. https://doi.org/10.1017/S0031182016000160

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

Figure captions

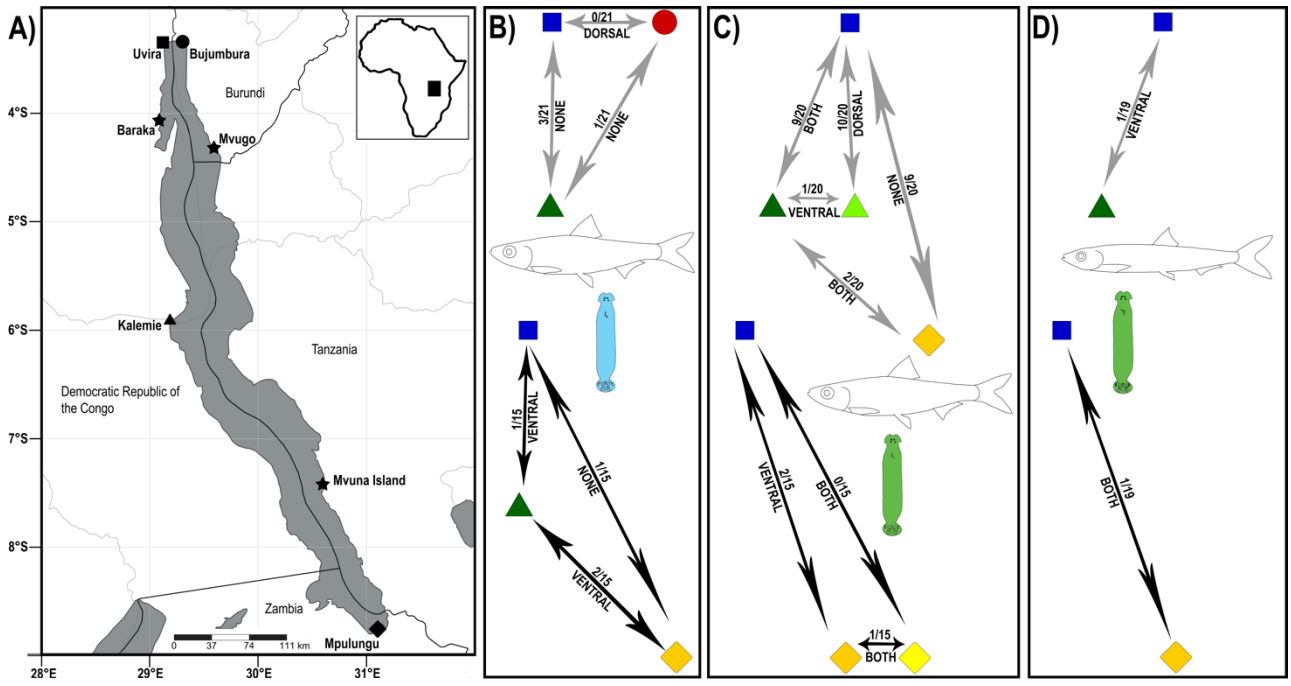


Fig. 1: Sampling sites in Lake Tanganyika with an overview of significant results of morphometric (above arrow) and geomorphometrics (below arrow) analyses between the specimens of *Kapentagyrus* spp. from the respective sampling sites. A) *K. limnotrissae*, B) *K. tanganicanus* ex *L. miodon* and C) *K. tanganicanus* ex *S. tanganicae*. The morphometric results are presented as the number of variables which differ between the respective sampling sites (before the dash) vs the total number of variables analysed (after the dash). In the case of geomorphometrics, the difference in either ventral, dorsal, both or none of the anchors is indicated. Shapes of signs correspond with the sampling site origin of specimens in the respective analyses. Colours of arrows refer to the separation on spatial (grey – ethanol preserved specimens) and spatio-temporal (black – fresh specimens) datasets. Map created using SimpleMappr software v7.0.0. (available at http://www.simplemappr.net. Accessed January 20, 2019).

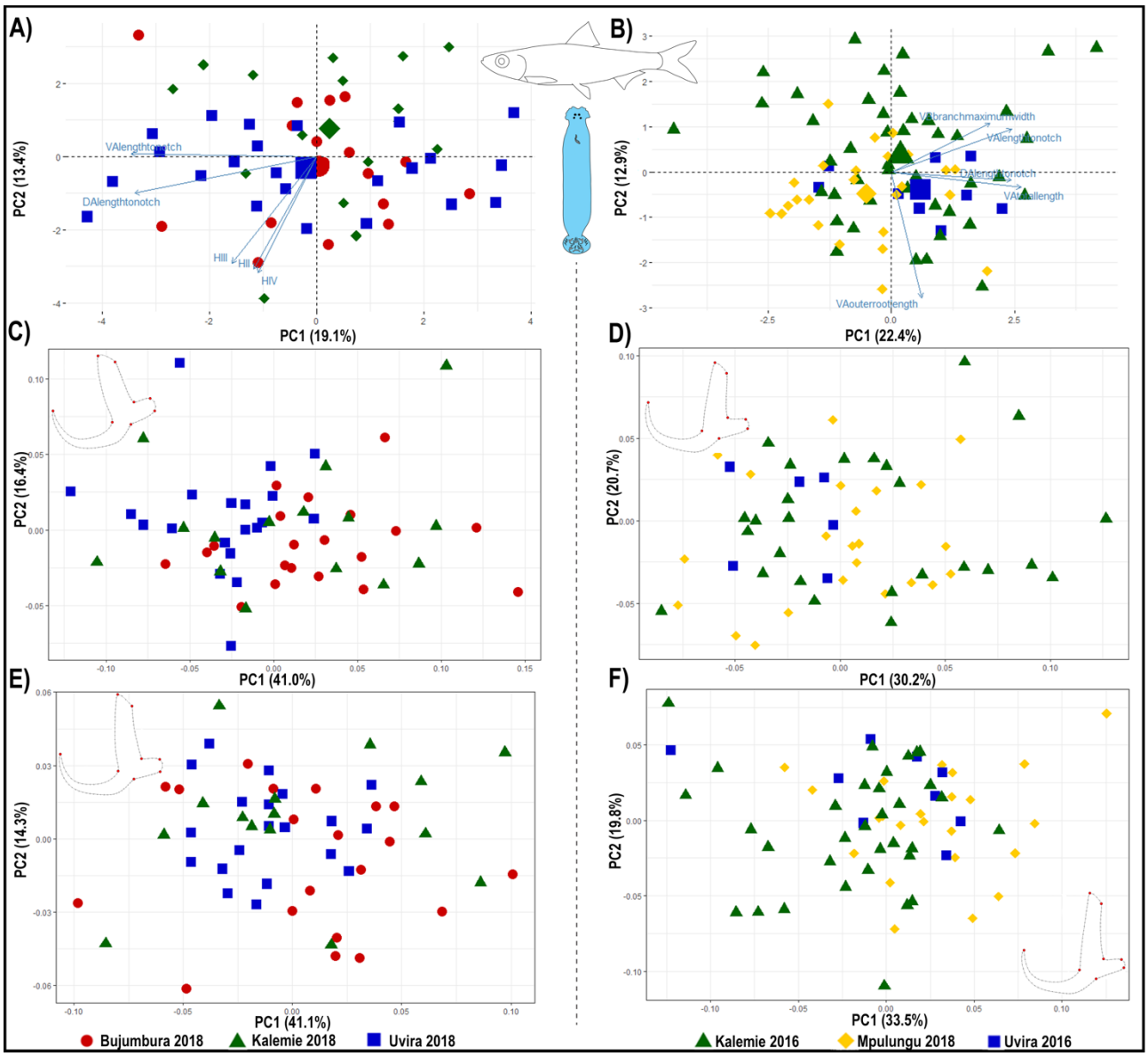


Fig. 2: **Biplots showing the variation in haptoral structures of *K. limnotrissae*. Only the first two axes are shown.** A) PCA of haptoral measurements with the five most contributing variables indicated by arrows, ethanol-preserved specimens (fifth and seventh pair of marginal hooks excluded due to missing data, the average position for each group is indicated by a larger size of the symbol); B) PCA of haptoral measurements with the five most contributing variables indicated by arrows, fresh specimens (second to seventh pair of marginal hooks excluded due to missing data, the average position for each group is indicated by a larger size of the symbol); C) PCA based on Procrustes distances of eight fixed landmarks describing the shape of the dorsal anchor, ethanol preserved specimens; D) PCA based on Procrustes distances of eight fixed landmarks

describing the shape of the dorsal anchor, fresh specimens; E) PCA based on Procrustes distances of eight fixed landmarks describing the shape of the ventral anchor, ethanol preserved specimens; F) PCA based on Procrustes distances of eight fixed landmarks describing the shape of the ventral anchor, ethanol preserved specimens. Consensus anchor shape for the respective analysis is shown.

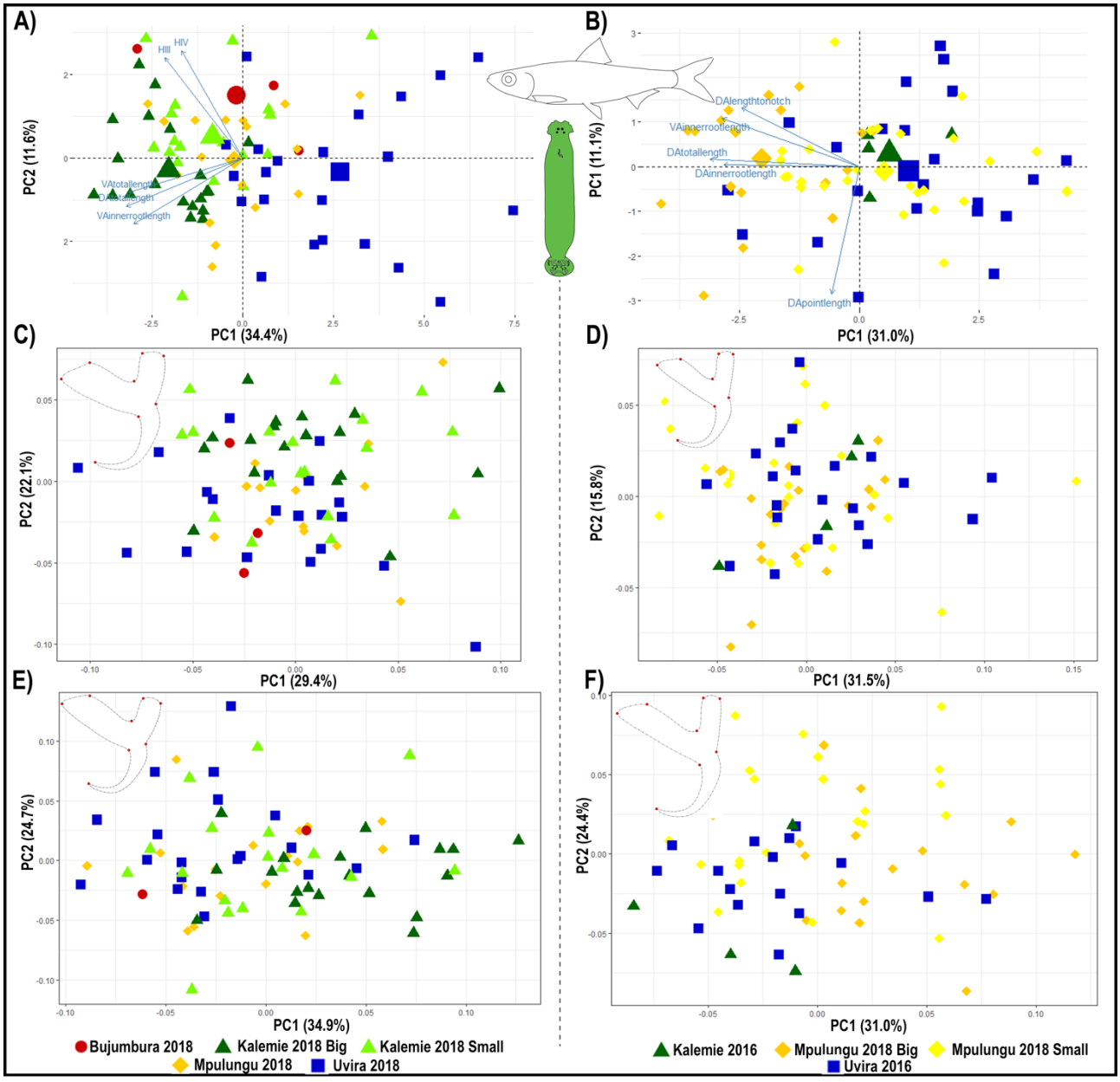


Fig. 3: Biplots showing the variation in haptoral structures of *K. tanganicanus* collected from *L. miodon* in this study. Only the first two axes are shown. A) PCA of haptoral measurements with the five most contributing variables indicated by arrows, ethanol-preserved specimens (fifth to

seventh pair of marginal hooks excluded due to missing data, the average position for each group, is indicated by a larger size of the symbol); B) PCA of haptoral measurements with the five most contributing variables indicated by arrows, fresh specimens (second to seventh pair of marginal hooks excluded due to missing data, the average position for each group is indicated by a larger size of the symbol); C) PCA based on Procrustes distances of eight fixed landmarks describing the shape of the dorsal anchor, ethanol preserved specimens; D) PCA based on Procrustes distances of eight fixed landmarks describing the shape of the dorsal anchor, fresh specimens; E) PCA based on Procrustes distances of eight fixed landmarks describing the shape of the ventral anchor, ethanol preserved specimens; F) PCA based on Procrustes distances of eight fixed landmarks describing the shape of the ventral anchor, ethanol preserved specimens. Consensus anchor shape for the respective analysis is shown.

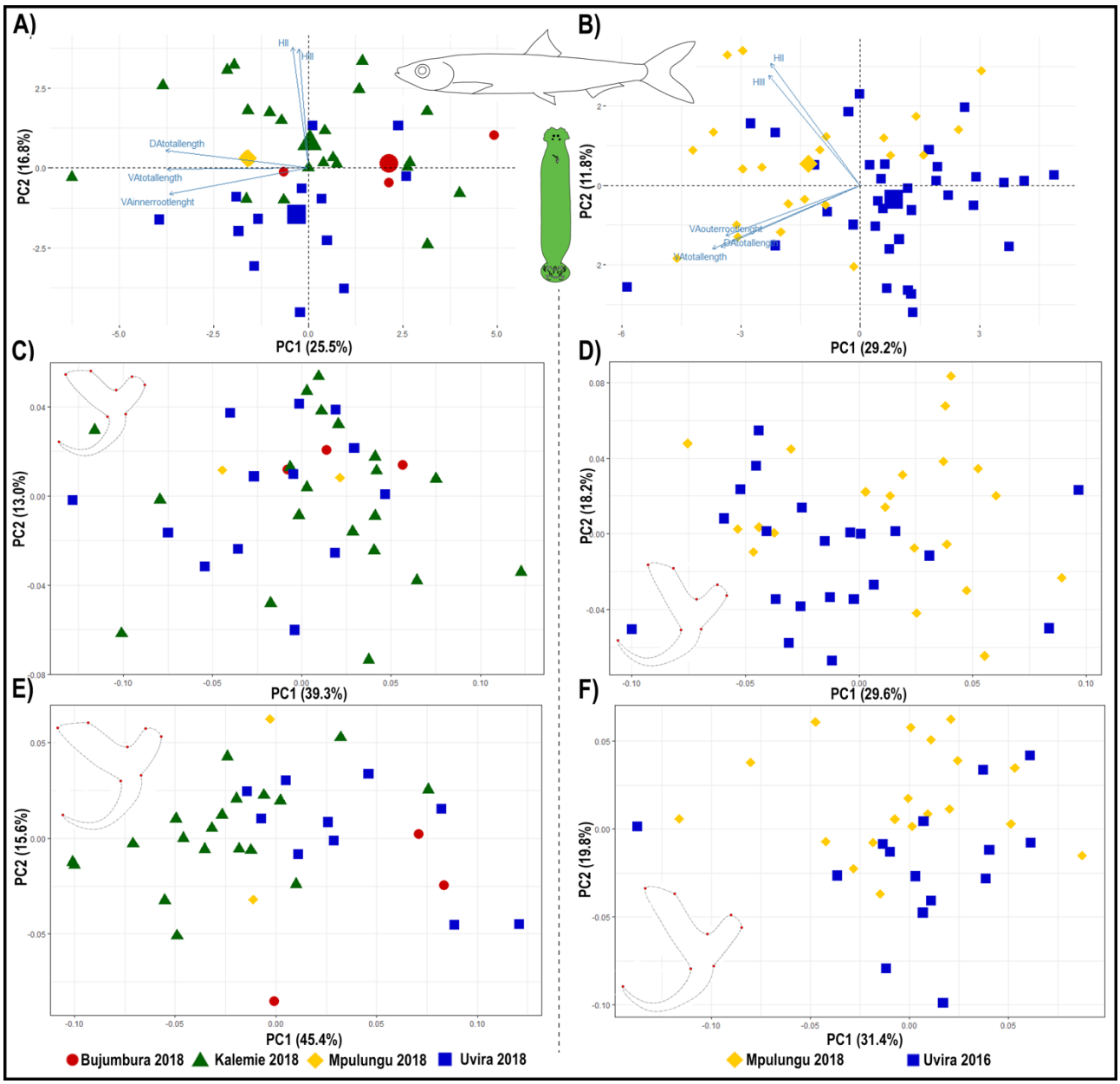
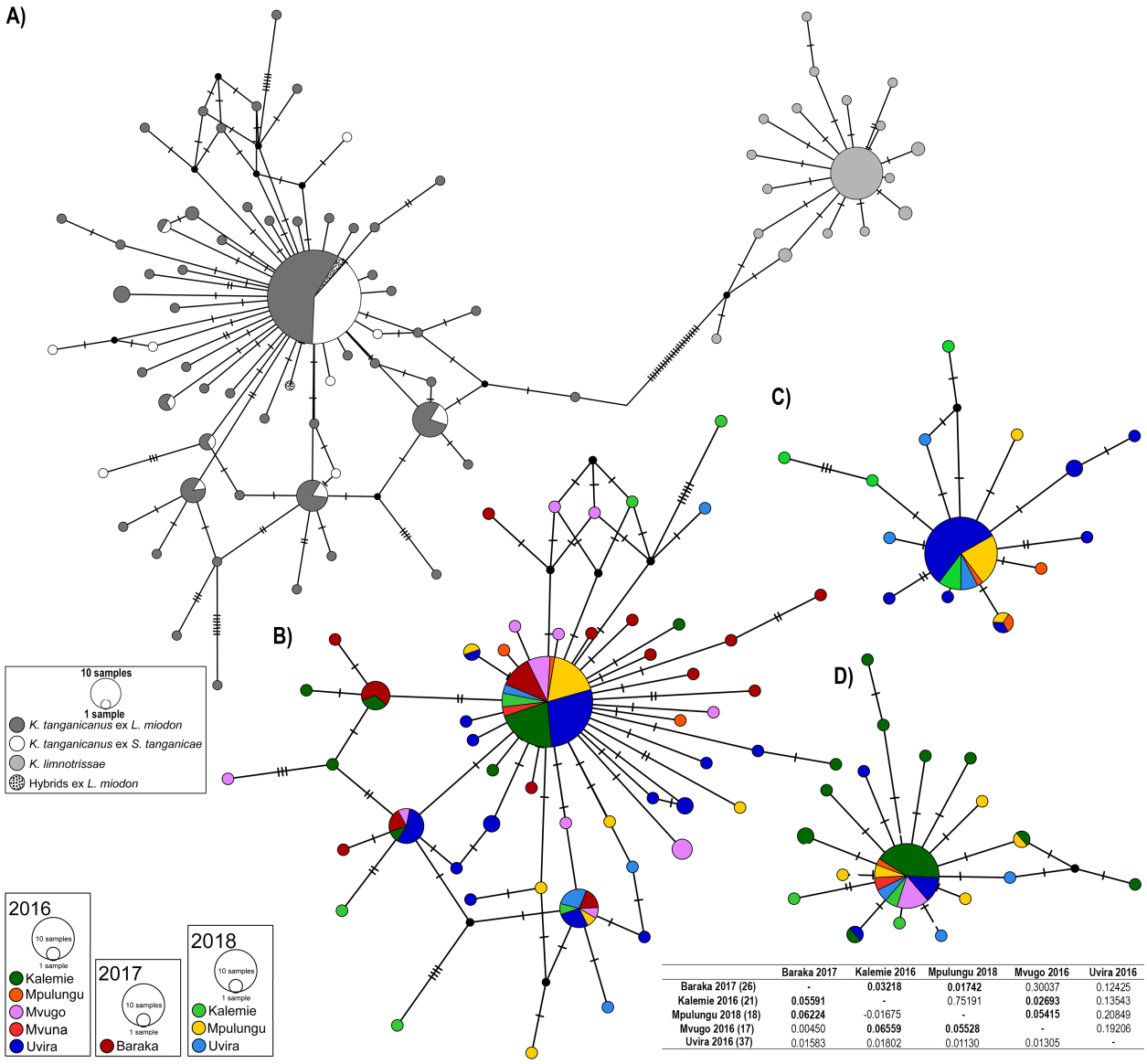


Fig. 4: Biplots showing the variation in haptoral structures of *K. tanganicanus* collected from *S. tanganicae* in this study. Only the first two axes are shown. A) PCA of haptoral measurements with the five most contributing variables indicated by arrows, ethanol-preserved specimens (fifth and seventh pair of marginal hooks excluded due to missing data, the average position for each group is indicated by a larger size of the symbol); B) PCA of haptoral measurements with the five most contributing variables indicated by arrows, fresh specimens (sixth and seventh pair of marginal hooks excluded due to missing data, the average position for each group is indicated by a larger size of the symbol); C) PCA based on Procrustes distances of eight fixed landmarks

describing the shape of the dorsal anchor, ethanol preserved specimens; D) PCA based on Procrustes distances of eight fixed landmarks describing the shape of the dorsal anchor, fresh specimens; E) PCA based on Procrustes distances of eight fixed landmarks describing the shape of the ventral anchor, ethanol preserved specimens; F) PCA based on Procrustes distances of eight fixed landmarks describing the shape of the ventral anchor, ethanol preserved specimens. Consensus anchor shape for the respective analysis is shown.



	Baraka 2017	Kalemie 2016	Mpulungu 2018	Mvugo 2016	Uvira 2016
Baraka 2017 (26)	-	0.03218	0.01742	0.30037	0.12425
Kalemie 2016 (21)	0.05591	-	0.75191	0.02693	0.13543
Mpulungu 2018 (18)	0.06224	-0.01675	-	0.05415	0.20849
Mvugo 2016 (17)	0.00450	0.06559	0.05528	-	0.19206
Uvira 2016 (37)	0.01583	0.01802	0.01130	0.01305	-

Fig. 5: Genetic population structure of *Kapentagyrus* spp. based on COI sequences (415 bp). Median joining haplotype network of A) *K. tanganicanus* and *K. limnotrissae* with hybrid individuals; B) *K. tanganicanus* ex *L. miodon*; C) *K. tanganicanus* ex *S. tanganicae*; D) *K.*

limnotrissae. The circles represent different haplotypes with their size proportional to the number of individuals represented. Haplotypes are connected with lines, indicating the number of mutations. Small black circles indicate hypothetical haplotypes, predicted by the model. Colours represent sampling events and host species, respectively, as mentioned in the legends. Genetic differentiation among geographically pre-defined subpopulations of *K. tanganicanus* ex *L. miodon* listed in the enclosed table. Pairwise F_{ST} values and corresponding P-values are shown below and above the diagonal, respectively. Significant results with α=0.05 are marked in bold. Number of monogenean individuals in brackets.

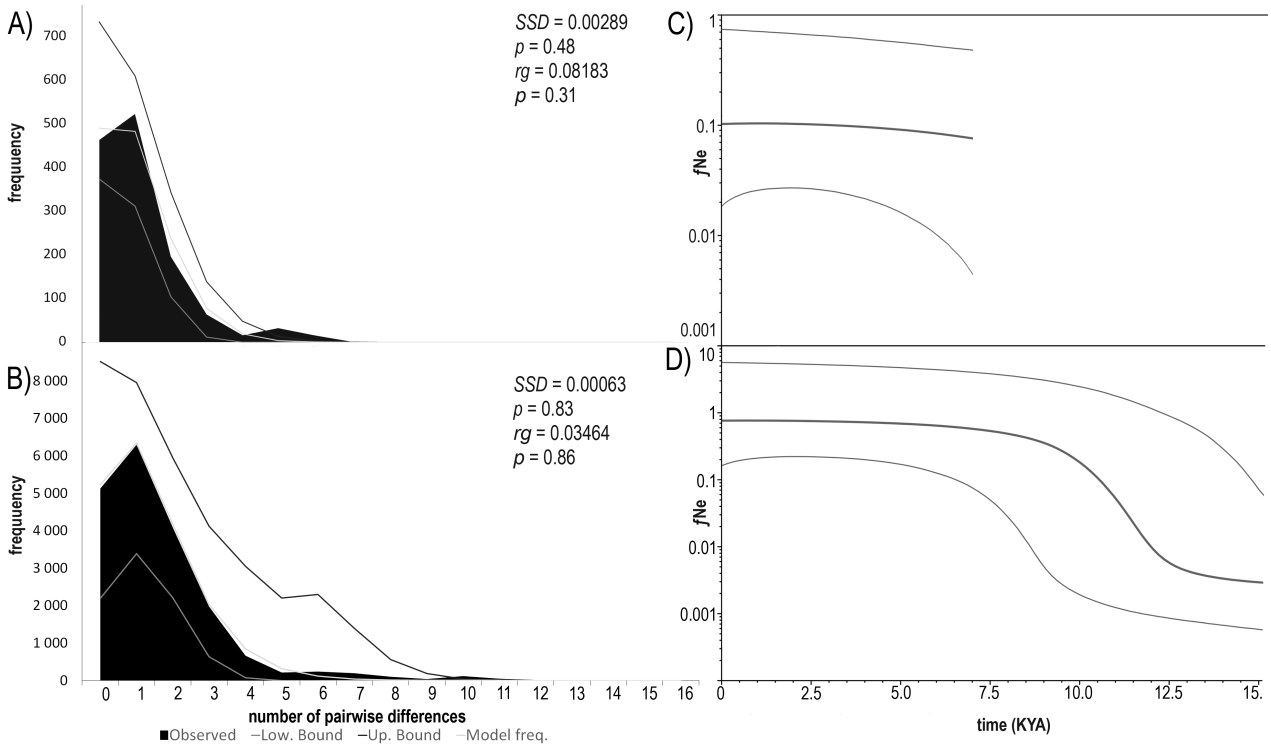


Fig. 6: Demographic history of *Kapentagyrus* spp. Mismatch distribution for A) *K. limnotrissae* and B) *K. tanganicanus*. The black bars show the observed frequency of pairwise differences. The grey lines refer to the expected distribution based on parameter estimates (plus 95% confidence limits) under a model of population growth. The sum of squared differences (SSD) and the raggedness index (rg) and their respective p-values are given to describe the fit of the observed distribution to the expectations based on growth parameter estimates, as well as τ, the modal value of the

mismatch distribution. Bayesian Skyline plot (BSP) of C) *K. limnotrissae* based on 415 base pairs of COI sequences and D) *K. tanganicanus* based on 415 base pairs of COI sequences. BSPs show the effective populations size through time, assuming a substitution rate of 10% per site per million years in *Kapentagyrus* spp. The thick line represents the median values; the thin lines denote 95% highest posterior density (HPD) intervals. The y-axis represents the population size parameter (product of female effective population size, ƒNe, and mutation rate, μ).

1099 Table 1: Number of fish specimens of (a) *Limnothrissa miodon* and (b) *Stolothrissa tanganicae* examined for monogenean parasites along with sampling

1100 site, basin and infection parameters. Values for *Kapentagyrus limnotrissae* and *Kapentagyrus tanganicanus* are shown before and after the dash,

1101 respectively).

Sampling site (geographic coordinates, date, year)	Locality – basins (Danley et al. 2012)	Number of fish specimens	Number of monogenean individuals	Prevalence (%)	Infection intensity	Abundance (range)	Number of COI haplotypes (Genbank a.n)	Number of microscopic slides (a.n. in HU)
(a) *Limnothrissa miodon*								
Baraka (4°05'S–29°06'E; 29.7.2017)	The northern basin	24	10/63	16.7/41.7	2.5/5.4	0.4 (0–4)/2.1 (0–15)	–/26 (MK598222–47)	–/–
Bujumbura (3°23'S–29°22'E; 10.4.2018)	The northern basin	30	108/4	83/10	4.3/1.3	3.6 (0–17)/ 0.1 (0–2)	–/–	X.4.13–37/ XII.2.10–13
Kalemie (5°56'S–29°12'E; 12.8.2016)	The central basin	10	55/5	80/33	6.9/1.7	5.5 (0–15)/0.5(0–2)	23 (MK598078–100)/21 (MK598145–65)	XI.1.11–2.12/ XI.3.49–50, 4.01–2, 19–20
Kalemie (12.4.2018)	The central basin	20	24/204	25/70	4.8/14.6	1.2 (0–11)/ 10.2 (0–37)	4 (MK598125–28)/8 (MK598137–44)	X.3.45–4.12/ XI.4.21, XII.1.01–38
Mpulungu (8°46'S–31°07'E; 19.8.2016)	The southern basin	2	1/3	50/50	1/3	0.5(0–1)/1.5(0–3)	1 (MK598114)/3 (MK598248–50)	XI.2.21–22/–
Mpulungu (7.4. – 21.4.2018)	The southern basin	81	60/452	28/63	2.6/9	0.7 (0–8)/ 5.6 (0–42)	6 (MK598115–20)/18 (MK598251–68)	X.3.26–44/ XI.4.22–50, XII.2.10–28
Mvugo (4°18'S–29°34'E. 4.8.2016)	The northern basin	6	9/25	50/100	3/4.2	1.5 (0–3)/4.2 (1–10)	2 (MK598112–13)/17 (MK598205-21)	XI.2.13–20/ XI.4.03–18
Mvuna Island (7°26'S–30°36'E 1.4.2015)	The southern basin	6	11/5	50/50	3.7/1.7	1.8 (0–8)/0.8 (0–3)	5 (MK598101–5) /2 (MK59817–8)	XI.2.33–37/–
Uvira (3°22' S–29°08'E; 11.8.2016)	The northern basin	41	12/28	35/40	1.7/3.5	0.6 (0–3)/1.4(0–9)	6 (MK598106–11)/37 (MK598166–76, 79–204)	XI.1.11–20/ XI.3.26–48
Uvira (12.4.2018)	The northern basin	30	43/70	53/76.7	2.7/3.0	1.4 (0–7)/ 2.3 (0–8)	4 (MK598121-24)/8 (MK598129-36)	X.4.38–50, XI.1.01–10/ XII.1.39–2.09
(b) *Stolothrissa tanganicae*								
Bujumbura (10.4.2018)	The northern basin	30	5	16.7	1	0.17 (0–1)	–	XII.3.48–3.50
Kalemie (11.8.2016)	The central basin	33	0	0	0	0	–	-
Kalemie (12.4.2018)	The central basin	30	44	66.7	2.2	1.5 (0–5)	7 (MK598317–23)	XII.3.26–3.47
Mpulungu (19.8.2016)	The southern basin	18	3	16.7	1	0.17 (0–1)	3 (MK598269, 81–82)	XII.4.24–25
Mpulungu (7.4. – 21.4.2018)	The southern basin	84	107	52.4	2.4	1.3 (0–13)	11 (MK598270–80)	XII.3.15–3.25, 4.01–2

Uvira (11.8.2016)	The northern basin	27	31	44	2.6	1.1 (0–6)	29 (MK598283–311)	XII.2.38–3.10
Uvira (12.4.2018)	The northern basin	25	12	28	1.7	0.5 (0–3)	5 (MK598312–16)	XII.4.03–15

Table 2: Genetic diversity indices of species of *Kapentagyrus*, and their hosts *Limnothrissa miodon* and *Stolothrissa tanganicae* (De Keyzer et al., 2019) inferred from the COI mtDNA region.

Species	N	H	S	Hd	π	Max. divergence (%)
K. limnotrissae	51	19	18	0.6329±0.0800	0.002241±0.001742	1.2
K. tanganicanus	195	60	68	0.7341±0.0348	0.003800±0.002515	3.1
K. tanganicanus* ex *L. miodon	140	53	64	0.8066±0.0339	0.004499±0.002869	3.1
K. tanganicanus* ex *S. tanganicae	55	14	17	0.4983±0.0838	0.001991±0.001604	1.4
L. miodon	69	38	45	0.9250±0.0236	0.008909±0.004799	3.2
S. tanganicae	96	46	48	0.8583±0.0337	0.003543±0.002174	1.2

N sample size, H number of haplotypes, S number of polymorphic sites, Hd haplotype diversity, π nucleotide diversity.