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Weak population structure and recent demographic expansion of the monogenean parasite Kapentagyrus spp. infecting clupeid fishes of Lake Tanganyika, East Africa Peer-reviewed author version

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1	Weak population structure and expansive demographic history of the monogenean
2	parasite Kapentagyrus spp. infecting clupeid fishes of Lake Tanganyika
3	Nikol Kmentová <sup>1,2,3*</sup> , Stephan Koblmüller <sup>4</sup> , Maarten Van Steenberge <sup>3,4,5,6</sup> , Joost A.M.
4	Raeymaekers <sup>7</sup> , Tom Artois <sup>2</sup> , Els L.R. De Keyzer <sup>3,8</sup> , Leona Milec <sup>7</sup> , Fidel Muterezi Bukinga <sup>9</sup> , Théophile
5	Mulimbwa N'sibula <sup>9</sup> , Pascal Masilya Mulungula <sup>9</sup> , Gaspard Ntakimazi <sup>10</sup> , Filip A.M. Volckaert <sup>3</sup> ,
6	Milan Gelnar <sup>1</sup> , Maarten P.M. Vanhove <sup>1,2,3,11</sup>
7	<sup>1</sup> Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37
8	Brno, Czech Republic
9	<sup>2</sup> Hasselt University, Centre for Environmental Sciences, Research Group Zoology: Biodiversity &
10	Toxicology, Agoralaan Gebouw D, B-3590 Diepenbeek, Belgium
11	<sup>3</sup> Laboratory of Biodiversity and Evolutionary Genomics, Department of Biology, University of
12	Leuven, Ch. Deberiotstraat 32, B-3000 Leuven, Belgium
13	<sup>4</sup> Institute of Biology, University of Graz, Universitätsplatz 2, A-8010 Graz, Austria
14	<sup>5</sup> Operational Directorate Taxonomy and Phylogeny, Royal Belgian Institute of Natural Sciences,
15	Vautierstraat 29, B-1000 Brussels, Belgium
16	<sup>6</sup> Biology Department, Royal Museum for Central Africa, Leuvensesteenweg 13, 3080, Tervuren,
17	Belgium
18	<sup>7</sup> Faculty of Biosciences and Aquaculture, Nord University, N-8049 Bodø, Norway
19	<sup>8</sup> Capacities for Biodiversity and Sustainable Development (CEBioS), Operational Directorate Natural
20	Environment, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000, Brussels,
21	Belgium
22	<sup>9</sup> Centre de Recherche en Hydrobiologie, Département de Biologie, B.P. 73 Uvira, Democratic
23	Republic of Congo

24	<sup>10</sup> Département de Biologie, Université du Burundi, Campus Mutanga, B.P. 2700, Bujumbura,
25	Burundi
26	<sup>11</sup> Zoology Unit, Finnish Museum of Natural History, University of Helsinki, P.O.Box 17, Helsinki Fl-
27	00014, Finland
28	*corresponding author: kmentovan@mail.muni.cz, Laboratory of Parasitology, Department of
29	Botany and Zoology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
30	Note: Supplementary data associated with this article
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#### 46 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 58 along a north-south axis. This lack of geographical population structure was also reflected by a 59 60 large proportion of shared haplotypes, and a pattern of seemingly unrestricted gene flow between 61 populations. Significant morphological and genetic differentiation between some populations might reflect temporal differentiation rather than pure geographical isolation. Overall, the shallow 62 population structure of both species of *Kapentagyrus* reflects the near-panmictic population 63 structure of both host species reported in previous studies. Morphological differences related to 64 65 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 66 linked to paleohydrological events. Finally, hybridisation between species of Kapentagyrus was 67 68 found, representing the first case in dactylogyrid monogeneans.

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

#### 72 **1. Introduction**

The pelagic realm of the African Great Lakes harbours a lower species diversity than the littoral 73 74 habitat. This might be attributed to the lower number of niches and a lack of barriers to gene flow 75 (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) 76 and their latid fish predators. The two clupeids make up 65% (in mass) of the total catch in Lake 77 Tanganyika, making them a key component of the local fishery andan important factor for the 78 79 food security in the countries bordering the lake (Mölsä et al., 1999). Clupeids play an important 80 role in the food chain, because they are a link between the plankton and the piscivores (Coulter, 81 1991). Lake Tanganyika clupeids are parasitized by two species of Kapentagyrus Kmentová, Gelnar 82 & Vanhove, 2018 (Monogenea, Dactylogyridae), Kapentagyrus limnotrissae (Paperna, 1973) and Kapentagyrus tanganicanus Kmentová, Gelnar & Vanhove, 2018. Both parasite species have a 83 84 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 85 86 and S. tanganicae. In K. tanganicanus, two distinct morphotypes related to sardine species identity have been observed (Kmentová et al., 2018). 87

88 Clupeids in Lake Tanganyika are short-lived species with a lifespan of usually one year and 89 maximally three years. Other biological characteristics include schooling behaviour and a diurnal 90 vertical migration that follows that of zooplankton (Coulter, 1991; Mulimbwa and Shirakihara, 91 1994). Migration and population connectivity of clupeids in the lake are poorly understood, but 92 are thought to be linked to seasonal changes in the plankton distribution (Kurki et al., 1999; 93 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, 94 such as data storage tags (DST) and passive physical tags are no option for clupeids because of 95

96 their fragility (James et al., 1988). Hence, a combination of biological markers such as 97 morphometry, parasites, otolith elemental profiles, and molecular markers appears to be a more 98 promising approach (Svedäng et al., 2010). Lake-wide genome screening of both clupeids in Lake 99 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 100 composition of otoliths in both species (Sako et al., 2005) and a pattern of isolation by distance 101 along a north-south gradient in S. tanganicae (De Keyzer et al., 2019) pointed to restricted long-102 103 distance migration.

A combination of host- and parasite genetics has been proposed as an integrative approach to 104 reconstruct host population structure (Catalano et al., 2014) or stock structure over small 105 106 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 107 108 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 109 generation time, monogeneans may accumulate genetic changes more rapidly than their hosts 110 111 (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; 112 Nieberding and Olivieri, 2007), and therefore parasites have been proposed to act as a 113 114 "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 115 thymalli Žitňan, 1960 combined with dehydrogenase subunit 5 to indirectly infer barriers to gene 116 117 flow in grayling (*Thymallus thymallus* L.). Monogenean genetics was also used to track the 118 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). 119

120 Several steps have to be considered before using parasites as tags for host population structure, 121 including parasite species identification, the availability of more than one genetic marker to verify 122 cryptic species, and temporal stability in the presence of the parasite species across the host's 123 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 124 shown to vary along a north-south gradient (Kmentová et al., 2018), the species of Kapentagyrus 125 are proposed as candidates for unravelling the clupeids' population structure in Lake Tanganyika. 126 Moreover, several periods of draught in the past led to low lake levels and at times even 127 separation into up to four paleolakes corresponding with the current subbasins (Danley et al., 128 2012; Sturmbauer et al., 2017). Such events repeatedly caused periods of population separation 129 followed by periods of secondary admixture across the north-south gradient. These left their 130 signature in the genetics of various animal taxa (Sturmbauer et al., 2001) and influenced their 131 132 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 133 134 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 135 136 history of cichlid fishes and their respective monogenean species in a similar way (Kmentová et al., 137 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.

#### 144 **2. Material and methods**

### 145 2.1. Sampling design

146 In total, 380 monogenean individuals collected from 497 host specimens were morphologically 147 analysed in this study. We used samples listed in Kmentová et al. (2018) as well as new specimens 148 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, 149 150 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 151 152 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 153 154 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 155 156 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 157 158 genetically (see Table 1).

All host specimens were either bought at fish markets in the above-mentioned cities or caught 159 with gills nets during experimental fishing. Fishes were identified to species level in situ. Voucher 160 161 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 162 specimens were placed on a slide in a drop of glycerine ammonium picrate solution (GAP) in 1:1 163 164 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 165 monogenean species were identified as either K. limnotrissae or K. tanganicanus. Infection 166 parameters are listed in Table 1. Voucher specimens of *Kapentagyrus* spp. are deposited in the 167

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

Morphological variation on a lake wide geographical scale was inferred via both morphometric and geomorphometric approaches. Haptoral and male copulatory hardparts of the two species of *Kapentagyrus* were measured and photographed using an Olympus BX51 microscope with incorporated phase contrast at a magnification of 1000x (objective x100 immersion, ocular x10) 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

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,

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

190 Tissue Genomic DNA kit following the manufacturer's instructions. The extracted DNA was eluted191 in a volume of 50 µl.

192 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'-

195 TTTTTTGGGCATCCTGAGGTTTAT-3') (Littlewood et al., 1997) and Schisto3 (5'-

196 TAATGCATMGGAAAAAAAAAAA3') (Lockyer et al., 2003) primers in 24 μl of PCR mix (one unit of *Taq* 

197 Polymerase, 1X buffer containing 2 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.8 mM of each primer) for a total

198 reaction volume of 25 μl. It was carried out under the following conditions: initial denaturation at

199 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

200 elongation for 7 min at 72°C. The nested PCR with ASmit1 and ASmit2 (5'-

201 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

203 purified using 1  $\mu$ l of ExoSAP-IT reagent and 2.5  $\mu$ l of PCR product under the following conditions:

204 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<sup>®</sup> Cycle Sequencing Kit v3.1 (ThermoFisher Scientific),

following the manufacturer's recommendations. The fragments were cleaned up using the BigDye

207 XTerminator<sup>®</sup> Purification Kit (ThermoFisher Scientific) and visualized on an ABI 3130 capillary

208 sequencer (Applied Biosystems).

209 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.

211 *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

213 universal primer combination HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and LCO1490

214 (5'-GGTCAACAAATCATAAAGATATTGG-3') (Folmer et al., 1994). PCR reaction was performed in 24

215 μl of PCR mix (12.5μl MyTaq HS mix (2x) (Bioline, London, UK), 10.5μl H2O and 1μl primer mix 216 (20µM of each primer) to which 1 µl of purified DNA was added for a total reaction volume of 25 217 µl (Handy et al., 2011). It was carried out under the following conditions: initial denaturation at 218 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 219 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 220 221 BigDye Terminator<sup>®</sup> Cycle Sequencing Kit v3.1 (ThermoFisher Scientific), following the manufacturer's recommendations. The fragments were purified CleanDTR beads (CleanNA, GC 222 Biotech) and visualized on an ABI 3500XL capillary sequencer (Applied Biosystems). 223 DNA sequences were visually inspected and corrected using MEGA v7 (Kumar et al., 2016) and 224 aligned using MUSCLE (Edgar, 2004) under default distance measures as implemented in MEGA. 225 226 COI sequences of *Kapentagyrus* spp. are deposited in NCBI GenBank under the accession numbers 227 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 228 229 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 230 from Stolothrissa tanganicae), MK521656-MK521659 (28S rDNA portion of K. limnotrissae 231 232 individuals identified as hybrids) and MK521661-MK521664 (18S and ITS-1 rDNA portion of K. 233 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 234 235 from different localities covering all three major subbasins of Lake Tanganyika (see Table S1). 236 237 2.4. Data analysis

238

# 239 2.4.1. Morphological differentiation

240 To avoid any possible influence of ethanol fixation on the size and shape of sclerotised structures, 241 the samples were subdivided into spatial (ethanol-preserved) and spatio-temporal (fresh) data 242 sets. To evaluate intraspecific and intrahost variation, data sets were further subdivided into six 243 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 244 geomorphometric analyses of b) dorsal and c) ventral anchors. Samples in all sample sets and 245 subsequent analyses were grouped by sampling site to check for possible geographical structure in 246 247 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 248 analysed as two groups using 12 cm of host standard length (SL) as a cut off value (referred to as 249 250 Kalemie 2018 Big and Kalemie 2018 Small, respectively). As the same pattern was discovered with 251 the fresh samples from Mpulungu, a threshold in SL of host specimens was set to 10 cm (referred 252 to as Mpulungu 2018 Big and Mpulungu 2018 Small, respectively).

Morphometrics - Principal component analysis (PCA) of haptoral morphometric parameters 253 standardised to unit scale was performed in the R package adegenet (Jombart, 2008). Missing data 254 were replaced by the average value for each morphological character. To increase the resolution 255 of the resulting pattern, morphological characters with more than 50% missing data were 256 excluded prior to the analysis. Then, linear or generalised linear models were calculated in the R 257 258 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 259 statistics, respectively. In case of an overall significant effect of sampling site, post hoc Welsh's t 260 261 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 262 the analyses. 263

264 Geomorphometrics - Configurations of fixed landmarks were superimposed using Generalized Full 265 Procrustes Analyses (Cox and Cox, 1989; Zelditch et al., 2012), under the Least Squares criterion to 266 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 267 268 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 269 270 shape of both anchors, captured using fixed landmarks and semi-landmarks, was analysed using 271 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 272 with insufficient number of specimens (<6 as in the case of K. tanganicanus ex L. miodon from 273 274 Bujumbura 2018 and K. tanganicanus ex S. tanganicae from Bujumbura 2018 and Mpulungu 2018 275 in spatial sample sets) were excluded from the analyses.

276 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, 277 278 2013). This was done for each sample set and within the respective groups. All sample sets were 279 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 280 of variance among groups within each sample set was assessed by Levene's tests in the R package 281 282 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). 283

284 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).

# 290 The genealogy of the COI haplotypes for the parasites were inferred by means of a Median Joining 291 network in *PopART* v1.71 (Leigh and Bryant, 2015). Differentiation among pre-defined populations 292 was estimated by F<sub>ST</sub> in Arlequin v3.5 (Excoffier and Lischer, 2010): for K. tanganicanus collected 293 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 294 295 (AMOVA) based on F-statistics was used to test for significant population structure of K. 296 tanganicanus at the level of subbasins within Lake Tanganyika. Sample size for K. limnotrissae was 297 generally too low to allow for any meaningful population genetic analyses. 298 2.4.3. Demographic history 299 To test for signals of past population expansion in both species of monogeneans and their host 300 species, two different neutrality test statistics, Tajima's D (Tajima, 1989) and Fu's F<sub>s</sub> (Fu, 1997), were calculated in Arlequin v3.5 (Excoffier and Lischer, 2010). 301 302 The demographic history of *Kapentagyrus* spp. was further assessed by mismatch distribution 303 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 304 305 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., 306 2005), as implemented in BEAST v1.8.2. (Suchard et al., 2018). The substitution rate was set to 307 308 10% per million years, which is lower than the rates previously used for viviparous gyrodactylid 309 monogenean species characterised by asexual multiplication (Meinilä et al., 2004), and should 310 take into account the assumed comparatively longer generation time and lower reproductive capacity of Kapentagyrus as oviparous dactylogyrid monogeneans (Tinsley, 2004). Two 311

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

317 3. Results

318 3.1. Morphological variation

319 3.1.1. Kapentagyrus limnotrissae ex Limnothrissa miodon

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

Haptoral structures - PCA did not reveal any clear geographical separation based on haptoral 324 325 morphometric parameters in K. limnotrissae along any of the PC axes in any of the two sample sets (Fig. 2A&B). However, differentiation was visible between the specimens from Mpulungu 2018 326 327 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 significantly. The length of the outer root of the ventral anchor was the only morphological 329 330 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 clearer differentiation between Uvira and Bujumbura 2018 for the dorsal than for the ventral 332 333 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 ( $x_{2,57} = 9.98$ , 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 ( $x_{2,61} = 5.34$ , P = 0.02) in the spatio-temporal sample set.

#### 345 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.

350 Haptoral structures - The first PC axis of haptoral morphometric parameters revealed that

351 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

353 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

357 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 358 359 anchors, based on a fixed landmark geomorphometric approach, reflected the gradient visible in 360 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 361 362 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 363 2018, where no difference was found (see also Table S3). Moreover, the shape of the ventral 364 365 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) 366 confirmed the pattern obtained via PCA (Fig. S3). 367

*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 ( $x_{23,77} = 10.81$ , P = 0.001) in the case of spatio-temporal sample set.

## 373 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.

402 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).

410 3.3. Parasite population genetics

First, there was no evident clustering of *K. tanganicanus* according to host species (see Fig. 5A). 411 However, significant F<sub>ST</sub> value were found between K. tanganicanus infecting different host 412 413 species collected in Uvira 2016 (F<sub>ST</sub> = 0.0668; P = 0.0112). Haplotype networks indicated neither geographic, nor school-related structure in either of the monogenean species. All networks 414 415 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 FST values 416 were also recorded in K. tanganicanus ex L. miodon between several sampling sites on a temporal 417 scale (see enclosed table in Fig. 5). AMOVA calculated for K. tanganicanus ex L. miodon showed 418 419 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. 420

421 3.4. Demographic history

422 Signatures of population expansion were detected in both monogenean species and their host

423 species. Recent population growth was suggested by significantly negative values of Fu's F<sub>s</sub> in *K*.

424 *limnotrissae* (-20.98; P < 0.001), in *K. tanganicanus* (-27.90; P < 0.001), in *L. miodon* (24.09; P <

425 0.001) and in *S. tanganicae* (-61.64; P < 0.001) as well as Tajima's D in *K. limnotrissae* (-2.48; P <

426 0.001), in *K. tanganicanus* (-2.41; P < 0.001) and in *S. tanganicae* (-2.41; P < 0.001). In *L. miodon*,

427 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

431 to 17.6 KYA in *K. tanganicanus* (95% CI: 3.3–30.1 KYA).

432 Based on Bayesian Skyline Plot analysis, the start of population growth for *K. tanganicanus* was

433 estimated around 12 KYA (see Fig. 6D) and the time to the most recent common ancestor (TMRCA)

434 around 70.9 KYA (95% HDP: 15.6–143.1 KYA). Due to the insufficient number of haplotypes, BSP

435 could not track past changes of effective population size back to more than 7 KYA in the case of *K*.

436 *limnotrissae* (see Fig. 6C). No sign of population growth was observed and the TMRCA was

437 estimated at 14.4 KYA (95% HDP: 6.5–24.1 KYA).

438 3.5. Nuclear-mitochondrial discordance

439 Based on the comparison of rDNA markers published in Kmentová et al. (2018) and the obtained

440 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

442 cases, morphological vouchers are available (specimens on slides deposited under X.4.04 and

443 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.* 

### 446 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.

455 Monogeneans as tags for the geographical population structure of clupeids

The pelagic environment, promoting dispersal, and the large effective population size constrain 456 457 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 458 459 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 460 hand, population differentiation in the pelagic realm might be facilitated by the presence of 461 462 physical barriers such as currents (Podsetchine and Huttula, 2000) or geographical distance 463 (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 464 465 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). 466

Morphological variation - Based on our comprehensive study, morphometrics of monogenean 467 468 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 469 morphological character that was unambiguously specific to a particular sampling site in neither of 470 471 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 472 structures provided sufficient resolution to resolve the geographic origin of a monogenean 473 individual. Significantly different morphological characters between the sampling sites in 2016 474

were not found between the specimens from the same localities in 2018. This suggests 475 dependency of phenotypic differentiation on environmental conditions rather than fidelity to a 476 477 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), 478 pollutants (Beaumont, 1997) or other environmental factors (Cable and Harris, 2002; Olstad et al., 479 2009) influence the morphology of monogeneans. The morphological differences of *Kapentagyrus* 480 spp. observed between some of the sampling sites might hence be attributed to environmental 481 factors directly influencing the parasites' morphology or indirectly through host morphology. 482 These may induce geographical patterns via restricted host - parasite migration, or via similar 483 environmental conditions in geographically isolated locations. This might explain the clustering of 484 geographically isolated specimens of *K. tanganicanus* from Mpulungu and Kalemie (Fig. 3A). 485 486 Interestingly, spatio-temporal variation in sample sets of fresh supported the hypothesis of 487 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 488 489 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 490 mostly by wind conditions (Hecky et al., 1978; Langenberg et al., 2003). However, no experimental 491 492 data for representatives of Kapentagyrus are currently available to attribute observed 493 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 sizedependent schools (Misund, 1993). Host-size dependent intensity of infection between

Kapentagyrus spp. collected from L. miodon was observed (K. limnotrissae being more prevalent 499 500 on smaller L. miodon and vice versa, own unpublished results). Therefore, observed discrepancy in 501 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 502 different morphological characters between the groups of K. tanganicanus ex S. tanganicae was 503 lower compared to the individuals collected from L. miodon. Such a pattern might be related to 504 the difference in ecology between the host species as *S. tanganicae* displays more pelagic life style 505 compared to L. miodon (Mannini et al., 1996; Mulimbwa and Mannini, 1993). However, this result 506 might also be influenced by the small number of specimens collected from S. tanganicae. 507 508 Genetic population structure - Generally, the genetic structure of parasites is strongly connected 509 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 510 511 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 512 network of none of the three monogenean/host species combinations, we suggest geographical 513 panmixia with temporal effects in both species of monogeneans infecting Lake Tanganyika 514 clupeids. This result corresponds with the general biology of clupeids, with assumed lake-wide 515 migration patterns (De Keyzer et al., 2019; Hauser et al., 1998; Junker et al., 2019; Mulimbwa and 516 517 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 518 Zardoya, 2007; Kinsey et al., 1994) nor fish tags (Clark, 1945). Nevertheless, and despite the shared 519 520 COI haplotypes, significant genetic divergence among some of the pre-defined populations of K. tanganicanus from L. miodon was detected based on F<sub>ST</sub>. Such temporal genetic structure without 521 clear evidence for a geographical pattern could be explained by restricted migration of clupeid 522

523 hosts, random genetic drift across generations or cohorts related to overfishing of declining 524 clupeid populations in the lake (Mölsä et al., 1999) or recruitement-dependent population 525 fluctuations in r-strategic fish stocks (Watanabe et al., 1995). Interestingly, a recent study by 526 Junker et al. (2019) suggested that population structure in *L. miodon* is linked to chromosomal 527 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 528 529 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 530 depends on effective population size (Nei and Tajima, 1981). Given the observed variability in 531 prevalence and infection intensity of Kapentagyrus spp. (see Table 1), it is hard to estimate 532 population size relative to their clupeid hosts and subsequently evaluate the effect of host 533 534 population fluctuations. However, no temporal differentiation was observed in neither of the 535 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 536 537 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 538 539 dactylogyrid monogeneans (Harris, 1983; Scott and Nokes, 1984; Xiaoqin et al., 2000). Indeed, 540 multiple spawning events per year were reported for both species of Tanganyika clupeids 541 (Mulimbwa and Shirakihara, 1994). Together with their short life span, this may erase the 542 expected effect of a faster molecular evolution in parasites. Alternatively, monogenean reproduction in the pelagic habitat connected with planktonic larval dispersal might cause 543 differences in local genetic diversity of parasites. This is known as fluctuating genetic patchiness 544 545 (Hellberg et al., 2002). A similar mechanism was suggested for populations of several monogenean 546 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). 547

We need to know more about the population dynamics of the hosts and parasites to identify the 548 549 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 compared with similar data on the host species as this study is limited by the single genetic marker 551 552 being used. A promising approach to clarify the true nature of the interaction between environment, host and parasite are waterscape genomic and transcriptomic studies (Grummer et 553 al., 2019). Here, the peculiarities of the aquatic environment are taken in consideration in the 554 555 analysis of populations. Promising examples are the highly resolved population structure of welldispersing taxa (Clucas et al., 2018) and dual transcriptomic studies in host and parasite (Feis et al., 556 2018). 557

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

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

Interestingly, morphological differentiation of *K. tanganicanus*, influenced by the host species and detected in a previous study (Kmentová et al., 2018), was supported by genetic differentiation of the specimens sampled off Uvira in 2016. Our results indicate genetic differentiation of *K. tanganicanus* with respect to the clupeid host species. Most probably it happened after a recent

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 preypredator 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).

## 578 Demographic history

Haplotype structure pointed to a recent population expansion for both species of *Kapentagyrus* 579 580 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 581 582 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 583 the demographic history of monogeneans (Wang et al., 2016; Yan et al., 2016). We suggest that 584 expansion and population growth of *Kapentagyrus* spp. are linked to rising lake levels. We assume 585 586 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 587 (Koblmüller et al., 2019) and their monogenean parasite C. casuarinus (Kmentová et al., 2016). The 588 589 onset of population expansion and the time to the most recent common ancestor was estimated 590 for both species of Kapentagyrus to have been more recent than for C. casuarinus (using the same 591 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. 592

593 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 596 597 identical to other individuals of K. limnotrissae, which excludes that they are F1 hybrids of 598 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. 599 600 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 601 contemporary hybridisation. Nevertheless, as no intermediate nuclear haplotype was captured, 602 the presence of a mitochondrial genome of one species in the nuclear environment of another 603 species suggests mitochondrial introgression followed by recurrent backcrossing into the paternal 604 species. Eventually, the introgression resulted in dilution and loss of alleles inherited from the 605 maternal species (Okamoto et al., 2010). A hybridisation event would support the above-606 607 mentioned scenario of a recent host switch of K. tanganicanus followed by temporal 608 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 609 610 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 611 612 dactylogyrid monogeneans. The poor documentation of hybridisation in monogeneans might be 613 related to the lack of studies combining morphology, nuclear and mitochondrial markers. In 614 general, hybridisation is considered a major driver of evolution (Franssen et al., 2015; Hedrick, 615 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). 616

617 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 attributed to similar environmental conditions in geographically isolated sampling sites, in 623 combination with restricted host migration. Moreover, significant genetic differentiation was 624 found between some of the parasite populations. Serial sampling and genomic data should 625 increase spatio-temporal resolution to track host migration. Some evidence for incipient 626 627 speciation in K. tanganicanus according to host species was found based on mitochondrial data, despite uniformity in nuclear gene portions. Our findings provide additional support for the impact 628 of historical lake level changes also on organisms inhabiting the lake's pelagic zone. Finally, 629 630 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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Fig. 1: Sampling sites in Lake Tanganyika with an overview of significant results of morphometric 1018 1019 (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. 1020 miodon and C) K. tanganicanus ex S. tanganicae. The morphometric results are presented as the 1021 1022 number of variables which differ between the respective sampling sites (before the dash) vs the 1023 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 1024 1025 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-1026 temporal (black – fresh specimens) datasets. Map created using SimpleMappr software v7.0.0. 1027 1028 (available at http://www.simplemappr.net. Accessed January 20, 2019).



1030 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 1031 1032 indicated by arrows, ethanol-preserved specimens (fifth and seventh pair of marginal hooks 1033 excluded due to missing data, the average position for each group is indicated by a larger size of 1034 the symbol); B) PCA of haptoral measurements with the five most contributing variables indicated 1035 by arrows, fresh specimens (second to seventh pair of marginal hooks excluded due to missing 1036 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, 1037 ethanol preserved specimens; D) PCA based on Procrustes distances of eight fixed landmarks 1038

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

1048 seventh pair of marginal hooks excluded due to missing data, the average position for each group, 1049 is indicated by a larger size of the symbol); B) PCA of haptoral measurements with the five most 1050 contributing variables indicated by arrows, fresh specimens (second to seventh pair of marginal 1051 hooks excluded due to missing data, the average position for each group is indicated by a larger 1052 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 1053 1054 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 1055 preserved specimens; F) PCA based on Procrustes distances of eight fixed landmarks describing 1056 1057 the shape of the ventral anchor, ethanol preserved specimens. Consensus anchor shape for the respective analysis is shown. 1058



1059

Fig. 4: Biplots showing the variation in haptoral structures of *K. tanganicanus* collected from *S.* 1060 tanganicae in this study. Only the first two axes are shown. A) PCA of haptoral measurements 1061 1062 with the five most contributing variables indicated by arrows, ethanol-preserved specimens (fifth 1063 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 1064 1065 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 1066 1067 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.



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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

1077 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 1078 of individuals represented. Haplotypes are connected with lines, indicating the number of 1079 mutations. Small black circles indicate hypothetical haplotypes, predicted by the model. Colours 1080 represent sampling events and host species, respectively, as mentioned in the legends. Genetic 1081 differentiation among geographically pre-defined subpopulations of K. tanganicanus ex L. miodon 1082 1083 listed in the enclosed table. Pairwise F<sub>ST</sub> values and corresponding P-values are shown below and 1084 above the diagonal, respectively. Significant results with  $\alpha$ =0.05 are marked in bold. Number of 1085 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

- 1093 mismatch distribution. Bayesian Skyline plot (BSP) of C) *K. limnotrissae* based on 415 base pairs of
- 1094 COI sequences and D) *K. tanganicanus* based on 415 base pairs of COI sequences. BSPs show the
- 1095 effective populations size through time, assuming a substitution rate of 10% per site per million
- 1096 years in *Kapentagyrus* spp. The thick line represents the median values; the thin lines denote 95%
- 1097 highest posterior density (HPD) intervals. The y-axis represents the population size parameter
- 1098 (product of female effective population size, fNe, and mutation rate,  $\mu$ ).

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

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, vear)	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 mi	odon		•	· · ·
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 tang	anicae		· · · · ·	
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

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1103 Table 2: Genetic diversity indices of species of Kapentagyrus, and their hosts Limnothrissa miodon and Stolothrissa tanganicae (De Keyzer et al.,

1104 2019) inferred from the COI mtDNA region.

Species	N	н	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

1105 N sample size, H number of haplotypes, S number of polymorphic sites, Hd haplotype diversity,  $\pi$  nucleotide diversity.