Co-introduction of Dolicirroplectanum lacustre, a monogenean gill parasite of the invasive Nile perch Lates niloticus: intraspecific diversification and mitonuclear discordance in native versus introduced areas

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1   **Title**

2   Intraspecific diversification and mitonuclear discordance in native versus introduced areas: co-introduction of

3   *Dolicirroplectanum lacustre*, a monogenean gill parasite of the invasive Nile perch *Lates niloticus*

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46 **Abstract**

47 The Nile perch (*Lates niloticus*) is a notorious invasive species. The introductions of Nile perch into several lakes

48 and rivers in the Lake Victoria region led to the impoverishment of the trophic food webs, particularly well

49 documented in Lake Victoria. Along with the introductions of the Nile perch, its parasites were co-introduced.

50 *Dolicirroplectanum lacustre* (Monogenea, Diplectanidae) is a gill parasite of latid fishes (*Lates* spp.) inhabiting

51 several major African freshwater systems. We examined the intra-specific diversification of *D. lacustre* from *L.*

52 *niloticus* in Lake Albert (native range) and Lake Victoria (introduced range) by assessing morphological and

53 genetic differentiation, and microhabitat preference. We expected reduced morphological and genetic diversity

54 for *D. lacustre* in Lake Victoria compared to Lake Albert, as a result of the historical introductions.

55 *Dolicirroplectanum lacustre* displays high morphological variability within and between African freshwaters.

56 Mitonuclear discordance within the morphotypes of *D. lacustre* indicates an incomplete reproductive barrier

57 between the morphotypes. The diversification in the mitochondrial gene portion is directly linked with the

58 morphotypes, while the nuclear gene portions indicate conspecificity. Based on our results, we reported reduced

59 genetic and morphological diversity, potentially being a result of a founder effect in Lake Victoria.

60

61

62 **Keywords**

64 **Introduction**

65 The Nile perch *Lates niloticus* (Linnaeus, 1758) is considered one of the world's most invasive species by the

66 Invasive Species Specialist Group of the International Union for Conservation of Nature (IUCN) (Lowe et al.

67 2000). The introductions of the non-native Nile perch into several of the lakes and rivers in the Lake Victoria

68 region, led to the reduction of species and functional diversity, and as a result dramatically restructured the ecology

69 of the area (Pringle 2011). These introductions were particularly well documented in Lake Victoria (Ogutu-

70 Ohwayo and Hecky 1991; Reynolds et al. 1995; Pringle 2005). In the 1950s and 1960s, the Nile perch was

71 repeatedly introduced into Lake Victoria by the release of captured individuals from Lake Albert and Lake

72 Turkana (Pringle 2005). In addition to the introductions of non-native tilapias, eutrophication, and overfishing,

73 the introductions of predatory Nile perch lead to the depletion of most, and the extinction of some endemic fish

74 species, causing impoverishment of the trophic food webs (Ogutu-Ohwayo and Hecky 1991; Seehausen et al.

75 1997; Goudswaard et al. 2006; van Zwieten et al. 2016). The introductions transformed the local artisanal fishery

76 in Lake Victoria into a global industrial fishery, with dramatic consequences for the local livelihoods (Pringle

77 2011).

78 Following Pringle (2005), the source population of Nile perch is located in Lake Albert. In Lake Albert, *L.

79 niloticus* occurs in sympatry with *Lates macrophthalmus* Worthington, 1929. While *L. niloticus* is larger and

80 mainly occurs inshore, *L. macrophthalmus* is a smaller, offshore species (Holden 1967). The limited genetic

81 evidence based on allozymes suggests that these are indeed distinct species, and that Nile perch in Lake Victoria

82 are largely represented by individuals of *L. niloticus* from Lake Albert, but the presence of *L. macrophthalmus* in

83 Lake Victoria cannot be excluded (Hauser et al. 2022).

84 The threats of alien species have been highlighted many times, but the co-introduction of their possibly co-invasive

85 parasites deserves more attention, as its consequences are usually underestimated (Peeler et al. 2004; Lymbery et

86 al. 2014; Kmentová et al. 2018; Šimková et al. 2018). *Co-introduced* parasites are parasites that have been

87 transported with an alien host to a new locality outside their natural range. There, they become established by

88 survival, reproduction and dispersal within the alien hosts. In case parasites were transported but have not

89 established in the introduced range, it is unlikely they are ever recorded (Lymbery et al. 2022). *Co-invasive*

90 parasites are those that have been co-introduced, and then have spread to new, native hosts (Lymbery et al. 2014),

91 so-called spill-over event(s) (Prenter et al. 2004; Goedknegt et al. 2016, 2017). The invasive potential, and success

92 of parasite establishment is affected by several ecological factors, such as the size of the founder population of

93 the host and consequently that of the parasite (Anderson and May 1991; Sakai et al. 2001; Dlugosch and Parker

4

94   2008), the parasite's life cycle, and environmental biotic and abiotic conditions (Taraschewski 2006; Lymbery et

95   al. 2014). Hence, co-invasion is not a straightforward consequence of co-introduction. Along with the

96   introductions of the Nile perch, some of its parasites were co-introduced, more specifically, the monogenean

97   (Platyhelminthes, Monogenea) parasite *Dolicirroplectanum lacustre* (Thurston & Paperna, 1969), and possibly,

98   the copepods *Ergasilus kandti* van Douwe, 1912 and *Dolops ranarum* (Stuhlmann, 1892) (Thurston and Paperna

99   1969), the myxosporean *H. ghaffari* Ali, 1999, and the nematodes *Contracaecum multipapillatum* (Drasche, 1882)

100   and *Cucullanus* spp. Müller, 1777 (Outa et al. 2021).

101   Monogeneans are parasitic flatworms that can be identified morphologically by their hard parts: the attachment

102   organ (haptor) and the male copulatory organ (MCO) (Woo 2006). These ectoparasites have a direct life cycle in

103   which short-living, ciliated larvae (oncomiracidia) hatch from eggs and attach to their single host, presumably

104   synchronised with host behaviour (Kearn 1973). More specifically, members of Diplectanidae metamorphose on

105   their single host into an adult with a functional male reproductive system, and later a fully mature hermaphrodite

106   that delivers eggs after cross-fertilisation (Whittington et al. 1999). Monogeneans usually exhibit high host

107   specificity (i.e., restricted to one particular host group or host species) (Odening 1976). Exceptions have been

108   recorded, such as *Neobenedenia melleni* (MacCallum, 1927), a pathogenic species which has the broadest host

109   specificity of all monogenean species, recorded from over 100 host species (Bullard et al. 2000; Whittington and

110   Horton 2022).

111   Various speciation processes (e.g., co-speciation and intrahost speciation) are proposed to affect monogenean

112   diversity (Šimková et al. 2004; Vanhove et al. 2015). Parasites tend to diversify faster than their hosts due to their

113   short generation times and faster mutation rates (Nieberding et al. 2004; Nieberding and Olivieri 2007). This

114   should be especially apparent in comparison with long-lived and large hosts, as would be expected for *D. lacustre*

115   from its latid hosts (Kmentová et al. 2020a). The study of parasite population structure may increase the resolution

116   for understanding host population structure, phylogeography (Nieberding et al. 2004), and introduction pathways

117   (Ondračková et al. 2012). Following (Buchmann and Lindenstrøm 2002), host species selection is a consequence

118   of dynamic interactions between the parasite and its host over time. Selection acts on the scale of microhabitats

119   on the gills of a single host species, through differential microhabitat preferences (Koskivaara et al. 1992;

120   Buchmann and Uldal 1997; Raymond et al. 2006). Purportedly, the morphology of the attachment organs is an

121   important adaptation to the monogenean's host, and to specific microhabitats within the host, since these structures

122   could influence the ability of the parasite to infect, feed, and reproduce on a specific host species (Šimková et al.

123   2002).

124 *Dolicirroplectanum lacustre* (Monogenea, Diplectanidae) is the single known monogenean gill parasite of four

125 species of lates perches (Perciformes, Latidae) in African freshwaters (*L. niloticus, Lates microlepis* Boulenger,

126 1898*, Lates angustifrons* Boulenger, 1906*,* and *Lates mariae* Steindachner, 1909) that have been examined for

127 parasites (Kmentová et al. 2020a). *Dolicirroplectanum lacustre* displays a high morphological variation: a "wide

128 range of shapes and sizes" (Thurston and Paperna 1969). In the original description of *D. lacustre*, Thurston &

129 Paperna (1969) noted these differences and identified a so-called 'slender form', with a well delineated haptor at

130 the posterior end of the body, and a usually longer 'gravid form' which is proportionally wider and has an

131 embedded haptor. In accordance with Thurston & Paperna (1969), Kmentová et al. (2020) reported distinct

132 morphotypes of *D. lacustre* in Lake Albert and continuous variation across several African freshwater systems.

133 Although *D. lacustre* showed high morphological variation, the genetic differentiation between parasites of

134 allopatric host species was not as high as typically associated with distinct diplectanid species (Kmentová et al.

135 2020a).

136 In this study, we investigate the fine-scale morphological and genetic differentiation of *D. lacustre* from Nile

137 perch in its native (Lake Albert) and introduced range (Lake Victoria). We hypothesise that: (1) a founder effect

138 had taken place in Lake Victoria. Hence, we expect reduced genetic and morphological diversity within *D.*

139 *lacustre* in its introduced range in comparison with its native population in Lake Albert; (2) we expect high

140 phenotypic variation and low genetic differentiation in Lake Albert, as supported by the presence of distinct

141 morphotypes in earlier studies (Thurston and Paperna 1969; Kmentová et al. 2020a); (3) there has been a niche

142 shift among the populations in Lake Albert that resulted in differential gill microhabitat preferences which led to

143 morphological changes of *D. lacustre*, producing an (imperfect) reproductive barrier between the morphotypes.

144

145 **Materials and methods**

146 **Sampling of the Nile perch and its gill parasites**

147 Fish samples of *L. niloticus* from Lake Victoria and Lake Albert were examined (Table 1). Fresh specimens were

148 obtained from local fishermen in Kaiso, Uganda (Lake Albert) and Kikondo, Uganda (Lake Victoria) during a

149 field expedition in 2019 (Figure 1). In total, the gills of 15 fish specimens (8 samples from Lake Albert, 7 samples

150 from Lake Victoria) were dissected and preserved in ethanol (99% EtOH). The pairs of gills from 12 randomly

151 chosen specimens (5 specimens from Lake Albert, 7 specimens from Lake Victoria) were examined following the

152 standard protocol of Ergens and Lom (1970) using a Leica EZ4 stereomicroscope. Monogenean gill parasites were

153 extracted, some of the individuals were cut in three parts with the posterior and anterior parts mounted on slides
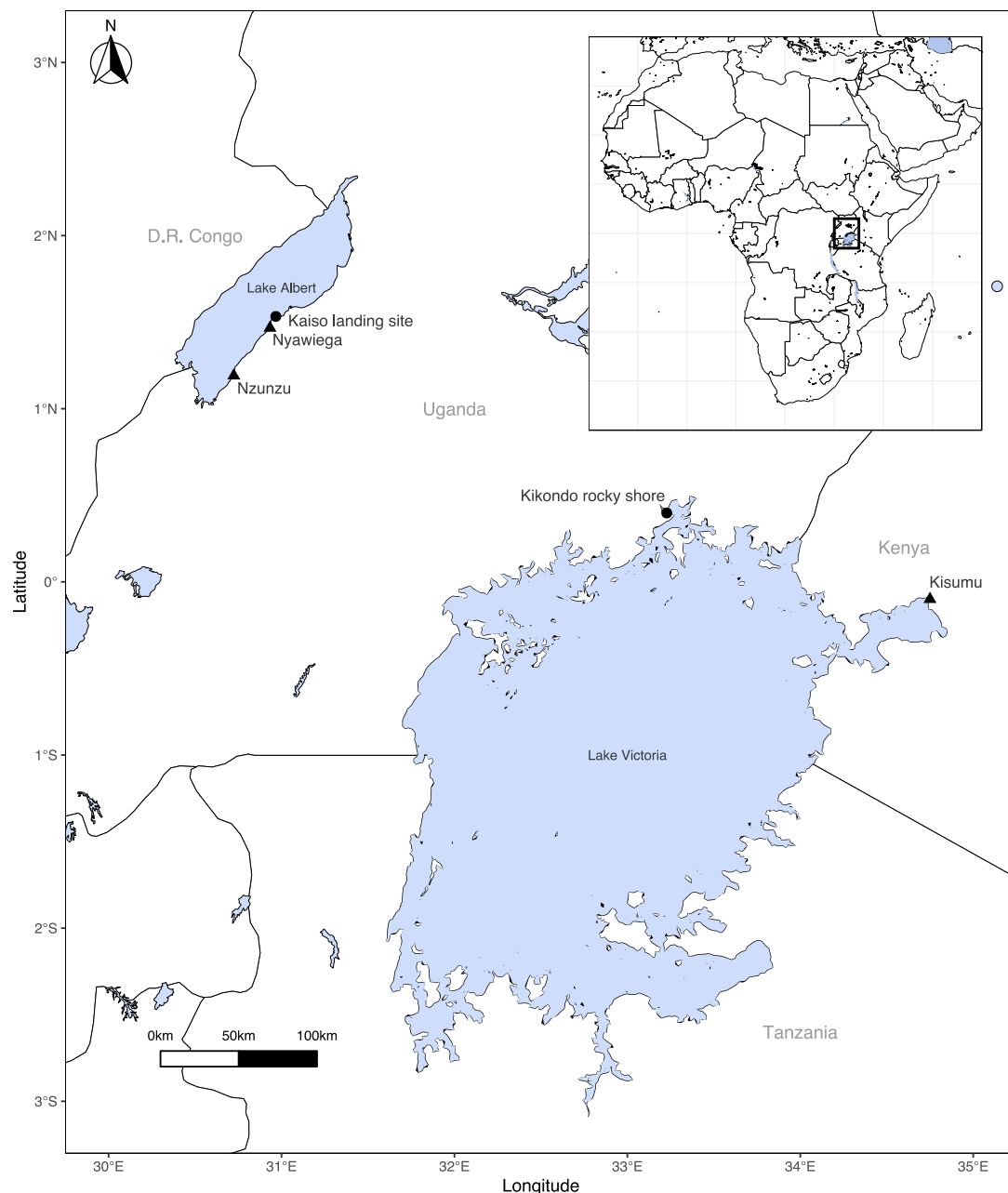
154

Fig. 1 Sampling localities of *Lates niloticus* at Lake Albert and Lake Victoria: Kaiso landing site, Lake Albert, Uganda (N 00°23'52.0", E 33°13'39.6") (n = 8) and Kikondo rocky shore, Lake Victoria, Uganda (N 01°31'59.3", E 30°58'00.9") (n = 7). Sampled localities from Kmentová et al. (2020a) depicted by triangles: Kisumu, Lake Victoria, Kenya (00°06'S, 34°45'E; n = 3) and Nzunzu, Lake Albert, Uganda (N 01°11'27.6", E 30°43'26.4"; n = 12) and Nyawiega, Lake Albert (01°28'N, 30°56'E; n = 3). We used the geographical databases 'Global Lakes and Wetlands Database' (WWF, Lehner and Dooll) and the 'High Resolution World Vector Map' from package rnaturalearth (South 2021) to map the lakes, and countries respectively. The map was created using the packages sf (Pebesma 2018), ggspatial (Dunnington 2021) and ggplot 2 (Wickham 2016) in R Studio v 1.4.1106 (RStudio Team 2020).

7

164   (using Hoyer's medium as fixative) for morphological characterisation, and the central part preserved in ethanol

165   (99% EtOH) for genetic analyses. In addition, several individuals were mounted on slides as entire specimens and

166   fixed (Hoyer's medium). Whole mounted parasite specimens were drawn by group according to locality and

167   morphotype (Thurston & Paperna 1969): Lake Albert (gravid), Lake Albert (slender), Lake Victoria using a Leica

168   DM2500 optical microscope at a magnification of 1000x (objective x100 1.32 oil XHC PL FLUOTAR, ocular

169   x10) and a reMarkable® graphical tablet. Drawings were edited in Adobe Photoshop® 2021 (v 22.3.1).

170   Infection parameters prevalence (P) and mean infection intensity (MI) were calculated following Bush et al.

171   (1997). Prevalence was determined as the proportion of host specimens infected with *D. lacustre*. The mean

172   infection intensity was calculated as the ratio of the total number of parasite specimens of *D. lacustre* to the total

173   number of host specimens infected by *D. lacustre*. During extraction of the parasites, the microhabitat of each

174   parasite specimen on the gill was recorded by subdivision of each gill arch into nine microhabitats. Following

175   Gobbin et al. (2021) each gill arch was subdivided along the longitudinal axis (dorsal, median, ventral) and

176   transversal axis (proximal, central, distal; from gill bar to tips of gill filaments).

177

178   *Morphometrics of the parasites*

179   Measurements of the hard parts, total body size and distances between the two pairs of eyespots were obtained

180   from 71 specimens at a magnification of 1000x (objective x100/1.32 oil XHC PL FLUOTAR, ocular x10) with

181   differential interference contrast on a Leica DM2500 optical microscope using Las X software v3.6.0.20104. A

182   total of 20 parameters of the hard parts of the haptor and MCO were measured (Figure 2). The terminology of

183   (Justine and Henry 2010) was followed. To investigate morphological differentiation in haptor morphology

184   between localities and morphotypes, raw haptor measurements were analysed by multivariate statistical

185   techniques in RStudio® (R Core Team 2020). A Principal Component Analysis (PCA) was performed with 23

186   measurements from 56 individuals (combined with measurements of 22 individuals from Kmentová et al. (2020))

187   using the package FactoMineR (Lê et al. 2008). Individuals were selected for the PCA when more than 50% of

188   the measured characteristics were obtained. Missing values for measurements were imputed by the variable mean

189   as default in the package factoextra (Kassambara and Mundt 2017). Results were visualised using the packages

190   ggplot2 (Wickham, 2016), reshape2 (Wickham, 2007), factoextra (Kassambara and Mundt 2017), tidyr (Wickham

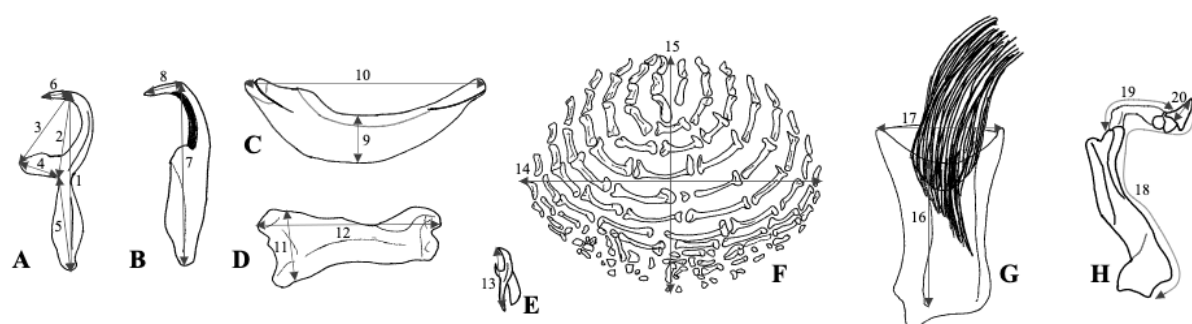191   et al. 2021), and MASS (Venables & Ripley 2002).

192

Fig. 2 Measurements for sclerotized structures of haptor and reproductive organs of *Dolicirroplectanum lacustre*: A, Ventral anchor: 1, total length; 2, length to notch; 3, length to inner root, 4, inner root length, 5, outer root length, 6, point length; B - Dorsal anchor: 7, total length, 8, point length; C - Ventral bar: 9, maximum width, 10, straight length; D - Dorsal bar: 11, maximum width, 12, straight length; E - Hook: 13, hook length; F - Squamodisc: 14, squamodisc width; 15, squamodisc length; G - Male copulatory organ: 16, copulatory tube length; 17, copulatory tube width; H - Vagina: 18, total length; 19, tube length; 20, point length. Structures drawn of *D. lacustre* from host specimen HP4318 from Lake Victoria.

To investigate the relationship between groups per lake and morphotype, and level of differentiation in the measurements of the MCO, parametric tests were applied. Analysis of Variance (ANOVA) was applied for overall effects; for pairwise comparisons, unpaired t-tests were applied, implemented in the package stats (R Core Team 2020). Assumptions of normality and homogeneity of variance were tested by Shapiro-Wilk's W tests from the package stats (R Core Team 2020) and Levene's tests from the car package (Fox and Weisberg 2019), respectively, as well as by graphical examination of the residuals.

Molecular characterisation of the parasites

Whole genomic DNA was extracted from the central part of 64 monogenean parasite individuals (20 from Lake Victoria, 44 from Lake Albert) that were preserved in 99% ethanol using the following protocol. The sample was spinned down and ethanol was removed. 195 µL of TNES buffer (400 mM NaCl, 20 mM EDTA, 50 mM Tris (pH 8, 0.5% SDS)), and 5 µL of proteinase *K* (20 mg/mL) were added to the sample. After 45 min of incubation at 55 °C, 2 µL of Invitrogen™ yeast RNA (10 mg/mL) was added as a carrier. Next, 65 µL of NaCl (5 M) and 290 µL of 96% ethanol were added, and the sample was cooled for 60 min at -20 °C. The sample was spinned down for 15 min at 18,000 rcf to a small pellet. Supernatant was removed and substituted by 1 mL of chilled 70% ethanol.

218   Next, the samples were centrifuged for 5 min at 18,000 rcf. The ethanol rinse step (removal of supernatant,

219   addition of ethanol and centrifugation) was repeated once again. The ethanol was removed, and the DNA was

220   eluted in 100 µL of 0.1X TE (0.02 % tween-20). The DNA extract was placed at 4 °C for resuspension overnight,

221   and stored at a temperature of -20°C.

222   Sequences of a portion of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene, and nuclear gene portions

223   from the small and large ribosomal subunit gene (18S rDNA and 28S rDNA) and internal transcriber spacer 1

224   (ITS-1) were obtained following PCR. The three nuclear markers evolve at different rates and are suitable to

225   assess genetic divergence at the interspecific level (Vanhove et al. 2013). COI is the current standard marker for

226   intraspecific genetic differentiation in many monogenean taxa (Kmentová et al. 2020b) due to its relatively high

227   rate of molecular evolution in comparison to rDNA. Part of the mitochondrial COI gene was amplified using

228   ASmit1 (5'-TTTTTTGGGCATCCTGAGGTTTAT-3') (Littlewood et al. 1997) combined with Schisto3 (5′-

229   TAATGCATMGGAAAAAAAACA-3′)   (Lockyer   et   al.   2003),   and   with   Asmit2   (5′-

230   TAAAGAAAGAACATAATGAAAATG-3′) (Littlewood et al. 1997) in a nested PCR following Vanhove et al.

231   (2015). For both primer combinations, the amplification reaction contained 24 µL of PCR mix (0.2 µL of 1 unit

232   Invitrogen Taq polymerase, 2.5 µL Invitrogen PCR buffer, 2 µL of 2 mM MgCl$_2$, 0.5 µL of 0.2 mM dNTPs, 2µL

233   of 0.8 µM of each primer, 14.80 µL of dd H$_2$O) with 1 µL of isolated DNA (concentration was not measured) in

234   a total reaction volume of 25 µL and was performed under the following conditions for the first reaction: initial

235   denaturation at 95 °C for 5 min, 40 cycles of 1 min at 94 °C, 1 min at 44 °C and 1 min at 72 °C, and a final 7 min

236   at 72 °C. The second (nested) reaction was performed under the following conditions: 5 min at 95 °C, 40 cycles

237   of 1 min at 94 °C, 1 min at 50 °C and 1 min at 72 °C, and a final 7 min at 72 °C. Primers C1 (5′-

238   ACCCGCTGAATTTAAGCAT-3′) and D2 (5′-TGGTCCGTGTTTCAAGAC-3′) (Hassouna et al. 1984) were

239   used for amplification of the partial 28S rDNA gene. Each PCR reaction contained 1 unit of Q5® Hot Start High-

240   Fidelity DNA Polymerase, 1X PCR buffer containing 0.1 mg/mL bovine serum albumin (BSA), 0.2 mM dNTPs,

241   0.5 µM of each primer and 2 µL of isolated DNA (concentration not measured) in a total reaction volume of 25

242   µL. The reaction proceeded under the following conditions: 2 min at 95 °C, 39 cycles of 20 s at 94 °C, 30 s at 65

243   °C, 1 min 30 s at 72 °C, and 10 min. at 72 °C. Partial 18S rDNA and ITS-1 were amplified using the S1 (5′-

244   ATTCCGATAACGAACGAGACT-3′) (Sinnappah et al. 2001) and IR8 (5′-GCTAGCTGCGTTCTTCATCGA-

245   3′) (Šimková et al. 2003) primers. Each reaction contained 1 unit of Q5® Hot Start High-Fidelity DNA

246   Polymerase, 1X PCR buffer containing 0.1 mg/mL BSA, 0.2 mM dNTPs, 0.5 µM of each primer and 2 µL of

247   isolated DNA (concentration not measured) in a total reaction volume of 25 µL under the following conditions: 2

248   min at 95 °C, 39 cycles of 1 min at 94 °C, 1 min at 64 °C and 1 min 30 s at 72 °C, and a final elongation of 10

249   min at 72 °C. PCR amplification success was verified by agarose gel electrophoresis. A volume of 10 μl of the

250   PCR product was enzymatically purified for positive samples, using 4 μL of ExoSAP-IT reagent under the

251   following conditions: 15 min at a temperature of 37 °C, and 15 min at 80 °C. Purified PCR products were sent out

252   to Macrogen Europe B.V. for Sanger sequencing; amplification primers were used for sequencing reactions. The

253   acquired sequences for each marker were visually inspected and trimmed using the software Geneious v2021.1.1.

254   Sequences were aligned with previously published sequences of *D. lacustre* from Kmentová et al. (2020a) using

255   MUSCLE (Edgar 2004) under default settings as implemented in Geneious v2021.1.1.

256

257   *Genetic differentiation and phylogeography*

258   Per marker, uncorrected pairwise genetic distances (p-distances) among sequences were computed in Geneious

259   Prime v2021.1.1. Already available sequences of the four genetic markers of *D. lacustre* from Lake Albert

260   (GenBank accession numbers: MK908145.1-MK908196.1, MK937576.1, MK937579.1-MK937581.1,

261   MK937574.1, MK937575.1) were included in the analyses. Haplotype networks were constructed using Median

262   Joining networks following Bandelt et al. (1999) in PopART v1.7104 (Leigh and Bryant 2015) for each marker

263   separately. The analyses of population structure and demographic history within *D. lacustre* were based on a 325

264   bp fragment of COI. This allows for the detection of recent evolutionary events, such as possible incipient

265   speciation as a result of host preference (Kmentová et al. 2016). Genetic diversity of COI was assessed by the

266   number of haplotypes and polymorphic sites, haplotype diversity (h) and nucleotide diversity (π), calculated in

267   Arlequin v3.5.2.2 (Excoffier and Lischer 2010). Differentiation among pre-defined populations (according to

268   locality and morphotype) was estimated by F_{ST}. Analysis of Molecular Variance (AMOVA) based on F-statistics

269   was applied to test for a significant population structure of *D. lacustre* at the level of locality (non-native and

270   native). To test for signals of past population expansion, two different neutrality tests, Tajima's D and Fu's F_S

271   were calculated in Arlequin v3.5.2.2 (Excoffier and Lischer 2010) for the two hypothetical populations. First, the

272   neutrality tests were calculated for the specimens from Lake Albert. Second, the Lake Victoria individuals were

273   added.

274

275   *Microhabitat description*

276   Parasite microhabitats were visualised by the combination of the spatial distribution of *D. lacustre* on each pair

277   of gills (left and right gills per host individual) into a single spatial distribution. Per lake/morphotype, the spatial

278   distribution of all individuals was summarised in a heat map using the geom_tile function as part of the ggplot2

279   package (Wickham, 2016). As replicates per population were limited, no statistical effects could be verified.

280

281   **Results**

282   A total of 124 monogenean gill parasites were retrieved from the 8 host specimens examined in this study (Table

283   1). The parasite specimens were morphologically identified as *Dolicirroplectanum lacustre*. Diplectanid-specific

284   haptoral characteristics included the presence of ventral and dorsal squamodiscs, and three transversal bars

285   connected to pairs of ventral hooks and dorsal hooks. *Dolicirroplectanum lacustre* can be distinguished from other

286   diplectanids by the observed characteristics: a longer outer root in the ventral anchors (Thurston and Paperna

287   1969); a wide, robust and barrel-shaped MCO formed by two anteriorly oriented nested tubes, one encasing the

288   other (Kmentová et al. 2020a); the presence of an accessory piece; squamodiscs composed of variable concentric

289   rows of bone shaped rodlets forming open rings; reduced inner roots of the ventral anchor. Additional

290   characteristics include: a simple prostatic reservoir; seminal vesicle as an expansion of vas deferens; intercaecal,

291   pre-testicular ovary encircles right caecum (Thurston and Paperna 1969; Kmentová et al. 2020a). The dorsal bars

292   are large, bone-shaped structures with a broad base pointing towards the centre of the haptor. The ventral bar has

293   an elongated, oval shape with tapering ends. A sclerotized vagina was observed in 29 out of the 71 measured

294   specimens. Additional observed characteristics include two pairs of eyespots, of which the posterior eyespots are

295   larger and closer together, a thin tegument covering the body, and 14 rudimentary hooklets of equal sizes.

296   Infection parameters varied per location, with greater prevalence (P) and mean infection intensity (MI) in Lake

297   Albert compared to Lake Victoria (Table 1). We observed co-infections (infection by both morphotypes of *D.*

298   *lacustre* on a single host individual of *L. niloticus*) of the morphotypes on two host individuals in Lake Albert

299   (HP4094, HPAlb.X).

300

301   **Table 1** Overview of host species examined for monogenean parasites and infection parameters per locality.

Locality (Geographic coordinates, year)	Number of fish specimens examined	Number of infected fish specimens	Number of monogenean individuals	P/MI
Kaiso landing site, Lake Albert, Uganda (N 0°23'52.0", E 33°13'39.6")	5	5	90	1/18
Kikondo rocky shore, Lake Victoria, Uganda (N 1°31'59.3", E 30°58'00.9")	7	3	34	0.43/11.3

302   P, prevalence; MI, Mean infection intensity

303

304   *Morphotypes and morphometric variation*

305   During the screening procedure, clear size differences in total body length and haptoral sclerotised structures were

306   apparent between individuals of *D. lacustre* from Lake Albert. Therefore, we classified the specimens from Lake

307   Albert under the gravid morphotype (n=9) and slender morphotype (n=42) following Thurston and Paperna

308   (1969). Specimens of the gravid morphotype (Fig. 3a) were identified by the following haptoral features:

309   proportionally larger body, longer ventral anchors with a longer outer root, longer dorsal anchors with a shorter

310   tip, long and narrow ventral and dorsal bars, larger ventral and dorsal squamodiscs (Table 2). The MCO for gravid

311   individuals was longer, as was the vagina (Table 2). Also, the distance between the eyespots of both pairs was

312   larger in the gravid morphotype. Conversely, specimens of the slender morphotype (Fig. 3b) were identified by a

313   proportionally smaller body, shorter ventral anchors with a shorter outer root, shorter dorsal anchors with a longer

314   tip, wider but shorter ventral and dorsal bars, and smaller squamodiscs. The copulatory tube for the slender

315   morphotype was usually shorter, as was the vagina total length and tube length (Table 2; Table S1). The

316   individuals retrieved from Lake Victoria had sclerotized structures of the slender morphotype (Fig. 3c). However,

317   these individuals were smaller in total body size, they had shorter ventral and dorsal anchors, narrower ventral

318   and dorsal bars, and squamodiscs were less wide in comparison with both morphotypes in Lake Albert. The

319   specimens from Lake Victoria had a shorter copulatory tube and vagina in comparison with the specimens in Lake

320   Albert (Table 2).

321   Variation in morphology of the measured characteristics was summarised in a biplot through PCA of 17

322   parameters (Table 2; Fig. 4). The PCA biplot (Fig. 4a) depicts the variation in morphometric measurements where

323   PC1 explains 46.67%, and PC2 explains 14.79% of the variation with main contributors: distance between the

324   eyespots of the larger pair, total body width, total length of the dorsal anchor, maximum width of the ventral bar,

325   and straight length of the ventral bar. The density plot of PC1 scores (Fig. 4b), in accordance with the biplot,

326   indicates a clearly distinctive morphometry in haptoral structures between two morphotypes present in Lake

327   Albert. The morphometry of the slender morphotype coincides with the morphometry of the specimens from Lake

328   Victoria (Fig. 4b). We observed similar morphometric variation for the slender morphotype from Lake Albert and

329   the specimens from Lake Victoria. The variation was larger for the gravid morphotype, although this could be an

330   artefact of sample size in Fig. 4 (since the 95 % C.I. is larger when less observations are included for the ellipse).

331   The separation of the gravid morphotype along the PC1 axis from the two other groups can be explained by larger

332   distance between the eyespots of the larger pair, a wider body, and a narrower ventral bar (Fig. 4a, Table 2).

Table 2. Overview of morphometric measurements performed on haptoral and genital sclerotised structures of *Dolicirroplectanum lacustre*. Values depict the average measurements ± the standard deviations; min. - max. values, and *n*, the number of individuals.

Parameters (µm)	*L. niloticus*, Lake Victoria	*L. niloticus*, Lake Albert (slender morphotype)	*L. niloticus*, Lake Albert (gravid morphotype)
Total length	533.4 ± 47.0 (475.9–603.7); n=6	776.6 ± 97.1 (601.3–899.3); n=8	1384.0; n=1
Total width	298.6 ± 61.1 (222.2–417.4); n=12	361.6 ± 91.7 (214.6–555.4); n=24	947.8 ± 129.5 (843.7–1245.0); n=8
Ventral anchor			
Total length	47.8 ± 2.4 (44.2–51.6); n=26	51.1 ± 3.8 (45.4–59.3); n=25	63.2 ± 4.0 (58.9–69.1); n=8
Length to notch	22.2 ± 0.74 (20.6–23.5); n=26	21.1 ± 1.8 (17.9–24.5); n=25	24.5 ± 0.8 (23.0–25.7); n=8
Length to inner root	25.4 ± 1.2 (22.4–27.8); n=26	25.5 ± 1.3 (23.1–28.8); n=25	28.8 ± 1.3 (26.5–30.4); n=8
Inner root length	12.4 ± 0.9 (11.2–14.8); n=26	12.8 ± 0.7 (11.1–14.0); n=25	14.9 ± 0.9 (13.2–16.0); n=8
Outer root length	28.5 ± 2.2 (22.5–32.9); n=26	31.7 ± 3.3 (26.03–37.6); n=25	40.9 ± 4.1 (36.5–46.5); n=8
Point length	9.8 ± 0.9 (8.1–11.9); n=26	9.5 ± 0.7 (7.9–11.0); n=25	10.6 ± 1.3 (8.5–12.0); n=8
Dorsal anchor			
Total length	42.3 ± 2.1 (37.2–44.9); n=26	45.0 ± 2.6 (39.5–52.6); n=25	56.5 ± 3.1 (50.4–59.7); n=8
Point length	8.6 ± 1.3 (4.0–10.5); n=26	9.3 ± 0.9 (7.8–10.5); n=25	6.5 ± 0.8 (5.2–8.0); n=8
Ventral bar			
Straight length	65.7 ± 5.2 (55.7–74.1); n=24	68.9 ± 11.7 (37.4–94.6); n=25	80.4 ± 10.0 (66.9–95.2); n=8
Maximum width	16.2 ± 1.5 (11.7–18.1); n=24	16.8 ± 2.3 (11.4–19.5); n=25	6.1 ± 0.6 (5.5–6.8); n=8
Dorsal bar			
Straight length	44.8 ± 3.9 (33.1–52.5); n=26	51.6 ± 6.1 (34.1–61.5); n=25	56.4 ± 6.6 (45.3–61.7); n=8
Maximum width	16.9 ± 2.1 (13.2–23.0); n=26	17.6 ± 2.7 (11.0–22.3); n=25	11.4 ± 2.6 (6.7–14.2); n=8
Ventral squamodisc			
Width	69.3 ± 13.5 (51.0–102.2); n=23	80.4 ± 18.9 (50.1–125.4); n=19	115.9 ± 29.8 (83.9–156.1); n=8
Length	63.5 ± 11.9 (43.6–78.9); n=23	57.1 ± 14.1 (28.4–86.9); n=19	108.7 ± 39.5 (69.5–191.0); n=8
Dorsal squamodisc			
Width	59.2 ± 11.8 (39.5–89.1); n=23	69.1 ± 17.7 (34.2–102.5); n=22	99.3 ± 27.7 (55.3–135.4); n=8
Length	55.5 ± 12.8 (35.9–76.9); n=23	57.0 ± 14.6 (26.7–84.2); n=18	71.6 ± 14.1 (58.7–103.3); n=8
Hook			
Length	11.9 ± 1.2 (10.5–16.6); n=24	11.0 ± 1.1 (8.4–13.5); n=24	13.3 ± 0.6 (12.1–14.0); n=8
Male copulatory organ (MCO)			
Straight length	52.2 ± 13.3 (32.0–70.7); n=14	64.0 ± 10.2 (46.9–88.2); n=27	91.5 ± 7.8 (77.2–100.6); n=7
Straight width	27.4 ± 7.8 (12.4–40.8); n=16	36.1 ± 5.6 (19.2–44.9); n=27	40.4 ± 3.8 (34.9–45.1); n=7
Vagina			
Total length	36.3 ± 3.0 (32.9–41.6); n=6	43.2 ± 8.2 (32.3–57.8); n=18	75.1 ± 18.5 (54.3–97.8); n=4
Tube length	5.8 ± 1.1 (3.7–7.1); n=7	8.5 ± 1.1 (7.2–11.4); n=17	17.3 ± 8.3 (7.9–26.8); n=4
Point length	7.6 ± 2.0 (3.9–10.4); n=8	8.3 ± 1.6 (6.4–11.6); n=19	11.6 ± 2.0 (10.1–14.5); n=4
Eyespots			
Smaller pair distance	38.2 ± 9.3 (16.9–49.5); n=28	44.8 ± 10.6 (18.6–66.0); n=26	95.4 ± 15.6 (67.1–111.6); n=7
Larger pair distance	20.2 ± 6.7 (6.1–33.1); n=31	27.4 ± 10.9 (13.3–65.9); n=28	93.3 ± 15.9 (64.8–113.7); n=8

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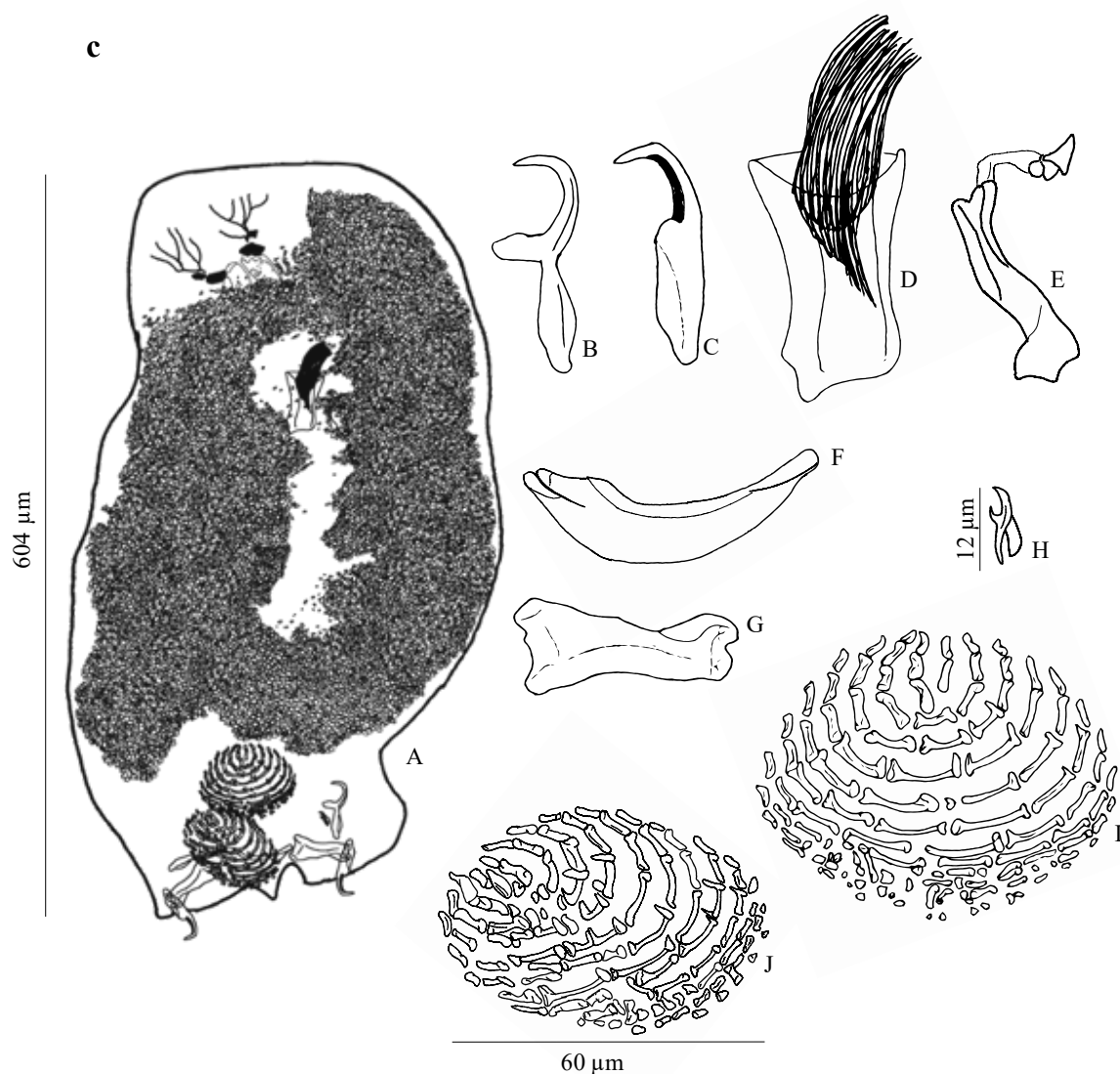335

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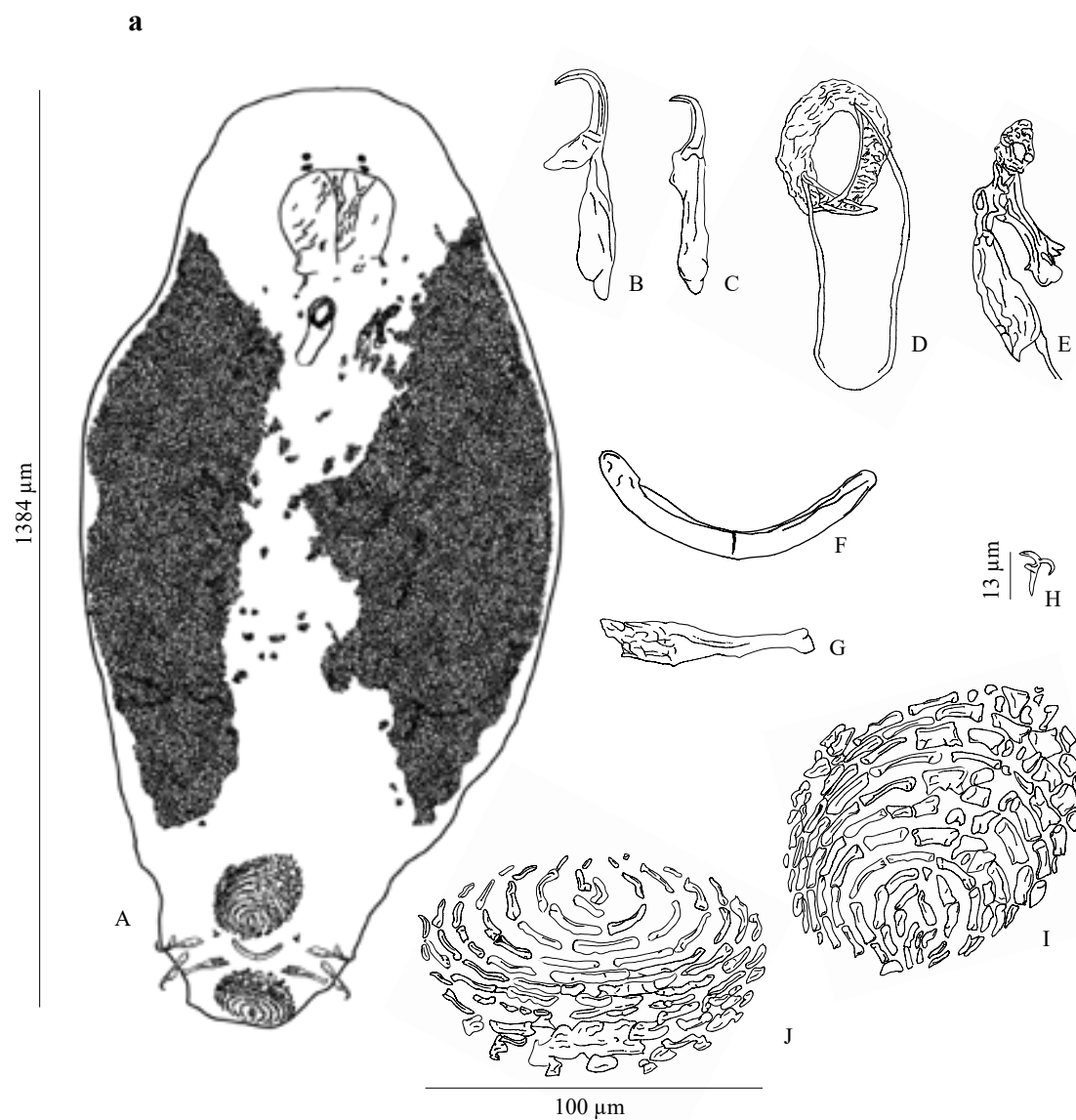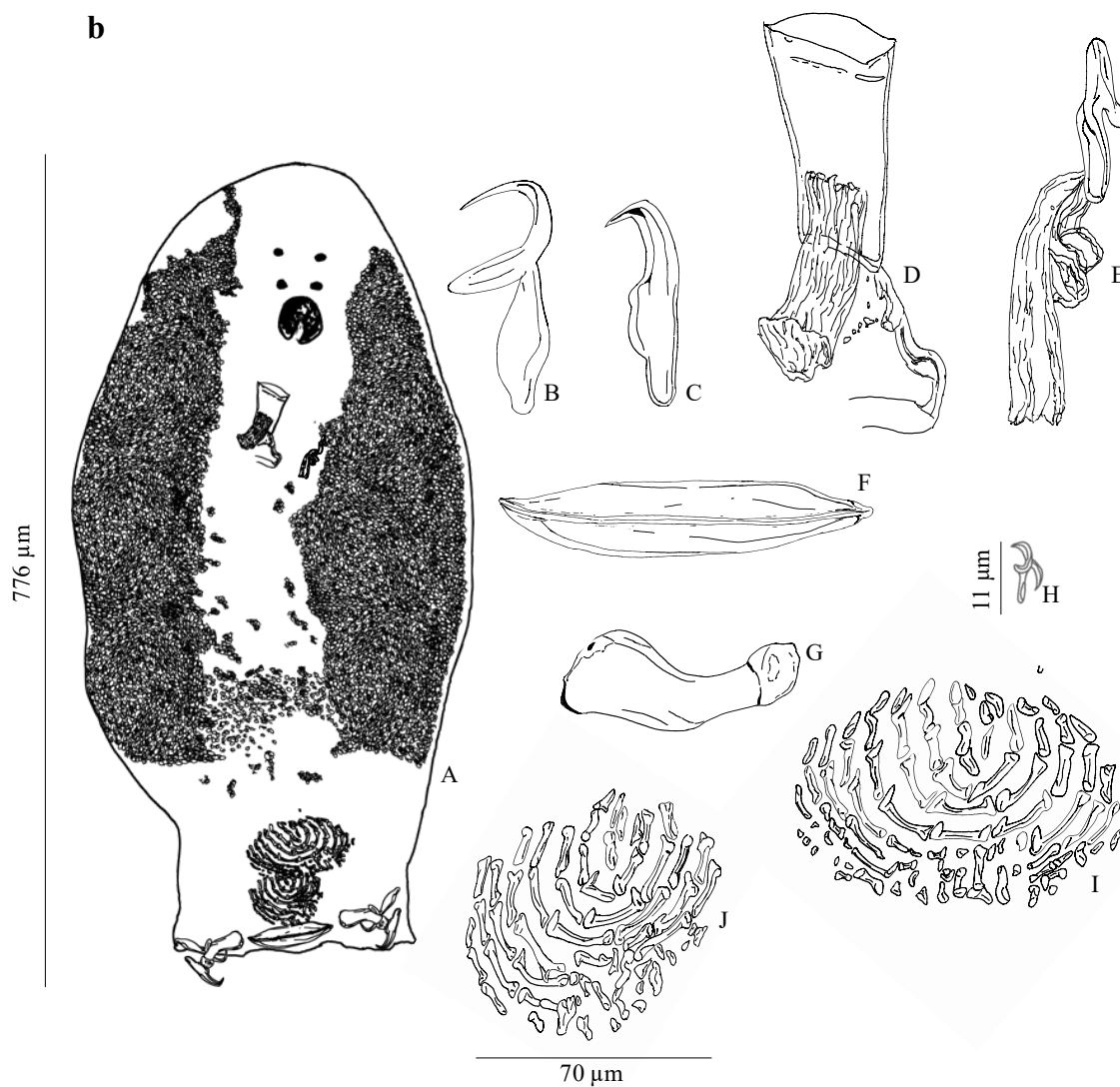
Fig. 3 Morphotypes of *Dolicirroplectanum lacustre* collected from *Lates niloticus*. Specimens drawn from a dorsal view with Hoyer's medium as fixative. a *Dolicirroplectanum lacustre* gravid morphotype from Lake Albert (host specimen HP4094); (b) *Dolicirroplectanum lacustre* slender morphotype from Lake Albert (host specimen HP4099); (c) *Dolicirroplectanum lacustre* from Lake Victoria (host specimen HP4318). Drawings from the dorsal view with A, whole mount; B, ventral anchor; C, dorsal anchor; D, male copulatory organ; E, vagina; F, ventral bar; G, dorsal bar; H, hook; I, ventral squamodisc; J, dorsal squamodisc.
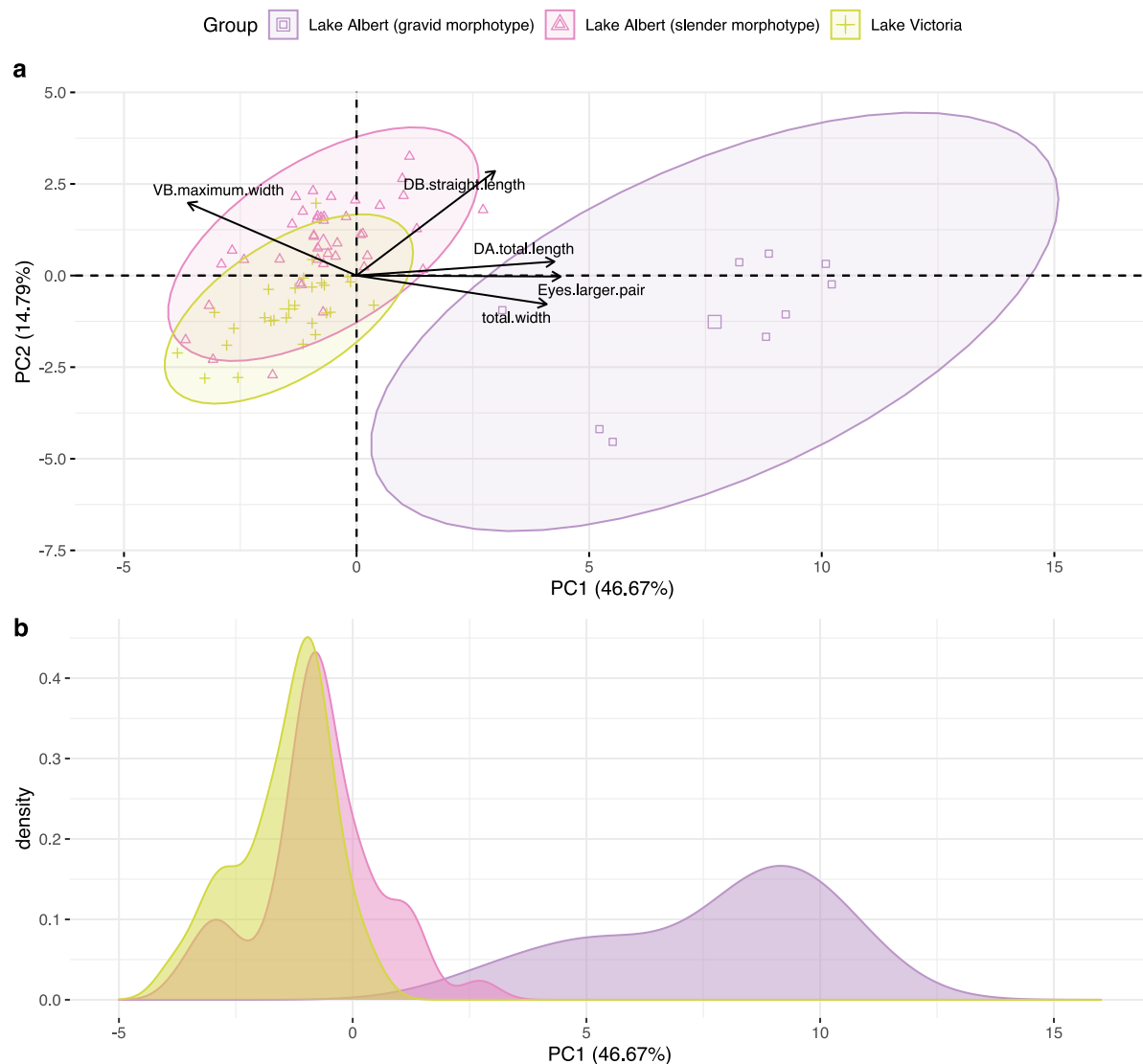
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Fig. 4 Morphometric variation of haptoral structures and body size in *Dolicirroplectanum lacustre*. (a) Biplot based on PCA from measured characteristics with gravid specimens (n= 9) and slender specimens (n = 42) from Lake Albert, and specimens from Lake Victoria (n= 27) with 95% C.I. ellipses; (b) Density plots depicting the morphometric variation of haptoral structures across populations, summarised in PC1 that explains 46.67% of the haptoral variation. Colours and symbols depict different populations. Vectors indicate the 5 most influential haptoral and body size measurements (DB, dorsal bar; VB, ventral bar; DA, dorsal anchor).

MCO straight length (ANOVA, df = 2, F = 40.36, p = 0.0000000000028) and straight width (ANOVA, df = 2, F = 19.638, p = 0.00000019) were found to differ significantly among the three groups (Fig 5). Overall, MCO length and width were largest for the gravid morphotype (n = 8) from Lake Albert, and smallest in the specimens from Lake Victoria (n = 19). All pairwise comparisons revealed significant differences between the three studied groups (unpaired t-test with Bonferroni correction) (Fig. 5).
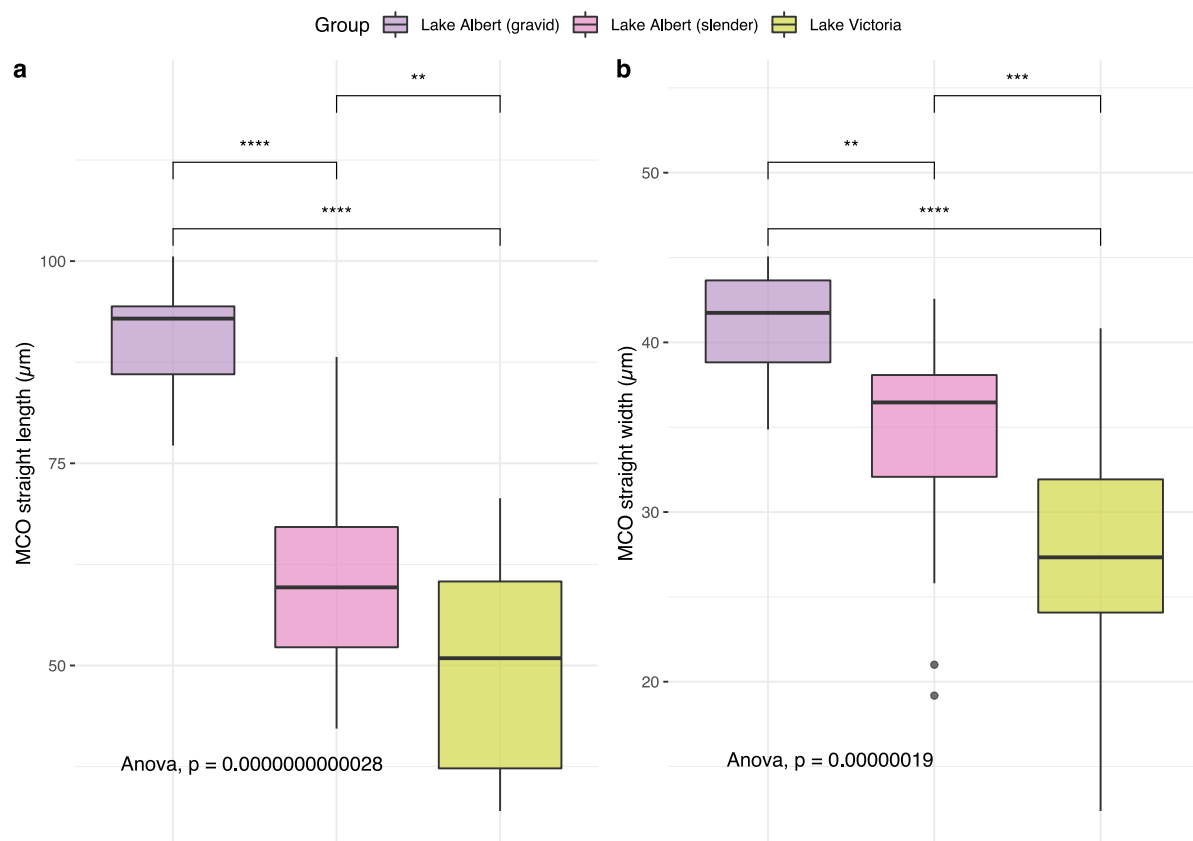
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Figure 5. Morphometric variation of the male copulatory organ (MCO) of *Dolicirroplectanum lacustre*. (a) variation in MCO straight length (μm) between morphotypes; (b) variation in MCO straight width (μm) between morphotypes. Colours depict the morphotype from which the MCO measurements were obtained. Following One-way Anova, the p-values are depicted per MCO measurement, with pairwise comparisons following t-tests with Bonferroni correction for multiple testing (ns, p > 0.05; * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001; **** p ≤ 0.0001). Box plots with statistical entries: minimum, first quartile, median, third quartile, maximum, outliers. Data from this study combined with measurements of Kmentová et al. (2020a).

Genetic diversity and phylogeography

We found two haplotypes for the 18S rDNA marker (443 bp), two haplotypes for the 28S rDNA marker (778 bp), five haplotypes for the ITS-1 marker (493 bp), and nine haplotypes in the COI marker (325 bp). Uncorrected p-distances varied up to 0.7% for the 18S rDNA marker, and the two haplotypes were shared between lakes and morphotypes. Accordingly, two haplotypes were shared between lakes and morphotypes for 28S rDNA, with uncorrected p-distances up to 2.1%. For the ITS-1 marker, one haplotype was shared between lakes, and uncorrected p-distances varied between 0.2 - 20.6% with a total of 17 indels between haplotypes. Between the morphotypes, no haplotypes were shared for the ITS-1 marker, and uncorrected p-distances varied between 1.5 -

377   17.2%. For the mitochondrial COI marker, four haplotypes were shared between the lakes, and uncorrected p-

378   distances varied between 0.3 - 13.5%. No haplotypes were shared between the morphotypes, and uncorrected p-

379   distances varied between 12.5 - 13.5% in the COI haplotypes. As all ITS-1 haplotypes could be aligned, our

380   hypothesis that a single diplectanid species was examined is supported (Wu et al. 2007; Poisot et al. 2011). The

381   uncorrected p-distance over the COI fragment does not reach the ''best-compromise threshold" (Meier et al. 2006)

382   for barcoding of 14.5% proposed by Vanhove et al. (2013), which indicates that the specimens are conspecific,

383   and all belong to *D. lacustre*.

384   We observed no genetic separation between specimens from different lakes. Genetic separation was observed

385   between the morphotypes of *D. lacustre* from Lake Albert in the COI marker, where the gravid morphotype was

386   represented by a single, exclusive haplotype (Fig. 6). There was haplotype sharing between the slender

387   morphotype and the Lake Victoria specimens in the COI marker, and three haplotypes were dominant, surrounded

388   by several satellite haplotypes, separated by single mutations. For the ITS-1 marker, a single haplotype was shared

389   between the gravid population and the Lake Victoria population. The ITS-1 marker did not show the same pattern

390   as the other nuclear markers. Marker incongruence was observed in both lakes. In Lake Albert, one specimen with

391   a gravid morphotype had a COI haplotype dominant in other individuals showing a gravid morphotype, but the

392   nuclear haplotypes were the same as the dominant haplotypes in the slender morphotype. Conversely, in Lake

393   Victoria, two specimens of the slender morphotype and COI haplotype, but with haplotypes of nuclear gene

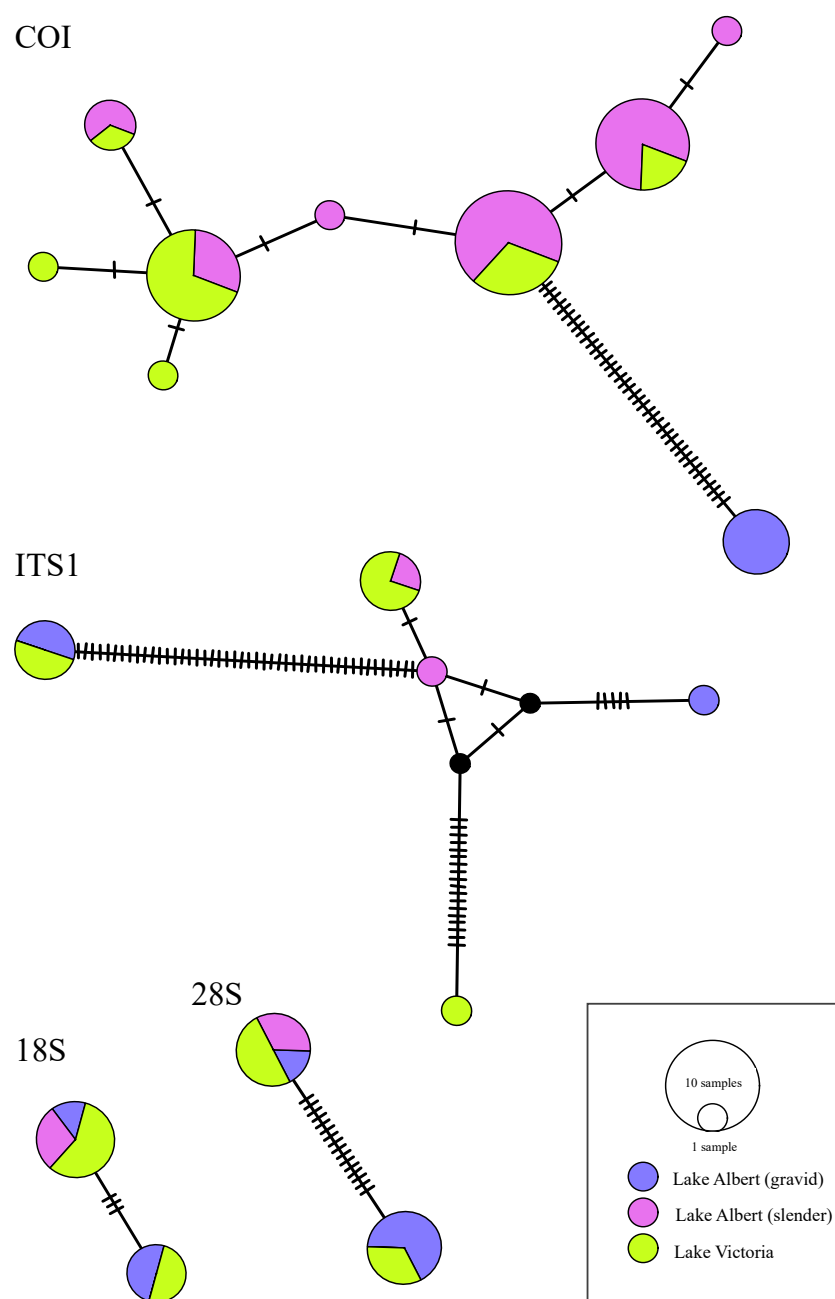394   portions corresponding with the gravid morphotype were characterised.

395

Fig. 6 Haplotype networks of *Dolicirroplectanum lacustre* from Lake Albert and Lake Victoria based on sequences of COI mtDNA (n=45), ITS1 rDNA (n=11), 28S rDNA (n=12), 18S rDNA (n=11) combining data generated during this study and by Kmentová et al. (2020a). Circles represent different haplotypes with the size proportional to the number of individuals sharing the haplotype. Colours correspond to the different populations. Mutation steps as hatch marks.

The genetic diversity in the COI mtDNA gene portion was largest in Lake Albert (Table 3). The number of polymorphic sites in COI was 46 (n=29) in Lake Albert, and six (n=16) in Lake Victoria. Five sites were

405   polymorphic (n=24) in the slender morphotype, and for the gravid morphotype only a single haplotype was

406   represented in five individuals. We observed similar levels of nucleotide and haplotype diversity between *D.*

407   *lacustre* in both lakes, however values for these diversity measures were larger in Lake Albert. F_{ST} values were

408   significant between the Lake Albert morphotypes (F_{ST} = 0.97049, p-value = 0.00000). Likewise, the gravid

409   morphotype and the specimens from Lake Victoria were genetically differentiated (F_{ST} = 0.97024, p-value =

410   0.00000). Conversely, low F_{ST} values (F_{ST} = 0.16794, p-value = 0.01802) for the Lake Victoria specimens and the

411   slender specimens from Lake Albert indicate higher genetic similarity between these populations. Following

412   AMOVA between specimens from Lake Albert and Lake Victoria (F_{ST} = 0.05983, p-value = 0.03812), most of

413   the variation was present within the lakes (94.02%) in comparison with 5.98% among-lake variation.

414

415   *Demographic history*

416   No signatures of population expansion could be detected for *D. lacustre* from the studied lakes. Neutrality test

417   statistics were not significant for Lake Albert alone (Tajima's D = 0.537, p = 0.774; Fu's F_S = 9.872, p = 0.995),

418   as for Lake Albert and Lake Victoria combined (Tajima's D = -0.230, p = 0.453; Fu's F_S = 7.137, p = 0.981).

419

420   *The gill microhabitats*

421   Individuals of *D. lacustre* were predominantly attached to the median-central and median-distal microhabitats on

422   the gills (Fig. 7). In Lake Victoria, parasites were mostly retrieved from the right gill chamber (2 left, 18 right).

423   In Lake Albert, most specimens were retrieved from the left gill chamber (slender: 50 left, 22 right; gravid: 8 left).

424   In co-infections, both morphotypes occupied the same microhabitats, but several specimens of the gravid

425   morphotype were mostly found on the median-ventral portion of the gill (Fig. 7). Whereas specimens from Lake

426   Victoria were attached to the proximal-ventral portion of the gill, this was uncommon in Lake Albert.

427

428   **Table 3.** Genetic diversity indices of *Dolicirroplectanum lacustre* in Lake Albert (per population) and Lake

429   Victoria inferred from a 325 bp portion of the mitochondrial cytochrome *c* oxidase subunit I (COI) region.

Population	*n*	H	S	Maximum uncorrected p-distance	Hd	π
Lake Albert	29	7	46	13.2 %	0.8079 ± 0.0405	0.041228 ± 0.021246
Lake Victoria	16	6	6	1.2 %	0.7667 ± 0.0839	0.004949 ± 0.003462
Lake Albert (slender)	24	6	5	1.5 %	0.7536 ± 0.0562	0.004515 ± 0.003159
Lake Albert (gravid)	5	1	0	/	/	/

430   *n*, sample size; H, number of haplotypes; S, number of polymorphic sites; Hd, haplotype diversity; π, nucleotide diversity
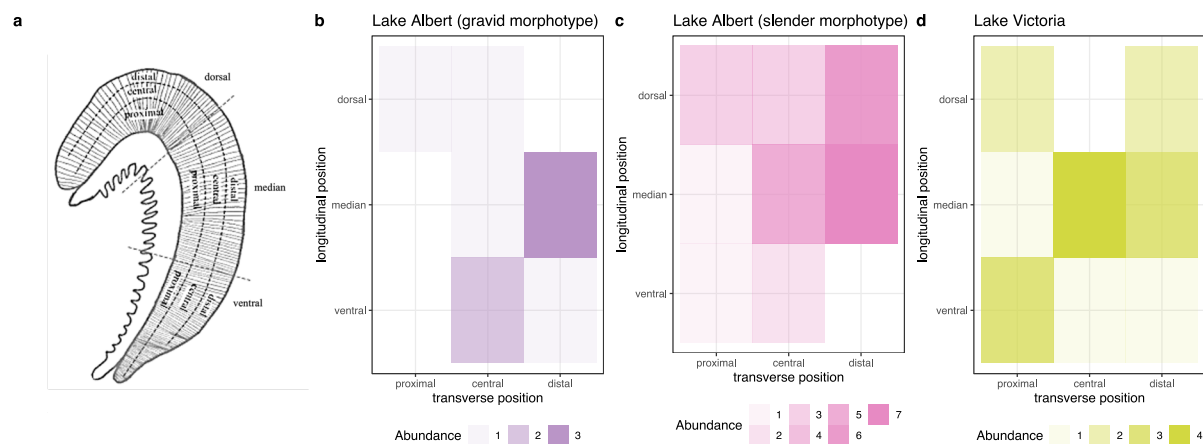
Fig. 7. Microhabitat distribution of *Dolicirroplectanum lacustre* visualised in heat plots. (a) division of the fish gill into nine microhabitats across a longitudinal and transversal axis; (b) Lake Victoria; (c) gravid morphotype from Lake Albert; (d) slender morphotype from Lake Albert.

Discussion

In Lake Albert we morphologically identified two distinct morphotypes of *Dolicirroplectanum lacustre*, a monogenean parasite infecting latid fishes across the African freshwater system. A single morphotype (slender) is suggested to be co-introduced in Lake Victoria with the historical introductions of the Nile perch. The variation in haptoral structures of the slender morphotype coincides with the variation in specimens from Lake Victoria, with the latter showing slightly less phenotypic and genetic variation. We observed spatial similarity in the microhabitat distribution between the specimens of the slender morphotype from both lakes. Co-infections with both morphotypes were observed in Lake Albert. The two morphotypes were genetically separated in the COI mtDNA region, but genetic differentiation did not meet the level typically associated with species-delineation. Patterns in the ITS-1 region differed in the slender specimens between Lake Victoria and Lake Albert. Mitonuclear marker incongruence was observed within the morphotypes of *D. lacustre*.

A host species harbours the greatest diversity of parasites in the area where it resided longest

The haplotype sharing between studied locations supports the co-introduction of *D. lacustre* with *L. niloticus* to Lake Victoria from Lake Albert (Pringle 2005). This agrees with host data provided by Hauser et al. (1998), which confirmed the presence of *L. niloticus* from Lake Albert in Lake Victoria and excluded the successful establishment of introduced Nile perch from Lake Turkana. We observed large variation in haptoral morphology in Lake Albert (native range) and identified the two morphotypes as in earlier studies (Thurston and Paperna 1969;

454 Kmentová et al. 2020a). The presence of different species of Nile perch (Hauser et al. 1998), that occur in sympatry

455 in Lake Albert may have provided *D. lacustre* with different gill habitats and could account for the morphological

456 variation between morphotypes in Lake Albert. Under this scenario, the observations of co-infections would

457 require secondary contact and host switching or hybridisation between the two host taxa. The reduced phenotypic

458 variation (and presence of a single morphotype) in Lake Victoria may be an indication for a founder effect. In

459 accordance, we have observed reduced genetic diversity in the specimens from Lake Victoria in comparison with

460 Lake Albert. When relatively few parasite specimens were co-introduced, a 'founder effect' would be expected

461 (Mayr 1942). Accordingly, a host species is expected to harbour the greatest diversity of parasites in the area

462 where it resided longest (Manter 1966). This argument is supported by the low number of introduced hosts (n =

463 382) into Lake Victoria (Hauser et al. 1998).

464

465 *Phenotypic plasticity: discordant variation and mitonuclear discordance*

466 The observed variation in the morphology and resulting morphotypes, in combination with below species-level

467 genetic differentiation, suggests phenotypic plasticity of the species *D. lacustre* following the definition of DeWitt

468 et al. (1998): species with "the potential to produce a range of different, relatively fit phenotypes in multiple

469 environments". Discordance between molecular and morphological differentiation has been observed for other

470 diplectanid monogenean species, namely *Lamellodiscus* spp. (Diplectanidae) (Desdevises et al. 2000; Poisot et al.

471 2011). Following Desdevises et al. (2000), these inconsistencies could be attributed to phenotypic variability of

472 some characters related to the environment and the host (Villar-Torres et al. 2019). Moreover, the observed

473 phenotypic variability and the genetic structuring of the morphotypes observed in the COI marker could be an

474 indication for ongoing sympatric speciation or diversification processes (Poulin 2002) of *D. lacustre* in Lake

475 Albert. Parasites with direct life cycles, like monogeneans, are expected to experience more favourable conditions

476 for sympatric speciation, especially when infecting long-living hosts (Brooks and McLennan 1993), such as the

477 Nile perch. Similarly, the observed morphological differentiation in the MCO could be an indication of ongoing

478 diversification or incipient speciation (Šimková et al. 2002), explained by the 'reinforcement hypothesis' (Rohde

479 1991): "reinforcement of reproductive barriers is one of the main factors resulting in niche segregation".

480 Although the presented population structure based on the COI marker indeed agrees with the reproductive

481 isolation of the gravid morphotype, the mitonuclear discordance gives a different picture, and indicates an

482 incomplete reproductive barrier between the two morphotypes. Potentially, this discordance may be explained by

483 intraspecific gene flow by hybridisation between the morphotypes in Lake Albert, or incomplete lineage sorting

24

484   (Després 2019) the former has earlier been linked with the evolution of invasiveness (Culley and Hardiman 2008).

485   Therefore, the observed mitonuclear discordance in Lake Victoria deserves particular attention in future research.

486

487   *A 'failure to diverge' in Lake Tanganyika versus ongoing diversification in Lake Albert*

488   Although former studies identified two different morphotypes of *D. lacustre* in Lake Albert, no genetic

489   differentiation between the morphotypes had been investigated. In Kmentová et al. (2020a), a 'failure to diverge'

490   was observed for *D. lacustre*, as the species had not diversified over the variety of African freshwater systems,

491   even when infecting different host species in Lake Tanganyika. This was hypothesised to be attributed to a low

492   rate of molecular evolution. Our data supports the severely reduced genetic diversity in Lake Tanganyika in

493   comparison to other studied areas (Table 5). Moreover, the haplotype network for COI mtDNA indicates that the

494   gravid lineage from Lake Albert is genetically more distinctive from the slender lineage in Lake Albert and Lake

495   Victoria, compared to the specimens from Lake Tanganyika (Fig. 8). The reduced diversification in *D. lacustre*

496   from Lake Tanganyika may be explained by the recently estimated timing of colonisation by *L. niloticus* and

497   divergence (1.27-1.76 MYA) of the four extant *Lates* spp. in Lake Tanganyika (Koblmüller et al. 2021). The

498   observation of greater morphological and genetic diversity in Lake Albert agrees with this hypothesis.

499

500   *Future perspectives*

501   Although this study sheds light on the population-scale differentiation and the co-introduction of *D. lacustre*,

502   several questions on its co-introduction and diversification mechanisms remain. We suggest: (1) to investigate

503   whether the diversification patterns after co-introduction, observed in this study, occur across different freshwater

504   systems, we should increase the number of samples over various sampling sites within the entire native range

505   (including Lake Turkana) and introduced range (Lake Kyoga) of Nile perch. By sample acquisition from historical

506   collections, broader spatial and temporal patterns of diversification after co-introduction could be examined; (2)

507   with sample collection note should be taken of the host (species) identity, in particular in Lakes Albert and

508   Turkana, where two sympatric species could occur, since host identity may contribute to the observed

509   diversification patterns of *D. lacustre*; (3) the application of Next Generation Sequencing approaches to study

510   genome-wide intraspecific variation in *D. lacustre*, in order to identify the determinants of the observed diversity

511   and mitonuclear discordance; (4) to undertake a more detailed study on the gill environment of Nile perch and

512   microhabitat selection of *D. lacustre* in Lake Albert to determine whether microhabitat preference plays a role in

513   the ongoing morphological and genetic diversification and possible sympatric speciation of *D. lacustre*.

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729   Author contributions

730   All authors contributed to the study conception and design. Material preparation, and data collection were

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738   Compliance with Ethical Standards

739   The authors declare that they have no competing interests.

740

741   Data availability

742   All data generated or analysed during this study are included in this published article [and its supplementary

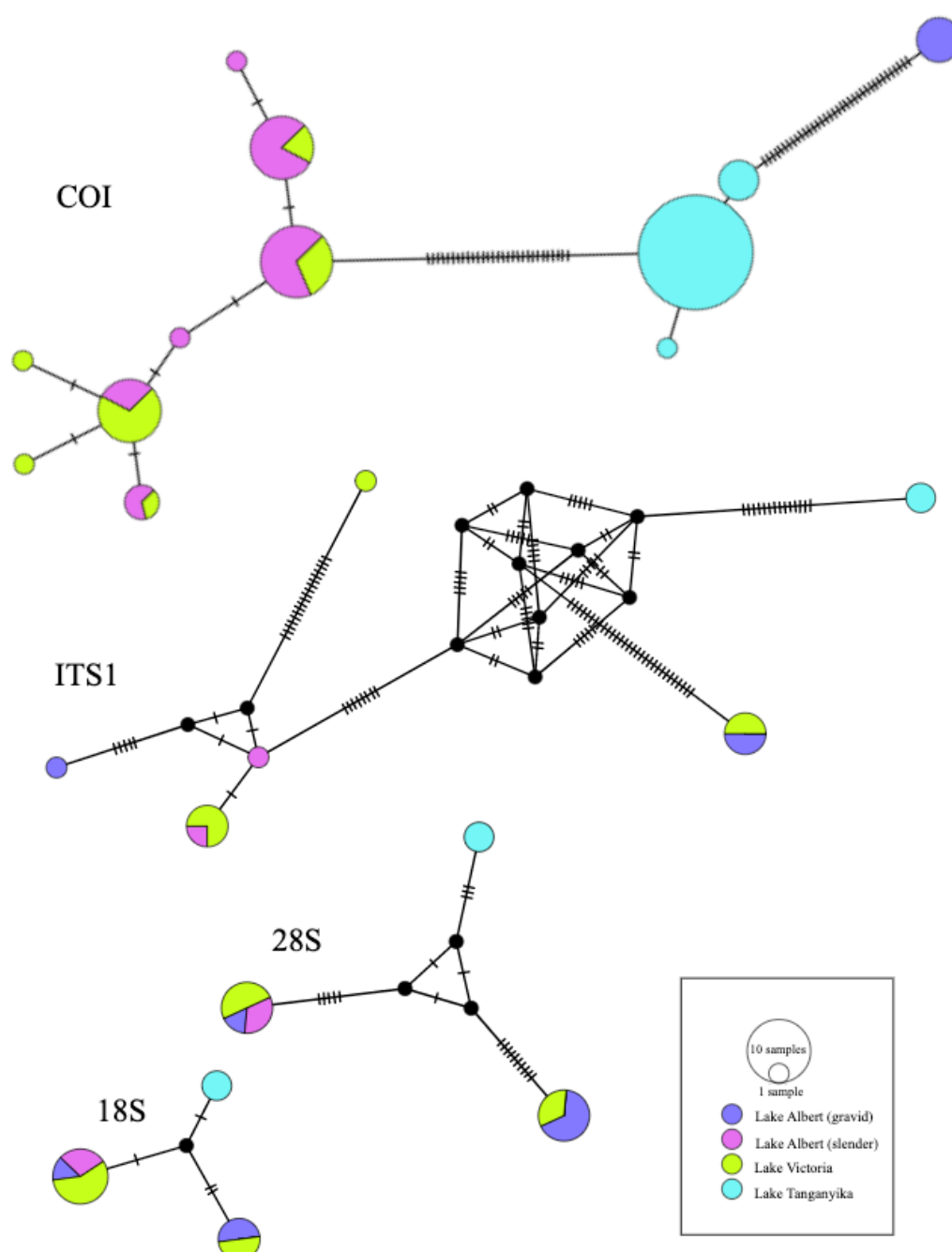743   information files].

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Supplementary data

Fig. 8 Haplotype networks of *Dolicirroplectanum lacustre* from Lake Albert, Lake Victoria and Lake Tanganyika based on COI mtDNA sequences (n=83), ITS1 rDNA (n=13), 28S rDNA (n=14), 18S rDNA (n=13) combining data generated during this study and Kmentová et al. (2020a). Circles represent different haplotypes with the size proportional to the number of individuals sharing the haplotype. Colours correspond to the different populations. Mutation steps as hatch marks.

755 **Table 5** Genetic diversity indices of *Dolicirroplectanum lacustre* in Lake Albert (per population) and Lake

756 Victoria inferred from a 325 bp portion of the mitochondrial cytochrome c oxidase subunit I (COI) region.

Population	n	H	S	Maximum uncorrected p-distance	Hd	π
Lake Albert	29	7	46	13.2 %	0.8079 ± 0.0405	0.041228 ± 0.021246
Lake Victoria	16	6	6	1.2 %	0.7667 ± 0.0839	0.004949 ± 0.003462
Lake Albert slender	24	6	5	1.5 %	0.7536 ± 0.0562	0.004515 ± 0.003159
Lake Albert gravid	5	1	0	/	/	/
Lake Tanganyika	38	3	2	0.3 %	0.2404 ± 0.0858	0.000757 ± 0.000976

757 *n*, sample size; H, number of haplotypes; S, number of polymorphic sites; Hd, haplotype diversity; π, nucleotide diversity