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1 **Intraspecific morphological variation in *Cichlidogyrus* (Monogenea) parasitizing two cichlid hosts from**
2 **Lake Tanganyika exhibiting different dispersal capacities**
3
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16 **Abstract**

17 As parasites depend on their hosts and play a significant role in their ecology and evolution, we hypothesized an
18 association between the host dispersal capacity and the intraspecific variability of their host-specific parasites. We
19 investigated the morphological variability of the gill monogeneans *Cichlidogyrus gistelincki* and *C. milangelnari*
20 parasitizing the Tanganyika cichlids '*Ctenochromis*' *horei* and *Cyprichromis microlepidotus*, respectively. The
21 profound ecological and behavioural differences between these species allowed us to assume that the former is a
22 good- and the latter a poor disperser. Specimens of monogeneans were collected from cichlids inhabiting different
23 locations at the northern end of Lake Tanganyika. Sequences of the 28S rDNA gene were used to confirm parasite
24 conspecificity. Dorsal and ventral anchors of the attachment organ of parasite specimens were used to evaluate
25 variability in shape. Geomorphometric analyses revealed that populations of *C. milangelnari*, which parasitize
26 poorly-dispersing cichlids, are more differentiated than populations of *C. gistelincki* infecting well-dispersing
27 hosts. Both anchors showed significant shape variation between populations of *C. milangelnari*. In *C. gistelincki*,
28 anchors were highly similar in comparisons of populations from nearby, and from distant locations.

29 **Keywords**

30 Attachment organ, Cichlids, Cyprichromini, fish dispersal, gill parasites, monogeneans, Tropheini.

31 **Introduction**

32 Monogenea van Beneden, 1858 is a cosmopolitan group of flatworms (or platyhelminths) parasitizing
33 mainly aquatic vertebrates. They particularly attach to fish gills, fins and scales, but some of them also infect the
34 eyes, nostrils or internal organs. They are highly diverse with an estimated 25 000 species and exceptionally host-
35 specific (Cribb, 2002; Theisen et al., 2017). In contrast to other parasitic flatworms that require one or more
36 intermediate hosts to complete their lifecycle, monogeneans are characterized by a single-host life cycle, a feature
37 that considerably reduces barriers that could preclude to infect their hosts (Gussev, 1995; Huyse et al., 2003).

38 The posterior end of all monogeneans bears a highly characteristic structure, the attachment organ, also
39 called haptor. It comprises sclerotized hard parts such as marginal hooks, connective bars, clamps or anchors.
40 Unsurprisingly, the haptor exhibits huge differentiation within the group (Roberts & Janovy, 2009). The various
41 forms of attachment organ structures have been interpreted as adaptations to the host species that have influenced
42 the specialization of these parasites and considerably contributed to their host specificity (Šimková et al., 2006;
43 Olstad et al., 2009; Bueno-Silva et al., 2011). Monogeneans are nowadays considered one of the best model
44 systems for addressing fundamental ecological and evolutionary questions related to fish-parasite interactions
45 (Šimková et al., 2001; Olstad et al., 2009; Bueno-Silva & Boeger, 2019). Their simple life cycle, species diversity
46 and host specificity make them the first choice for investigating diversity and speciation in parasites of closely
47 related hosts (Pariselle et al., 2003, Šimková et al., 2004; Mendlová et al. 2012; Šimková et al., 2013). A lot of
48 consideration has been given to the shape variation of the monogenean haptoral sclerites (see for instance Rohde
49 & Watson, 1985a, 1985b; Huyse & Volckaert, 2002; Jarkovský et al., 2004; Olstad et al., 2009; Khang et al.,
50 2016). Intraspecific variation in the shape and size of the haptoral hard parts were previously reported in a few
51 monogenean groups, of which dactylogyrids (Vignon & Sasal, 2010; Khang et al., 2016, Kmentová et al., 2016,
52 2020a) and diplectanids (Vignon & Sasal, 2010; Kmentová et al., 2020b) were investigated using a
53 geomorphometric approach. Compared to the marginal hooks and the connective bars of the attachment organ,
54 intra-specific geographic variation seems to be especially present in the shape of the anchors (Vignon & Sasal,
55 2010; Rodríguez-González et al., 2017; Kmentová et al., 2020a, b).

56 *Cichlidogyrus* Paperna, 1960 is the most common, most species-rich and most host-specific gill flatworm
57 genus known from African cichlids (Pariselle et al., 2011), with over 25 new species discovered only during the
58 past two years (Rahmouni et al., 2017, 2018; Jorissen et al., 2018a,b; Geraerts et al. 2020). Considering the host
59 specificity, direct life cycle, limited dispersal ability, and vicariance of monogeneans, the biogeographical

60 distribution of *Cichlidogyrus* species seems to follow the patterns of their cichlid hosts (Pariselle et al., 2011).
61 Therefore, these parasites were proposed as a tool to better understand the adaptive radiations driving rapid
62 speciation in cichlid assemblages (see the review by Vanhove et al., 2016). Few studies have focused on the
63 morphological evolution of the hard parts of the attachment organ in species of *Cichlidogyrus*. Mendlová et al.
64 (2012) investigated species of *Cichlidogyrus* of West African cichlids and suggested a link between phylogeny
65 and morphological adaptation of these host-specific parasites, whereas Messu Mandeng et al. (2015) suggested an
66 adaptive component to the haptoral morphology of species of this genus.

67 Lake Tanganyika (LT) is one of the main hotspots for cichlid adaptive radiations in African freshwaters
68 and represents an important model system to understand biological diversity and mechanisms of diversification
69 (Salzburger, 2018; Meyer et al., 2019). This lake contains a highly diverse assemblage of approximately 250
70 species of cichlids that are subdivided into 12 to 16 tribes (Poll, 1986; Takahashi, 2003; Takahashi 2014), which
71 are supported by phylogenetic analyses (Koblmüller et al., 2008; Takahashi & Sota, 2016). Species of two tribes,
72 Cyprichromini Poll, 1986 and Tropheini Poll, 1986 were selected for this study. We investigated *Cyprichromis*
73 *microlepidotus* (Poll, 1956) (Cyprichromini) and '*Ctenochromis*' *horei* (Günther, 1894) (Tropheini) and their
74 respective gill monogeneans, *C. milangelnari* Rahmouni, Vanhove & Šimková, 2017 and *Cichlidogyrus gistelincki*
75 Gillardin, Vanhove, Pariselle, Huyse & Volckaert, 2011. The two cichlid species differ strongly in life history
76 traits, behaviour, geographical distribution and dispersal capacity. '*Ctenochromis*' *horei* is widely distributed
77 throughout LT, and the most common cichlid species living in shallow intermediate and vegetated habitats
78 (Konings, 2015). Although belonging to the endemic LT tribe Tropheini, '*C.*' *horei* has a generalist morphology
79 that deviates from most other tropheines, which are more specialized. It has a very broad dietary range (Muschick
80 et al., 2012) and a broad ecological tolerance. Besides its preferences for vegetated patches, this cichlid is also
81 commonly observed at other habitats along the Lake's shoreline, including rocky coasts (Sturmbauer et al., 2008).
82 Given its broad ecological tolerance, '*C.*' *horei* encounters few barriers for dispersal. Hence, it also occurs out of
83 LT in the Lukuga (Kullander & Roberts, 2011), Malagarasi, and Rusizi Rivers (De Vos et al., 2001; Konings,
84 2015). Additionally, in contrast to most other mouthbrooding cichlids, neither males, nor females of '*C.*' *horei*
85 possess well-defined territories. This because the hierarchy between males, rather than the defence of mating
86 territories, determines the mating system of the species (Ochi, 1993). Finally, this species does not harbour any
87 known differentiation into colour morphs, and exhibits low intraspecific genetic divergence (Van Steenberge et
88 al., 2015). In view of the above, we consider the species to be a good disperser. *Cyprichromis microlepidotus*,
89 however, shows only a weak dispersal ability. Although species of *Cyprichromis* have evolved several adaptations

90 allowing them to live and spawn in the open water with a capacity to catch pelagic prey, they remained strongly
91 dependant on deep rocky shores for shelter (Konings, 2015). As this habitat is distributed in a patchy way along
92 the shoreline of LT, populations of *Cyprichromis* are geographically isolated. Therefore, species of *Cyprichromis*
93 contain many colour variants that evolved due to the geographic isolation. This specifically holds for *C.*
94 *microlepidotus,* which only occurs at the Lake's northern half, and which contains several geographically-isolated
95 (colour) variants.

96 Cyprichromine cichlids were investigated for monogenean parasites only recently by Rahmouni et al.
97 (2017) who described the first species of *Cichlidogyrus* infecting a member of this tribe: *C. microlepidotus*. In
98 contrast, among the endemic cichlids living in LT, Tropheini is the group that was most extensively studied for
99 their gill monogenean fauna, with over 15 nominal species of *Cichlidogyrus* recognized (see the overview
100 published by Rahmouni et al. (2017) and Rahmouni et al. (2018)). In addition, only a few molecular studies on
101 monogenean parasites of LT cichlids have been carried out (Vanhove et al., 2011, 2015; Kmentová et al., 2016).

102 As a high richness of host species could lead to a high richness of parasites, we would expect that the
103 extraordinary diversity of cichlids in LT would bring about a high parasite diversity and diversification (see the
104 review by Vanhove et al., 2016). Additionally, monogeneans could also diversify within cichlid hosts. Such
105 diversification would be hampered by dispersal and gene flow between populations of host-specific monogeneans,
106 which, in turn, depends on the dispersal of the fish hosts (Criscione & Blouin, 2004). Gene flow caused by dispersal
107 among subpopulations of fish hosts affects genetic variation (Pettersen et al., 2015) whereas low gene flow
108 contributes to high levels of genetic differentiation within parasite species (Mazé-Guilmo et al., 2016). We
109 hypothesize in the present study that cichlid dispersal capacity in LT would drive the diversity of their parasite
110 assemblages, and that limited dispersal ability precluding gene flow between cichlid populations could promote
111 the differentiation of their monogenean assemblages (Grégoir et al., 2015). Hitherto, relatively little attention has
112 been given to the effect of cichlid host dispersal capacity on genetic and morphological differentiations within
113 *Cichlidogyrus* species. Such research has, until now, only been carried out on intraspecific phenotypic variability
114 in a Tanganyikan monogenean that infects a few species of deep-water cichlids (Kmentová et al., 2016).

115 In this study, we hypothesize that there is a link between host dispersal capacity and the intraspecific
116 diversity in gill monogeneans. More specifically, we hypothesized that, because of low gene flow in parasites,
117 cichlid species with limited dispersal capacity will harbour more morphologically differentiated monogenean

118 populations in terms of haptoral morphology (measured by shape variation in the anchors), than populations of
119 well-dispersing cichlids.

120 **Material and methods**

121 Fish and parasite collections

122 Cichlid hosts were sampled from the northern part of LT (Fig. 1a). Twelve specimens of '*C.*' *horei* (Fig.
123 1b) and fifteen specimens of *C. microlepidotus* (Fig. 1c) were collected from the Burundese and Congolese
124 shorelines in 2013 and 2016. The following localities were sampled for '*C.*' *horei* (Burundi): Magara (3° 44′ S,
125 29° 19′ E; *n* = 4), Mukuruka (4° 14′ S, 29° 33′ E; *n* = 1), and Nyaruhongoka (3° 41′ S, 29° 20′ E; *n* = 7). Specimens
126 of *C. microlepidotus* were sampled from Nyaruhongoka (*n* = 3), and Kalundo (3° 26′ S, 29° 07′ E; *n* = 12) (see
127 Fig. 1a). The geographical distance between localities was calculated using Geographic Distance Matrix Generator
128 software v. 1.2.3 (Ersts, 2014). Only 3.5 km separate Magara and Nyaruhongoka, while 79.4 km separate Magara
129 and Mukuruka. There is 82.5 km between Nyaruhongoka and Mukuruka, and only 22 km between Nyaruhongoka
130 and Kalundo (Fig. 1a). The protocols used for dissecting the cichlid fish, as well as for isolating, fixing and drawing
131 gill-infecting monogeneans (Fig. 1d and 1e), follow Rahmouni et al. (2017, 2018). Basic epidemiological data, i.e.
132 prevalence, mean abundance, minimum and maximum intensity of infection, were calculated for each monogenean
133 species according to Bush et al. (1997). Host nomenclature follows FishBase (Froese & Pauly, 2019), except with
134 respect for the use of '' (single quotation) in '*C.*' *horei*, where Konings (2015) is followed. This notation is used
135 as Takahashi (2003) showed that '*C.*' *horei* is not closely related with the nominal species of the genus:
136 *Ctenochromis pectoralis* Pfeffer, 1893. All applicable institutional, national and international guidelines for the
137 care and use of animals were followed. Sampling was carried out under mission statements 022/MINEURS/CRH-
138 U/2013 and 031/MINRST/CRH-U/2016 from the Centre de Recherche en Hydrobiologie-Uvira. In the absence of
139 relevant animal welfare regulations in the D.R. Congo or Burundi, the same strict codes of practice enforced within
140 the European Union were applied.

141 Molecular characterization and genetic analysis

142 To confirm the conspecificity of parasites infecting the respective host species, a fragment of the 28S
143 rDNA region was amplified and sequenced for 18 parasite specimens collected from all sampling localities and
144 host species. Ribosomal DNA regions such as 28S are highly conservative, which makes them suitable and
145 commonly used for species recognition in flatworms (Vanhove et al., 2013; see for instance the studies of Šimková
146 et al., 2006; Mendlová et al., 2012; Mendlová & Šimková, 2014; Messu Mandeng et al., 2015). These 18 specimens

147 were cut into half using fine needles under a dissecting microscope during the fieldtrip. The reproductive organs
148 were fixed on slides (see below) whereas the other half of the body was placed in 96% ethanol for DNA extraction.
149 As this half contained the haptor with sclerotized anchors, specimens used for molecular analyses could not be
150 used for the morphological part of the study. The universal primers C1 (F: 5′-ACCCGCTGAATTTAAGCAT-3′)
151 and D2 (R: 5′-TCCGTGTTTCAAGACGG-3′) (Hassouna et al., 1984) were used, following the protocol published
152 in Rahmouni et al. (2017). Sequences were edited using the Sequencher® software v. 5.0 (Gene Codes
153 Corporation, Ann Arbor, MI USA), aligned using the ClustalW algorithm (Thompson et al., 1994) implemented
154 in MEGA X (Kumar et al., 2018) and deposited in GenBank under accession numbers: MK860914-16.
155 Uncorrected *p*-distances were calculated between specimens and populations, using the same software.

156 Geomorphometrics

157 Variation in anchor shape between parasite populations of various localities was analysed using landmark-
158 based (LM) geometric morphometrics. We analyzed dorsal and ventral anchors (DA and VA), two sclerotized
159 structures of the posterior haptoral apparatus of the worms (Fig. 1e). The number of DA and VA analyzed
160 (nDA:nVA) for parasite specimens collected from '*C*'. *horei* per locality was as follows: (17:17, Magara); (16:16,
161 Mukuruka); and (30:32, Nyaruhongoka). Parasite specimens were collected from *C. microlepidotus* from two
162 opposite locations and nDA:nVA per locality was as follows: (27:27, Nyaruhongoka) and (10:7, Kalundo). We
163 used only monogenean specimens wholly body mounted on slides with a drop of glycerine ammonium picrate
164 (GAP) (Malmberg, 1957). Anchors were then photographed by using an Olympus BX51 phase-contrast
165 microscope, under magnifications 20X and 40X, and using Olympus Stream Image Analysis v. 1.9.3 software.
166 Voucher specimens of parasite species from each sampling locality were deposited in the Muséum National
167 d'Histoire Naturelle (MNHN, Paris, France) under the accession numbers (*Cichlidogyrus gistelincki*: MNHN
168 HEL1195-1202; *Cichlidogyrus milangelnari*: MNHN HEL1203-05 and MNHN HEL1223). The anchor shape
169 variables were obtained using nine homologous LMs based on the studies of Vignon & Sasal (2010) and Kmentová
170 et al. (2016) (Fig. 1f). Landmark terminology follows Rodríguez-González et al. (2015): (LM1) anchor point;
171 (LM2) inner point base; (LM3) inner shaft base; (LM4) most convex point base; (LM5) most proximal point of
172 inner root; (LM6) notch between inner and outer roots; (LM7) mean point of outer root; (LM8) outer shaft base;
173 and (LM9) outer point base. Dorsal and ventral anchors were aligned by their vertical axis, which is defined by
174 LM2 and LM9. Digitalization of the LMs was performed using tpsDig2 software (Rohlf, 2006). The LM
175 coordinates were forwarded to MorphoJ v. 1.06 (Klingenberg, 2011). We performed a Procrustes fit by aligning
176 the coordinates using Generalized Procrustes Analysis (GPA). The Procrustes method removes all information

177 related to size and orientation and superimposes LM configurations to achieve an overall best fit. We generated
178 covariance matrices for each of the two parasite species, which were used in further analyses, i.e. principal
179 component analyses (PCA) and canonical variate analyses (CVA). Principal component analyses were used to
180 visualise the variation in the datasets whereas canonical variate analyses were performed to investigate whether
181 anchor shape could differentiate between *a priori* defined groups (i.e. localities) (Klingenberg & Monteiro, 2005).
182 The latter analysis computes the axes of variance by minimizing differences within groups and maximizing
183 differences between groups. We tested whether different populations from the same species differed
184 morphologically by computing the Procrustes distances between specimens and by using a permutation test (Good,
185 2001) with 10 000 randomizations (significance level α = 0.05). For this, *p*-values were adjusted using Holm-
186 Bonferroni correction (Holm, 1979). Phenotypic change patterns in DA and VA for the main axes of PCA and
187 CVA were visualised in MorphoJ with respect to a consensus using a wireframe scheme (Klingenberg, 2011).

188 **Results**

189 Species identification and genetic characterization

190 The morphological identification of species of *Cichlidogyrus* was based on their haptoral and reproductive
191 sclerites using the original descriptions. Specimens collected from '*C.*' *horei* and *C. microlepidotus* were assigned
192 to *C. gistelincki* (Fig. 1e) and *C. milangelnari*, respectively. Only a single species of dactylogyrid monogenean
193 species was found on the gills of '*C.*' *horei*, as was also the case in the study of Gillardin et al. (2012). The same
194 held for the gills of *C. microlepidotus*, in accordance with Rahmouni et al. (2017). These parasite species have
195 never been recorded from any other cichlid hosts so far and are, thus, considered as strict specialists (Mendlová &
196 Šimková, 2014). All specimens of '*C.*' *horei* were infected by *C. gistelincki* (100%), the mean abundance was 18.3
197 ± 8.2 and the intensity of infection ranged from 4 to 33 monogeneans per infected host. *Cichlidogyrus milangelnari*
198 parasitized 10 out of 15 specimens (66.6%), the mean abundance was 7.6 ± 12.2 and the intensity of infection
199 ranged from 1 to 39 monogeneans per infected host. From three to five specimens from each sampling locality
200 were successfully sequenced. Using the partial sequences of 28S rDNA, the conspecificity of all monogeneans
201 from each respective host species was confirmed. The sequence of the partial 28S rDNA was 682 bp long for *C.*
202 *gistelincki* and 591 bp long for *C. milangelnari*. No variability was observed within the sequences obtained from
203 specimens of *C. gistelincki*. However, the 28S rDNA sequences of *C. milangelnari* showed weak differentiation
204 among the populations from localities on opposite lake shores (a single nucleotide; 0.2%). Thus, two 28S rDNA
205 sequences representing each of the populations of *C. milangelnari* and a single sequence for *C. gistelincki* were

206 deposited in GenBank (see accession numbers in material and methods section). The genetic distance between

207 *C. milangelnari* and *C. gistelincki* ranged from 3% to 3.2%.

208 Anchor shape variation in populations of *C. milangelnari* parasitizing *C. microlepidotus*

209 Results from the PCA performed on the DA and VA datasets of populations of *C. milangelnari* are shown

210 in Fig. 2. Regarding the DA (Fig. 2a), PC1 explained 38%, and PC2 18.1% of the variation. Concerning the VA

211 (Fig. 2b), 35.9% of the variation was explained by PC1 and 17.5% by PC2. Samples from the two localities

212 overlapped in the scatter plots for DA and VA. However, specimens from Nyaruhongoka had, on average, higher

213 values for PC2 in the DA dataset, and lower values for PC1 in the VA dataset. The changes along the first PC

214 corresponded with a DA having a slightly broader and more pronounced inner root, and a broader, more curved

215 shaft base, and a more elevated convex point. The second PC corresponded with a DA having a broader and more

216 pronounced inner root, a deeper notch, and more reduced outer root and outer shaft base (Fig. 2a). For the VA

217 dataset, specimens with high values for PC1 had a VA with a narrower and more pronounced inner root, and a

218 thinner, shorter shaft base. The highest contribution to PC2 was a change in the inner root that had a broader and

219 more pronounced shape (Fig. 2b).

220 The frequencies of the distribution of the samples across the CV axes are represented in Fig. 2c,d. In the

221 case of the DA, CVA almost completely separated the two populations (Fig. 2c) whereas a complete separation

222 was obtained in the VA dataset (Fig. 2d). The shape changes in DA along the CV corresponded with a thinner base

223 and a more pronounced outer root (Fig. 2c). For VA, the CV corresponded with having a more pronounced distance

224 between the roots with a longer and slightly broader shaft (Fig. 2d). The permutation tests using Procrustes

225 distances revealed significant differences in shape for both DA and VA (Table 1). The differences remained

226 significant after Holm-Bonferroni correction.

227 Anchor shape variation in populations of *C. gistelincki* parasitizing '*C.*' *horei*

228 Principal component analyses were performed on morphometrical landmarks of the DA (Fig. 3a) and VA

229 (Fig. 3b) of *C. gistelincki* (Fig. 3). The first two PC axes accounted for 44.7% of the total DA shape variation

230 (27.9% and 16.8%) and for 45.4% of the total VA shape variation (26.1% and 19.3 %). On the scatter plots of both

231 datasets, there was a complete overlap between groups. Variations in the shape of anchors associated to each of

232 the PCs are shown next to their corresponding axis (Fig. 3a,b). For the DA, a high value for PC1 mostly

233 corresponded with reduced inner roots with a relatively reduced distance between the inner shaft base and the most

234 convex point of the anchor base, resulting from a shift of the latter. A high value for PC2 corresponded to anchors

235 with a broader base and a more upturned inner and outer point base, with a more conspicuous inner root in terms
236 of length and angle that forms the notch (Fig. 3a). For VA, higher values for PC1 corresponded with a more
237 reduced point associated to the inner and outer roots with redressed point base and narrow base. Variations related
238 to VA along PC2 corresponded to anchors with relatively longer point, thinner and more curved shaft with broader
239 inner root and more pronounced outer roots (see Fig. 3b).

240 Canonical variate analyses only partially separated the samples among localities for both haptoral sclerites
241 and a considerable amount of overlap remained. For DA (Fig. 3c), CV1, explaining 69.8% of the total shape
242 variation, partially separated monogenean populations of Nyaruhongoka and Mukuruka. Meanwhile, CV2,
243 explaining 30.1% of the variation, partially separated those parasites of Magara from those of the other two
244 localities. For VA (Fig. 3d), CV1 (60.9% of variation) partially separated populations of Nyaruhongoka and
245 Magara from Mukuruka. The second CV (39% of variation), partially separated populations from Magara and
246 Nyaruhongoka. For both anchors, CV axes corresponded with different shape variations than those observed for
247 PC axes. The first CV axis on the DA plot described anchors having a less curved shaft and longer inner root with
248 a deeper notch. Along the second CV axis, specimens of *C. gistelincki* showed a DA with thinner base and slightly
249 shorter inner root and wider outer root, as well as a thinner shaft with a more pronounced shaft-point (Fig. 3c). For
250 the VA, variations along CV1 corresponded to a reduced notch, a thinner base with wider shaft at its base, and a
251 narrower shaft point. Along CV2, anchors mainly displayed a thinner base with a relatively more reduced inner
252 root, and notch between the roots (Fig. 3d).

253 The pairwise Procrustes distances, as well as the results of the permutation tests, are shown in Table 1.
254 Surprisingly, the smallest Procrustes distance for the DA dataset was found between the Mukuruka and Magara
255 populations. In the VA dataset, samples from Nyaruhongoka and Magara were the most similar in shape. The
256 highest distances were found between the Mukuruka and Nyaruhongoka populations for the DA, and between the
257 Magara and Mukuruka populations for the VA dataset. However, the difference in morphology was only
258 significant for the DA between the Mukuruka and Nyaruhongoka populations and for the VA only between the
259 Magara and Mukuruka populations. Only the *p*-value of the first comparison (Magara and Nyaruhongoka)
260 remained significant after Holm-Bonferroni adjustment for multiple testing.

261 **Discussion**

262 We investigated shape variation of the anchors in the attachment organ of *C. gistelincki* and *C.*
263 *milangelnari*, monogeneans that parasitise two endemic Tanganyika cichlids that are expected to differ in dispersal

264 capacities, '*C.*' *horei* and *C. microlepidotus*, respectively. We collected specimens of '*C.*' *horei* - a tropheine,
265 which disperses easily in the Lake, and *C. microlepidotus*, a cyprichromine, which shows restricted dispersal
266 ability. Our goal was to investigate the association between the host's dispersal capacity and the morphological
267 differentiations in cichlid-specific monogeneans, i.e. species of *Cichlidogyrus*. We specifically studied the shape
268 variability of the haptoral sclerites, as these are linked to adaptations to the fish host (Šimková et al., 2006; Olstad
269 et al., 2009; Bueno-Silva et al., 2011). We hypothesized that, because of the limited gene flow, cichlids with
270 restricted dispersal capacity will harbour morphologically more differentiated parasite populations compared to
271 good dispersers. This morphological approach focused on landmarks-based data by evaluating intraspecific anchor
272 shape variations.

273 Host dispersal is assumed to drive the genetic structure and the diversity of parasites (Mazé-Guilmo et
274 al., 2016). The results obtained from the morphological data of *C. milangelnari* populations infecting *C.*
275 *microlepidotus* agreed with our hypothesis. Geomorphometric results obtained for *C. milangelnari* reflected
276 differentiation between two relatively distant populations. Using a geomorphometric approach, differences in the
277 shape of their anchors (DA and VA) were found between specimens from Nyaruhongoka and Kalundo. In contrast,
278 geomorphometric patterns did not reveal differentiation among the studied populations of *C. gistelincki* infecting
279 '*C.*' *horei*, a well dispersing tropheine. It should be noted, however, that populations of *C. microlepidotus* stem
280 from opposite sides of the Rusizi River, a known barrier for many cichlid species, whereas those of '*C.*' *horei* all
281 originate from the same side. However, as '*C.*' *horei* thrives in vegetated areas and is even found more upstream
282 in this river (De Vos et al., 2001), we don't expect this to influence our results. Although one significant difference
283 in shape was revealed, geomorphometric data of anchors of *C. gistelincki* showed profound shape overlap among
284 distant and neighbouring populations for both the DA and VA datasets (see results section). The lack of a clear
285 geographical trend in the shape of haptoral structures of *C. gistelincki* can also be explained by other factors that
286 are known to influence morphological diversification in monogeneans, such as historical and local environmental
287 factors (Ergens & Gelnar, 1985; Dávidová et al., 2005; Bueno-Silva and Boeger, 2019). As we only used a genetic
288 maker that is highly conserved in monogenean species, we cannot say whether the patterns of morphological
289 variation in anchors of *C. gistelincki* and *C. milangelnari* are also reflected in the genomes of these monogenean
290 parasites. Hence, additional sampling, supplemented by the analyses of multi-locus data, would help us in the
291 future to investigate the population structure of each of the studied cichlid species and their monogeneans across
292 geographical scales.

In our study, the fifth landmark, which corresponded to the inner root, was the most variable in all analyses. In monogeneans, anchors are often supported by other sclerotized structures of the haptoral apparatus such as ventral and dorsal bars, or accessory sclerites (Roberts & Janovy, 2009). In *Cichlidogyrus*, the inner roots of the anchors are more closely situated to the bars than the outer roots. Possibly, the high variation in the inner roots is due to morphological changes in the other sclerotized parts of the haptor. The study of Rodríguez-González et al. (2015) supported this hypothesis as they connected shape variability in the DA and VA displayed by species of *Ligophorus* Euzet & Suriano, 1977 with that of the dorsal and ventral bars. So far, there are no detailed studies focusing on the functional role of the sclerotized structures of the attachment organ in *Cichlidogyrus*. Therefore, further studies are necessary to study shape variability in other cichlid gill flatworms using cichlid gill flatworms. However, we did not investigate the pattern of the shape variation in other haptoral sclerites as, compared to anchors, the marginal hooks and the connective bars are less suited for a study of two-dimensional landmarks. The marginal hooks are commonly prone to modifications from the flattening and/or the fixation processes. Similarly, the connective bars are thick, which make them less easily flattened and more exposed to distortion during fixation and mounting (Vignon & Sasal, 2010).

Conclusion

Despite the estimation of a high diversity of cichlid monogeneans in LT, studies on the intraspecific morphological and genetic variability of these cichlid-specific parasites remain scarce. Only few studies focused on intraspecific differences in the haptoral hard parts of gill-infecting monogeneans. Here, we showed that higher morphological differentiation is found in host-specific monogenean species that infect a poorly dispersing cichlid than in those that infect a good disperser. This indicates that the ecology of a host lineage influences diversification and therefore potentially speciation of its parasite fauna.

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528

529 **Figure captions**

530 **Fig. 1** Design of the study. **(a)** Map of northern Lake Tanganyika with sampling localities at the Burundese and 531 Congolese shorelines (edited with http://www.simplemappr.net and Photoshop v. 13.0), **(b)** '*Ctenochromis*' 532 *horei,* **(c)** *Cyprichromis microlepidotus* (photos Radim Blažek, Burundi 2013), **(d)** whole view of *Cichlidogyrus* 533 sp. (exemplified by *Cichlidogyrus longicirrus* Paperna, 1965), **(e)** sclerotized structures of *Cichlidogyrus* 534 *gistelincki* (DA, dorsal anchor; VA, ventral anchor; DB, dorsal bar; VB, ventral bar; HI-HVII, marginal 535 hooks; MCO, male copulatory organ; He, heel; Ap, accessory piece), **(f)** location of the nine analysed 536 landmarks on the right anchor (example DA of *Cichlidogyrus gistelincki*).

537 **Fig. 2** Geomorphometric analyses on DA and VA of *Cichlidogyrus milangelnari*. Scatter plots of the PCA of 538 DA (a) and VA (b) datasets, shape changes next to each PC are shown by wireframes with starting shapes 539 (consensus, value 0) in light blue, and target shapes (changes) associated with extreme values (value +0.1) in dark 540 blue. Scatter plots of the CVA of DA (c) and VA (d), shape changes next to CV axes are shown by the wireframes 541 associated with extreme values (+4).

542 **Fig. 3** Geomorphometric analyses on DA and VA of *Cichlidogyrus* gistelincki. Scatter plots of the PCA of DA 543 (a) and VA (b) datasets, shape changes next to each PC are shown by wireframes with starting shapes (consensus) 544 in light blue, and target shapes (changes) associated with extreme values (+0.1) in dark blue. Scatter plots of the 545 CVA of DA (c) and VA (d), shape changes next to CV axes are shown by the wireframes associated with extreme 546 values (+5).

547 **Table captions**

548 **Table 1** Matrix of Procrustes distances and p-value from permutation test with 10 000 randomizations (**in bold**) 549 among localities using the DA (left side of the diagonal) and VA (right side of the diagonal) observations of 550 *Cichlidogyrus gistelincki*. Distances for *Cichlidogyrus milangelnari* are represented on the right side of the table 551 with the left column for DA and right column for VA. "*" indicates a statistically significant *p*-value after Holm-552 Bonferroni correction.

Figure 1

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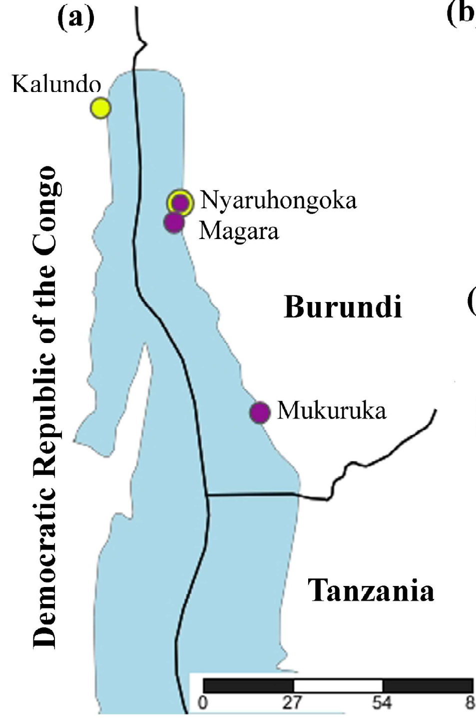

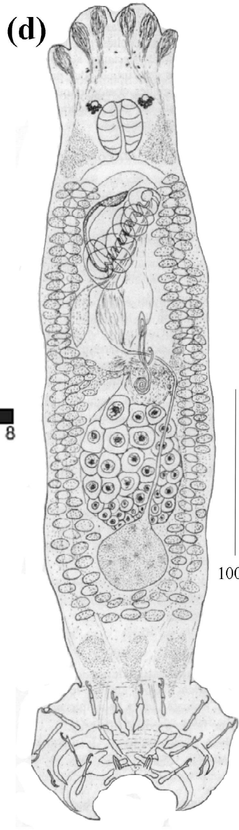

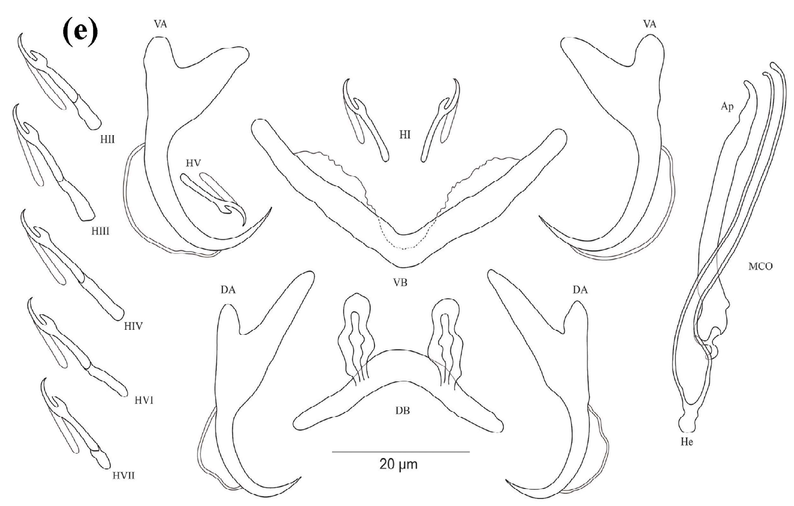

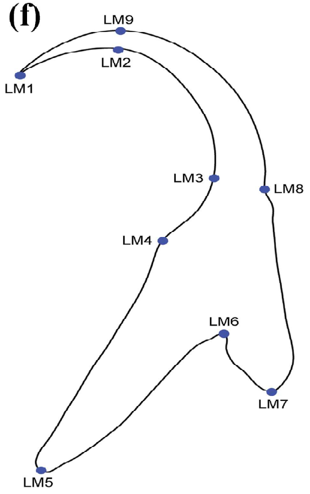

Figure 2

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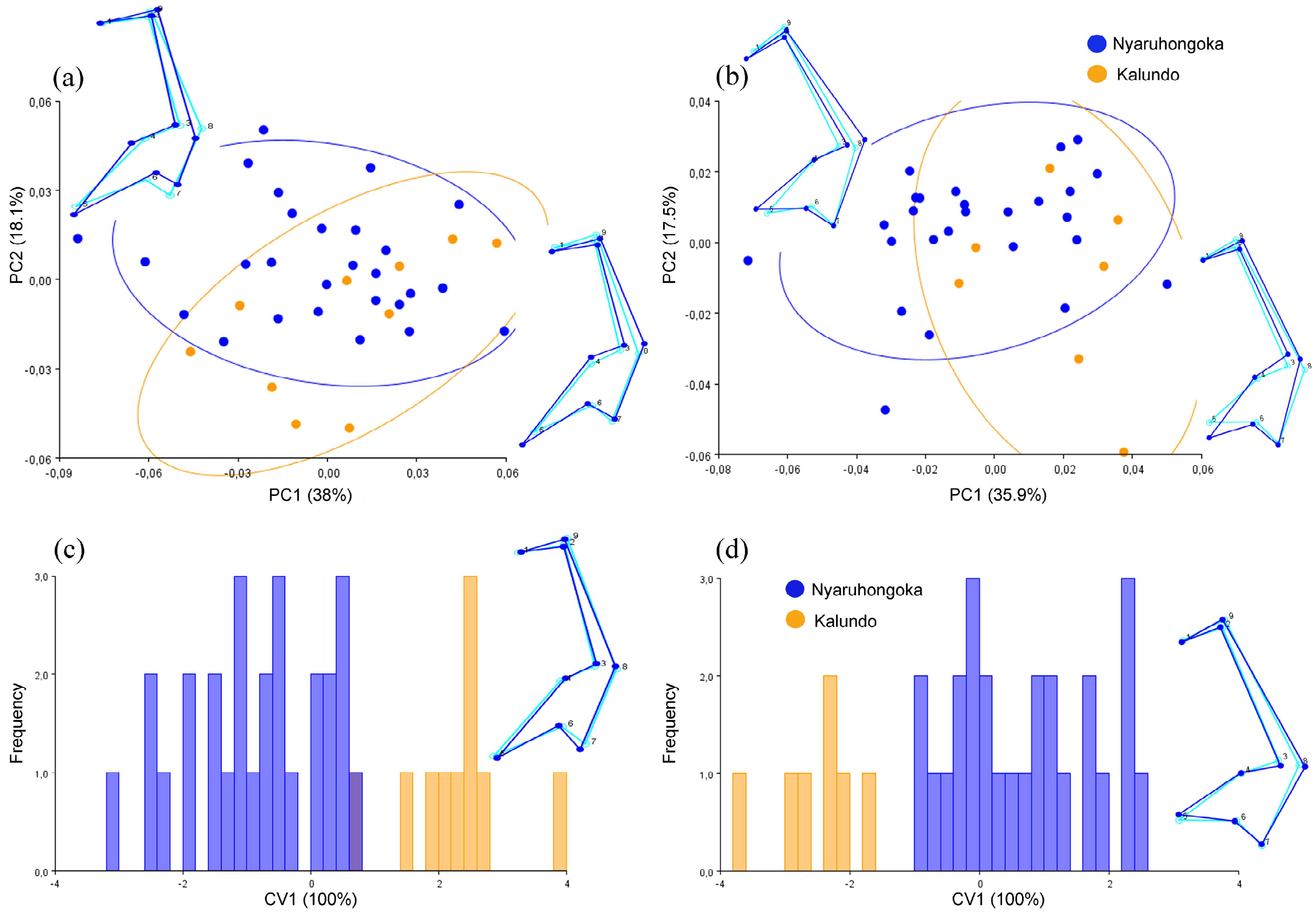

Figure 3

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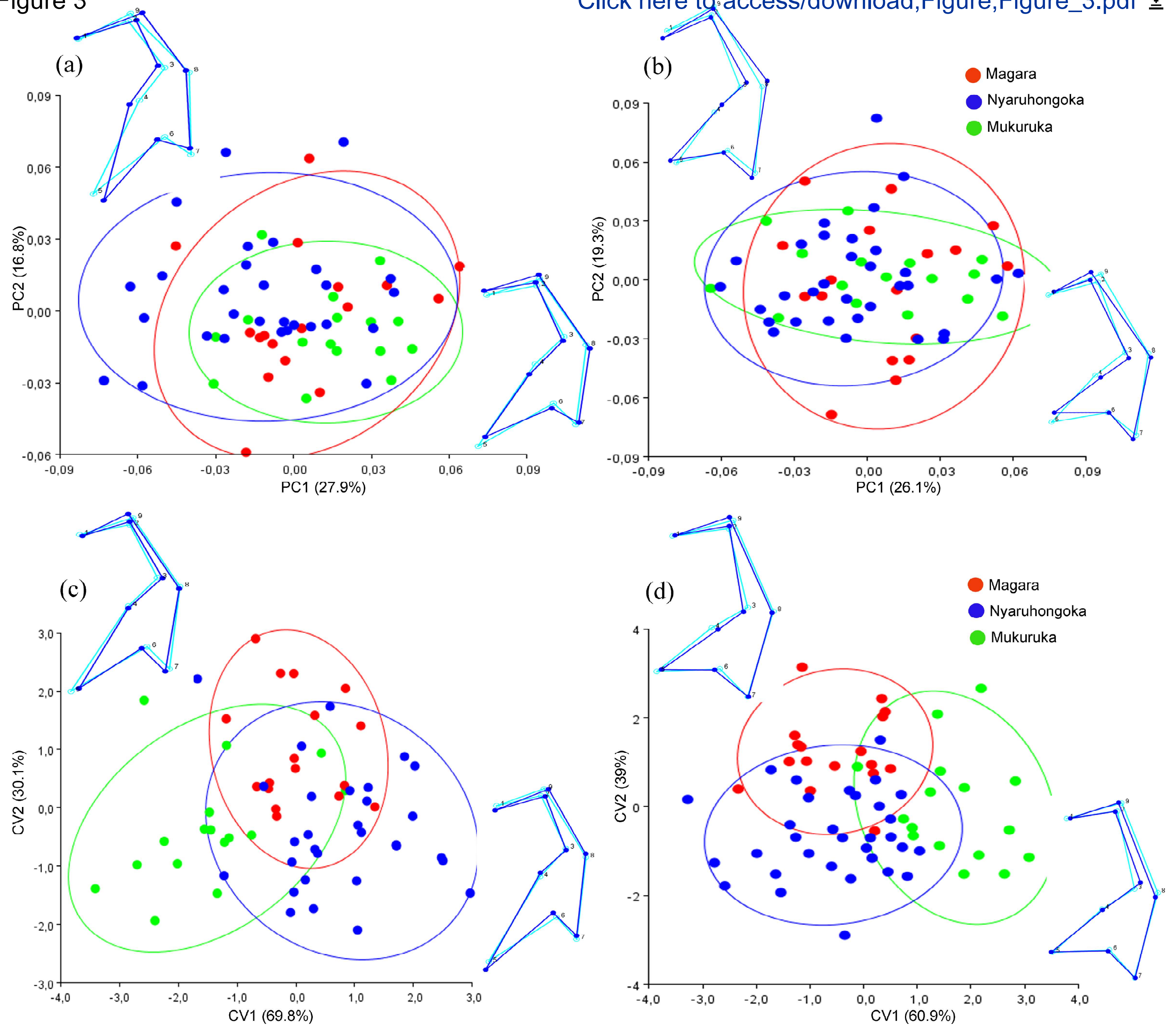

Table 1 Click here to access/download;Table;Table 1_RESUBMISSION.docx

Table 1 Matrix of Procrustes distances and p-value from permutation test with 10 000 randomizations (in bold) among localities using the DA (left side of the diagonal) and VA (right side of the diagonal) observations of *C. gistelincki*. Distances for *C. milangelnari* are represented in the right side of the table with the left column for DA and right column for VA. "*" indicates a statistically significant *p*-value after Holm- Bonferroni correction.

Species	*C. gistelincki*				*C. milangelnari*	
Localities	Magara	Mukuruka	Nyaruhongoka	Magara	Kalundo	
Mukuruka	0.0214	-	0.0246	0.0295	-	-
	0.2866	-	**0.0577**	**0.0383**	-	-
Nyaruhongoka	0.0235	0.0331	-	0.0218	0.0320	0.0299
	0.1248	**0.0022***	-	**0.1469**	**0.0179***	**0.038***