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Morphological and Genetic Divergence in a Gill Monogenean Parasitizing Distant Cichlid Lineages of Lake Tanganyika: Cichlidogyrus nshomboi (Monogenea: Dactylogyridae) from Representatives of Boulengerochromini and Perissodini Peer-reviewed author version

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1	Morphological and genetic divergence in a gill monogenean parasitizing distant cichlid lineages of Lake
2	Tanganyika: Cichlidogyrus nshomboi (Monogenea: Dactylogyridae) from representatives of
3	Boulengerochromini and Perissodini
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13 ABSTRACT

14 As hosts constitute the resource for parasites, an adaptive radiation in a host can drive one in a parasite. 15 In Lake Tanganyika, the diversification of cichlids has often led to a diversification of their Cichlidogyrus 16 monogeneans. Hitherto, Cichlidogyrus nshomboi was known only from Boulengerochromis microlepis, the sole 17 member of Boulengerochromini. Surprisingly, we retrieved this monogenean from Perissodus microlepis, P. 18 straeleni and Haplotaxodon microlepis, belonging to Perissodini. We sequenced the nuclear 18S, 28S, ITS1 rDNA, 19 and the mitochondrial COI genes and studied the morphology of the male copulatory organ (MCO) and the anchors 20 of the attachment organ. This confirmed the conspecificity of the specimens. The occurrence of C. nshomboi on unrelated host lineages could be explained by inheritance from a common ancestor, or by host-switching. We 21 22 further investigated the genetic and morphological variation across taxonomic (host tribes and species) and 23 geographical scales. Results revealed divergence in ITS1 and COI between parasites infecting different tribes, 24 which could indicate incipient speciation. Additionally, morphological differentiation in the shape and size of 25 anchors was found between these groups, which could be attributed to phenotypic plasticity or to adaptation. 26 Monogeneans from large-bodied B. microlepis had significantly larger anchors, whereas only two of the four 27 measurements differed for the MCO. Unexpectedly, no morphological variation was observed between specimens 28 infecting different species of Perissodini from nearby localities. However, differences were found between C. 29 nshomboi infecting P. microlepis from different parts of the lake, which could be linked to the population genetic 30 structure of the host.

31 KEYWORDS

32 Boulengerochromis microlepis, Perissodus microlepis, Perissodus straeleni, anchors, MCO, parasitism.

33

34 Introduction

35 The large variation in diversification and speciation rates at all levels of biological classification form a hallmark of the tree of life. Hence, why certain lineages diversify, and others fail to do so remains one of the 36 37 fundamental questions in speciation research. This especially holds true for parasites, which form a large part of 38 metazoan diversity. Indeed, as most free-living organisms are infected by several species of parasites, parasitism 39 can be considered the most common life strategy on earth (Windsor 1998). Traditionally, parasitic helminths are 40 classified according to their specificity (Humphery-Smith 1989), i.e. the extent to which a parasite's distribution is limited to certain host species ('host range' sensu Lymbery, 1989) (Poulin and Mouillot 2003), and their life 41 42 cycle (Roberts and Janovy 2009). Species with direct life cycles infect only a single host, while those with indirect life cycles need several hosts to reproduce (Gussev 1995). Whereas differences in life cycle appear to be 43 44 evolutionarily highly conserved, a large variation can exist regarding specificity, even among closely related 45 parasite taxa (Mendlová and Šimková 2014). It remains largely unstudied how transitions between a specialist and 46 more generalist lifestyles evolved and how evolutionarily flexible host specificity is (see for instance Agosta et al. 47 (2010)).

48 Fundamental questions in parasitology have been addressed by looking at species flocks (Hablützel et al. 49 2017; Karvonen and Seehausen 2012 and references herein). These are large assemblages of closely related, 50 endemic species that have evolved rapidly within a specific area (Ribbink 1984). They often arose via explosive 51 speciation and adaptive radiation, i.e. the evolution of a diversity of ecological roles and adaptations within a 52 lineage (Givnish 1997). As species flocks are phylogenetically and geographically confined, and as adaptive radiations can create evolutionary replicates, these systems form prime natural experiments to investigate 53 fundamental questions in ecology, evolution, and speciation research (Kocher et al. 1995; Kornfield and Smith 54 55 2000). From a parasitological point of view, however, most of the studies have looked at the potential effect of 56 parasites on the diversification of the hosts, and less on the effect of diversification of the hosts on that of parasites. 57 As hosts provide the resources for parasites, an adaptive radiation in a host lineage can trigger an adaptive radiation 58 in that of a parasite. For species-specific parasites, this process would naturally be obtained via co-speciation, in 59 which the speciation events in hosts trigger those in parasites, after which within-host speciation can increase the 60 number of parasite species even more (Vanhove et al. 2015). However, in some cases, failure to co-speciate has been recorded in species flocks (Kmentovà et al. 2016; Gobbin et al. 2020). Hence, these species-rich systems can 61 62 provide insights as to when parasite speciation overtakes, follows, or lags the diversification of the hosts. Here, we

will study this using <u>parasitic flatworms</u> belonging to *Cichlidogyrus* <u>Paperna</u>, <u>1960</u> (Monogenea,
 <u>Plathyhelminthes</u>) infecting phylogenetically distantly related species of Lake Tanganyika cichlids as a model.

65 The species flocks of cichlids in the Great East African Lakes form the most prominent examples of 66 adaptive radiation (Kocher 2004) and explosive speciation (Salzburger et al. 2014; Schwarzer et al. 2017; 67 Seehausen 2015; Sturmbauer et al. 2011) in vertebrates. The oldest of these lakes (9-12 Ma) (Cohen et al. 1997), 68 Lake Tanganyika, holds the most ancestral and the morphologically, behaviourally, ecologically, and genetically 69 most diverse lacustrine cichlid assemblage, albeit not the most species-rich one (Snoeks 2000). Its 241 species of 70 cichlids (Ronco et al. 2020) can be classified into fourteen to sixteen tribes, which range from containing a single 71 to about hundred species (Poll 1986; Takahashi 2003; Koblmüller et al. 2008). Generally speaking, these tribes 72 occupy one, or a few gross ecological niches (Koblmüller et al. 2008). This certainly holds for the tribes 73 investigated herein: Boulengerochromini Takahashi, 2008 and Perissodini Poll, 1986.

74 Monogeneans are a group of (mostly) ectoparasitic gill parasites of aquatic vertebrates (Roberts and 75 Janovy 2009). They are diverse in terms of species number (Cribb et al. 2002; Theisen et al. 2017), host specificity, 76 morphology, reproductive strategy, and ecology (Whittington et al. 2000). With regard to host specificity, Šimková 77 et al. (2006) classified them into five categories ranging from strict specialists (i.e., a parasite species restricted to 78 a single host species) to real generalists (parasitizing phylogenetically unrelated host species). As monogeneans 79 have a direct, single-host life cycle, they form an ideal model system to investigate host-parasite interactions 80 (Poulin 2002). This especially holds for groups with purely sexual modes of reproduction, as for these species, 81 each monogenean specimen represents an independent infection of a host. This is case for *Cichlidogyrus*, which 82 is, with 131 valid species, the most species-rich monogenean genus parasitising African, Malagasy or Levantine 83 cichlids (Pariselle and Euzet 2009; Řehulková et al. 2018). Morphological identification in *Cichlidogyrus* is mostly 84 based on sclerotised structures of the haptor and the male copulatory organ (MCO). The latter consists of a 85 copulatory tube that is often coupled with a simple or complicated structure called the accessory piece, which normally extends from the bulb of the tube towards its distal end. The haptor is a highly characteristic attachment 86 87 organ located at the posterior end of the worm (Roberts and Janovy 2009). In members of Cichlidogyrus, it contains 88 two sets of anchors (dorsal and ventral) that are connected by transversal bars and surrounded by marginal hooks. 89 As the anchor prevents physical dislodging, its morphology reflects adaptations to the gills of the fish host (Rohde and Watson 1985; Šimková et al. 2002; Dávidová et al. 2005). Additionally, haptoral morphology has also been 90 shown to represent phylogenetic (Vignon and Sasal 2010; Khang et al. 2016) and geographic signals in 91 92 monogeneans (Lim and Gibson, 2009; Rahmouni et al., 2020).

93 Mirroring its cichlids, Lake Tanganyika contains a unique Cichlidogyrus assemblage that can be classified 94 into several morphological lineages. Hitherto, 39 species of these monogeneans have been described from 15 95 species of Lake Tanganyika cichlids from various tribes (Rahmouni et al. 2018; Řehulková et al. 2018). If this 96 number were to be extrapolated, it could be said that these gill flatworms are even more diverse than the cichlids 97 themselves, especially as many of them were found to be highly species-specific (Van Steenberge et al. 2015; 98 Vanhove et al. 2015). However, a large amount of variation exists in Cichlidogyrus with regards to host specificity, 99 which seems linked with the ecology and the phylogenetic position of the host lineage. Indeed, in the mostly rock 100 dwelling, and often stenotopic members of Tropheini Poll, 1986, host specificity was remarkably high (Van 101 Steenberge et al. 2015; Vanhove et al. 2015). Alternatively, only a single monogenean species, C. casuarinus 102 Pariselle, Muterezi Bukinga & Vanhove, 2015 was found infecting several representatives of the ancestral deep-103 water lineage of Bathybathini Poll, 1986, suggesting a generally lower host specificity, and a lower parasite 104 diversity in the deep-water realms (Kmentová et al. 2016). A species morphologically similar to C. casuarinus, C. 105 nshomboi Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle, 2012, was found to parasitize the Lake's top 106 predator cichlid and the sole representative of Boulengerochromini: Boulengerochromis microlepis (Boulenger, 107 1988).

108 A recent field survey revealed the presence of specimens of *Cichlidogyrus* that could be identified as C. 109 nshomboi based on morphological criteria on all examined cichlid species belonging to Perissodini. This was 110 unexpected as Perissodini and Boulengerochromini are only distantly related: B. microlepis represents the earliest 111 diverging lineage of the East African radiation, in which Perissodini are deeply nested (Ronco et al. 2021). 112 Additionally, members of these tribes also have profoundly different ecologies. Boulengerochromis microlepis is 113 a substrate-brooder that ranks amongst the world's largest cichlids. It is a highly mobile top-predator that occupies 114 the lake's bentho-pelagic habitat (Meyer et al. 2015; Konings 2019). Perissodini contains nine species that are all 115 mouth-brooders, but that belong to two groups with different ecologies (Koblmüller et al. 2007, Takahashi et al. 116 2007). One of these contains the two species of Haplotaxodon Boulenger, 1906: Haplotaxodon microlepis 117 Boulenger, 1906 and Haplotaxodon trifasciatus Takahashi & Nakaya, 1999 (considered synonyms by Konings 118 (2019)). These are open-water predators that feed on juveniles of pelagic fishes and on zooplankton. The second 119 group consists of six representatives of Perissodus Boulenger, 1898 and of Xenochromis hecqui Boulenger, 1899. 120 These species have a lepidophagous, or scale-eating, diet, a spectacular feeding mode that is not found in other 121 Lake Tanganyika tribes. It should be noted, however, that the generic position of Xenochromis hecqui Boulenger, 122 1899 is debated and that it has, at least, a less strict lepidophagous diet than the other members of the group (Takahashi et al. 2007, Konings 2019). Most members of this scale-eating group occur in deeper waters, and little
is known about their ecology and behaviour. *Perissodus microlepis* Boulenger, 1898 and, to a lesser degree, *Perissodus straeleni* (Poll, 1948), however, are found in shallower waters (Konings 2019). These sister species are
relatively recent offshoots of the perissodine radiation. The two species differ in hunting technique with *P. microlepis* being a highly mobile scale-eating cichlid, and *P. straeleni* behaving more as an ambush predator
(Yanagisawa et al. 1990).

129 In this study, we used a combined molecular and morphological approach to study Cichlidogyrus infecting 130 boulengerochromine and perissodine cichlids sampled at different localities along Lake Tanganyika. We studied 131 the morphology of the MCO and anchors using traditional morphometric and geomorphometric (Adams et al. 132 2014) techniques, respectively. While the former is based on lengths, widths, and angles, the latter focuses on 133 shape as a whole (Bonhomme et al., 2014). We focussed on the anchors as these were shown to be more appropriate 134 for geomorphometric studies than other haptoral structures as they are more resistant to deformation when mounted 135 on a slide (Vignon and Sasal, 2010). We further sequenced three nuclear genes (18S, ITS1 and 28S rDNA) and 136 one protein-coding mitochondrial gene (COI). The latter gene is nowadays the standard marker for DNA barcoding 137 (Hebert et al. 2003), both in vertebrate, and in several invertebrate taxa (Evans and Paulay 2012). However, it is 138 not a suitable marker for barcoding in parasitic flatworms given its high rate of molecular evolution (Hillis and 139 Dixon 1991), often even necessitating taxon-specific primers (Moszczynska et al. 2009; Vanhove et al. 2013). 140 Hence, it was used to answer population genetic questions, whereas the three nuclear markers were used for species 141 recognition and delineation. The 28S and 18S rDNA genes are conservative markers commonly used to confirm 142 the taxonomic status of dactylogyrids and to infer their phylogeny (Plaisance et al. 2005; Šimková et al. 2006; 143 Mendlová et al. 2012; Vanhove et al. 2013; Messu Mandeng et al. 2015; Benovics et al. 2017, 2018; Rahmouni et 144 al. 2020). The ITS1 rDNA is somewhat more variable allowing it to be used as a species delineation marker in 145 monogeneans (Zietara and Lumme 2002; 2003). If the representatives of Cichlidogyrus parasitizing members of 146 the two cichlid tribes would prove to be conspecific, our approach would allow us to contrast genetic and 147 morphological differentiation in a single parasite species across a taxonomic (tribe, genus, and species), ecological 148 (predator vs. scale eater) and a geographical (localities) gradient of cichlid hosts.

We hypothesize herein that <u>a</u>) a single species of *Cichlidogyrus* infects boulengerochromine and perissodine cichlids. Additionally, we hypothesize that <u>b</u>) the genetic and morphological differentiation between monogeneans mirror_the phylogenetic and ecological distinctness of the hosts, and <u>c</u>) that larger differences are found between monogeneans infecting heterospecific host populations than between monogeneans infectingconspecific host populations.

154 Materials and Methods

155 Study area and cichlid hosts

156 Four cichlid species: B. microlepis, P. microlepis, P. straeleni and H. microlepis were examined for gill 157 monogeneans (Table 1, Fig. 1a). The former three were collected during various field trips in Tanzania (2008), the 158 DR Congo (2010) (see Van Steenberge et al. 2011), and Burundi (2013). Specimens of B. microlepis were acquired 159 from a fish market, whereas those of P. microlepis and P. straeleni were caught while snorkelling (Fig. 1a). 160 Although the catch locality is less specific for *B. microlepis*, all specimens stem from small-scale capture fishes. 161 As such, these fishes were caught relatively close to the market and acquired their parasites naturally. Although 162 no known geographic variation is present in the sample of *B. microlepis*, this does hinder the study design as this 163 species does not harbour any known geographical variation in the lake (Koblmüller et al. 2015), in contrast to P. 164 microlepis (Koblmüller et al. 2009). Gill samples from H. microlepis stem from historical specimens stored in the 165 ichthyological collections of the Royal Museum for Central Africa (RMCA, Tervuren, Belgium). For freshly 166 collected specimens, both gill chambers were examined for parasites. On historical samples, only the right gill 167 chamber was examined, following museum policy. In total, the cichlid hosts stem from 12 sites along the 168 Tanganyikan lakeshores, of which five are situated in the northern (NB), five in the central (CB), and two in the 169 southern basin (SB) (Danley et al. 2012). Of these, six localities were included in further analyses (see below). 170 The abbreviations used herein for the cichlid hosts combined to their sampling sites across the three sub-basins of 171 the lake are presented in Table 1. Cichlids were identified to species level in situ and in the laboratory by 172 comparison with type material and using the literature (Poll 1986). Freshly collected specimens were immediately 173 transported to a field laboratory for dissection. Hereafter, they were preserved in formalin and deposited in the 174 RMCA (accession numbers available after acceptance). Fish nomenclature follows the catalog of Fishes (Fricke 175 et al. 2021).

176 Monogenean isolation and identification

The gills of *H. microlepis* from Murega and Mukumba were free of monogeneans. Aditionally, only a
small number of monogenean specimens could be isolated from *P. microlepis* from Bulumba Island (NB, n=2), *H. microlepis* from Luhunga (NB, n=3) and *P. straeleni* from both Mtosi and Mtoto (respectivly, CB and SB, n=2)
(Table 1, Fig. 1a). Therefore, these samples were included in the morphological and/or genetic characterization,

181 but not in the geomorphometric study (see below). Adult specimens of Cichlidogyrus were detached from the gill 182 arches and isolated according to Musilová et al. (2009) using an MST130 stereoscopic microscope. Monogenean 183 specimens used for DNA analyses were cut in half using fine needles under a dissecting microscope, the haptoral 184 part of the body was placed in 96% ethanol for genomic DNA extraction. The remaining part containing the 185 reproductive organs was completely flattened under a coverslip and fixed following the basic mounting protocol 186 (Rahmouni et al., 2017; 2018) with glycerine ammonium picrate (GAP) (Malmberg 1957). As monogenean 187 specimens isolated from the gills of H. microlepis stem from formaline-fixed specimens, they were mounted in 188 Hoyer's medium (Humason 1979), which is considered as strong clearing agent (Fankoua et al. (2017). This 189 fixation medium is better-suited for monogenean tissues that, due to lengthy fixation, could have become opaque, 190 hindering the imaging of diagnostic hard parts. In view of the difference in fixation, specimens isolated from H. 191 *microlepis* were neither included in the genetic nor in the morphological analyses.

All monogeneans were morphologically identified following the original description of Muterezi Bukinga et al. (2012). The terminology of dorsal (DA) and ventral anchors (VA) of the haptoral apparatus follows Gussev (1983). For the analyses, parasites were grouped by host species and location. Basic epidemiological data, i.e. prevalence, mean abundance, minimum and maximum intensity of infection, were calculated according to Bush et al. (1997).

197 Genetic characterization

198 We sequenced and amplified three nuclear and one mitochondrial marker for the examined monogeneans, 199 following Kmentová et al. (2016). The domains C1-D2 of the partial 28S rDNA region were sequenced using 200 universal primers (Hassouna et al. 1984). As this marker is highly conservative and commonly used for species 201 recognition in flatworms (Vanhove et al. 2013, Rahmouni et al., 2020), we used it to confirm the conspecificity of our specimens. We further obtained sequences of the partial 18S, the entire ITS1 rDNA using the primers S1-IR8 202 203 (Šimková et al. 2003), as well as the partial mitochondrial COI gene for a selection of samples using ASmit1 and 204 Schisto3 (Littlewood et al. 1997), with ASmit2 as internal primers for the nested PCR (Plaisance et al. 2008). 205 Sequence data was checked manually for sequencing errors, edited using Sequencher® v.5.0 (Gene Codes 206 Corporation, Ann Arbor, MI USA), and aligned using ClustalW (Thompson et al. 1994) implemented in MEGA 207 X (Kumar et al. 2018). The sequence fragments from the nuclear genes were subjected to NCBI BLAST (Altschul 208 et al. 1990) in order to compare them with all previously submitted sequences of Cichlidogyrus. The CLC 209 Sequence Viewer v. 8.0 (CLC Bio-Qiagen, 2016) was used to visualize the nucleotide substitutions in the nuclear and the mitochondrial alignments. Nucleotide sequences were deposited in GenBank under accession numbers:
(available after acceptance). Uncorrected *p*-distances were calculated between specimens and groups using
MEGA X. For the sequences of COI, DnaSP v. 5.10.01 (Librado and Rozas 2009) was used to study genetic
diversity by calculating the number of polymorphic sites, nucleotide diversity, number of haplotypes, sequence
conservation, and average number of nucleotide differences.

215 Geomorphometric analyses and size of the anchors

216 We used a landmark-based technique to investigate the morphological variation in the DA and VA 217 between the groups of Cichlidogyrus for which enough freshly collected material was available (Table 1). 218 Landmarks (LMs) are discrete anatomical loci observed at the same place in all specimens. For this, all slides were 219 photographed using an Olympus BX51 phase-contrast microscope and Olympus Stream Image Analysis v. 1.9.3. 220 Voucher specimens of monogenean species from each cichlid host and sampling locality were deposited in the 221 RMCA under the accession numbers (available after acceptance). Landmarks placed on photographs of DA and 222 VA were analysed separately. We used only specimens for which the whole body was mounted on a slide, and we 223 selected the anchors located at the right side of the worm. Photos were inputted into tps-Util v.1.76 and LMs were 224 digitalized using tpsDig2 (Rohlf 2006). Nine LMs were used for both DA and VA following the terminology of 225 Rodríguez-González et al. (2015) and Rahmouni et al. (2020) (Fig. 1b). All landmarks were placed by one person 226 (C. Rahmouni). Coordinate files were imported into MorphoJ v. 1.06 (Klingenberg 2011) for further analysis. 227 Here, raw LM coordinates of all specimens were superimposed using Procrustes' fit (Zelditch et al. 2012) and 228 aligned by the axis defined by LM2 and LM9 (Fig. 1b).

229 To visualize the variation at taxonomic and geographical levels, Principal Component Analyses (PCA) 230 were performed on the covariance matrices of the DA and VA datasets. Additionally, canonical variate analyses 231 (CVA) were performed to maximize the differentiation in anchor shape between groups. Procrustes distances 232 between groups were calculated using a permutation test (Good 2001) with 10,000 randomizations ($\alpha = 0.05$). The 233 main axes of the PCA and CVA were visualized using wireframe diagrams (Klingenberg 2011). To investigate 234 the effect of the size on the shape of the anchors (allometry), linear regressions between PCs of haptoral sclerites 235 and the log-transformed centroid size (log CS) were performed. Using the Mahalanobis distances between 236 groups, Neighbour-Joining (NJ) trees were constructed with 1,000 bootstrap replicates using PAST (Hammer et 237 al. 2001). The size, measured as log CS, of both DA and VA, was compared between groups using Mann-Whitney U tests, followed by post-hoc Holm-Bonferroni correction ($\alpha = 0.05$) in PAST, as Shapiro-Wilk 238

anormality tests indicated that the conditions of normality were not met. Statistica v. 13.5 (TIBCO Inc., 2018) was

240 used to visualize the distribution of the anchors size.

241 Morphometrics of the male copulatory organ

242 Four measurements (in micrometres, µm) of the MCO were taken at a magnification of ×1000 (objective 243 $\times 100$ oil immersion, ocular $\times 10$), using similar microscope and software as above. These are: the total length (TL), 244 and the lengths of the copulatory tube (PE), the accessory piece (AP) and the heel (HE) (Fig. 1c). The features of the MCO were drawn using the same microscope equipped with a drawing tube and edited with a graphic tablet 245 246 compatible with Adobe Illustrator CS6 v. 16.0.0 and Adobe Photoshop v. 13.0. All measurements were normally 247 distributed, based on Shapiro-Wilk normality tests, except for that of the heel (HE). Therefore, both parametric 248 (one-way analysis of variance ANOVA followed by post-hoc Tukey's HSD test) and non-parametric tests (Mann-249 Whitney U test followed by post-hoc Holm-Bonferroni test) were applied to compare measurements between 250 groups. The data was also checked for homogeneity of variances using Levene's test (significance level $\alpha = 0.05$ for all tests). All statistical analyses were carried out using PAST v.3.23 (Hammer et al. 2001). The graphical 251 252 representation of the distributional size of MCO features per cichlid host and locality of sampling was made using 253 Statistica.

254 Results

255 All monogenean specimens sampled from the gills of boulengerochromine and perissodine hosts were 256 morphologically identified based on their sclerotized structures (haptor and MCO) as C. nshomboi following the 257 original description of Muterezi Bukinga et al. (2012) (Fig. S1). Cichlidogyrus nshomboi parasitized 38 out of 47 258 boulengerochromine and perissodine specimens; the overall prevalence and mean abundance were 80.8% and 4.08 \pm 14.2, respectively. The lowest prevalence and mean abundance were recorded on *H. microlepis* from historical 259 260 samples and were 14.2% and 0.42 ± 1.1 , respectively. It should be noted, however, that only one gill chamber was 261 examined for *H. microlepis*, which could explain the lower parasite infection. Additionally, Tthis could be related 262 to the quality of the gills, which were conserved in same medium for over 60 years. Boulengerochromis microlepis 263 showed a prevalence of 50% and 24.5 ± 49 mean abundance, while all specimens of *P. microlepis* and *P. straeleni* 264 were infected (100%) with 2.96 ± 3.05 and 1.7 ± 1.05 mean abundance, respectively. The total intensity of infection 265 ranged from 1 to 98 monogeneans per infected host, the highest number of specimens of C. nshomboi was found 266 on the gills of B. microlepis, followed by P. microlepis and P. straeleni and then H. microlepis with the lowest 267 intensity of infection.

268 Genetic divergence of the nuclear and mitochondrial genes

269 The conspecificity of the parasite specimens was molecularly confirmed using the sequences of the partial 270 28S, and 18S plus the entire ITS1 rDNA regions. In total, we obtained 22 sequences of 28S rDNA and 15 sequences 271 of 18S rDNA and ITS1 (Table 1). The sequences of the partial 28S rDNA were about 770 bp before trimming, and 272 the final alignment was 756 bp long. The raw sequences of partial 18S rDNA, together with ITS1 were 860 bp 273 long, with 481 bp for 18S and 368 bp for ITS1 rDNA after trimming. The 18S and 28S rDNA sequences were all 274 identical. For ITS1 rDNA, a differentiation between C. nshomboi specimens parasitizing B. microlepis and those 275 infecting perissodine hosts occurred at four nucleotide positions (see Fig. S2a), resulting in a sequence divergence 276 of 1.1% (uncorrected p-distance). However, no genetic variation was found between C. nshomboi specimens 277 parasitizing hosts of the same tribe. All sequences were entered in BLAST in order to compare them with all 278 previously submitted sequences of *Cichlidogyrus*. The 18S sequences were identical to those of *C. casuarinus*, 279 whereas those of the 28S rDNA were 99.87% similar. For the ITS1 rDNA, the monogenean specimens isolated 280 from boulengerochromines and perissodines revealed 96.45% of genetic similarity to C. casuarinus.

281 The mitochondrial COI gene was successfully sequenced for seven specimens of C. nshomboi parasitizing 282 B. microlepis and three parasitizing P. microlepis, all from Nyaruhongoka (Table 1). Sequences were about 450 283 bp long, and 444 bp after trimming. Respective nucleotide and haplotype diversities were 0.06 and 0.86, and 6 284 haplotypes were detected. The number of variable sites (mutations) was 56. The genetic divergence ranged from 285 11.7% to 12.2% between C. nshomboi specimens parasitizing species of different tribes. All COI sequences 286 obtained from specimens of C. nshomboi of P. microlepis were identical, wherase intrapopulation genetic distance 287 between monogenean specimens from B. microlepis was 0.5% at most (2 bp) (see substitutions in Fig. S2b).

288

Geomorphometry of dorsal and ventral anchors

289 In total, 75 and 76 images of DA and VA were analysed. Of these, 26 (DA and VA) were from C. 290 nshomboi specimens from of boulengerochromine and 49 (DA) and 48 (VA) from perissodine hosts. Five (DA, 291 VA) images stem from C. nshomboi infecting P. straeleni. Finally, 44/45 (DA/VA) images stem from C. nshomboi 292 infecting *P. microlepis* collected at four different locations (Table 1).

293 Shape and size of the dorsal anchors (DA)

294 The PCA of landmark data on the DA is visualized in Fig. 2. The three most informative PCs together 295 accounted for 77.70% of the total variance. The first PC explained 60.21% of the total variance and fully separated 296 the C. nshomboi that infected B. microlepis from those infecting perissodines. The main shape changes along PC1 297 corresponded with DA having a reduced outer root and less curved shaft (Fig. 2a). The second PC, accounting for 298 10.81% of total variance failed to separate any of the groups. The shape changes on the positive side of PC2 299 corresponded with DA having a more pronounced point-border, a relatively thinner base with shorter inner root 300 and a reduced notch, plus a longer outer root (Fig. 2a). The third PC accounted for 6.68% of the variance and 301 separated specimens of C. nshomboi infecting perissodine hosts from the two northernmost sites, from those of 302 the rest of the Lake (Fig. 2b). Changes along this axis corresponded with DAs having a shorter point, a longer 303 shaft, and a relatively wider inner root with a reduced notch (Fig. 2b).

In order to check whether the separations obtained in PC1 and PC3 were independent of the size of the anchors, we plotted these axes against log CS. Multivariate regression analysis revealed a significant correlation between the scores of log CS and PCs (r = -0.341, p < 0.001 for PC1 (Fig. 2c)) and (r = -0.044, p = 0.006 for PC3 (Fig. 2d)), although *r* indicated only a small effect size for the latter. Additionally, within the group of *C. nshomboi* parasitizing only perissodine hosts, there did not seem to be any correlation between the shape and the size of anchors along PC1, indicating that the differences observed in PC1 were not due to allometry.

In the CVA performed on the DA dataset of all studied groups of *C. nshomboi*, over 95% of the variation was explained by CV1 and CV2 (85.80 % and 11.26%, respectively). As PC1, CV1 fully separated specimens of *C. nshomboi* at the level of the host tribe. Similarly, CV1 corresponded with a change in the length of the outer root and the curvature of the shaft (Fig. 2e). As for PC3, CV2 separated *C. nshomboi* parasitizing perissodines at a geographical scale. This CV corresponded with a change in the length of the shaft and the thickness of the base (Fig. 2e).

316 Pairwise Procrustes distances for DA and results of permutation tests are shown in Table 2. The highest 317 Procrustes distances, as well as shape variability of DA were found between specimens of C. nshomboi infecting 318 hosts of different tribes (NB1 vs. all other localities). Within tribes, no difference was found in DA morphology 319 between specimens of C. nshomboi from P. straeleni and from P. microlepis. Within C. nshomboi parasitizing 320 geographically distinct groups of *P. microlepis*, however, the shape of the DA differed significantly between some 321 of the northern and the central/southern groups (NB2 vs. CB1 and SB1, NB3 vs. SB1, Table 2). No difference was 322 found between the pairs of northern (NB2, NB3), and central/southern groups (CB1, SB1, CB2) (Table 2). In the 323 NJ tree (Fig. 3a) based on Mahalanobis distances (Table S1) all clusters were well-supported (\geq 95%). The tree 324 indicated a separation of the group of C. nshomboi parasitizing B. microlepis from all groups hosted by the two perissodine cichlids. Within the perissodine hosts, specimens of *C. nshomboi* clustered following a geographical
pattern. The groups of northern *P. microlepis* branched off first and monogeneans of both *P. microlepis* and *P. straeleni* in the central (western) part of Lake Tanganyika were separated from the groups of *C. nshomboi*parasitizing *P. microlepis* in the southern (eastern) part of the lake.

329 The differences in log CS of DA between the groups of C. nshomboi are presented in Fig. 3b. Mann-330 Whitney U tests revealed that the values for C. nshomboi parasitizing B. microlepis were significantly higher than 331 those of specimens infecting species of Perissodini, indicating bigger anchors in the former (NB1 vs. all the 332 remaining localities, Table S2). Within Perissodini, no clear pattern of size variability was observed. The log CSs 333 of DA of C. nshomboi parasitizing P. straeleni were only higher than those parasitizing the P. microlepis group of 334 the northernmost locality (CB2 vs. NB2), and then a group of a neighbouring central locality (CB2 vs. CB1). 335 Within the groups of C. nshomboi on P. microlepis, a geographical pattern was found as smaller anchors were 336 found in northern locations compared to more southern localities (SB1 vs. NB2 and NB3 and NB3 vs. CB1). The 337 remaining pairwise groups of C. nshomboi did not show any differences in the size of DA.

338 Shape and size of the ventral anchors (VA)

339 The first three PCs of the PCA performed on the VA dataset explained 69.05% of the total variance (Fig. 340 4). As with DA, PC1 (40.98%) separated specimens of C. nshomboi parasitizing B. microlepis from those 341 parasitizing perissodine hosts and this axis again corresponded with a reduction of the outer root. The third PC 342 (12.66%) was again more informative than PC2 (15.41%), which did not separate any of the groups. The second 343 PC corresponded with a VA having shorter point and thinner shaft base and inner root (Fig. 4a). The third PC 344 separated specimens of C. nshomboi parasitizing northern and more southern perissodine populations. This axis 345 corresponded with a change in the width of the shaft-base (Fig. 4b). As in the DA dataset, PC1 and PC3 were 346 significantly correlated with the anchor size (r = -0.3121, p < 0.001 for PC1 (Fig. 4c) and r = -0.1382, p < 0.001347 for PC3 (Fig. 4d)).

In the CVA performed on the VA dataset, the first two CVs axes explained 96.89% of the variance. These two axes fully separated the parasite specimens from the two tribes and from the two main geographical groupings identified above (Fig. 4e). The shape changes along CV1 (60.98%) and CV2 (35.94%) mostly corresponded to those obtained with DA. The first CV corresponded to a change in the length of the outer root and the curvature of the shaft, whereas CV2 corresponded with a change in the length of the point, the curvature of the shaft and the thickness of the base (Fig. 4e). 354 The pairwise Procrustes distances and permutation tests for the VA dataset are shown in Table 2. As for 355 the DA, the highest distances were found between the groups of C. nshomboi parasitizing boulengerochromine 356 and those parasitizing perissodine hosts, followed by those between parasites from perissodines from northern and 357 central/southern parts of the lake (Table 2). Permutation test conducted on the VA dataset of C. nshomboi revealed 358 similar patterns of shape variation at tribal and intraspecies levels as obtained with the DA, except for additional 359 significant differences in the shape of the VA between groups parasitizing P. microlepis from northern and central 360 localities (NB3 vs. CB1), and between those from P. straeleni and one of the northern groups on P. microlepis 361 (NB2 vs. CB2). In the NJ tree constructed with the Mahalanobis distances of the VA (Table S1), similar well-362 supported clusters (\geq 95%) were obtained, to those based on the DA dataset. Hence similar tribe-specific and 363 geographical patterns were found (Fig. 3a).

A visualisation of log CSs of the VA of *C. nshomboi* along the studied groups of boulengerochromine and perissodine hosts is shown in Fig. 3b. The size of the VA showed similar patterns among the studied monogenean groups as obtained with the DA dataset. The only exception was that log CS was not significantly different between groups of *C. nshomboi* on populations of *P. microlepis* and *P. straeleni* from neighbouring central localities (CB1 vs. CB2).

369 Morphometrical intraspecific variability of the male copulatory organ

The lengths (µm) of MCO structures from specimens of *C. nshomboi* are presented in Table 3 and their distribution is shown in Fig. S3. Although both parametric and non-parametric tests showed significant intraspecific size variation between the groups, no clear pattern of differentiation across taxonomic or geographic scales was obtained (Table S3-S6). However, specimens of *C. nshomboi* infecting *B. microlepis* had shorter copulatory tubes than the other groups (Table S4). Additionally, at northern locations, specimens of *C. nshomboi* from *B. microlepis* had shorter heels than those collected of perissodines.

376

377 Discussion

We studied monogenean flatworms parasitizing the gills of cichlids belonging to two phylogenetically distant Tanganyika tribes: Boulengerochromini represented by the predator *B. microlepis* and Perissodini represented by the planctivorous *H. microlepis* and the scale eaters *P. microlepis* and *P. straeleni*. We hypothesised (a) that a single species of *Cichlidogyrus* infected boulengerochromine and perissodine cichlids. All studied cichlid hosts indeed carried a single monogenean species that we could identify as *C. nshomboi*. The conspecificity was 383 confirmed based on the morphology of the haptoral and reproductive organs and by sequence identity in 18S and 384 28S rDNA. The MCO, commonly used to delineate species in Monogenea (Mendlová et al. 2012; Pouyaud et al. 385 2006; Van Steenberge et al. 2015), was highly similar between specimens parasitizing different host species. The 386 most variable part of the ITS region (ITS1), showed only limited differentiation (1.1%), as 1% is suggested as a 387 cut-off between inter- and intra-specific variation for the entire ITS (including its less variable parts) (Zietara & 388 Lumme 2003). Previously, C. nshomboi was only known from B. microlepis from the central-western part of Lake 389 Tanganyika (Muterezi Bukinga et al. 2012). This study hence represents the first record of a species of 390 Cichlidogyrus parasitizing members of the endemic Lake Tanganyika tribe Perissodini.

391 The presence of C. nshomboi on distantly-related hosts renders it a generalist gill flatworm following the 392 classification of Šimková et al. (2006), Mendlová and Šimková (2014) and Kmentová et al. (2016). Its wide host 393 distribution can either be explained by an inheritance from a common ancestor, or from a host-switching event. 394 The former scenario seems, however, unlikely given the phylogenetic distinction between the two host tribes and 395 the genetic similarity between the parasite populations infecting them. Yet, when following a less strict 396 interpretation, an origin through a common ancestor could still have led to the pattern seen today. Hybridisation is 397 known to have driven the speciation of East African cichlid assemblages (Kornfield and Smith 2000; Seehausen 398 2015), and a past intertribal hybridization between ancestors of Boulengerochromini and Perissodini has already 399 been suggested (Meyer et al. 2017). Although the evolutionary circumstances and environmental conditions that 400 would have allowed this hybridization are still unidentified, it could have allowed for the transfer of a parasite 401 species from one of the parents into the hybrid lineage. Despite the fact that our data could be considered as 402 additional evidence of a shared past evolutionary history of the Boulengerochromini and Perissodini, an anchored 403 phylogenomic approach did not support this hybridization event and suggested a more complex scenario of 404 introgression (Irisarri et al. 2018). Previous studies have demonstrated, however, that hybridization of host lineages 405 facilitates parasite transmission resulting in the presence of specific monogeneans of both parental species 406 (Krasnovyd et al. 2017, 2020).

The second <u>scenario</u> is that the presence of *C. nshomboi* on two distinct Tanganyikan cichlid tribes is due to a past host-switching event. <u>Although the probability of host-switching decreases with increasing phylogenetic</u> distance between donor and recipient hosts (Charleston and Robertson 2002), host-switches in monogeneans have been documented between distantly related hosts, including across fish families (Benovics et al. 2018; Huyse et al. 2003; Šimková et al. 2017; Ziętara and Lumme 2002). In *Cichlidogyrus*, <u>such a distant host switch was</u> evidenced in *C. amieti* Birgi & Euzet, 1983, <u>which expanded its range from cichlids to nothobranchiids</u> (Birgi and 413 Euzet 1983; Messu Mandeng et al., 2015). Moreover, the evolutionary history and phylogenetic isolation of B. 414 microlepis could have facilitated a distant host switch. Although different species of Cichlidogyrus can coexist on 415 a single cichlid host, a range expansion of C. nshomboi could have been facilitated by the extinction of the original 416 Cichlidogyrus fauna of B. microlepis. Parasite extinctions were reported from the Lake Tanganyika Cichlidogyrus-417 cichlid system (Vanhove et al. 2015). Mitochondrial genealogies of B. microlepis suggested a population 418 expansion following a decline at around 60 kya (Koblmüller et al. 2015). If this species' numbers dropped below 419 the persistence threshold of Cichlidogyrus (Gubbins et al. 2015), B. microlepis could have undergone parasite 420 extinction. Such an event could also have been caused by stochasticity in host availability, driven by the high 421 fecundity and mobility of B. microlepis. Without closely-related species available, this would have facilitated a 422 colonisation by a *Cichlidogyrus* species infecting a distantly related lineage, such as the Perissodini.

423 We hypothesised (b) that the genetic and morphological differentiation within C. nshomboi would mirror 424 the phylogenetic and ecological distinctness of its hosts. This was indeed found between groups of C. nshomboi 425 infecting Perissodini and Boulengerochromini, but not between those infecting different perissodine hosts (see 426 below). The differentiation across host tribes suggests incipient speciation in C. nshomboi. Although incipient 427 speciation has not been recorded in Cichlidogyrus so far, it is known for monogeneans such as Kapentagyrus 428 tanganicanus Kmentová, Gelnar & Vanhove 2018, which parasitizes Tanganyikan clupeids (Kmentová et al. 2018, 429 2020), and in gyrodactylids parasitizing sympatric callichthyids (Bueno-Silva et al. 2011). Conversely, in a congeneric generalist that is morphologically highly similar to C. nshomboi, Cichlidogyrus casuarinus, no 430 431 indications for incipient speciation were found. For this parasite species, no link between host species and 432 divergence in COI and in nuclear rDNA was found (Kmentová et al. 2016). The COI divergence in C. nshomboi 433 is also higher than in C. casuarinus (Kmentová et al. 2016) and is in the same order of magnitude as found between 434 different species of Cichlidogyrus infecting conspecific tropheine cichlids (Vanhove et al. 2015). This suggests a 435 relatively strong separation of the lineages of C. nshomboi infecting different tribes

436 Specimens of *C. nshomboi* infecting *B. microlepis* had larger anchors than those infecting all perissodines.
437 This could be linked to the body size of the host as *B. microlepis* is the largest cichlid inhabiting Lake Tanganyika
438 measuring over 40 cm in standard length (Ochi and Yanagisawa 1998), whereas *P. microlepis* and *P. straeleni* are
439 relatively small and have a maximum total length of about 12 cm and 15 cm, respectively (Konings 2019). A
440 positive correlation between the body size of the host and the size of haptoral sclerites in *Cichlidogyrus* was also
441 revealed by Mendlová and Šimková (2014). Contrariwise, specimens of *C. nshomboi* infecting *B. microlepis*442 possessed a shorter copulatory tube compared to those found on perissodines. <u>These differences in anchor size, as</u>

well as the differences in shape could be due to morphological plasticity. In generalist parasites, morphological
plasticity can be a specialization to the resources provided by different host species (Kaci-Chaouch et al. 2008).
Several studies showed how generalists exhibited higher morphometric and shape variations in the attachment
organ than specialists (Jarkovský et al. 2004; Kaci-Chaouch et al. 2008). Yet, given the genetic differentiation, we
cannot say to what degree the morphological variation reflects plasticity or adaptation. As specimens of *B. microlepis* were more heavily infected, our results support the positive correlation between infection intensity and
host body size as found for, for instance, dactylogyrids from anguillids (Buchmann 1989).

450 In contrary to our hypothesis ((c) (larger differences between monogeneans infecting heterospecific host 451 populations than between monogeneans infecting conspecific host populations), larger differences were found 452 between groups of C. nshomboi infecting conspecific host populations from distant locations than between those 453 infecting heterospecific host populations from nearby locations. Indeed, none of the statistical analyses on the 454 geomorphometric and morphological data revealed any -differentiation between groups of C. nshomboi infecting 455 P. microlepis and P. straeleni from the central basin. However, differentiation was present between groups of C. 456 nshomboi infecting P. microlepis from different regions of the Lake. This suggests that at least within the same 457 host genus, geography has most probably a larger effect than host taxonomy. The lack of parasite_differentiation 458 across host species represents a case of 'failure to diverge' (sensu Brooks 1979). It also represents 'reversed 459 specialisation asymmetry', where the host community is more diverse than the parasite community, unlike what 460 is to be expected in host-parasite communities (Vázquez et al. 2005). Sharing of parasites can be explained by 461 evolutionary, geographical, and ecomorphological traits of the hosts (Cruz-Laufer et al. 2021). Indeed, P. 462 microlepis and P. straeleni are closely related, co-occur at the rocky shores, and have similar ecomorphological 463 specializations. Perissodus straeleni and P. microlepis are sibling species and relatively recent offshoots of the 464 perissodine radiation (Koblmüller et al. 2007). Hence, it remains to be verified to what degree the lack of intra-465 tribal differentiation holds across the monogeneans infecting the rest of Perissodini. Unfortunately, samples 466 collected from H. microlepis were preserved differently and could therefore not be included in the 467 geomorphometric analyses.

The morphological differentiation between groups of *C. nshomboi* parasitizing different populations of *P. microlepis* can be linked to population genetic structure of the host. *Perissodus microlepis* occurs along the rocky littoral zone, which has a patchy distribution along the lake's shoreline (Koblmüller et al. 2009). Population genetic studies on southern populations of *P. microlepis* showed that habitat fragmentation by environmental barriers restricted gene flow in this species, resulting in low levels of within-population genetic diversity (Koblmüller et al. 2009). Geographically structured cichlid species often contain distinct colour morphs in Lake
Tanganyika. Although Konings (2019) did not report geographical variants in *P. microlepis*, Yanagisawa et al.
(1990) observed differences in the coloration of the anal fin in distinct populations of *P. straeleni*. They interpreted
this as an adaptation to their preys, facilitating the scale eater to approach it. Unfortunately, no genetic data (COI)
was available for *C. nshomboi* occurring on *P. microlepis* across this geographical gradient. Additionally, we did
not study geographical differentiation on *C. nshomboi* infecting less-geographically structured species such as *B. microlepis* (Koblmüller et al. 2014) and the deep-water species of Perissodini.

480 Morphological and genetic evidence showed that C. nshomboi belonged to a Lake Tanganyika lineage of 481 Cichlidogyrus with a low host specificity. We found high genetic similarity between C. casuarinus and C. 482 nshomboi, and both species also exhibit a MCO with straight heel and a copulatory tube ending distally in a spirally 483 coiled thickening. Cichlidogyrus casuarinus was previously recognized as intermediate generalist given its 484 occurrence on several species of phylogenetically-related members of the Bathybatini (Kmentová et al. 2016). 485 Variation in copulatory tube length and sclerite morphology was also found in this species, although no 486 geographical variation was observed. The low host specificity in C. casuarinus was explained by the occurrence 487 of its hosts in the deep-water realm. As host density is lower in this habitat, this reduces the potential for 488 specialisation in gill parasites. A similar explanation could support the low host specificity in C. nshomboi as 489 most representatives of Perissodini are deep-water specialists (Konings 2019) and as B. microlepis is benthopelagic. The low host specificity of C. nshomboi, combined with the co-occurrence of B. microlepis and several 490 491 perissodine species could have facilitated a host range expansion of the parasite. Although none of the deep-water 492 species of Perissodini were hitherto screened for gill flatworms, we hypothesize that their gills will harbour C. 493 nshomboi or a highly similar species. In accordance with Mendlová and Šimková (2014) for a West African 494 cichlid-Cichlidogyrus system, our results do not support the hypothesis of an irreversible "dead-end" in the 495 evolution of specificity. However, it would be interesting to map the host specificity of Cichlidogyrus known from 496 Tanganyikan cichlids onto a phylogenetic tree and accurately identify which type of host-specificity represents the 497 ancestral state in these gill flatworms. The study of C. nshomboi and its cichlid hosts can shed light on the 498 evolutionary patterns of Tanganyikan cichlids and their monogeneans. Additionally, this host-parasite system 499 represents a promising model to investigate fundamental questions in host-parasite interactions and 500 evolutionary dynamics.

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527 Author contributions

528 CR, MVS, MPMV and AŠ designed the study. MVS, MPMV and AŠ supervised the work. MPMV co-organised 529 the fieldtrips, parasite collection, preparation of specimens and provided scientific background in the field. CR 530 performed the lab work and data analysis, CR and MVS wrote the manuscript. All authors read and approved the 531 final manuscript.

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533	Conflict of interest
534	The authors declare no conflict of interest.
535	Data availability
536	Genetic sequence data is publicly available from the NCBI database or from the author upon request. The voucher
537	specimens of monogeneans are housed in the Royal Museum for Central Africa (RMCA, Belgium) (see materials
538	and methods for details on repositories and accession numbers).
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817 Figures captions

818 Fig. 1 (a) map of Lake Tanganyika with sampling localities along of the investigated cichlid species (edited on 819 http://www.simplemappr.net and Photoshop v. 13.0). Shape in black and white indicates the cichlid species which 820 were free of monogeneans, and the groups that were included for the morphological identification only. Photos by 821 Van Steenberge M., Konings A. and Gessl W. (b) anchor with the nine digitized landmarks (LM1-LM9) following Rodríguez-González et al. (2015). (c) Morphometric characters of the Male Copulatory Organ (MCO) of C. 822 823 nshomboi parasitizing Perissodus microlepis sampled from Nyaruhongoka (NB2), with: TL: total straight length; 824 PE: copulatory tube curved length; HE: heel straight length and AP: accessory piece straight length. 825 Fig. 2 Geomorphometric analyses on the DA dataset of C. nshomboi specimens parasitizing

boulengerochromine and perissodine hosts. Scatter plot showing the variation in shape of DA along PC1 and PC2
(a), and PC1 and PC3 (b). Wireframe graphs illustrating the shape changes from overall mean next to each PC,

with starting shapes (consensus) in black and target shapes (changes ± 0.1) in grey. Results from PCA and multivariate regression of PC1 (d) and PC3 (e) against log centroid size (CS) (c). The shape changes along PC1 (r = -0.341, p < 0.0001) and PC3 (r = -0.044, p < 0.0058) were correlated with anchor size. Scatter plot showing the variation in shape along CV1 and CV2 (e). Wireframe graphs illustrating the shape changes from the overall mean based on CVA are next to each PC, with starting shapes (consensus) in black and target shapes (changes ± 0.7) in grey.

Fig. 3 Projection of Neighbour-Joining tree (NJ) generated from the morphometric Mahalanobis distance matrix
(a) and distribution of log centroid sizes (CS) of DA (purple) and VA (orange) of *C. nshomboi* parasitizing against
the host populations (b). The bootstrap values are indicated above the nodes for DA (in bold) and VA dataset for
the groups of *C. nshomboi*.

838 Fig. 4 Geomorphometric analyses on the VA dataset of C. nshomboi specimens parasitizing 839 boulengerochromine and perissodine hosts. Scatter plot showing the variation in shape of VA along PC1 and PC2 840 (a), and PC1 and PC3 (b). Wireframe graphs illustrating the shape changes from overall mean based on PCA are 841 next to each PC, with. starting shapes (consensus) in black and target shapes (changes +0.1) in grey. Results from 842 PCA and multivariate regression of PC1 (d) and PC3 (e) against log centroid size (CS) (c). The shape changes along PC1 (r = -0.3121, p < 0.0001) and PC3 (r = -0.1382, p < 0.0001) were correlated with anchor size. Scatter 843 844 plot showing the variation in shape along CV1 and CV2 (e). Wireframe graphs illustrating the shape changes from 845 overall mean based on CVA are next to each PC, with starting shapes (consensus) in black and target shapes 846 (changes +0.7) in grey.

847 Table captions

848Table 1List of cichlid species with dates of sampling, sampling localities and the abbreviations used in849the study (see also Van Steenberge et al., 2011). Specimens of *Cichlidogyrus* parasitizing the cichlid species850sampled from the localities highlighted in bold were targeted for the geomorphometric analyses, their sampling851size (number of observations) for DA and VA is also shown in bold. Accession numbers for the DNA sequences852corresponding to the specimens of a given parasite populations are included (the numbers in parentheses indicate853numbers of sequenced specimens). Monogenean specimens from *Haplotaxodon microlepis* were used only for854morphological characterization, * denotes that the specimens derive from small-scale fisheries.

855 Table 2 Procrustes distance matrix obtained from DA (right of the diagonal) and VA (left of the diagonal)
856 dataset among the studied populations of *C. nshomboi* based on nine LMs, with Procrustes distances (in bold) and

- 857 *p*-values from permutation test with 10 000 randomizations (right of the diagonal) (significant *p*-values after Holm-
- 858 Bonferroni correction are indicated by '*'). The sampling sites are as in Table 1
- 859 Table 3 Comparison of measurements of male copulatory organ (MCO) aspects among the studied
- 860 groups of *C. nshomboi* from Boulengerochromini and Perissodini. All measurements are given in micrometres as
- the average \pm standard deviation, the range in parentheses (min-max), and the number of specimens measured.
- The sampling sites are as in Table 1.







Table 1 List of cichlid species with dates of sampling, localities in Lake Tanganyika and their abbreviations used in the study (see also Van Steenberge et al., 2011). Specimens of *Cichlidogyrus* parasitizing the cichlid species sampled from the localities highlighted in bold were targeted for the geomorphometric analyses, their sampling size (number of observations) for DA and VA is also shown in bold. Accession numbers for the DNA sequences corresponding to the specimens of a given parasite populations are included (the numbers in parentheses indicate numbers of sequenced specimens). Monogenean specimens from *Haplotaxodon microlepis* were used only for morphological characterization.

NА	26	20	6	6	7		5	2	2			3
٨Ū	9	03	_	_		-					-	
)I mitDNA	675241-47 2	675248-51 2	5	5	v	·	<i>u</i> ,	N	N	•		61
rDNA CC	1O 06-88788.	38791-93 OL	88794-96 -	- 88797-800	ı	88801-04 -	88805-08 -	- 88809	I			'
-ITS1 28S VA 28S	675289-91 OL6	675292-94 OL6	675295-97 OL6	575298-300 OL6		9T0	675301-02 OL6	675303 OL6	ı	•	'	•
<i>n</i> parasites 18S rD1	TO 86	30 OT	6 0	15 OL	e -	2	4 OF	2 OL	2 -	-	, ,	3
GPS Coordinates	3°23'S, 29°22'E	3°41'S, 29°20'E	3°44'S, 29°19'E	7°35′S, 30°38′E	6°46'S, 29°33'E	3°46'S, 29°07'E	6°56'S, 29°42'E	6°58'S, 29°43′E	7°35'S, 30°38'E	5°38'S, 29°23'E	6°56'S, 29°42′E	3°31'S, 29°08'E
Abbreviations	B. microlepis_NB1	P. microlepis_NB2	P. microlepis_NB3	P. microlepis_SB1	P. microlepis_CB1	ı	P. straeleni_CB2	ı	ı		ı	
LT basin	Northern	Northern	Northern	Southern	Central	Northern	Central	Central	Southern	Central	Central	Northern
Locality of sampling	Bujumbura fish market	Nyaruhongoka	Magara	Mtosi	Mugayo	Bulumba Island	Mukumba	Mtoto	Mtosi	Murega	Makumba	Luhanga
Date	05/09/2013	09/09/2013	09/09/2013	24/04/2008	11/05/2010	25/03/2010	14/05/2010	15/04/2010	24/04/2008	24/04/2010	1997	1957
<i>n</i> cichlids	04	05	03	11	01	90	07	02	01	02	02	03
Cichlid species	B. microlepis	P. microlepis					P. straeleni			H. microlepis		
Cichlid tribe	Boulengerochromini	Perissodini								-		

Cichlid tribe	Cichlid species	Locality	NB1	NB2	NB3	CB1	SB1	CB2
Boulengerochromini	B. microlepis	NB1		0.1343	0.1230	0.1205	0.1362	0.1251
-	-			<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
Perissodini	P. microlepis	NB2	0.1010		0.0223	0.0492	0.0465	0.0438
			<.0001*		0.4163	0.0002*	0.0002*	0.0252
		NB3	0.1002	0.0211		0.0462	0.0475	0.0426
			<.0001*	0.6889		0.0209	0.0029*	0.1207
		CB1	0.1146	0.0760	0.0721		0.0333	0.0208
			<.0001*	<.0001*	0.0001*		0.3205	0.9549
		SB1	0.1146	0.0733	0.0683	0.0360		0.0362
			<.0001*	<.0001*	0.0001*	0.2778		0.3446
	P. straeleni	CB2	0.1210	0.0654	0.0616	0.0321	0.369	
			<.0001*	0.0056*	0.0616	0.8323	0.6608	

Table 2 Procrustes distance matrix (left of the diagonal) obtained from DA (in bold) and VA dataset among the studiedpopulations of C. nshomboi based on nine LMs, with p-values from permutation test with 10 000 randomizations (right of thediagonal) (significant p-values after Holm-Bonferroni correction are indicated by '*'). The sampling sites are as in Table 1.

Table 3 Comparison of measurements of male copulatory organ (MCO) aspects (p < .0001 for all measurements) among the studied groups of *C. nshomboi* from Boulengerochromini and Perisodini. All measurements are presented in micrometres and given as the average \pm standard deviation, the range in parentheses (min-max) followed by the number of specimens measured. The sampling sites are as in Table 1.

Cichlid Tribe	Cichlid species	Locality	MCO total straight	Copulatory tube	Heel straight length	Accessory piece
			length (TL)	curved length (PE)	(HE)	straight length (AP)
Boulengerochromini	B. microlepis	NB1	$49\pm1.94~(46\text{-}53)_{20}$	$24 \pm 1.22 \; (21\text{-}26)_{20}$	$24\pm1.14\ (22\text{-}26)_{20}$	$20 \pm 1.86~(17\text{-}14)_{20}$
Perissodini	P. microlepis	NB2	$48 \pm 3.61 \ (43-53)_{14}$	$27 \pm 0.82 \ (26-28)_{14}$	$19 \pm 2.87 \ (14-24)_{14}$	$22 \pm 1.38 \ (19\text{-}24)_{14}$
		NB3	$47\pm1.87~(44\text{-}49)_8$	$26 \pm 0.35 \ (26 - 27)_8$	$19 \pm 0.99 \ (18-21)_8$	$21 \pm 2.10 \ (17 - 24)_8$
		CB1	$49\pm2.08~(46\text{-}51)_4$	$27 \pm 2.50 \ (24-30)_4$	$19 \pm 1.29 \ (17 - 20)_4$	$19 \pm 2.50 \ (16-22)_4$
		SB1	$53 \pm 1.81 (50-56)_7$	$28 \pm 1.57 \ (25-30)_7$	$26 \pm 0.89 \ (25-27)_7$	$18\pm1.25~(16 ext{-}20)_7$
	P. straeleni	CB2	$53 \pm 2.42 \ (48-54)_6$	$27 \pm 2.50 \ (22-29)_6$	$25 \pm 1.75 \ (24-28)_6$	$17 \pm 2.13 \ (14-20)_6$