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Molecular phylogeny and speciation patterns in host-specific monogeneans (Cichlidogyrus, Dactylogyridae) parasitizing cichlid fishes (Cichliformes, Cichlidae) in Lake Tanganyika Peer-reviewed author version

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Highlights

- Cichlidogyrus species from most East African cichlids are monophyletic.
- Host switching followed by duplications as the most frequent coevolutionary event.
- Strict specialist species of *Cichlidogyrus* possess primitive character states.

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15 Abstract

16 Cichlidogyrus (including Scutogyrus) is the most speciose dactylogyridean monogenean genus known 17 from African and Levantine cichlid fishes (Cichlidae). While its taxonomy is well established, little is known about 18 the phylogenetic relationships and evolutionary history of this ectoparasite, especially from hosts belonging to one 19 of the most impressive vertebrate radiations, the cichlid fishes from the East African Great Lakes and surrounding 20 hydrological systems. Phylogenetic inference based on DNA sequences of the nuclear 18S, ITS1 and 28S rDNA 21 genes revealed that Cichlidogyrus parasitizing mainly West African cichlid tribes is paraphyletic with respect to 22 species parasitizing hosts belonging to the East African cichlid radiation, which constitute a well-supported 23 monophylum. Members of Cichlidogyrus from tylochromine and oreochromine hosts that colonised Lake 24 Tanganyika (LT) only recently, cluster with their non-LT relatives, indicating that they colonized LT with their 25 current host species, and did not jump over from any of the many cichlid species already present in the lake. The 26 diversification of Cichlidogyrus in LT seems to be driven by failure to diverge in old lineages of cichlids, 27 cospeciation in more recently evolved ones, and host switching followed by parasite duplication at the level of the 28 various host tribes. Evaluation of host specificity and structural evolution of haptoral and reproductive organs in 29 LT Cichlidogyrus revealed that strict specialist species with larval hook size represent the ancestral state of haptor 30 configuration, suggesting that members of Cichlidogyrus in this system evolved from a very simple form to a more 31 complex one like their West African congeners. Generalist species among Cichlidogyrus with a sclerotized vagina 32 parasitizing ancient LT lineages seem to have developed a different hook configuration, most probably to ensure 33 successful colonization of new, phylogenetically unrelated hosts.

34 Keywords African Great Lakes, Cichlidae, Monogenea, vagina, haptor, hooks, cophylogeny, host range.

35 Introduction

Parasites and their hosts may show intimate ecological interactions due to their common evolutionary histories over long time spans (Klassen, 1992). Parasites do not randomly infect hosts, as they have to synchronize their life cycle with that of their hosts to ensure optimal individual growth, survival and fecundity. Thus, they are expected to coevolve with their hosts to achieve maximal fitness in their new environment (Garamszegi, 2009). The continuous evolution of parasites over time_a gaining and/or loosing hosts_a considerably₃ shapes the evolutionary trajectories of both host and parasite lineages (Nylin et al., 2018).

42 Monogenean flatworms are well suited for evaluating host-parasite coevolutionary relationships for 43 several reasons. These parasites are highly diverse in terms of species number (Řehulková et al., 2018), 44 morphology (forms of attachment organs), and ecology (mainly gill and fin ectoparasites exhibiting host and microhabitat specificity) (Rohde, 1989; Euzet and Combes, 1998; Šimková et al., 2006; Woo, 2006). The 45 46 evolutionary success of monogeneans is presumed to be related to the diversity of their attachment organ (haptor), 47 and their adaptation to hosts and infection sites (Whittington and Chisholm, 2008). With a direct life cycle, 48 monogeneans further show a strong colonization ability, through either physical contacts between hosts or by the 49 ciliated infectious stage (oncomiracidium) (Cable and Harris, 2002). Monogenean species are known to parasitize 50 either a single host species (strict specialists), a narrow group of congeneric host species (intermediate specialists), 51 phylogenetically closely related non-congeneric hosts (intermediate generalists), or phylogenetically unrelated 52 host species (generalists) (Desdevises et al., 2002; Šimková et al., 2006; Thatcher, 2006; Franceschini et al., 2018; 53 Kuchta et al., 2020).

54 From an evolutionary perspective, perfect cospeciation at the macroevolutionary level seems to be rare in 55 parasitic flatworms and was shown to be restricted to higher taxonomical levels due to geographical isolation of particular hosts and parasite lineages (Boeger and Kritsky, 1997). Under cospeciation, a congruence between host 56 57 and parasite phylogenies is expected (Stammer, 1957; Brooks and McLennan, 1993). Host switching, parasite 58 duplication and sorting events are coevolutionary events in which parasites fail to respond to host speciation 59 (Johnson et al., 2003; Poulin, 2008), hence host and parasite phylogenies show incongruences (Brooks and 60 McLennan, 1991; Page, 1993). These events were often suggested in scenarios of speciation and diversification of 61 dactylogyridean monogeneans in freshwater fish hosts (Šimková et al. 2004; Mendlová et al., 2012; Šimková et 62 al. 2013; Benovics et al. 2020a). Host switching tends to occur more often between closely related host species (Charleston and Robertson, 2002; De Vienne et al., 2013). Host-parasite associations in marine diplectanids were 63 64 inferred to be driven by ecological factors, which facilitated host switching rather than cospeciation (Desdevises

et al., 2002). Moreover, sympatric speciation of monogeneans on closely related hosts by host switching was found in marine gyrodactylids (<u>Huyse and Volckaert, 2002</u>; Huyse et al., 2003). In this <u>latter</u> group of viviparous monogeneans, speciation by geographic isolation (allopatric mode), host switching and instant isolation by host specificity were further revealed (Meinilä et al., 2004).

69 African and Levantine cichlid fishes (Cichlidae Bonaparte, 1835) are known to host representatives of six 70 dactylogyridean genera, plus a single gyrodactylid genus. This includes endoparasitic (mesoparasitic) genera found 71 in the stomach and urinary bladder, i.e., Enterogyrus Paperna, 1963 and Urogyrus Bilong, Birgi & Euzet, 72 1994, respectively, and ectoparasitic genera found on fish gills: Onchobdella Paperna, 1968, Scutogyrus Pariselle 73 and Euzet 1995, Cichlidogyrus Paperna, 1960, and Gyrodactylus von Nordmann, 1832, the latter of which also 74 occurs on fins (Pariselle and Euzet, 2009; Dos Santos et al., 2019). The sixth and final dactylogyridean genus 75 Insulacleidus Rakotofiringa and Euzet, 1983 is an ectoparasite genus restricted to Madagascar (Rakotofiringa and 76 Euzet, 1983). Of the above-listed genera, Cichlidogyrus is the most speciose and mostly restricted to cichlid fishes. 77 The monophyly of the group Cichlidogyrus-Scutogyrus and the paraphyly of Cichlidogyrus is well established (Pouyaud et al., 2006; Mendlová et al., 2010, 2012; Messu Mandeng et al., 2015; Caña-Bozada et al., 2021). To 78 79 date, 130 species of Cichlidogyrus have been reported from 112 cichlid species inhabiting Africa and the Levant 80 (Pariselle and Euzet, 2009; Vanhove et al., 2016; Rahmouni et al., 2018; Geraerts et al., 2020). Although rarely, a 81 few representatives of this genus were reported from non-cichlid hosts like the pupfish (Cyprinodontidae Gill, 82 1865 (Birgi and Euzet, 1983)) and leaffish (Nandidae Bleeker, 1852 (Birgi and Lambert, 1986)). To account for 83 this, host switches from cichlid hosts to distant fish families were then suggested, but only a single case was 84 supported by molecular data (Messu Mandeng et al., 2015). Host switching was also recently documented 85 following the introduction of African mainland cichlids to Madagascar, where native Malagasy cichlids and 86 aplocheilids were infected by non-native species of Cichlidogyrus (Šimková et al., 2019). 87 Cichlidogyrus bear highly characteristic sclerotized structures in their attachment organ and reproductive

organs. The haptoral parts are characteristic for major phylogenetic lineages, while the reproductive organs, especially the male copulatory organ (MCO), seem to be important for species-level identification (Pouyaud et al., 2006; Mendlová et al., 2012; Van Steenberge et al., 2015). The MCO consists of two main and highly morphologically diverse parts, the copulatory tube and accessory piece. Similarly, the vagina in *Cichlidogyrus* can be sclerotized or not. The haptor comprises two pairs of anchors (or gripi) (one dorsal and one ventral), two transversal bars (dorsal bar with two typical auricles and a V-shaped ventral bar), and seven pairs of hooks (or uncinuli) (Paperna, 1960; Pariselle and Euzet, 2009). Using molecular data and geomorphometrics of mainly West African species of *Cichlidogyrus*, Vignon et al. (2011) recognized four main haptoral groups <u>based on the</u>
 configuration (size and shape) of hook pairs.

97 Certain morphotypes within Cichlidogyrus appear typical to a certain host lineage across distant 98 freshwater systems. This was repetitively evidenced by monogeneans sharing characteristic morphological 99 features in a range of host species of Tylochromini (Pariselle and Euzet, 1994; Muterezi Bukinga et al., 2012). 100 Host specificity of species of Cichlidogyrus, contrariwise, varies considerably across the African continent. In 101 southern and Central African systems, it ranges from strict specialists, to generalists parasitizing several tilapiine 102 and haplochromine hosts (Geraerts et al., 2020). Likewise, the host specificity in West Africa includes strict 103 specialists on heterotilapiines (Pariselle and Euzet, 1998), intermediate specialists like those restricted to tylochromines (Pariselle and Euzet, 1994; Pariselle et al., 2014; Jorissen et al., 2018), and generalists parasitizing 104 105 a range of cichlid species representing distinct host lineages (Paperna and Thurston, 1969; Paperna, 1979; Pariselle 106 et al., 2003; Pouyaud et al., 2006; see also Mendlová and Šimková (2014)). Some species of Cichlidogyrus exhibit . 107 variability in local host specificity (Mendlová and Šimková, 2014). For example, C. zambezensis Douëllou, 1993 108 was reported as an intermediate specialist restricted to haplochromine hosts in the Bangweulu-Mweru ecoregion 109 (Jorissen et al., 2017), whereas it was reported as generalist in Lake Kariba parasitizing haplochromine and 110 oreochromine hosts (Douëllou, 1993). Mendlová and Šimková (2014) showed that intermediate specialists 111 represent the ancestral state of host specificity for West African species of Cichlidogyrus. Their study also showed 112 a weak correlation between morphometry of haptoral sclerites and host specificity in Cichlidogyrus and 113 Scutogyrus, and a link between host specificity and host phylogeny, whilst host specificity was independent of 114 parasite phylogeny (Mendlová and Šímková, 2014).

115 The focus of the present study is on Lake Tanganyika (LT), the deepest and oldest lake in Africa (Cohen 116 et al., 1997; Lezzar et al., 2002), harbouring a number of different teleost lineages that radiated within the confines 117 of the lake and show very high levels of endemism (Salzburger et al., 2014). Cichlids are the by far most species 118 rich fish family in this lake and well established as model system in evolutionary biology (e.g. Kocher, 2004; 119 Seehausen, 2006). Compared to the other East African rift lakes, the cichlid assemblages in LT are genetically, 120 morphologically, ecologically and behaviourally the most diverse (Snoeks, 2000; Koblmüller et al., 2008). 121 Roughly 240 cichlid species belonging to 16 tribes occur in the lake (Ronco et al. 2020). While some lineages are 122 very species-rich (e.g. Lamprologini), others count a single representative in the lake (Tylochromini and 123 Boulengerochromini) (Koblmüller et al., 2008). Whereas most tribes evolved in situ within a short period of time; 124 (their rapid initial diversification was likely facilitated by hybridization at the base of the radiation (Irisarri et al., 2018), single representatives of other tribes like Oreochromini and Tylochromini, have colonized the lake only
recently (Klett and Meyer, 2002; Koch et al., 2007). Recent phylogenomic studies have clarified the phylogenetic
relationships not only among cichlid tribes, but also among all known (valid plus yet undescribed) species from
Lake Tanganyika (<u>Takahashi and Sota, 2016; Irisarri et al., 2018; Ronco et al., 2021)</u>, and thus provided an
invaluable resource for comparative follow-up studies on this system.

130 Mirroring the cichlid diversity in LT, various lineages of Cichlidogyrus inhabit this freshwater system. 131 To date, 39 species are known from a total of 36 cichlid species of 12_distinct tribes (Rahmouni et al., 2018). In 132 LT, the study of species of Cichlidogyrus parasitizing tropheine cichlids revealed phylogenetic congruence 133 between parasites and hosts, and geographically-dependent diversification (Vanhove et al., 2015). Moreover, it 134 was hypothesized that representatives of Cichlidogyrus infecting species belonging to Ectodini and Tropheini 135 evolved either by intrahost speciation or by host switching (Vanhove et al., 2011; Pariselle et al., 2015). However, 136 so far, there is no phylogenetic study inferring the origin and relationships of host-specific Cichlidogyrus species . 137 parasitizing cichlids representing LT tribes other than Tropheini. The phylogenetic relationship between West 138 African species of Cichlidogyrus and their congeners parasitizing East African cichlids (including the LT 139 radiation), and the evolution of haptoral and reproductive organ morphologies in species of Cichlidogyrus 140 parasitizing LT cichlids, are still unknown. Concerning host specificity, Kmentová et al. (2016b) provided an 141 overview on host specificity of Cichlidogyrus in LT cichlids, which included host representatives of only few LT 142 cichlid tribes. Lake_Tanganyika species of Cichlidogyrus include strict or intermediate specialists parasitizing . 143 members of Tropheini (Vanhove et al., 2015), intermediate generalists parasitizing a wide range of deep-water 144 bathybatines(Kmentová et al., 2016b, 2021), or true generalists parasitizing species of unrelated cichlid 145 lineages Ectodini and Cyphotilapiini (Rahmouni et al., 2018).

146 This study aimed at (i) investigating the phylogenetic position of species of Cichlidogyrus parasitizing . 147 East African cichlid lineages inhabiting one of the main biodiversity hotspots in Africa (LT), in relation to 148 congeners outside of the lake, (ii) assessing whether the phylogeny of Cichlidogyrus from LT follows their cichlid 149 host phylogeny at tribal level, (iii) identifying the role of coevolutionary processes in the diversification of 150 Cichlidogyrus parasitizing LT cichlids, (iv) investigating whether there is a relationship between parasite 151 phylogeny and morphological adaptation based on attachment (hook pairs) and reproductive organs (sclerotization 152 in the vagina), and (v) discussing local host specificity in Cichlidogyrus in the Tanganyika system and its potential 153 link to specific morphological characters in monogeneans.

155 Material and methods

156 Fish and parasite collection

157 In total, 185 cichlid individuals belonging to 23 species representing the majority of LT tribes were 158 sampled across the northern part of Lakes Tanganyika (Burundi and the Democratic Republic of the Congo 159 (DRC)), Cohoha (Burundi) and Kivu (DRC) between 2008 and 2016, and from Chitili and Kalambo Falls River 160 (Zambia) in 2015 (Table 1). The fish were obtained by local fishermen, caught using gill nets or during snorkelling 161 or diving. Cichlids were identified on site by ichthyologists based on available keys/literature and dissected using 162 standard methods described by Ergens and Lom (1970). Protocols used for isolating and fixing gill-infecting 163 monogeneans follow Rahmouni et al. (2017a, 2018a). Parasite determination was performed on the basis of 164 morphology and size of the sclerotized parts of haptor and reproductive organs following original descriptions. 165 Some individuals of each collected monogenean species were cut into half using fine needles using a dissecting 166 microscope during the collection. The anterior part of the worm, which contains the sclerotized parts of the 167 reproductive organs, was fixed on microscopic slides using a mixture of glycerine and ammonium picrate (GAP) 168 (Malmberg, 1957), whereas the remaining half of the body was placed in 96% ethanol for DNA extraction.

169 DNA extraction, amplification and sequencing

170 The conspecificity of parasites infecting the respective host species was confirmed using whole 171 individuals mounted on slides based on their hard parts, and fragments of the small and large subunits of ribosomal 172 DNA (18S and 28S rDNA), plus the entire first internal transcribed spacer (ITS1). Parasite individuals were 173 removed from ethanol and dried using an Eppendorf 5301 concentrator under vacuum conditions at 30 °C. 174 Genomic DNA was extracted using the DNeasy™ blood and tissue Kit (QIAGEN) following the manufacturer's 175 instructions. The partial 28S fragment (D1-D2) was amplified using forward primer C1 (5'-ACCCGCTGAATT 176 TAAGCAT-3') and reverse D2 (5'-TGGTCCGTGTTTCAAGAC-3') (Hassouna et al., 1984), or alternatively 177 using forward ANCY55 (5'-GAGATTAGCCCATCACCGAAG-3') (Plaisance et al., 2005) and reverse D2 if the 178 first primer combination was unsuccessful. The partial 18S rRNA gene and the entire ITS1 region were amplified 179 using the forward primer S1 (5'-ATT CCGATAACGAACGAGACT-3') (Sinnappah et al., 2001) and reverse 180 primer IR8 (5'- GCTAGCTGCGTTCTTCATCGA-3') (Šimková et al., 2003), or alternatively using forward S1 181 and reverse LIG5.8 (5'-GATACTCGAGCCGAGTGATCC-3') primers (Blasco-Costa et al., 2012) if unsuccessful 182 with the first combination of primers. Because of its utility in previously published multi-marker phylogenetic 183 trees of representatives of Cichlidogyrus (Vanhove et al., 2015; Cruz-Laufer et al., 2021) we also included a 184 fragment of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene. The partial COI gene was amplified 1185 for selected samples using forward_ASmit1 (5'-TTTTTTGGGCATCCTGAGGTTTAT-3') (Littlewood et al., 186 1997) and Schisto3 (5'-TAATGCATMGGAAAAAAAAAAA (Lockyer et al., 2003), with reverse ASmit2 (5'-187 TAA AGA AAG AAC ATA ATG AAA ATG-3') (Littlewood et al., 1997) as internal primer for the nested PCR. 188 For DNA amplification, we followed the protocols published by Plaisance et al. (2008), Mendlová et al. (2012), 189 and Benovics et al. (2020). The PCR products were electrophoresed using a 1% agarose gel and then purified by either the High Pure PCR product purification kit™ (Roche, Mannheim, Germany), or treated with ExoSAP-IT 190 191 (Ecoli, Bratislava, SK) according to the manufacturer's instructions. Bi-directional sequencing of PCR amplicons 192 using the BigDye® Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems by Thermo Fisher Scientific, 193 Prague, Czech Republic) and the PCR primers was performed. After purification of sequencing products with the 194 BigDye XTerminator® Purification Kit (Applied Biosystems by Thermo Fisher Scientific, Prague, Czech 195 Republic), sequences were visualised on an ABI 3130 Genetic Analyzer (Applied Biosystems).

196 Alignment and phylogenetic analyses

197 Raw sequences were edited using the Sequencher® software v. 5.0 (Gene Codes Corporation, Ann Arbor, 198 MI USA) and aligned with ClustalW (Thompson et al., 1994) as implemented in MEGA X (Kumar et al., 2018). 199 In addition, previously published sequences of species of Cichlidogyrus and Scutogyrus (Pouyaud et al., 2006; Wu 200 et al., 2007; Mendlová et al., 2010, 2012; Messu Mandeng et al., 2015; Kmentová et al., 2016a, b, 2018; Šimková 201 et al., 2019) (Table 2), were downloaded from GenBank and included in the analyses to determine the position of 202 Cichlidogyrus from LT cichlids in relation to Cichlidogyrus and Scutogyrus from the rest of Africa. Of West 203 African monogeneans, a few sequences downloaded from GenBank correspond to species of Cichlidogyrus 204 sampled from introduced cichlids in Asia and Madagascar, and a single species. C. amieti Pariselle & Euzet, 1995. 205 known from Aphyosemion spp. (Nothobranchiidae). Sequences representing C. mbirizei Muterezi Bukinga, 206 Vanhove, Van Steenberge & Pariselle, 2012 described from the LT species Oreochromis tanganicae 207 (Günther), 1894 were obtained from introduced cichlids in Asia (see Table 2 and below). GBlocks v. 0.91b 208 (Talavera and Castresana, 2007) was applied to remove unreliably aligned sequences (gaps and ambiguously 209 aligned regions) using less stringent parameters under the following criteria (i) smaller final blocks, (ii) gap positions within the final blocks, and (iii) less strict flanking positions. Final alignment length (after trimming) 210 211 was 585 bp for 28S rDNA (raw sequences of ~775 bp), 389 bp for 18S rDNA and 301 bp for ITS1 (raw sequences 212 of ~820 bp for these two adjacent regions). All newly obtained sequences were deposited in GenBank (see Table 213 1 for accession numbers). Phylogenetic relationships were inferred using Maximum Likelihood (ML) and 214 Bayesian Inference (BI). For each locus, the best fitting model of molecular evolution was inferred based on the 215 corrected Akaike Information Criterion (AICc) (Sugiura, 1978; Hurvich and Tsai, 1989) in jModelTest v. 2.1.10 216 (Guindon et al., 2010; Darriba et al., 2012): TVM +I+ Γ for 28S rDNA with a gamma shape parameter of 0.91, 217 K80+I for 18S rDNA, and TPM2uf+ Γ for ITS1 with a gamma shape parameter of 0.64. To assess the phylogenetic 218 content of the dataset, likelihood mapping based on quartet puzzling (Strimmer and Von Haeseler, 1997) 219 implemented in TREE-PUZZLE v. 5.2 (Schmidt et al., 2002) was used. Since the combined alignment showed 220 relatively high phylogenetic content (96.2% fully resolved, 2.4% partly resolved and 1.2% unresolved quartets), a 221 concatenated dataset (1275 bp) containing a total of 65 sequences from species of Cichlidogyrus was generated 222 using SeaView v. 5.0.4 (Gouy et al., 2010) and used for phylogenetic tree inference. Cichlidogyrus pouyaudi 223 Pariselle & Euzet, 1994 parasitizing Tylochromis intermedius (Boulenger, 1916) from West Africa and C. 224 mulimbwai Muterezi Bukinga, Vanhove, Van Steenberge and Pariselle, 2012, from the Lake Tanganyika species 225 Tylochromis polylepis (Boulenger, 1900) (Tylochromini) were used as outgroup following Mendlová et al. (2012) and references therein. ML trees were inferred using IQ-TREE v. 1.5.5 (Nguyen et al., 2015), employing the best 226 227 fit substitution model for each alignment (see above) and a SPR branch-swapping algorithm. The branch support 228 (bootstrap support, BS) was estimated using ultrafast bootstrap approximation (Minh et al., 2013) with 1 000 229 replicates. Selected models which are not implemented in MrBayes were substituted by the closest over-230 parameterized model (Huelsenbeck and Rannala, 2004). The analysis was performed using MrBayes v. 3.2.1 231 (Ronquist et al., 2012) with two independent Markov Chain Monte Carlo (MCMC) simulations (6 chains, 2×106 232 generations, sampling frequency 100, 25% burn-in). Chain stationarity and parameter convergence were assessed 233 in TRACER v. 1.7.1 (Rambaut et al., 2018), with effective samples sizes (ESS) always > 200 for all parameters, 234 and via the average standard deviation of split frequencies (always well below 0.01), and post burn-in trees were 235 summarized in a 50% majority rule consensus tree. The ML and BI trees were visualized in FigTree v. 1.4.4 236 (Rambaut, 2009). It should be noted that as the incongruence length difference (ILD) test performed in the 237 WinClada program (Farris, 1995) with 1000 replicates revealed heterogenous gene fragments (p=0.004), 238 sequences of the COI gene were not included in the phylogenetic and cophylogenetic analyses for lack of sufficient 239 DNA data (sequencing not successful for all species). Recent study by Jorissen et al. (2021), however, indicated 240 the utility of COI sequences in barcoding and species delineation within Cichlidogyrus, hence these sequence data 241 are a valuable resource for future research on representatives of this genus. Sequences were deposited in GenBank 242 (see Table 1 for accession numbers).

243 Cophylogenetic analyses

244	To study the coevolutionary associations between species of Cichlidogyrus and their LT cichlid hosts, the
245	ML tree topology was used. For the cichlid hosts, the recently published phylogenomic tree by Ronco et al. (2021)
246	was downloaded from https://datadryad.org/stash/dataset/doi:10.5061/dryad.9w0vt4bbf. The outgroup taxa were
247	removed with TreeGraph v. 2.15 (Müller and Müller, 2004) and the host tree was rooted using T. polylepis as the
248	representative of the earliest diverging tribe in LT (see for instance Dunz and Schliewen (2013)). We investigated
249	whether the phylogeny of species of Cichlidogyrus follows the tribal level classification of LT cichlids using
250	Mesquite v. 3.61 (Maddison and Maddison, 2019). Prior to the mapping, the ML tree of Cichlidogyrus was reduced
251	with TreeGraph to include only LT species, with C. mulimbwai as an outgroup. The tanglegram illustrating host-
252	parasite associations was inferred using TreeMap v. 3.0b (Charleston, 2012). Two different methods were
253	employed to assess the level of cophylogenetic signal in host-parasite associations. The distance-based method
254	ParaFit (Legendre et al., 2002) implemented in CopyCat (Meier-Kolthoff et al., 2007) was used with 9999
255	permutations to assess the statistical significance of global fit and individual coevolutionary links. Jane v. 4.0
256	(Conow et al., 2010) was applied to analyse host-parasite associations using an event-based approach. This method
257	compares two tree topologies (considering branch lengths) and optimally fits the parasite tree onto the host tree by
258	mixing different coevolutionary events with predefined costs. Costs were attributed for the five coevolutionary
259	events that can be inferred in this software package: (i) cospeciation (joint parasite and host speciation); (ii)
260	duplication (multiple parasites diversify within the same host species); (iii) duplication with host switch (parasites
261	diverge and then transfer from one host species to another); (iv) losses (loss of parasite); and (v) failure to diverge.
262	The latter event occurs when parasite speciation misses host speciation, resulting in the presence of the same
263	parasite species on new host species. A cost is attributed to each type of event, and the algorithm searches the
264	reconstruction with the lowest global cost. Eleven models with different cost schemes were applied for the
265	cophylogenetic analyses performed using 500 generations and a population size of 100 as parameters of the genetic
266	algorithm to assess the influence of each evolutionary event. Following Deng et al. (2013), the Jane default model,
267	TreeMap default model (Charleston, 1998) and TreeFitter default model (Ronquist, 1995) were included in our
268	analyses. Each of these default models states that cospeciation has the lowest cost, which corresponds to the most
269	common evolutionary event. Seven additional models were incorporated in the cophylogenetic analyses, of which
270	TreeFitter models adjusted for codivergence and host switch, respectively, with equal weights for coevolutionary
271	events following Mendlová et al. (2012). Five other models, where each event is alternatively extremely penalized
272	(cost of specific event set to 10 and all others to 1, following Deng et al. (2013) and Benovics et al. (2020b), were
l 273	further used. Statistical tests were computed using 500 randomizations with random parasite trees (Table 3).

274 Mapping of morphological characters onto the phylogeny of Cichlidogyrus

275 The ML tree including only species of Cichlidogyrus parasitizing LT cichlid hosts was used to evaluate 276 the structural evolution of haptoral and reproductive organs in this system. Unambiguous morphological character 277 states exhibited by species of Cichlidogyrus were mapped onto the parasite phylogeny. The mapping was 278 performed using maximum parsimony ancestral state reconstruction in Mesquite. The following morphological 279 characters were evaluated: (i) hook pair configuration: haptoral groups in terms of size of hook pairs, and (ii) the 280 sclerotization in the vagina (present or absent). A previous study of Vignon et al. (2011) and the overview in 281 Rahmouni et al. (2017) mentioned the following haptoral groups, i.e., states for hook pair configuration: group A 282 for species of Cichlidogyrus with short hook pairs I-IV, VI and VII (pair V with larval size); group B for species 283 of Cichlidogyrus exhibiting long hook pair I (pair V with larval size) and short pairs II-IV, VI and VII; group C 284 for species of Cichlidogyrus with short hook pair I (pair V with larval size) and longer pairs II-IV, VI and VII; and 285 group D for species of Cichlidogyrus showing long hook pairs I-VII, except larval-sized pair V. The numbering 286 of hook pairs (Roman letters I-VII) is that recommended by Mizelle (1936). Regarding the vagina, we followed 287 the classification elaborated by Pariselle and Euzet (2003) and applied in Rahmouni et al. (2017).

288 Host specificity in Cichlidogyrus from Lake Tanganyika

289 Host specificity in species of Cichlidogyrus from LT cichlids investigated herein was recorded. We 290 considered host specificity for 35 species of Cichlidogyrus based on previous records from LT cichlids (Kmentová 291 et al., 2016b) and our data. With regard to the index of host specificity (IS), the classification of species of 292 Cichlidogyrus follows that of Šimková et al. (2006) and applied by Kmentová et al. (2016b) for Cichlidogyrus: (i) 293 strict specialists parasitizing a single cichlid host species, (ii) intermediate specialists parasitizing two or more 294 congeneric cichlid species, (iii) intermediate generalists parasitizing heterogeneric cichlid species from the same 295 tribe, and (iv) true generalists parasitizing cichlid species belonging to different tribes. Host range was expressed 296 as the total number of LT cichlid species parasitized by a given species of Cichlidogyrus.

297 RESULTS

298 Monogenean phylogeny

The phylogenetic tree (Fig. 1) based on concatenated nuclear genes (18S, ITS1 and 28S rDNA) included a total of 63 species of *Cichlidogyrus* and three *Scutogyrus* spp.: 29 species of *Cichlidogyrus* and *Scutogyrus* parasitizing West African cichlid hosts, plus a single species from a South African haplochromine, and 36 species of *Cichlidogyrus* from LT itself. The alignment comprised sequences of ten undescribed species of *Cichlidogyrus* from Haplochromini (*Cichlidogyrus* sp. 1 and *Cichlidogyrus* sp. 2) inhabiting East African freshwater systems 304 outside of LT_(see Table 1), and from LT lamprologines (Cichlidogyrus sp. 3 to Cichlidogyrus sp. 10). Maximum 305 Likelihood (Fig. 1) and BI trees showed identical topologies when considering the well-supported nodes. 306 Cichlidogyrus amphoratus Pariselle & Euzet, 1996 and C. sclerosus Paperna & Thurston, 1969 constituted the 307 sister group of a well-supported LT cluster of Cichlidogyrus including three main clades (3, 4 and 5 in Fig. 1). 308 However, two species of Cichlidogyrus from LT_a C. mbirizei and C. mulimbwai parasitizing oreochromine and 309 tylochromine cichlids, respectively, belonged to lineages branching off earlier than the clade including species of 310 Cichlidogyrus from the East African radiation (Fig. 1). A weakly supported clade 1, formed by species of 311 Scutogyrus, plus C. falcifer Dossou & Birgi, 1984, C. longicirrus Paperna, 1965 and C. dracolemma Řehulková, 312 Mendlová & Šimková, 2013 parasitizing West African Hemichromis spp., and C. amieti from Aphyosemion 313 cameronense (Boulenger, 1903), was sister to the group including Cichlidogyrus from coptodonine, hemichromine 314 and oreochromine host lineages with high support (BS = 81, PP = 0.98). Within clade 2, the phylogenetic position of C. philander Douëllou, 1993 parasitizing the Southern African haplochromine Pseudocrenilabrus philander 315 316 (Weber, 1897) was weakly supported only by BS. The Lake Tanganyika species C. mbirizei from O. tanganicae 317 was sister to West African C. cirratus Paperna, 1964 from Oreochromis niloticus (Linnaeus, 1758), and both 318 species together with C. njinei Pariselle, Bilong Bilong & Euzet, 2003 from Sarotherodon galilaeus 319 (Linnaeus, 1758) formed a moderately supported group (only by BS). With high to low support values (BS = 98, 320 PP = 0.71), clade 2 also included a group of C. nageus Řehulková, Mendlová & Šimková, 2013, C. acerbus 321 Dossou, 1982, and C. halli Price & Kirk, 1967 from S. galilaeus and O. niloticus, in addition to a well-supported 322 subclade (BS = 91, PP = 1) clustering species of Cichlidogyrus restricted to coptodonines, from African Coptodon 323 guineensis (Günther, 1862) and C. rendalli (Boulenger, 1897) introduced to Madagascar, but including also C. 324 douellouae Pariselle, Bilong Bilong & Euzet, 2003_from the oreochromine S. galilaeus. Within Cichlidogyrus 325 from LT, three clades (3 to 5) were recognized. Clade 3 (BS = 81 and PP = 0.76) represented the <u>earliest diverging</u> 326 lineage, including species of Cichlidogyrus from the six LT cichlid tribes Bathybatini, Benthochromini, 327 Boulengerochromini, Cyphotilapiini, Ectodini and Perissodini (Fig. 2). The group of species of Cichlidogyrus 328 from Ophthalmotilapia nasuta (Poll & Matthes, 1962) (Ectodini), C. aspiralis, C. glacicremoratus and C. 329 rectangulus Rahmouni, Vanhove & Šimková, 2017, was paraphyletic, just like C. nshomboi Muterezi Bukinga et 330 al., 2012 (from Boulengerochromis microlepis (Boulenger, 1899), Boulengerochromini, plus Perissodus 331 microlepis Boulenger, 1898, and P. straeleni Poll, 1948, both Perissodini), whereas C. pseudoaspiralis and C. 332 discophonum Rahmouni, Vanhove & Šimková, 2017 from Aulonocranus dewindti (Boulenger, 1899) (Ectodini) 333 formed a monophyletic group (Fig. 1, 2). With high support values (BS = 98 and PP = 0.95), the first subclade

334 within clade 3 (Fig. 1, 2) harboured C. attenboroughi Kmentová et al., 2016 from the benthochromine 335 Benthochromis horii Takahashi, 2008 as sister species to C. glacicremoratus and C. rectangulus Rahmouni, 336 Vanhove & Šimková, 2017, both from ectodines (see above). The second subgroup was represented by seven 337 species, C. discophonum and C. pseudoaspiralis as sister species parasitizing A. dewindti (BS = 100 and PP = 1), 338 and a monophyletic group including C. aspiralis from the ectodine O. nasuta, C. habluetzeli Rahmouni, Vanhove 339 <u>& Šimková, 2018</u> from Cyphotilapia frontosa (Boulenger, 1906) and Cardiopharynx schoutedeni Poll, 1942, C. 340 nshomboi, and C. casuarinus Pariselle, Muterezi Bukinga & Vanhove, 2015 parasitizing members of Bathybatini. 341 Clade 4 (BS = 99 and PP = 1) grouped undescribed species parasitizing lamprologines, C. brunnensis Kmentová 342 et al., 2016 from Trematocara unimaculatum Boulenger, 1901 (Trematocarini), and C. milangelnari Rahmouni, 343 Vanhove & Šimková, 2017 from Cyprichromis microlepidotus (Poll, 1956) (Cyprichromini) (Fig. 1, 2). Clade 5 344 (BS = 100 and PP = 1) included mainly two subclades grouping Cichlidogyrus from Eretmodini, Haplochromini 345 and Tropheini. Three species of Cichlidogyrus from haplochromine hosts including two undescribed ones formed 346 a monophyletic group with high support (BS = 100 and PP = 1), while species from tropheine hosts were 347 paraphyletic, with C. franswittei Pariselle & Vanhove, 2015 as sister group to Cichlidogyrus from the non-348 tropheine haplochromines (BS = 86 and PP = 0.97) (Fig. 1, 2). Two monogenean species, C. jeanloujustinei 349 Rahmouni, Vanhove & Šimková, 2017 from Eretmodus marksmithi Burgess, 2012 and C. evikae Rahmouni, 350 Vanhove & Šimková, 2017 from Tanganicodus irsacae Poll, 1950, both from Eretmodini, formed a highly . 351 supported lineage within species infecting members of Tropheini (BS = 86 and PP = 0.99). Concerning 352 monogenean species of members of Tropheini, species of Cichlidogyrus from Simochromis diagramma 353 (Günther, 1894) formed a highly supported monophyletic group (BS = 100 and PP = 1), while species of 354 Cichlidogyrus from congeneric Petrochromis spp. were polyphyletic; C. antoineparisellei Rahmouni, Vanhove & 355 Šimková, 2018 from Interochromis loocki (Poll, 1949) and C. masilyai Rahmouni, Vanhove & Šimková, 2018 356 from P. orthognathus Matthes, 1959 were sister to Cichlidogyrus from S. diagramma, with a polytomy of species 357 formed by C. salzburgeri Rahmouni, Vanhove & Šimková, 2018 from P. trewavasae Poll, 1948 and C. irenae 358 Gillardin et al., 2012 from 'Gnathochromis' pfefferi (Boulenger, 1898) (Fig. 1, 2). . 359 Mapping the cichlid host tribes (lineages) from LT onto the ML tree (Fig. 2) again showed that

Tylochromini is the host group for the earliest diverging lineage of *Cichlidogyrus*, followed by Oreochromini.
 Cichlidogyrus from six LT cichlid tribes are included in a single lineage <u>(clade 3 in Fig. 1)</u>, just like *Cichlidogyrus* from Lamprologini, Cyprichromini and Trematocarini <u>(clade 4 in Fig. 1)</u>, and monogenean species from members

363 of Haplochromini, Eretmodini and Tropheini (clade 5 in Fig. 1). Cichlidogyrus from the latter tribe represents the
 364 most derived lineage in LT.

365 Cophylogenetic analyses of cichlids and Cichlidogyrus in Lake Tanganyika

366 The tanglegram indicating associations between species of Cichlidogyrus and their cichlid fish hosts is 367 shown in Fig. 3A. A global test calculated in ParaFit revealed a highly significant overall cophylogenetic structure (ParaFitGlobal = 886.05494, p < 0.001for 9999 permutations). The ParaFitLink test showed that 31 host-parasite 368 369 links out of a total of 45 significantly contributed to the overall phylogenetic congruence. Significant host-parasite 370 links were inferred for species of Cichlidogyrus parasitizing cichlids belonging to Boulengerochromini, 371 Bathybatini, Eretmodini, Haplochromini, Lamprologini, Tropheini and Tylochromini. Species of Cichlidogyrus 372 parasitizing cichlid species belonging to the remaining tribes Benthochromini, Cyphotilapiini, Cyprichromini, 373 Ectodini, Perissodini and Trematocarini revealed statistically non-significant links.

The event-based method of Jane also recovered a global signal of congruence across the whole dataset (*p* < 0.001) (Table 3). The lowest total cost (27) was produced by the host switch- adjusted TreeFitter model, corresponding to six cospeciation events, 12 duplications, 17 duplications followed by host switch, nine failures to diverge and a single loss event (Fig. 3B). The scenarios with the highest total costs were presented by the FTD prohibitive, and the host switch prohibited models.

379 Mapping of morphological characters onto the phylogeny of Cichlidogyrus

380 Mapping of morphological characters of species of Cichlidogyrus in terms of hook pair configuration 381 (Fig. 4A) showed that short pairs I-IV, VI and VII (group A) represent the ancestral state in LT, and the acquisition 382 of thickness and/or length in the hooks represent derived character states that are not common in species of 383 Cichlidogyrus in LT. Derived hook configurations were mostly found in species of Cichlidogyrus of clade 3 and 384 only C. salzburgeri from clade 5. Mapping of sclerotization in the vagina (Fig. 4B) showed that sclerotization or 385 loss of this organ evolved multiple times during diversification of Cichlidogyrus in LT (i.e., this character state is 386 present in C. mbirizei from Oreochromini and some species within clades 3, 4 and 5). When considering both 387 morphological characters, the represented LT species of Cichlidogyrus belonging to haptoral group D of Vignon 388 et al. (2011) seem to have acquired or retained sclerotization in their vagina.

389 Host specificity in Lake Tanganyika Cichlidogyrus

390 The host range of species of *Cichlidogyrus* from LT varied from a single host species to five host species 391 (*Cichlidogyrus casuarinus* parasitizing 7 cichlid species of Bathybatini (Supplementary Table S1)). Strict 392 specialism is apparently the ancestral state of host specificity (but note that less than 15% of LT's cichlid species were included in this study), and other forms of host specificity (that were rarely reported) are derived. Lower host
specificity was found in some species of *Cichlidogyrus* belonging to the clades 3 and 5. When considering
morphological characters investigated herein, the haptoral group D was found in <u>the</u> true generalists *C. nshomboi*and *C. habluetzeli*, and in the intermediate generalist *C. casuarinus*. These species possess long hook pairs I-VII,
except<u>for</u> pair V which retained its larval size, and a sclerotized vagina (Fig. 4A, B).

398

399 Discussion

The present study was focused on monogeneans of *Cichlidogyrus* parasitizing cichlid hosts inhabiting
 LT, one of the main biodiversity hotspots in Africa. This system harbours various lineages of *Cichlidogyrus* in
 terms of morphology and genetics.

403 Phylogeny of Cichlidogyrus

404 First, we focussed on the phylogenetic relationships between Cichlidogyrus from LT and its members 405 outside of the lake. Cichlidogyrus outside of LT was represented mostly by West African species sampled from 406 three cichlid tribes, the coptodonines, hemichromines and oreochromines, and by a single species sampled from 407 each of a Southern African haplochromine and a nothobranchiid host (Mendlová et al., 2012; Mendlová and 408 Šimková, 2014; Messu Mandeng et al., 2015; Šimková et al., 2019). Cichlidogyrus pouyaudi parasitizing West 409 African tylochromines was previously recognized as an early diverging species of Cichlidogyrus in West Africa 410 by Mendlová et al. (2012). In line with their study, we showed that C. mulimbwai found solely on T. polylepis 411 (Tylochromini) which occurs LT and its tributaries (Stiassny, 1991, 1990) belongs to the same early diverging 412 lineage as C. pouyaudi, consistent with the phylogenetic placement of T. polylepis, and the tylochromines as a 413 whole, among African cichlids (Irisarri et al. 2018; Ronco et al. 2021). We also demonstrated that species of 414 Cichlidogyrus parasitizing West African hemichromines, coptodonines and oreochromines, together with C. 415 mbirizei from LT, were sister to the rest of the assemblage of Cichlidogyrus including its representatives from the 416 lake itself, consistent with the phylogenetic relationships among the hosts (Schwarzer et al., 2009; Irisarri et al., 417 2018). The position of C. amieti from a non-cichlid host within the monogenean species of hemichromines is as 418 previously shown by Messu Mandeng et al. (2015). Our results showed that, unlike Scutogyrus, Cichlidogyrus in 419 West Africa is not monophyletic, in accordance with results by Mendlová et al. (2012). A clade of C. amphoratus 420 and C. sclerosus collected from coptodonine and oreochromine cichlid hosts was sister to the well supported large 421 LT clade of Cichlidogyrus.

422 Phylogenetic position of Cichlidogyrus parasitizing Lake Tanganyika cichlids

423 In the present phylogenetic study, 35 species of Cichlidogyrus parasitizing 32 LT cichlids belonging to 424 14 endemic tribes of a total of 16 (Ronco et al., 2020) were included. This includes some species, such as 425 Cichlidogyrus sp. 1 and Cichlidogyrus sp. 2, sampled also from haplochromines inhabiting other East African 426 freshwater habitats. Our study provides the first molecular evidence for a polyphyletic assemblage of 427 Cichlidogyrus in LT, as C. mulimbwai infecting T. polylepis and C. mbirizei from O. tanganicae, both from LT, 428 were sister to C. pouyaudi from West African Tylochromis species, and a few species from oreochromine hosts, 429 respectively, both quite divergent from the rest of the LT species of Cichlidogyrus (Fig. 1). The centre of diversity 430 of Tylochromis Regan, 1920 and Oreochromis Günther, 1889 lies in West Africa and the Congo River system (Stiassny, 1990, 1991; Agnèse et al., 1997), and both T. polylepis and O. tanganicae colonized LT only fairly 431 432 recently (Klett and Meyer, 2002; Koch et al., 2007). Three well supported clades of Cichlidogyrus (3 to 5) 433 parasitizing distinct cichlid tribes from LT were found. Clades 3 and 4 appear to be restricted to species of 434 Cichlidogyrus parasitizing representatives of cichlid tribes occupying particular ecological niches - semi-pelagic 435 (Cyprichromini and some members of Ectodini and Lamprologini), and deep and benthopelagic habitats 436 (Bathybatini, Benthochromini, Boulengerochromini, Cyphotilapiini, a few species of Perissodini, and 437 Trematocarini) (Konings, 2019). Clade 5 is restricted to species of Cichlidogyrus from Eretmodini, Haplochromini 438 (both from within and outside of LT) and Tropheini, consistent with the close phylogenetic relationships of the host tribes (Takahashi and Sota, 2016; Irisarri et al., 2018; Ronco et al., 2021), but also indicating host switches 439 440 across tribes.

441

Speciation and diversification of Cichlidogyrus on Lake Tanganyika cichlids

442 Considering <u>the inferred phylogenetic relationships between species of *Cichlidogyrus* from LT and their 443 West African congeners, we can infer that cospeciation has a low contribution to the diversity of this genus. As 444 already mentioned, host switching of *Cichlidogyrus* between phylogenetically distant cichlid hosts in African 445 freshwater habitats is far from unusual and can be considered as a main coevolutionary event shaping the 446 diversification within this genus.</u>

The evolutionary associations between species of *Cichlidogyrus* and their LT cichlid hosts were investigated using both distance-based and event- based methods. The analyses confirmed significant global cophylogenetic structure in this system. <u>Overall, our analyses indicated that host switching followed by</u> <u>duplications is the most frequent coevolutionary event in most of the models (except for host switch prohibited</u> <u>models with highest penalization of host switch and TreeFitter default with higher penalization of host switch</u> <u>when compared to other events and zero costs for cospeciation and duplication). Most models revealed only one</u> 453 loss event. All models produced the same number of instances of failure to diverge. Few species of Cichlidogyrus 454 from early diverging lineages showed to have failed to diverge, while most species of Cichlidogyrus from LT 455 tribes have diversified either by host switching and/or duplications, like Cichlidogyrus from lamprologines for 456 instance, or by cospeciation as evidenced for a few species parasitizing ectodines, both monogenean species from 457 eretmodines and most species from tropheines. For the latter host tribe,- a similar scenario was reported by 458 Vanhove et al., (2015) (see below). Based on previous studies by Pariselle et al. (2015a) and Kmentová et al. 459 (2016b), we can assume a failure to diverge for the intermediate generalist C. casuarinus parasitizing a wide range 460 of bathybatines. Herein, a similar scenario was attributed to monogeneans parasitizing unrelated host lineages C. 461 habluetzeli from Ectodini and Cyphotilapiini (Rahmouni et al., 2018), C. nshomboi from the boulengerochromine 462 B. microlepis and heterogeneric perissodines (Muterezi Bukinga et al. 2012; Rahmouni, 2021), and to a lesser 463 degree to species of Cichlidogyrus from congeneric or closely related hosts, C. gillardinae Muterezi Bukinga et 464 al., 2012 and C. franswittei from Haplochromini, and Tropheini (Van Steenberge et al. 2015 and reference herein), 465 respectively. At the same time, we find host switching of Cichlidogyrus from tropheine to haplochromine hosts 466 (Fig. 3B). Several factors could have promoted to keeping gill ectoparasites behind the course of their cichlid hosts 467 diversification. Johnson et al. (2003) stated that a given parasite species most likely fails to speciate when gene 468 flow among diverging host populations is maintained. For C. casuarinus, most bathybatine hosts are good 469 dispersers with lake-wide distribution in deep-water habitats (Koblmüller et al. 2005; 2019). It is well known that 470 physical proximity can facilitate gene flow among populations, and that long-distance dispersal offers the 471 opportunity for long-distance gene flow, and thus high levels of gene flow among populations (Mitton, 2013). 472 Further, high gene flow limits interpopulation differentiation (Woodruff, 2001).

473 Our results partially correspond to the findings of Vanhove et al. (2015) who found significant congruence 474 between the phylogeny of Tropheini and their species of Cichlidogyrus, potentially suggesting divergence with 475 the initial radiation of this tribe, whereas host switching was rare. Generally speaking, cospeciation is encouraged 476 either by little interspecific contacts (Paterson et al., 2000) or by predominantly vertical transmission (Clark et al., 477 2000), which is not the case of dactylogyrideans parasitizing modern LT tribes investigated herein. The tropheine 478 S. diagramma sampled off the north-eastern lakeshore showed to host the same monogenean community as 479 reported by Van Steenberge et al. (2015) from opposite locations in north-eastern and southern parts of LT. In the 480 case of Haplochromini, A. burtoni (Günther, 1894) from LT was previously shown to be parasitized by C. 481 gillardinae, whereas the gills of this host inhabiting distinct East African freshwater systems (see material and 482 methods section and Table 1) harboured two different (undescribed) species of Cichlidogyrus (sequenced in this

study). It should be noted that the presence of *C. gillardinae* on *A. stappersii* (Poll, 1943) is reported herein for the first time.

485 Structural evolution and host specificity in Cichlidogyrus

486 Using the morphological delimitation of Vignon et al. (2011), based on Pariselle and Euzet (2003), for 487 species outside of LT, most-species of Cichlidogyrus from LT belonged to the haptoral group A characterized by . 488 the presence of larval (short) hooks, whilst species of Cichlidogyrus representing the remaining haptoral groups 489 were rarely reported. With regard to the vagina, most studied LT species of Cichlidogyrus exhibited a non-490 sclerotized one (Fig. 4B). Mendlová et al. (2012) examined the evolution of haptoral morphology in West African 491 species of Cichlidogyrus and, in accordance with our findings, they found that larval hook size represents the 492 ancestral character state of the haptoral hooks. In the LT system, multiple transitions from this configuration to 493 derived ones seem to have occurred. Mapping of morphological characters onto the phylogeny of West African 494 Cichlidogyrus also indicated a clade-specific morphology, which is not, however, the case for Cichlidogyrus from 495 LT. This discrepancy may be related to a potentially different age of Cichlidogyrus in West African riverine and 496 East African lake systems. It should be noted that the West African species C. amphoratus and C. sclerosus, the 497 sister group of the species from the East African cichlid radiation, show short hook pairs but both possess a 498 sclerotized vagina (Paperna and Thurston, 1969; Pariselle and Euzet, 1996; Mendlová et al., 2012). Generally 499 speaking, the acquisition of thickness/length in the hooks seems to be the result of adaptation to specific hosts. 500 Indeed, it is well known that to successfully parasitize large hosts, stronger (larger) haptoral sclerites are necessary. 501 This was already shown for dactylogyridean monogeneans (Šimková et al., 2006), and recently observed in 502 specimens of C. nshomboi parasitizing the world's biggest cichlid, B. microlepis, in LT. The specimens of C. 503 nshomboi on this cichlid have larger and differently shaped anchors when compared to specimens parasitizing 504 smaller-sized perissodines (Rahmouni, 2021). Sclerotization in the vagina seems to have evolved or been lost 505 multiple times.

506 The different levels of host specificity of species of *Cichlidogyrus* occurring in West African cichlids and 507 the high species richness on some cichlid species provided the motivation to investigate the processes that have 508 contributed to diversification in *Cichlidogyrus* (Pouyaud et al., 2006; Mendlová and Šimková, 2014). A narrow 509 host specificity (strict specialism) was previously attributed to LT species of *Cichlidogyrus*, whilst generalist 510 species were rare, with only *C. casuarinus* reported as an intermediate generalist (Kmentová et al., 2016b). In our 511 study, host specificity was evaluated for 35 species of *Cichlidogyrus* parasitizing LT cichlids. The strict specialist 512 lifestyle seems to constitute the ancestral state of host specificity in *Cichlidogyrus* from LT cichlid hosts, and

513 changes towards lower host specificity were rare, recorded in few species of the clades 3 and 5 only (Muterezi 514 Bukinga et al. 2012; Kmentová et al. 2016b; Rahmouni et al. 2018; Rahmouni et al. unpublished). This corresponds 515 to findings for West African systems (Vignon et al. 2011; Mendlová and Šimková, 2014). The degree of host 516 specificity at global scale was shown, indeed, to differ from that at local levels in Dactylogyrus Diesing, 1850 517 parasitizing European cyprinids (Šimková et al., 2006), and Cichlidogyrus parasitizing West African cichlids. Such 518 differences could potentially be explained by isolation of monogenean populations (Mendlová and Šimková, 519 2014). Reduced host specificity reported by Kmentová et al. (2016b) in deep-water monogeneans of LT 520 Bathybatini seems to also occur in C. nshomboi and C. habluetzeli parasitizing benthopelagic hosts - B. microlepis 521 occurring down to the limit of the oxygenated layer (~50-200 m), while C. frontosa and C. schoutedeni are 522 commonly found in coastal water along rocks, and in shallow sandy habitats, respectively (~30-50 m) (Konings, 523 2019). In marine Lamellodiscus Johnston & Tiegs, 1922 (Desdevises et al., 2002) and freshwater Dactylogyrus 524 (Šimková et al., 2006), strict specialism represents the ancestral state of host specificity. This may indicate that 525 specificity is not an evolutionary 'dead-end' as previously suggested by Simpson (1953).

526 When summarizing the output of our mapping of morphology onto the parasite phylogeny and 527 considering our assumptions regarding host specificity in the LT system, we can deduce that strict specialists with 528 larval hook size (group A) represent character states of the ancestral Cichlidogyrus in LT, indicating that 529 decreasing host specificity could be at least in some species related to the evolution of complex parasite 530 morphology (increased thickness and/or elongation of hooks). We can support this observation by a few examples 531 of species of Cichlidogyrus with typical morphology and low host specificity. This is the case, for instance, for the 532 type-species C. arthracanthus Paperna, 1960 described from Levantine C. zillii (Gervais, 1848) (Paperna, 1960). 533 At the global level, this species is recognized as a true generalist in view of its occurrence on genetically and 534 geographically distant cichlid hosts, i.e., oreochromines native to the Levantine region (Paperna, 1960), 535 coptodonines and tilapiines native to West Africa (Paperna, 1960; Pariselle and Euzet, 2009, 1996; Pouyaud et al., 536 2006). In terms of haptoral morphology, C. arthracanthus displays a unique hooks organization with massive hook 537 pairs I and VI compared to larval-shaped pair V (see Pariselle and Euzet 2003; Vignon et al. 2011). Overall, it is 538 still too premature to attribute an adaptive meaning to the morphological variations in the hooks and vagina, in 539 association to the decrease of host specificity in Cichlidogyrus.

540 Conclusion

541 The application of molecular phylogenetics provided the first data on the phylogenetic position of LT 542 species of *Cichlidogyrus* in relation to their relatives inhabiting freshwater habitats elsewhere in Africa, and also on the relationships among LT species. The phylogenetic tree mirrored the evolutionary history of LT cichlid tribes, on the one hand, and provided support for previous morphological observations reflecting phylogenetic relatedness among species of *Cichlidogyrus*. Various speciation mechanisms, ranging from failure to diverge to cospeciation, with host switches and duplications observed at host tribal level, were revealed to have been involved in the diversification of this ectoparasite genus. Further research is, however, needed to determine the exact evolutionary meaning of morphological features of the haptor and reproductive organs, in relation to parasite specialization.

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574 Author contributions

575 CR, MPMV and AŠ designed the study. AŠ and MPMV supervised the work. MPMV and SK co-organised the

576 fieldtrips, parasite collection, preparation of specimens and provided scientific background in the field. SK

577 identified the host specimens and provided scientific background on the system and the analyses. CR performed

- 578 the lab work and data analysis, CR, MPMV, SK and AŠ wrote and corrected the manuscript. All authors read and
- 579 approved the final manuscript.

580 Ethics declarations

581 Conflict of interest

- 582 The authors declare no conflict of interest.
- 583 Data availability

Genetic sequence data is available from the NCBI database (see Table 1 for accession numbers) or from the authors
 upon request. <u>Data associated with this manuscript is provided online as electronic supplementary material at:</u>
 <u>http://dx.doi.org/10.17632/fwpczwwsbs.</u>

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911 Figure captions

912 Fig. 1 Maximum Likelihood (ML) phylogram of species of Cichlidogyrus and Scutogyrus parasitizing Lake 913 Tanganyika cichlid hosts and lineages from the rest of Africa based on analysis of the 18S, ITS1 and 28S rDNA 914 sequences. Species of Cichlidogyrus from tylochromine hosts were selected to root the tree following Mendlová 915 et al. (2012). Values above branches indicate bootstrap values (BS) from ML and posterior probabilities (BP) from BI analyses. Values below 50 (ML) and 0.80 (BI) are shown as dashes. Clade numbers 1-5 refer to different 916 917 Cichlidogyrus lineages (including Scutogyrus). Clades in black cluster West African cichlid linages of 918 Cichlidogyrus, while those in red are for species parasitizing LT hosts. Branch lengths correspond to the expected 919 number of substitutions per site.

920 Fig. 2 Mapping of cichlid host lineages onto the ML tree of Cichlidogyrus from Lake Tanganyika.

921 Fig. 3 (A) Tanglegram of phylogenies of Lake Tanganyika cichlid hosts (left) and associated species of 922 Cichlidogyrus (right) obtained using ML tree inference for species of Cichlidogyrus and the cichlid tree topology 923 from Ronco et al. (2021). Green lines indicate statistically significant host-parasite links (p < .05) and red lines 924 indicate non-statistically significant links. (B) One of the possible cophylogenetic scenarios between cichlid hosts 925 and their specific Cichlidogyrus obtained using Jane software, with the lowest cost produced by the host switch-926 adjusted TreeFitter model (6 cospeciation events (hollow coloured circle), 12 duplications (solid coloured circle), 927 17 duplications followed by host switch (duplication with an arrow following the trajectory of the switching 928 species), nine failures to diverge (jagged line) and 1 loss event (dashed line)). Black branches represent the cichlid 929 phylogeny and blue branches represent the phylogeny of Cichlidogyrus. 930 Fig. 4 Mapping of (A) haptoral groups in term of hooks configuration and (B) sclerotization in the vagina onto the ML phylogenetic tree of species of Cichlidogyrus from Lake Tanganyika cichlid hosts. The haptoral groups 931

932 A-D are represented by hook pairs of C. milangelnari, C. aspiralis, C. rectangulus and C. nshomboi. The

933 represented vagina is that exhibited by C. salzburgeri.

specificity of species of *Cichlidogyrus* from Tanganyikan cichlids onto their phylogenetic tree. Based on Pariselle and Euzet (2003), Vignon et al. (2011) and Rahmouni et al. (2017), group A clusters species of *Cichlidogyrus* with short hook pairs I-IV, VI and VII, group B for species exhibiting long hook pair I (pair V with larval size) and short pairs II-IV, VI and VII, group C harbors species with shorter hook pair I (pair V with larval size) and longer pairs II-IV, VI and VII, and B received the context of the provided pairs (2017), group C harbors species with shorter hook pair I (pair V with larval size) and longer pairs II-IV, VI and VII, and B received the provided pairs (2017), group C harbors species with shorter hook pair I (pair V with larval size) and longer pairs II-IV, VI and VII, and group D for species with long hook pairs. local index of specificity (IS) and information regarding the presence of a sclerotized vagina and their haptoral groups in terms of size of hook pairs. Host range is expressed as strict specialists parasitizing a single cichlid host species, (ii) intermediate specialists parasitizing two or more congeneric host species, (iii) intermediate generalists from heterogeneric host species from the same tribe, and (iv) true generalists from phylogenetically unrelated cichlid species. This classification was adopted for mapping the host-List of species of Cichlidogyrus from Lake Tanganyika investigated in this study with their cichlid hosts classified by tribes, host range, the total number of Tanganyikan cichlid host species parasitized by a given species of Cichlidogyrus. The index of specificity (IS) follows that of Kmentová et al. (2016): (i) except larval-shaped pair V. Supplementary Table S1 935 936 937 937 938 939 940 941 942

Cichlidogyrus spp.	Cichlid host	Cichlid tribe	Host range	Index of specificity (IS)	Sclerotization in the Vagina	Haptoral groups (hooks configuration)
Cichlidogyrus antoineparisellei	Interochromis loocki	Tropheini	1	Strict specialist	Yes	Group A
Cichlidogyrus aspiralis	Ophthalmotilapia nasuta	Ectodini	1	Strict specialist	Yes	Group B
Cichlidogyrus attenboroughi	Benthochromis horii	Benthochromini	1	Strict specialist	No	Group A
Cichlidogyrus banyankimbonai	Simochromis diagramma	Tropheini	1	Strict specialist	No	Group A
Cichlidogyrus brunnensis	Trematocara unimaculatum	Trematocarini	1	Strict specialist	No	Group A
Cichlidogyrus casuarinus	Bathybates fasciatus	Bathybatini	7	Intermediate generalist	Yes	Group D
	Bathybates graueri					
	Bathybates hornii					
	Bathybates leo					
	Bathybates minor					
	Bathybates vittatus					
	Hemibates stenosoma					
Cichlidogyrus discophonum	Aulonocranus dewindti	Ectodini	1	Strict specialist	No	Group A
Cichlidogyrus evikae	Tanganicodus irsacae	Eretmodini	1	Strict specialist	No	Group A
Cichlidogyrus franswittei	Pseudosimochromis curvifrons	Tropheini	2	Intermediate specialist	No	Group A
	Pseudosimochromis marginatus					
Cichlidogyrus gillardinae	Astatotilapia burtoni	Haplochromini	2	Intermediate specialist	No	Group A
	Astatotilapia stappersii					
Cichlidogyrus gistelincki	'Ctenochromis' horei	Tropheini	-	Strict specialist	No	Group A

Group A	Group D		Group A	Group A	Group A	Group A	Group A	Group A	Group A	Group D				Group B	Group A	Group C	Group C	Group A	Group A	Group A	Group A	Group A	Group A	Group A	Group A	Group A	Group A
No	Yes		No	No	No	Yes	No	No	No	Yes				No	No	No	No	No	No	Yes	No	No	No	No	Yes	No	No
Strict specialist	True generalist		Strict specialist	Strict specialist	Strict specialist	Strict specialist	Strict specialist	Strict specialist	Strict specialist	True generalist				Strict specialist	Strict specialist	Strict specialist	Strict specialist	Strict specialist	Strict specialist	Strict specialist	Strict specialist	Strict specialist	Strict specialist	Strict specialist	Strict specialist	Strict specialist	Strict specialist
1	2		-	1	1	1	1	-	-	4				-	1	1	-		-	1	1	1	1	1		-	1
Ectodini	Cyphotilapiini	Ectodini	Tropheini	Eretmodini	Tropheini	Oreochromini	Cyprichromini	Tylochromini	Tropheini	Boulengerochromini	Perissodini			Ectodini	Tropheini	Ectodini	Tropheini	Haplochromini	Haplochromini	Lamprologini						Lamprologini	
Ophthalmotilapia nasuta	Cyphotilapia frontosa	Cardiopharymx schoutedeni	'Gnathochromis' pfefferi	Eretmodus marksmithi	Petrochromis orthognathus	Oreochromis tanganicae	Cyprichromis microlepidotus	Tylochromis polylepis	Simochromis diagramma	Boulengerochromis microlepis	Perissodus microlepis	Perissodus straeleni	Haplotaxodon microlepis	Aulonocranus dewindti	Simochromis diagramma	Ophthalmotilapia nasuta	Petrochromis trewavasae	Astatotilapia burtoni	Astatotilapia burtoni	Lamprologus callipterus						Neolamprologus fasciatus	
Cichlidogyrus glacicremoratus	Cichlidogyrus habluetzeli		Cichlidogyrus irenae	Cichlidogyrus jeanloujustinei	Cichlidogyrus masilyai	Cichlidogyrus mbirizei	Cichlidogyrus milangelnari	Cichlidogyrus mulimbwai	Cichlidogyrus muterezii	Cichlidogyrus nshomboi				Cichlidogyrus pseudoaspiralis	Cichlidogyrus raeymaekersi	Cichlidogyrus rectangulus	Cichlidogyrus salzburgeri	Cichlidogyrus sp. 1	Cichlidogyrus sp. 2	Cichlidogyrus sp. 3	Cichlidogyrus sp. 4	Cichlidogyrus sp. 5	Cichlidogyrus sp. 6	Cichlidogyrus sp. 7	Cichlidogyrus sp. 8	Cichlidogyrus sp. 9	Cichlidogyrus sp. 10

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Table 1 Cichlid hosts (classified into tribes) sampled along the Lake Tanganyika shoreline and in neighbouring freshwater habitats, with the number of specimens sampled, date and locality of sampling, the species of *Cichlidogyrus* associated with them, and GenBank accession numbers for <u>18S to 1TS1 rDNA, 28S rDNA and COI mtDNA</u> for the parasites obtained in the present study (see material and methods).

Cichlid Tribe	Cichlid host species	ц	Date of sampling	Sampling locality	Country	GPS coordinates	Cichlidogyrus species	18S-ITS1 rDNA	28S rDNA	COI
Boulengerochromini Takahashi, 2003	Boulengerochromis microlepis (Boulenger, 1899)	2	05/09/2013	Bujumbura fish market	Burundi	3°23'S, 29°22'E	<i>C. nshomboi_</i> I Muterezi Bukinga, Vanhove, Van Steenberge and Pariselle. 2012	OL675317	OL675266	OL704757
Cyphotilapiini Salzburger, Meyer, Baric, Verheyen and Shurmhauer 2002	Cyphotilapia frontosa (Boulenger, 1906)	7	07/08/2016	Makabola village	DRC	3°32′S, 29°9′E	<i>C. adkoningsi</i> Rahmouni, Vanhove and Šimková, 2018			
							C. habluetzeli Rahmouni, Venhova and Šimboué, 2018	OL675312	OL675271	OL704742
Cyprichromini Poll, 1986	Cyprichromis microlepidotus	3	23/09/2013	Nyaruhongoka	Burundi	3°41'S, 29°20'E	Vannove and Sunkova, 2018 C. milangelnari Rahmouni, Vanhova and Šimbová, 2017	OL675315	OL675263	OL704754
Ectodini Poll, 1986	(Poll, 1990) Ophthalmotilapia nasuta (Poll and Matthes, 1962)	4	07-09/09/2013	Magara	Burundi	3°44'S, 29°19'E	Valuove and Shinkova, 2017 C. aspiralis Rahmouni, Vanhove and Šimková 2017	OL675305	OL675254	
							C. glacicremoratus Rahmouni, Vanhove and Šimková, 2017	OL675311	OL675262	OL704753
							C. rectangulus Rahmouni, Vanhove and Šimková, 2017	OL675321	OL675270	OL704746
	Aulonocranus dewindti (Boulenger, 1899)	б	04-07/09/2013	Nyaruhongoka	Burundi	3°41'S, 29°20'E	<i>C. discophonum</i> Rahmouni, Vanhove and Šimková, 2017	OL675307	0L675257	OL704745
							C. pseudoaspiralis Rahmouni, Vanhove and Šimková 2017	OL675319	OL675268	
	Cardiopharynx schoutedeni Poll. 1942	9	07/08/2016	Mulongwe fish market	DRC	3°22'S, 29°6'E	<i>C. koblmuelleri</i> Rahmouni, Vanhove and Šimková 2018			
							<i>C. habluetzeli</i> Rahmouni, Vanhove and Šimková. 2018	OL675333	OL675286	ı
Eretmodini Poll, 1986	<i>Eretmodus marksmithi</i> Burgess, 2012	10	03/09/2013	Magara	Burundi	3°44′S, 29°19′E	C. jeanloujustinei Rahmouni, Vanhove and Šimková, 2017	OL675313	OL675256	OL704747
		12	04/09/2013	Nyaruhongoka	Burundi	3°41'S, 29°20'E				
		13	23/09/2013	Mukuruka	Burundi	4°14'S, 29°33'E				
	Tanganicodus irsacae	٢	20/09/2013	Mukuruka	Burundi	4°14′S, 29°33′E	C. evikae Rahmouni, Vanhove	OL675308	OL675258	OL704752
Haplochromini Trewavas, 1983	Astatotilapia burtoni Günther 1803)	6	25/09/2013	Lake Cohoha	Burundi		Cichlidogyrus sp. 1 (undescribed)	OL675322	OL675275	
6071		1	25/09/2013	Bujumbura fish market	Burundi	3°23'S, 29°22'E	<i>C. gillardinae</i> Muterezi Bukinga, Vanhove, Van Steenberge and Pariselle 2012	OL675309	OL675261	OL704748
		7	09/09/2013	Mulongwe fish market	DRC	3°22'S, 29°6'E	1 m 104 16, 74 17			
		11	16/09/2013	Kilomoni beach	DRC					
			07/08/2016	Chitili	Zambia	8°36'S, 31°11'E	Cichlidogyrus sp. 2 (undescribed)	OL675324	OL675276	ı
				Kalambo Falls Rivers	Zambia	8°38'S, 31°11'E				
	Astatotilapia stappersii Doll 1943	1	16/09/2013	Kilomoni beach	DRC	3°20'S, 29°10'E	C. gillardinae	OL675334	OL675288	OL753688
Lamprologini Poll, 1986	Lou, 1945 Lamprologus callipterus Boulenger, 1906	9	06-10/09/2013	Nyaruhongoka	Burundi	3°41′S, 29°20′E	Cichlidogyrus sp. 3 (undescribed)	OL675325	OL675284	

					ı	ı	OL704756			ı						OL704759					ı	OL704749		ı	OL704750	OL704760	OL704758
OL675277	OL675278	OL675279	OL675280	OL675281	OL675282	OL675283	OL675267			OL675285						OL675260					OL675272	OL675273	OL675274	OL675259		OL675255	OL675265
OL675326	OL675327	OL675328	OL675329	OL675330	OL675331	OL675323	OL675318			OL675332						OL675310					OL675304	OL675314		ı		OL675306	OL675316
Cichlidogyrus sp. 4 (undescribed)	Cichlidogyrus sp. 5 (undescribed)	Cichlidogyrus sp. 6 (undescribed)	Cichlidogyrus sp. 7 (undescribed)	Cichlidogyrus sp. 8 (undescribed)	Cichlidogyrus sp. 9 (undescribed)	Cichlidogyrus sp. 10	undescribed) C. <i>nshomboi</i> _2 Muterezi Bukinga, Vanhove, Van Steenbarre and Paricella 2012	occurrence and Fariscus, 2012		C. nshomboi_2			C. $nshomboi_2$			C. gistelincki Gillardin, Vanhove, Pariselle, Huyse and Volckaert,	1107				C. antoineparisellei Rahmouni, Vonkova and Šimková 2018	V aunove and Sunova, 2010 C. masilyai Rahmouni, Vanhove	diu Junkova, 2010 C. salzburgeri Rahmouni,	vannove and Sumkova, 2018 C. <i>franswitte</i> i Pariselle and Vanhove, 2015	<i>C. frankwillemsi</i> Pariselle and Vanhove. 2015	<i>C. banyankimbonai</i> Pariselle and Vanhove 2015	<i>C. muterezii</i> Pariselle and Vanhove, 2015
3°44′S, 29°19′E	4°14′S, 29°33′E	4°15'S, 29°34'E			8°43'S, 31°08'E	8°37'S, 31°12'E	3°44'S, 29°19'E	3°41′S, 29°20′E	3°37′S, 29°9′E	6°56'S, 29°42'E	6°58′S, 29°43′E	7°35′S, 30°38′E	3°31'S, 29°08'E	5°38′S, 29°23′E	6°56′S, 29°42′E	3°41'S, 29°20'E	4°14′S, 29°33′E	3°23'S, 29°22'E	3°44′S, 29°19′E	4°15'S, 29°34'E	3°37'S, 29°9'E	3°37'S, 29°9'E	3°37'S, 29°9'E	3°37'S, 29°9'E		3°44′S, 29°19′E	3°41'S, 29°20'E
Burundi	Burundi	Burundi	Burundi	Burundi	Zambia	Zambia	Burundi	Burundi	DRC	DRC	DRC	Zambia	DRC	DRC	DRC	Burundi	Burundi	Burundi	Burundi	Burundi	DRC	DRC	DRC	DRC		Burundi	Burundi
Magara	Mukuruka	Mvugo			Wonzye Point	Kalambo Lodge	Magara	Nyaruhongoka	Pemba	Mukumba	Mtoto	Mtosi	Luhanga	Murega	Makumba	Nyaruhongoka	Mukuruka	Bujumbura fish market	Magara	Mvugo	Pemba	Pemba	Pemba	Pemba		Magara	Nyaruhongoka
04/09/2013	17-19/09/2013	20/09/2013			23/08/2011	18/04/2008	09/09/2013	09/09/2013	08/08/2016	14/05/2010	15/04/2010	ı	1957	24/04/2010	1997	6-7/09/2013	17/09/2013	26/09/2013	06/09/2013	25/09/2013	08/08/2016	08/08/2016	08/08/2016	08/08/2016		05/09/2013	05-06/09/2013
7	9	4			3	,	ŝ	5	2	٢	2	1	3			٢	1	1	4	5	6	4	1	1		10	10
					Neolamprologus fasciatus	(DOUTCHERT, 1070)	Perissodus microlepis Boulenger, 1898			Perissodus straeleni Dolt 1048	FOIL, 1740		Haplotaxodon microlepis Boulencer 1006	DURINIZYI, 1700		' <i>Ctenochromis</i> ' horei Günther, 1893					Interochromis loocki	Petrochromis orthognathus Mothes 1050	Petrochromis trewavasae Poll,	1948 Pseudosimochromis curvifrons Poll, 1942		Simochromis diagramma (Ginther 1893)	
							Perissodini Poll, 1986									Tropheini Poll, 1986											

OL704755		OL704743	OL704744	OL704751
OL675269	OL675287	OL675264	ı	
OL675320			ı	
C. raeymaekersi Pariselle and Vanhove, 2015	C. franswittei Pariselle and Vanhove, 2015	C. mulimbwai Muterezi Bukinga, Vanhove, Van Steenberge and Pariselle, 2012	<i>C. muzumanii</i> Muterezi Bukinga, Vanhove, Van Steenberge and Pariselle, 2012	<i>C. sergemorandi</i> Rahmouni, Vanhove and Šimková, 2018
	3°37′S, 29°9′E	3°22′S, 29°6′E		
		DRC		
	Pemba	Mulongwe fish market		
	08/08/2016	09/09/2013		
	-	1		
	Pseudosimochromis marginatus (Poll, 1956)	Tylochromis polylepis (Boulenger, 1900)		
		Tylochromini Poll, 1986		

 Table 2
 List of previously published Cichlidogyrus spp. parasitizing West African and LT cichlids included in the present study, with cichlid host species, cichlid lineage/tribe, and GenBank accessions numbers for 18S, ITS1 and 28S rDNA sequences data.

Cichlidogyrus spp.	Cichlid host species	Cichlid lineage	18S rDNA-	28S rDNA
о с II	ľ	8	ITS1	
Cichlidogyrus acerbus	Sarotherodon galilaeus	Oreochromini	HE7927801	HQ010036 ²
Dossou, 1982	(Linnaeus, 1758)	Dunz and Schliewen, 2013		
Cichlidogyrus aegypticus	Coptodon guineensis	Coptodonini	HE7927811	HQ010021 ²
Ergens, 1981	(Günther, 1862)	Dunz and Schliewen, 2013		
Cichlidogyrus agnesi	Coptodon guineensis	Coptodonini	AJ920286 ³	-
Pariselle & Euzet, 1995	(Günther, 1862)	-		
Cichlidogyrus amieti	Aphyosemion cameronense	Non-cichlid	-	KT945076 ⁴
Birgi & Euzet, 1983	(Boulenger, 1903)			
Cichlidogyrus amphoratus	Coptodon guineensis	Coptodonini	HE7927821	HE792772 ¹
Pariselle & Euzet, 1996	(Günther, 1862)			
Cichlidogyrus arthracanthus	Coptodon guineensis	Coptodonini	HE7927831	HQ010022 ²
Paperna, 1960	(Günther, 1862)	-		
Cichlidogyrus attenboroughi	Benthochromis horii	Benthochromini	MH708153 ⁵	MH708146 ⁵
Kmentová et al., 2016	Takahashi, 2008	Takahashi, 2003		
Cichlidogyrus bilongi	Coptodon guineensis	Coptodonini	AJ920287 ³	-
Pariselle & Euzet, 1995	(Günther, 1862)	-		
Cichlidogyrus brunnensis	Trematocara unimaculatum	Trematocarini	MH708152 ⁵	MH708144 ⁵
Kmentová et al., 2016	Boulenger, 1901	Poll, 1986		
Cichlidogyrus casuarinus	Bathybates minor	Bathybatini	KX007795 ⁶	KX007822 ⁶
Pariselle, Muterezi Bukinga & Vanhove, 2015	Boulenger, 1906	Poll, 1986		
Cichlidogyrus cirratus	Oreochromis niloticus	Oreochromini	HE7927841	HE792773 ¹
Paperna, 1964	(Linnaeus, 1758)			
Cichlidogyrus cubitus	Coptodon guineensis	Coptodonini	HE7927851	HQ010037 ²
Dossou, 1982	(Günther, 1862)	1		ζ.
Cichlidogyrus digitatus	Coptodon guineensis	Coptodonini	HE792786 ¹	HQ010023 ²
Dossou, 1982	(Günther, 1862)			
Cichlidogyrus douellouae	Sarotherodon galilaeus	Oreochromini	HE792787 ¹	HE792774 ¹
Pariselle, Bilong Bilong & Euzet, 2003	(Linnaeus, 1758)			
Cichlidogyrus dracolemma	Hemichromis letourneuxi	Hemichromini	HE792794 ¹	HQ010027 ²
Řehulková, Mendlová & Šimková, 2013	Sauvage, 1880	Dunz and Schliewen, 2013		,
Cichlidogyrus ergensi	Coptodon guineensis	Coptodonini	HE7927881	HQ010038 ²
Dossou, 1982	(Günther, 1862)	<u>.</u>		~
Cichlidogyrus falcifer	Hemichromis fasciatus	Hemichromini	HE792789 ¹	HQ010024 ²
Dossou & Birgi, 1984	Peters, 1857			-
Cichlidogyrus philander	Pseudocrenilabrus	Haplochromini	MG250207*	MG279698*
Douëllou, 1993	philander (Weber, 1897)	-		
Cichlidogyrus flexicolpos	Coptodon guineensis	Coptodonini	AJ920283 ³	-
Pariselle & Euzet, 1995	(Günther, 1862)	-		
Cichlidogyrus gallus	Coptodon guineensis	Coptodonini	AJ920285 ³	-
Pariselle & Euzet, 1995	(Günther, 1862)	-		
Cichlidogyrus halli 1	Sarotherodon galilaeus	Oreochromini	HE7927901	HQ010025 ²
(Price & Kirk, 1967)	(Linnaeus, 1758)			
Cichlidogyrus halli 2	Oreochromis niloticus	Oreochromini	AJ920272 ³	-
(Price & Kirk, 1967)	(Linnaeus, 1758)			
Cichlidogyrus irenae	'Gnathochromis' pfefferi	Tropheini	KT692939 ⁷	MH708145 ⁷
Gillardin et al., 2012	(Boulenger, 1898)	Poll, 1986		
Cichlidogyrus longicirrus	Hemichromis fasciatus	Hemichromini	HE7927911	HQ010026 ²

Paperna, 1965	Peters, 1857			
Cichlidogyrus mbirizei	Oreochromis tanganicae	Oreochromini	MG030376*	MG030378*
Muterezi Bukinga et al., 2012	(Günther, 1894)			
Cichlidogyrus nageus	Sarotherodon galilaeus	Oreochromini	HE7927951	HQ010028 ²
Řehulková, Mendlová & Šimková, 2013	(Linnaeus, 1758)			
Cichlidogyrus njinei	Sarotherodon galilaeus	Oreochromini	HE7927921	HE7927751
Pariselle, Bilong Bilong & Euzet, 2003	(Linnaeus, 1758)			
Cichlidogyrus pouyaudi	Tylochromis intermedius	Tylochromini	HE7927931	HQ010039 ²
Pariselle & Euzet, 1994	(Boulenger, 1916)	Poll, 1986		
Cichlidogyrus sclerosus	Oreochromis niloticus	Oreochromini	DQ5373598	DQ157660 ⁸
Paperna & Thurston, 1969	(Linnaeus, 1758)			
Cichlidogyrus thurstonae	Coptodon rendalli	Coptodonini	AJ920274 ³	MH7674069
Ergens, 1981	(Boulenger, 1897)			
Cichlidogyrus tiberianus	Coptodon rendalli	Coptodonini	MH7674049	HE7927961
Paperna, 1960	(Boulenger, 1897)			
Cichlidogyrus tilapiae	Hemichromis fasciatus	Hemichromini	HE792797 ¹	HQ010029 ²
Paperna, 1960	Peters, 1857			
Cichlidogyrus yanni	Coptodon guineensis	Coptodonini	HE7927981	HE7927771
Pariselle & Euzet, 1996	(Günther, 1862)			
Scutogyrus bailloni	Sarotherodon galilaeus	Oreochromini	HE7927991	HE7927781
Pariselle & Euzet, 1995	(Linnaeus, 1758)			
Scutogyrus longicornis	Oreochromis niloticus	Oreochromini	HE7928001	HQ010035 ²
(Paperna & Thurston, 1969)	(Linnaeus, 1758)			
Scutogyrus minus	Sarotherodon melanotheron	Oreochromini	HE7928011	HE7927791
(Dossou, 1982)	Rüppell, 1852			

References: ¹Mendlová et al. (2012); ²Mendlová et al. (2010); ³Pouyaud et al. (2006), ⁴Messu Mandeng et al. (2015); ⁵Kmentová et al. (2018); ⁶Kmentová et al. (2016a); ⁷Kmentová et al. (2016b); ⁸Wu et al. (2007); ⁹Šimková et al. (2019) and . No references are available for sequences indicated by "*".









