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

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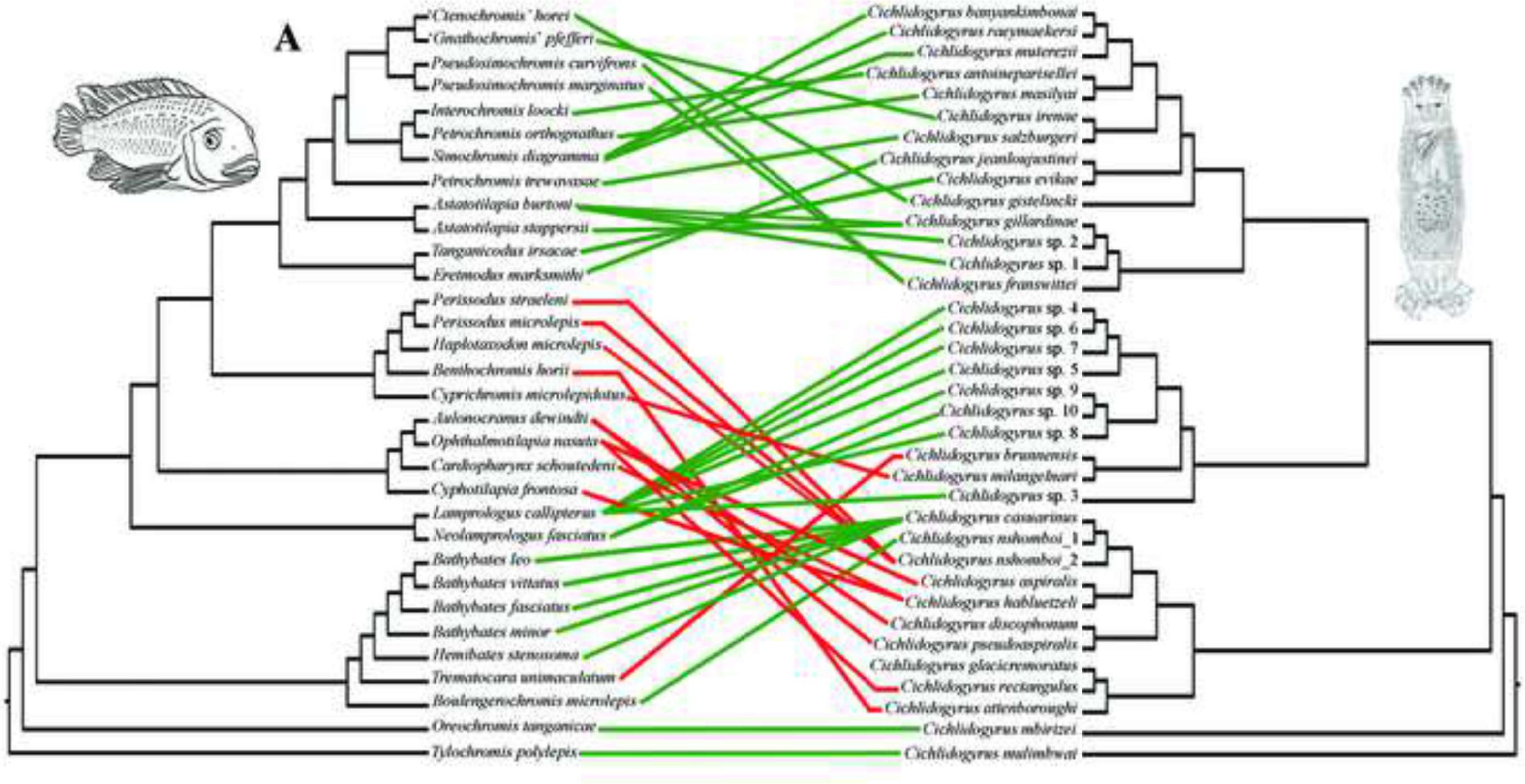
A
'Ctenochromis' horei
'Gnathochromis' pfefferi
Pseudosimochromis curvifrons
Pseudosimochromis marginatus
Interochromis loocki
Petrochromis orthognathus
Simochromis diagramma
Petrochromis trewavasae
Astatotilapia burtoni
Astatotilapia stappersii
Tanganicodus irsacae
Eretmodus marksmithi
Perissodus straeleni
Perissodus microlepis
Haplotaxodon microlepis
Benthochromis horii
Cyprichromis microlepidotus
Aulonocranus dewindti
Ophthalmotilapia nasuta
Cardiopharynx schoutedeni
Cyphotilapia frontosa
Lamprologus callipterus
Neolamprologus fasciatus
Bathybates leo
Bathybates vittatus
Bathybates fasciatus
Bathybates minor
Hemibates stenosoma
Trematocara unimaculatum
Boulengerochromis microlepis
Oreochromis tanganicae
Tylochromis polylepis
Cichlidogyrus banyankimbonai
Cichlidogyrus raeymaekersi
Cichlidogyrus muterezii
Cichlidogyrus antoineparisellei
Cichlidogyrus masilyai
Cichlidogyrus irenae
Cichlidogyrus salzburgeri
Cichlidogyrus jeanloujustinei
Cichlidogyrus evikae
Cichlidogyrus gistelincki
Cichlidogyrus gillardinae
Cichlidogyrus sp. 2
Cichlidogyrus sp. 1
Cichlidogyrus franswittei
Cichlidogyrus sp. 4
Cichlidogyrus sp. 6
Cichlidogyrus sp. 7
Cichlidogyrus sp. 5
Cichlidogyrus sp. 9
Cichlidogyrus sp. 10
Cichlidogyrus sp. 8
Cichlidogyrus brunnensis
Cichlidogyrus milangelnari
Cichlidogyrus sp. 3
Cichlidogyrus casuarinus
Cichlidogyrus nshomboi_1
Cichlidogyrus nshomboi_2
Cichlidogyrus aspiralis
Cichlidogyrus habluetzeli
Cichlidogyrus discophonum
Cichlidogyrus pseudoaspiralis
Cichlidogyrus glacicremoratus
Cichlidogyrus rectangulus
Cichlidogyrus attenboroughi
Cichlidogyrus mbirizei
Cichlidogyrus mulimbwai

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.

Molecular phylogeny and speciation patterns in host specific monogeneans (*Cichlidogyrus,* Dactylogyridae) parasitizing cichlid fishes (Cichliformes, Cichlidae) in Lake Tanganyika

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Abstract

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

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

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, gaining and/or loosing hosts, considerably, shapes the evolutionary trajectories of both host and parasite lineages (Nylin et al., 2018).

Monogenean flatworms are well suited for evaluating host-parasite coevolutionary relationships for several reasons. These parasites are highly diverse in terms of species number (Řehulková et al., 2018), 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 evolutionary success of monogeneans is presumed to be related to the diversity of their attachment organ (haptor), and their adaptation to hosts and infection sites (Whittington and Chisholm, 2008). With a direct life cycle, monogeneans further show a strong colonization ability, through either physical contacts between hosts or by the ciliated infectious stage (oncomiracidium) (Cable and Harris, 2002). Monogenean species are known to parasitize either a single host species (strict specialists), a narrow group of congeneric host species (intermediate specialists), phylogenetically closely related non-congeneric hosts (intermediate generalists), or phylogenetically unrelated host species (generalists) (Desdevises et al., 2002; Šimková et al., 2006; Thatcher, 2006; Franceschini et al., 2018; Kuchta et al., 2020).

From an evolutionary perspective, perfect cospeciation at the macroevolutionary level seems to be rare in 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 and parasite phylogenies is expected (Stammer, 1957; Brooks and McLennan, 1993). Host switching, parasite duplication and sorting events are coevolutionary events in which parasites fail to respond to host speciation (Johnson et al., 2003; Poulin, 2008), hence host and parasite phylogenies show incongruences (Brooks and McLennan, 1991; Page, 1993). These events were often suggested in scenarios of speciation and diversification of dactylogyridean monogeneans in freshwater fish hosts (Šimková et al. 2004; Mendlová et al., 2012; Šimková et 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 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 (Huyse and Volckaert, 2002; Huyse et al., 2003). In this latter 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).

African and Levantine cichlid fishes (Cichlidae Bonaparte, 1835) are known to host representatives of six dactylogyridean genera, plus a single gyrodactylid genus. This includes endoparasitic (mesoparasitic) genera found in the stomach and urinary bladder, i.e., *Enterogyrus* Paperna, 1963 and *Urogyrus* Bilong Bilong, Birgi & Euzet, 1994, respectively, and ectoparasitic genera found on fish gills: *Onchobdella* Paperna, 1968, *Scutogyrus* Pariselle and Euzet 1995, *Cichlidogyrus* Paperna, 1960, and *Gyrodactylus* von Nordmann, 1832, the latter of which also occurs on fins (Pariselle and Euzet, 2009; Dos Santos et al., 2019). The sixth and final dactylogyridean genus *Insulacleidus* Rakotofiringa and Euzet, 1983 is an ectoparasite genus restricted to Madagascar (Rakotofiringa and Euzet, 1983). Of the above-listed genera, *Cichlidogyrus* is the most speciose and mostly restricted to cichlid fishes. 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 date, 130 species of *Cichlidogyrus* have been reported from 112 cichlid species inhabiting Africa and the Levant (Pariselle and Euzet, 2009; Vanhove et al., 2016; Rahmouni et al., 2018; Geraerts et al., 2020). Although rarely, a few representatives of this genus were reported from non-cichlid hosts like the pupfish (Cyprinodontidae Gill, 1865 (Birgi and Euzet, 1983)) and leaffish (Nandidae Bleeker, 1852 (Birgi and Lambert, 1986)). To account for this, host switches from cichlid hosts to distant fish families were then suggested, but only a single case was supported by molecular data (Messu Mandeng et al., 2015). Host switching was also recently documented following the introduction of African mainland cichlids to Madagascar, where native Malagasy cichlids and aplocheilids were infected by non-native species of *Cichlidogyrus* (Šimková et al., 2019).

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 based on the configuration (size and shape) of hook pairs.

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

The focus of the present study is on Lake Tanganyika (LT), the deepest and oldest lake in Africa (Cohen et al., 1997; Lezzar et al., 2002), harbouring a number of different teleost lineages that radiated within the confines of the lake and show very high levels of endemism (Salzburger et al., 2014). Cichlids are the by far most species rich fish family in this lake and well established as model system in evolutionary biology (e.g. Kocher, 2004; Seehausen, 2006). Compared to the other East African rift lakes, the cichlid assemblages in LT are genetically, morphologically, ecologically and behaviourally the most diverse (Snoeks, 2000; Koblmüller et al., 2008). Roughly 240 cichlid species belonging to 16 tribes occur in the lake (Ronco et al. 2020). While some lineages are very species-rich (e.g. Lamprologini), others count a single representative in the lake (Tylochromini and Boulengerochromini) (Koblmüller et al., 2008). Whereas most tribes evolved *in situ* within a short period of time~~,~~ (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 (Takahashi and Sota, 2016; Irisarri et al., 2018; Ronco et al., 2021), and thus provided an invaluable resource for comparative follow-up studies on this system.

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

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

Material and methods

Fish and parasite collection

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

DNA extraction, amplification and sequencing

The conspecificity of parasites infecting the respective host species was confirmed using whole individuals mounted on slides based on their hard parts, and fragments of the small and large subunits of ribosomal DNA (18S and 28S rDNA), plus the entire first internal transcribed spacer (ITS1). Parasite individuals were removed from ethanol and dried using an Eppendorf 5301 concentrator under vacuum conditions at 30 °C. Genomic DNA was extracted using the DNeasy™ blood and tissue Kit (QIAGEN) following the manufacturer's instructions. The partial 28S fragment (D1–D2) was amplified using forward primer C1 (5′-ACCCGCTGAATTTAAGCAT-3′) and reverse D2 (5′-TGGTCCGTGTTTCAAGAC-3′) (Hassouna et al., 1984), or alternatively using forward ANCY55 (5′-GAGATTAGCCCATCACCGAAG-3′) (Plaisance et al., 2005) and reverse D2 if the first primer combination was unsuccessful. The partial 18S rRNA gene and the entire ITS1 region were amplified using the forward primer S1 (5′-ATT CCGATAACGAACGAGACT-3′) (Sinnappah et al., 2001) and reverse primer IR8 (5′- GCTAGCTGCGTTCTTCATCGA-3′) (Šimková et al., 2003), or alternatively using forward S1 and reverse LIG5.8 (5′-GATACTCGAGCCGAGTGATCC-3′) primers (Blasco-Costa et al., 2012) if unsuccessful with the first combination of primers. Because of its utility in previously published multi-marker phylogenetic trees of representatives of *Cichlidogyrus* (Vanhove et al., 2015; Cruz-Laufer et al., 2021) we also included a fragment of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene. The partial COI gene was amplified for selected samples using forward ASmit1 (5′-TTTTTGGGCATCCTGAGGTTTAT-3′) (Littlewood et al., 1997) and Schisto3 (5′-TAATGCATMGGAAAAAAACA-3′) (Lockyer et al., 2003), with reverse ASmit2 (5′-TAA AGA AAG AAC ATA ATG AAA ATG-3′) (Littlewood et al., 1997) as internal primer for the nested PCR. For DNA amplification, we followed the protocols published by Plaisance et al. (2008), Mendlová et al. (2012), 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 (Ecoli, Bratislava, SK) according to the manufacturer's instructions. Bi-directional sequencing of PCR amplicons using the BigDye® Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems by Thermo Fisher Scientific, Prague, Czech Republic) and the PCR primers was performed. After purification of sequencing products with the BigDye XTerminator® Purification Kit (Applied Biosystems by Thermo Fisher Scientific, Prague, Czech Republic), sequences were visualised on an ABI 3130 Genetic Analyzer (Applied Biosystems).

Alignment and phylogenetic analyses

Raw sequences were edited using the Sequencher® software v. 5.0 (Gene Codes Corporation, Ann Arbor, MI USA) and aligned with ClustalW (Thompson et al., 1994) as implemented in MEGA X (Kumar et al., 2018). In addition, previously published sequences of species of *Cichlidogyrus* and *Scutogyrus* (Pouyaud et al., 2006; Wu et al., 2007; Mendlová et al., 2010, 2012; Messu Mandeng et al., 2015; Kmentová et al., 2016a, b, 2018; Šimková et al., 2019) (Table 2), were downloaded from GenBank and included in the analyses to determine the position of *Cichlidogyrus* from LT cichlids in relation to *Cichlidogyrus* and *Scutogyrus* from the rest of Africa. Of West African monogeneans, a few sequences downloaded from GenBank correspond to species of *Cichlidogyrus* sampled from introduced cichlids in Asia and Madagascar, and a single species, *C. amieti* Pariselle & Euzet, 1995, known from *Aphyosemion* spp. (Nothobranchiidae). Sequences representing *C. mbirizei* Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle, 2012 described from the LT species *Oreochromis tanganicae* (Günther), 1894 were obtained from introduced cichlids in Asia (see Table 2 and below). GBlocks v. 0.91b (Talavera and Castresana, 2007) was applied to remove unreliably aligned sequences (gaps and ambiguously 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) was 585 bp for 28S rDNA (raw sequences of ~775 bp), 389 bp for 18S rDNA and 301 bp for ITS1 (raw sequences of ~820 bp for these two adjacent regions). All newly obtained sequences were deposited in GenBank (see Table 1 for accession numbers). Phylogenetic relationships were inferred using Maximum Likelihood (ML) and Bayesian Inference (BI). For each locus, the best fitting model of molecular evolution was inferred based on the corrected Akaike Information Criterion (AICc) (Sugiura, 1978; Hurvich and Tsai, 1989) in jModelTest v. 2.1.10 (Guindon et al., 2010; Darriba et al., 2012): TVM +I+ Γ for 28S rDNA with a gamma shape parameter of 0.91, K80+I for 18S rDNA, and TPM2uf+Γ for ITS1 with a gamma shape parameter of 0.64. To assess the phylogenetic content of the dataset, likelihood mapping based on quartet puzzling (Strimmer and Von Haeseler, 1997) implemented in TREE-PUZZLE v. 5.2 (Schmidt et al., 2002) was used. Since the combined alignment showed relatively high phylogenetic content (96.2% fully resolved, 2.4% partly resolved and 1.2% unresolved quartets), a concatenated dataset (1275 bp) containing a total of 65 sequences from species of *Cichlidogyrus* was generated using SeaView v. 5.0.4 (Gouy et al., 2010) and used for phylogenetic tree inference. *Cichlidogyrus pouyaudi* Pariselle & Euzet, 1994 parasitizing *Tylochromis intermedius* (Boulenger, 1916) from West Africa and *C. mulimbwai* Muterezi Bukinga, Vanhove, Van Steenberge and Pariselle, 2012, from the Lake Tanganyika species *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 fit substitution model for each alignment (see above) and a SPR branch-swapping algorithm. The branch support (bootstrap support, BS) was estimated using ultrafast bootstrap approximation (Minh et al., 2013) with 1 000 replicates. Selected models which are not implemented in MrBayes were substituted by the closest over-parameterized model (Huelsenbeck and Rannala, 2004). The analysis was performed using MrBayes v. 3.2.1 (Ronquist et al., 2012) with two independent Markov Chain Monte Carlo (MCMC) simulations (6 chains, 2×10^6 generations, sampling frequency 100, 25% burn-in). Chain stationarity and parameter convergence were assessed in TRACER v. 1.7.1 (Rambaut et al., 2018), with effective samples sizes (ESS) always > 200 for all parameters, and via the average standard deviation of split frequencies (always well below 0.01), and post burn-in trees were summarized in a 50% majority rule consensus tree. The ML and BI trees were visualized in FigTree v. 1.4.4 (Rambaut, 2009). It should be noted that as the incongruence length difference (ILD) test performed in the WinClada program (Farris, 1995) with 1000 replicates revealed heterogenous gene fragments (p=0.004), sequences of the COI gene were not included in the phylogenetic and cophylogenetic analyses for lack of sufficient DNA data (sequencing not successful for all species). Recent study by Jorissen et al. (2021), however, indicated the utility of COI sequences in barcoding and species delineation within *Cichlidogyrus*, hence these sequence data are a valuable resource for future research on representatives of this genus. Sequences were deposited in GenBank (see Table 1 for accession numbers).

Cophylogenetic analyses

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

Mapping of morphological characters onto the phylogeny of *Cichlidogyrus*

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

Host specificity in *Cichlidogyrus* from Lake Tanganyika

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

RESULTS

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 outside of LT (see Table 1), and from LT lamprologines (*Cichlidogyrus* sp. 3 to *Cichlidogyrus* sp. 10). Maximum Likelihood (Fig. 1) and BI trees showed identical topologies when considering the well-supported nodes. *Cichlidogyrus amphoratus* Pariselle & Euzet, 1996 and *C. sclerosus* Paperna & Thurston, 1969 constituted the sister group of a well-supported LT cluster of *Cichlidogyrus* including three main clades (3, 4 and 5 in Fig. 1). However, two species of *Cichlidogyrus* from LT, *C. mbirizei* and *C. mulimbwai* parasitizing oreochromine and tylochromine cichlids, respectively, belonged to lineages branching off earlier than the clade including species of *Cichlidogyrus* from the East African radiation (Fig. 1). A weakly supported clade 1, formed by species of *Scutogyrus*, plus C. *falcifer* Dossou & Birgi, 1984, *C. longicirrus* Paperna, 1965 and *C. dracolemma* Řehulková, Mendlová & Šimková, 2013 parasitizing West African *Hemichromis* spp., and *C. amieti* from *Aphyosemion cameronense* (Boulenger, 1903), was sister to the group including *Cichlidogyrus* from coptodonine, hemichromine 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* (Weber, 1897) was weakly supported only by BS. The Lake Tanganyika species *C. mbirizei* from *O. tanganicae* was sister to West African *C. cirratus* Paperna, 1964 from *Oreochromis niloticus* (Linnaeus, 1758), and both species together with *C. njinei* Pariselle, Bilong Bilong & Euzet, 2003 from *Sarotherodon galilaeus* (Linnaeus, 1758) formed a moderately supported group (only by BS). With high to low support values (BS = 98, PP = 0.71), clade 2 also included a group of *C. nageus* Řehulková, Mendlová & Šimková, 2013, *C. acerbus* Dossou, 1982, and *C. halli* Price & Kirk, 1967 from *S. galilaeus* and *O. niloticus*, in addition to a well-supported subclade (BS = 91, PP = 1) clustering species of *Cichlidogyrus* restricted to coptodonines, from African *Coptodon guineensis* (Günther, 1862) and *C. rendalli* (Boulenger, 1897) introduced to Madagascar, but including also *C. douellouae* Pariselle, Bilong Bilong & Euzet, 2003 from the oreochromine *S. galilaeus*. Within *Cichlidogyrus* from LT, three clades (3 to 5) were recognized. Clade 3 (BS = 81 and PP = 0.76) represented the earliest diverging lineage, including species of *Cichlidogyrus* from the six LT cichlid tribes Bathybatini, Benthochromini, Boulengerochromini, Cyphotilapiini, Ectodini and Perissodini (Fig. 2). The group of species of *Cichlidogyrus* from *Ophthalmotilapia nasuta* (Poll & Matthes, 1962) (Ectodini), *C. aspiralis*, *C. glacicremoratus* and *C. rectangulus* Rahmouni, Vanhove & Šimková, 2017, was paraphyletic, just like *C. nshomboi* Muterezi Bukinga et al., 2012 (from *Boulengerochromis microlepis* (Boulenger, 1899), Boulengerochromini, plus *Perissodus microlepis* Boulenger, 1898, and *P. straeleni* Poll, 1948, both Perissodini), whereas *C. pseudoaspiralis* and *C. discophonum* Rahmouni, Vanhove & Šimková, 2017 from *Aulonocranus dewindti* (Boulenger, 1899) (Ectodini) formed a monophyletic group (Fig. 1, 2). With high support values (BS = 98 and PP = 0.95), the first subclade within clade 3 (Fig. 1, 2) harboured *C. attenboroughi* Kmentová et al., 2016 from the benthochromine *Benthochromis horii* Takahashi, 2008 as sister species to *C. glacicremoratus* and *C. rectangulus* Rahmouni, Vanhove & Šimková, 2017, both from ectodines (see above). The second subgroup was represented by seven species, *C. discophonum* and *C. pseudoaspiralis* as sister species parasitizing *A. dewindti* (BS = 100 and PP = 1), and a monophyletic group including *C. aspiralis* from the ectodine *O. nasuta*, *C. habluetzeli* Rahmouni, Vanhove & Šimková, 2018 from *Cyphotilapia frontosa* (Boulenger, 1906) and *Cardiopharynx schoutedeni* Poll, 1942, *C. nshomboi*, and *C. casuarinus* Pariselle, Muterezi Bukinga & Vanhove, 2015 parasitizing members of Bathybatini. Clade 4 (BS = 99 and PP = 1) grouped undescribed species parasitizing lamprologines, *C. brunnensis* Kmentová et al., 2016 from *Trematocara unimaculatum* Boulenger, 1901 (Trematocarini), and *C. milangelnari* Rahmouni, Vanhove & Šimková, 2017 from *Cyprichromis microlepidotus* (Poll, 1956) (Cyprichromini) (Fig. 1, 2). Clade 5 (BS = 100 and PP = 1) included mainly two subclades grouping *Cichlidogyrus* from Eretmodini, Haplochromini and Tropheini. Three species of *Cichlidogyrus* from haplochromine hosts including two undescribed ones formed a monophyletic group with high support (BS = 100 and PP = 1), while species from tropheine hosts were paraphyletic, with *C. franswittei* Pariselle & Vanhove, 2015 as sister group to *Cichlidogyrus* from the non-tropheine haplochromines (BS = 86 and PP = 0.97) (Fig. 1, 2). Two monogenean species, *C. jeanloujustinei* Rahmouni, Vanhove & Šimková, 2017 from *Eretmodus marksmithi* Burgess, 2012 and *C. evikae* Rahmouni, Vanhove & Šimková, 2017 from *Tanganicodus irsacae* Poll, 1950, both from Eretmodini, formed a highly supported lineage within species infecting members of Tropheini (BS = 86 and PP = 0.99). Concerning monogenean species of members of Tropheini, species of *Cichlidogyrus* from *Simochromis diagramma* (Günther, 1894) formed a highly supported monophyletic group (BS = 100 and PP = 1), while species of *Cichlidogyrus* from congeneric *Petrochromis* spp. were polyphyletic; *C. antoineparisellei* Rahmouni, Vanhove & Šimková, 2018 from *Interochromis loocki* (Poll, 1949) and *C. masilyai* Rahmouni, Vanhove & Šimková, 2018 from *P. orthognathus* Matthes, 1959 were sister to *Cichlidogyrus* from *S. diagramma*, with a polytomy of species formed by *C. salzburgeri* Rahmouni, Vanhove & Šimková, 2018 from *P. trewavasae* Poll, 1948 and *C. irenae* Gillardin et al., 2012 from *'Gnathochromis' pfefferi* (Boulenger, 1898) (Fig. 1, 2).

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 (clade 3 in Fig. 1), just like *Cichlidogyrus* from Lamprologini, Cyprichromini and Trematocarini (clade 4 in Fig. 1), and monogenean species from members of Haplochromini, Eretmodini and Tropheini (clade 5 in Fig. 1). *Cichlidogyrus* from the latter tribe represents the most derived lineage in LT.

Cophylogenetic analyses of cichlids and *Cichlidogyrus* in Lake Tanganyika

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

Mapping of morphological characters onto the phylogeny of *Cichlidogyrus*

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

Host specificity in Lake Tanganyika *Cichlidogyrus*

The host range of species of *Cichlidogyrus* from LT varied from a single host species to five host species (*Cichlidogyrus casuarinus* parasitizing 7 cichlid species of Bathybatini (Supplementary Table S1)). Strict 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 the true generalists *C. nshomboi* and *C. hablueztzeli*, and in the intermediate generalist *C. casuarinus*. These species possess long hook pairs I-VII, except for pair V which retained its larval size, and a sclerotized vagina (Fig. 4A, B).

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.

Phylogeny of *Cichlidogyrus*

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

Phylogenetic position of *Cichlidogyrus* parasitizing Lake Tanganyika cichlids

In the present phylogenetic study, 35 species of *Cichlidogyrus* parasitizing 32 LT cichlids belonging to 14 endemic tribes of a total of 16 (Ronco et al., 2020) were included. This includes some species, such as *Cichlidogyrus* sp. 1 and *Cichlidogyrus* sp. 2, sampled also from haplochromines inhabiting other East African freshwater habitats. Our study provides the first molecular evidence for a polyphyletic assemblage of *Cichlidogyrus* in LT, as *C. mulimbwai* infecting *T. polylepis* and *C. mbirizei* from *O. tanganicae*, both from LT, were sister to *C. pouyaudi* from West African *Tylochromis* species, and a few species from oreochromine hosts, respectively, both quite divergent from the rest of the LT species of *Cichlidogyrus* (Fig. 1). The centre of diversity 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 recently (Klett and Meyer, 2002; Koch et al., 2007). Three well supported clades of *Cichlidogyrus* (3 to 5) parasitizing distinct cichlid tribes from LT were found. Clades 3 and 4 appear to be restricted to species of *Cichlidogyrus* parasitizing representatives of cichlid tribes occupying particular ecological niches – semi-pelagic (Cyprichromini and some members of Ectodini and Lamprologini), and deep and benthopelagic habitats (Bathybatini, Benthochromini, Boulengerochromini, Cyphotilapiini, a few species of Perissodini, and Trematocarini) (Konings, 2019). Clade 5 is restricted to species of *Cichlidogyrus* from Eretmodini, Haplochromini (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 across tribes.

Speciation and diversification of *Cichlidogyrus* on Lake Tanganyika cichlids

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

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

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

Structural evolution and host specificity in *Cichlidogyrus*

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

The different levels of host specificity of species of *Cichlidogyrus* occurring in West African cichlids and the high species richness on some cichlid species provided the motivation to investigate the processes that have contributed to diversification in *Cichlidogyrus* (Pouyaud et al., 2006; Mendlová and Šimková, 2014). A narrow host specificity (strict specialism) was previously attributed to LT species of *Cichlidogyrus*, whilst generalist species were rare, with only *C. casuarinus* reported as an intermediate generalist (Kmentová et al., 2016b). In our study, host specificity was evaluated for 35 species of *Cichlidogyrus* parasitizing LT cichlids. The strict specialist lifestyle seems to constitute the ancestral state of host specificity in *Cichlidogyrus* from LT cichlid hosts, and changes towards lower host specificity were rare, recorded in few species of the clades 3 and 5 only (Muterezi Bukinga et al. 2012; Kmentová et al. 2016b; Rahmouni et al. 2018; Rahmouni et al. unpublished). This corresponds to findings for West African systems (Vignon et al. 2011; Mendlová and Šimková, 2014). The degree of host specificity at global scale was shown, indeed, to differ from that at local levels in *Dactylogyrus* Diesing, 1850 parasitizing European cyprinids (Šimková et al., 2006), and *Cichlidogyrus* parasitizing West African cichlids. Such differences could potentially be explained by isolation of monogenean populations (Mendlová and Šimková, 2014). Reduced host specificity reported by Kmentová et al. (2016b) in deep-water monogeneans of LT Bathybatini seems to also occur in *C. nshomboi* and *C. habluetzeli* parasitizing benthopelagic hosts – *B. microlepis* occurring down to the limit of the oxygenated layer (~ 50–200 m), while *C. frontosa* and *C. schoutedeni* are commonly found in coastal water along rocks, and in shallow sandy habitats, respectively (~ 30–50 m) (Konings, 2019). In marine *Lamellodiscus* Johnston & Tiegs, 1922 (Desdevises et al., 2002) and freshwater *Dactylogyrus* (Šimková et al., 2006), strict specialism represents the ancestral state of host specificity. This may indicate that specificity is not an evolutionary ‘dead-end’ as previously suggested by Simpson (1953).

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

Conclusion

The application of molecular phylogenetics provided the first data on the phylogenetic position of LT 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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Author contributions

CR, MPMV and AŠ designed the study. AŠ and MPMV supervised the work. MPMV and SK co-organised the fieldtrips, parasite collection, preparation of specimens and provided scientific background in the field. SK identified the host specimens and provided scientific background on the system and the analyses. CR performed the lab work and data analysis, CR, MPMV, SK and AŠ wrote and corrected the manuscript. All authors read and approved the final manuscript.

Ethics declarations

Conflict of interest

The authors declare no conflict of interest.

Data availability

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

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Figure captions

Fig. 1 Maximum Likelihood (ML) phylogram of species of *Cichlidogyrus* and *Scutogyrus* parasitizing Lake Tanganyika cichlid hosts and lineages from the rest of Africa based on analysis of the 18S, ITS1 and 28S rDNA sequences. Species of *Cichlidogyrus* from tylochromine hosts were selected to root the tree following Mendlová 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 *Cichlidogyrus* lineages (including *Scutogyrus*). Clades in black cluster West African cichlid linages of *Cichlidogyrus*, while those in red are for species parasitizing LT hosts. Branch lengths correspond to the expected number of substitutions per site.

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

Fig. 3 (A) Tanglegram of phylogenies of Lake Tanganyika cichlid hosts (left) and associated *species of Cichlidogyrus* (right) obtained using ML tree inference for species of *Cichlidogyrus* and the cichlid tree topology from Ronco et al. (2021). Green lines indicate statistically significant host-parasite links ($p < .05$) and red lines indicate non-statistically significant links. (B) One of the possible cophylogenetic scenarios between cichlid hosts and their specific *Cichlidogyrus* obtained using Jane software, with the lowest cost produced by the host switch-adjusted TreeFitter model (6 cospeciation events (hollow coloured circle), 12 duplications (solid coloured circle), 17 duplications followed by host switch (duplication with an arrow following the trajectory of the switching species), nine failures to diverge (jagged line) and 1 loss event (dashed line)). Black branches represent the cichlid phylogeny and blue branches represent the phylogeny of *Cichlidogyrus*.

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 A-D are represented by hook pairs of *C. milangelnari, C. aspiralis, C. rectangulus* and *C. nshomboi.* The represented vagina is that exhibited by *C. salzburgeri.*

Supplementary Table S1 List of species of *Cichlidogyrus* from Lake Tanganyika investigated in this study with their cichlid hosts classified by tribes, host range, 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 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) 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-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 group D for species with long hook pairs, except larval-shaped pair V.

***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	1	Strict specialist	No	Group A

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

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 18S to ITS1 rDNA, 28S rDNA and COI mtDNA for the parasites obtained in the present study (see material and methods).

Cichlid Tribe	Cichlid host species	n	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	*C. nshomboi*_1 Muterezi Bukinga, Vanhove, Van Steenberge and Pariselle, 2012	OL675317	OL675266	OL704757
Cyphotilapiini Salzburger, Meyer, Baric, Verheyen and Sturmbauer, 2002	*Cyphotilapia frontosa* (Boulenger, 1906)	2	07/08/2016	Makabola village	DRC	3°32′S, 29°9′E	*C. adkoningsi* Rahmouni, Vanhove and Šimková, 2018	-	-	-
							C. hablueetzeli Rahmouni, Vanhove and Šimková, 2018	OL675312	OL675271	OL704742
Cyprichromini Poll, 1986	*Cyprichromis microlepidotus* (Poll, 1956)	3	23/09/2013	Nyaruhongoka	Burundi	3°41′S, 29°20′E	*C. milangelnari* Rahmouni, Vanhove and Šimková, 2017	OL675315	OL675263	OL704754
Ectodini Poll, 1986	*Ophthalmotilapia nasuta* (Poll and Matthes, 1962)	4	07-09/09/2013	Magara	Burundi	3°44′S, 29°19′E	*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)	3	04-07/09/2013	Nyaruhongoka	Burundi	3°41′S, 29°20′E	*C. discophonum* Rahmouni, Vanhove and Šimková, 2017	OL675307	OL675257	OL704745
							C. pseudoaspiralis Rahmouni, Vanhove and Šimková, 2017	OL675319	OL675268	-
	Cardiopharynx schoutedeni Poll, 1942	6	07/08/2016	Mulongwe fish market	DRC	3°22′S, 29°6′E	*C. koblmuelleri* Rahmouni, Vanhove and Šimková, 2018	-	-	-
							C. hablueetzeli Rahmouni, Vanhove and Šimková, 2018	OL675333	OL675286	-
Eretmodini Poll, 1986	*Eretmodus marksmithi* 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 Poll, 1950	7	20/09/2013	Mukuruka	Burundi	4°14′S, 29°33′E	*C. evikae* Rahmouni, Vanhove and Šimková, 2017	OL675308	OL675258	OL704752
Haplochromini Trewavas, 1983	*Astatotilapia burtoni* (Günther, 1893)	9	25/09/2013	Lake Cohoha	Burundi	-	*Cichlidogyrus* sp. 1 (undescribed)	OL675322	OL675275	-
		1	25/09/2013	Bujumbura fish market	Burundi	3°23'S, 29°22'E	*C. gillardinae* Muterezi Bukinga, Vanhove, Van Steenberge and Pariselle, 2012	OL675309	OL675261	OL704748
		2	09/09/2013	Mulongwe fish market	DRC	3°22′S, 29°6′E				
		11	16/09/2013	Kilomoni beach	DRC					
			07/08/2016	Chitili	Zambia	8°36′S, 31°11′E	*Cichlidogyrus* sp. 2 (undescribed)	OL675324	OL675276	-
				Kalambo Falls Rivers	Zambia	8°38′S, 31°11′E				
	Astatotilapia stappersii Poll, 1943	1	16/09/2013	Kilomoni beach	DRC	3°20′S, 29°10′E	*C. gillardinae*	OL675334	OL675288	OL753688
Lamprologini Poll, 1986	*Lamprologus callipterus* Boulenger, 1906	6	06-10/09/2013	Nyaruhongoka	Burundi	3°41′S, 29°20′E	*Cichlidogyrus* sp. 3 (undescribed)	OL675325	OL675284	-

		2	04/09/2013	Magara	Burundi	3°44′S, 29°19′E	*Cichlidogyrus* sp. 4 (undescribed)	OL675326	OL675277	-
		6	17-19/09/2013	Mukuruka	Burundi	4°14′S, 29°33′E	*Cichlidogyrus* sp. 5 (undescribed)	OL675327	OL675278	-
		4	20/09/2013	Mvugo	Burundi	4°15'S, 29°34'E	*Cichlidogyrus* sp. 6 (undescribed)	OL675328	OL675279	-
					Burundi		*Cichlidogyrus* sp. 7 (undescribed)	OL675329	OL675280	-
					Burundi		*Cichlidogyrus* sp. 8 (undescribed)	OL675330	OL675281	-
	Neolamprologus fasciatus (Boulenger, 1898)	3	23/08/2011	Wonzye Point	Zambia	8°43'S, 31°08'E	*Cichlidogyrus* sp. 9 (undescribed)	OL675331	OL675282	-
		-	18/04/2008	Kalambo Lodge	Zambia	8°37’S, 31°12'E	*Cichlidogyrus* sp. 10 (undescribed)	OL675323	OL675283	-
Perissodini Poll, 1986	*Perissodus microlepis* Boulenger, 1898	3	09/09/2013	Magara	Burundi	3°44′S, 29°19′E	*C. nshomboi*_2 Muterezi Bukinga, Vanhove, Van Steenberge and Pariselle, 2012	OL675318	OL675267	OL704756
		5	09/09/2013	Nyaruhongoka	Burundi	3°41′S, 29°20′E				
		2	08/08/2016	Pemba	DRC	3°37′S, 29°9′E				
	Perissodus straeleni Poll, 1948	7	14/05/2010	Mukumba	DRC	6°56′S, 29°42′E	*C. nshomboi*_2	OL675332	OL675285	-
		2	15/04/2010	Mtoto	DRC	6°58′S, 29°43′E				
		1	-	Mtosi	Zambia	7°35′S, 30°38′E				
	Haplotaxodon microlepis Boulenger, 1906	3	1957	Luhanga	DRC	3°31′S, 29°08′E	*C. nshomboi*_2	-	-	-
			24/04/2010	Murega	DRC	5°38′S, 29°23′E				
			1997	Makumba	DRC	6°56′S, 29°42′E				
Tropheini Poll, 1986	‘*Ctenochromis*’ *horei* Günther, 1893	7	6-7/09/2013	Nyaruhongoka	Burundi	3°41′S, 29°20′E	*C. gistelincki* Gillardin, Vanhove, Pariselle, Huyse and Volckaert, 2011	OL675310	OL675260	OL704759
		1	17/09/2013	Mukuruka	Burundi	4°14′S, 29°33′E				
		1	26/09/2013	Bujumbura fish market	Burundi	3°23'S, 29°22'E				
		4	06/09/2013	Magara	Burundi	3°44′S, 29°19′E				
		5	25/09/2013	Mvugo	Burundi	4°15'S, 29°34'E				
	Interochromis loocki (Poll, 1949)	9	08/08/2016	Pemba	DRC	3°37′S, 29°9′E	*C. antoineparisellei* Rahmouni, Vanhove and Šimková, 2018	OL675304	OL675272	-
	Petrochromis orthognathus Matthes, 1959	4	08/08/2016	Pemba	DRC	3°37′S, 29°9′E	*C. masilyai* Rahmouni, Vanhove and Šimková, 2018	OL675314	OL675273	OL704749
	Petrochromis trewavasae Poll, 1948	1	08/08/2016	Pemba	DRC	3°37′S, 29°9′E	*C. salzburgeri* Rahmouni, Vanhove and Šimková, 2018	-	OL675274	-
	Pseudosimochromis curvifrons Poll, 1942	1	08/08/2016	Pemba	DRC	3°37′S, 29°9′E	*C. franswittei* Pariselle and Vanhove, 2015	-	OL675259	-
							C. frankwillemsi Pariselle and Vanhove, 2015	-	-	OL704750
	Simochromis diagramma (Günther, 1893)	10	05/09/2013	Magara	Burundi	3°44′S, 29°19′E	*C. banyankimbonai* Pariselle and Vanhove, 2015	OL675306	OL675255	OL704760
		10	05-06/09/2013	Nyaruhongoka	Burundi	3°41′S, 29°20′E	*C. muterezii* Pariselle and Vanhove, 2015	OL675316	OL675265	OL704758

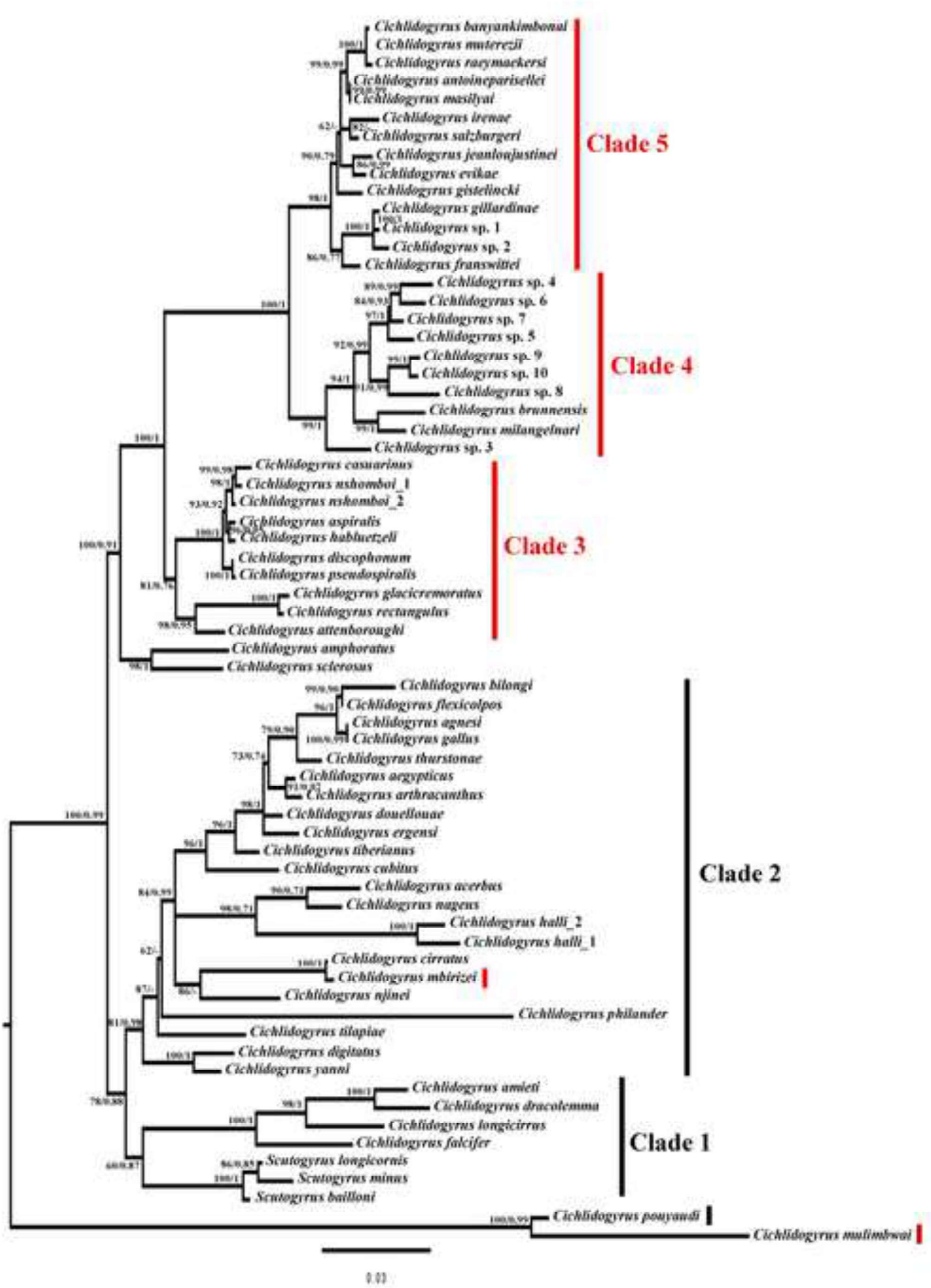
							C. raeymaekersi Pariselle and Vanhove, 2015	OL675320	OL675269	OL704755
	Pseudosimochromis marginatus (Poll, 1956)	1	08/08/2016	Pemba		3°37′S, 29°9′E	*C. franswittei* Pariselle and Vanhove, 2015	-	OL675287	-
Tylochromini Poll, 1986	*Tylochromis polylepis* (Boulenger, 1900)	1	09/09/2013	Mulongwe fish market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

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 sequence~~s~~ data.

***Cichlidogyrus* spp.**	**Cichlid host species**	**Cichlid lineage**	**18S rDNA-ITS1**	**28S rDNA**
Cichlidogyrus acerbus Dossou, 1982	*Sarotherodon galilaeus* (Linnaeus, 1758)	Oreochromini Dunz and Schliewen, 2013	HE792780[1]	HQ010036[2]
Cichlidogyrus aegypticus Ergens, 1981	*Coptodon guineensis* (Günther, 1862)	Coptodonini Dunz and Schliewen, 2013	HE792781[1]	HQ010021[2]
Cichlidogyrus agnesi Pariselle & Euzet, 1995	*Coptodon guineensis* (Günther, 1862)	Coptodonini	AJ920286[3]	-
Cichlidogyrus amieti Birgi & Euzet, 1983	*Aphyosemion cameronense* (Boulenger, 1903)	Non-cichlid	-	KT945076[4]
Cichlidogyrus amphoratus Pariselle & Euzet, 1996	*Coptodon guineensis* (Günther, 1862)	Coptodonini	HE792782[1]	HE792772[1]
Cichlidogyrus arthracanthus Paperna, 1960	*Coptodon guineensis* (Günther, 1862)	Coptodonini	HE792783[1]	HQ010022[2]
Cichlidogyrus attenboroughi Kmentová et al., 2016	*Benthochromis horii* Takahashi, 2008	Benthochromini Takahashi, 2003	MH708153[5]	MH708146[5]
Cichlidogyrus bilongi Pariselle & Euzet, 1995	*Coptodon guineensis* (Günther, 1862)	Coptodonini	AJ920287[3]	-
Cichlidogyrus brunnensis Kmentová et al., 2016	*Trematocara unimaculatum* Boulenger, 1901	Trematocarini Poll, 1986	MH708152[5]	MH708144[5]
Cichlidogyrus casuarinus Pariselle, Muterezi Bukinga & Vanhove, 2015	*Bathybates minor* Boulenger, 1906	Bathybatini Poll, 1986	KX007795[6]	KX007822[6]
Cichlidogyrus cirratus Paperna, 1964	*Oreochromis niloticus* (Linnaeus, 1758)	Oreochromini	HE792784[1]	HE792773[1]
Cichlidogyrus cubitus Dossou, 1982	*Coptodon guineensis* (Günther, 1862)	Coptodonini	HE792785[1]	HQ010037[2]
Cichlidogyrus digitatus Dossou, 1982	*Coptodon guineensis* (Günther, 1862)	Coptodonini	HE792786[1]	HQ010023[2]
Cichlidogyrus douellouae Pariselle, Bilong Bilong & Euzet, 2003	*Sarotherodon galilaeus* (Linnaeus, 1758)	Oreochromini	HE792787[1]	HE792774[1]
Cichlidogyrus dracolemma Řehulková, Mendlová & Šimková, 2013	*Hemichromis letourneuxi* Sauvage, 1880	Hemichromini Dunz and Schliewen, 2013	HE792794[1]	HQ010027[2]
Cichlidogyrus ergensi Dossou, 1982	*Coptodon guineensis* (Günther, 1862)	Coptodonini	HE792788[1]	HQ010038[2]
Cichlidogyrus falcifer Dossou & Birgi, 1984	*Hemichromis fasciatus* Peters, 1857	Hemichromini	HE792789[1]	HQ010024[2]
Cichlidogyrus philander Douëllou, 1993	*Pseudocrenilabrus philander* (Weber, 1897)	Haplochromini	MG250207*	MG279698*
Cichlidogyrus flexicolpos Pariselle & Euzet, 1995	*Coptodon guineensis* (Günther, 1862)	Coptodonini	AJ920283[3]	-
Cichlidogyrus gallus Pariselle & Euzet, 1995	*Coptodon guineensis* (Günther, 1862)	Coptodonini	AJ920285[3]	-
Cichlidogyrus halli 1 (Price & Kirk, 1967)	*Sarotherodon galilaeus* (Linnaeus, 1758)	Oreochromini	HE792790[1]	HQ010025[2]
Cichlidogyrus halli 2 (Price & Kirk, 1967)	*Oreochromis niloticus* (Linnaeus, 1758)	Oreochromini	AJ920272[3]	-
Cichlidogyrus irenae Gillardin et al., 2012	'*Gnathochromis*' *pfefferi* (Boulenger, 1898)	Tropheini Poll, 1986	KT692939[7]	MH708145[7]
Cichlidogyrus longicirrus	*Hemichromis fasciatus*	Hemichromini	HE792791[1]	HQ010026[2]

Paperna, 1965	Peters, 1857			
Cichlidogyrus mbirizei Muterezi Bukinga et al., 2012	*Oreochromis tanganicae* (Günther, 1894)	Oreochromini	MG030376*	MG030378*
Cichlidogyrus nageus Řehulková, Mendlová & Šimková, 2013	*Sarotherodon galilaeus* (Linnaeus, 1758)	Oreochromini	HE792795[1]	HQ010028[2]
Cichlidogyrus njinei Pariselle, Bilong Bilong & Euzet, 2003	*Sarotherodon galilaeus* (Linnaeus, 1758)	Oreochromini	HE792792[1]	HE792775[1]
Cichlidogyrus pouyaudi Pariselle & Euzet, 1994	*Tylochromis intermedius* (Boulenger, 1916)	Tylochromini Poll, 1986	HE792793[1]	HQ010039[2]
Cichlidogyrus sclerosus Paperna & Thurston, 1969	*Oreochromis niloticus* (Linnaeus, 1758)	Oreochromini	DQ537359[8]	DQ157660[8]
Cichlidogyrus thurstonae Ergens, 1981	*Coptodon rendalli* (Boulenger, 1897)	Coptodonini	AJ920274[3]	MH767406[9]
Cichlidogyrus tiberianus Paperna, 1960	*Coptodon rendalli* (Boulenger, 1897)	Coptodonini	MH767404[9]	HE792796[1]
Cichlidogyrus tilapiae Paperna, 1960	*Hemichromis fasciatus* Peters, 1857	Hemichromini	HE792797[1]	HQ010029[2]
Cichlidogyrus yanni Pariselle & Euzet, 1996	*Coptodon guineensis* (Günther, 1862)	Coptodonini	HE792798[1]	HE792777[1]
Scutogyrus bailloni Pariselle & Euzet, 1995	*Sarotherodon galilaeus* (Linnaeus, 1758)	Oreochromini	HE792799[1]	HE792778[1]
Scutogyrus longicornis (Paperna & Thurston, 1969)	*Oreochromis niloticus* (Linnaeus, 1758)	Oreochromini	HE792800[1]	HQ010035[2]
Scutogyrus minus (Dossou, 1982)	*Sarotherodon melanotheron* Rüppell, 1852	Oreochromini	HE792801[1]	HE792779[1]

References: [1]Mendlová et al. (2012); [2]Mendlová et al. (2010); [3]Pouyaud et al. (2006), [4]Messu Mandeng et al. (2015); [5]Kmentová et al. (2018); [6]Kmentová et al. (2016a); [7]Kmentová et al. (2016b); [8]Wu et al. (2007); [9]Šimková et al. (2019) and . No references are available for sequences indicated by "*".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99/0.99
Cichlidogyrus masilyai
62/-
Cichlidogyrus irenae
82/-
Cichlidogyrus salzburgeri
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Cichlidogyrus jeanloujustinei
86/0.99
Cichlidogyrus evikae
Cichlidogyrus gistelincki
98/1
Cichlidogyrus gillardinae
100/1
100/1
Cichlidogyrus sp. 1
Cichlidogyrus sp. 2
86/0.77
Cichlidogyrus franswittei
Clade 5
100/1
89/0.99
Cichlidogyrus sp. 4
84/0.93
Cichlidogyrus sp. 6
97/1
Cichlidogyrus sp. 7
Cichlidogyrus sp. 5
92/0.99
99/1
Cichlidogyrus sp. 9
Cichlidogyrus sp. 10
94/1
91/0.99
Cichlidogyrus sp. 8
Clade 4
Cichlidogyrus brunnensis
99/1
99/1
Cichlidogyrus milangelnari
Cichlidogyrus sp. 3
100/1
99/0.98
Cichlidogyrus casuarinus
98/1
Cichlidogyrus nshomboi_1
93/0.92
Cichlidogyrus nshomboi_2
Cichlidogyrus aspiralis
100/1
96/0.95
Cichlidogyrus habluetzeli
100/0.91
Cichlidogyrus discophonum
100/1
Cichlidogyrus pseudospiralis
Clade 3
81/0.76
100/1
Cichlidogyrus glacicremoratus
Cichlidogyrus rectangulus
98/0.95
Cichlidogyrus attenboroughi
Cichlidogyrus amphoratus
98/1
Cichlidogyrus sclerosus
99/0.98
Cichlidogyrus bilongi
96/1
Cichlidogyrus flexicolpos
79/0.98
Cichlidogyrus agnesi
100/0.99
Cichlidogyrus gallus
73/0.74
Cichlidogyrus thurstonae
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91/0.82
Cichlidogyrus arthracanthus
98/1
Cichlidogyrus douellouae
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79/1
Cichlidogyrus ergensi
96/1
Cichlidogyrus tiberianus
Cichlidogyrus cubitus
Clade 2
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98/0.71
Cichlidogyrus nageus
100/1
Cichlidogyrus halli_2
Cichlidogyrus halli_1
62/-
100/1
Cichlidogyrus cirratus
Cichlidogyrus mbirizei
87/-
86/-
Cichlidogyrus njinei
Cichlidogyrus philander
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Cichlidogyrus tilapiae
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Cichlidogyrus digitatus
Cichlidogyrus yanni
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98/1
Cichlidogyrus dracolemma
100/1
Cichlidogyrus longicirrus
Cichlidogyrus falcifer
Clade 1
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86/0.85
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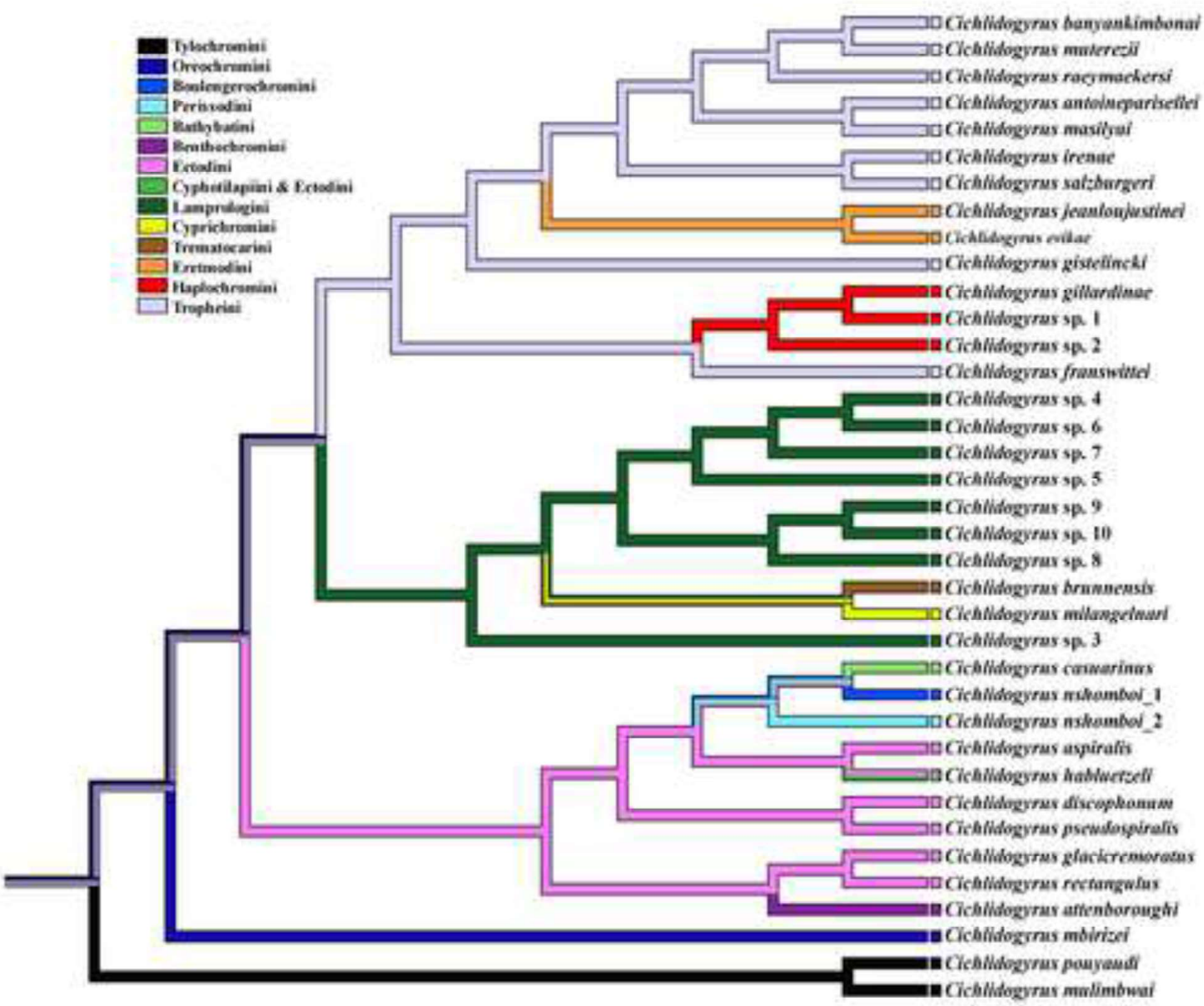
100/0.99
Cichlidogyrus pouyaudi
Cichlidogyrus mulimbwai
0.03

Figure2


Tylochromini
Oreochromini
Boulengerochromini
Perissodini
Bathybatini
Benthochromini
Ectodini
Cyphotilapiini & Ectodini
Lamprologini
Cyprichromini
Trematocarini
Eretmodini
Haplochromini
Tropheini
Cichlidogyrus banyankimbonai
Cichlidogyrus muterezii
Cichlidogyrus raeymaekersi
Cichlidogyrus antoineparisellei
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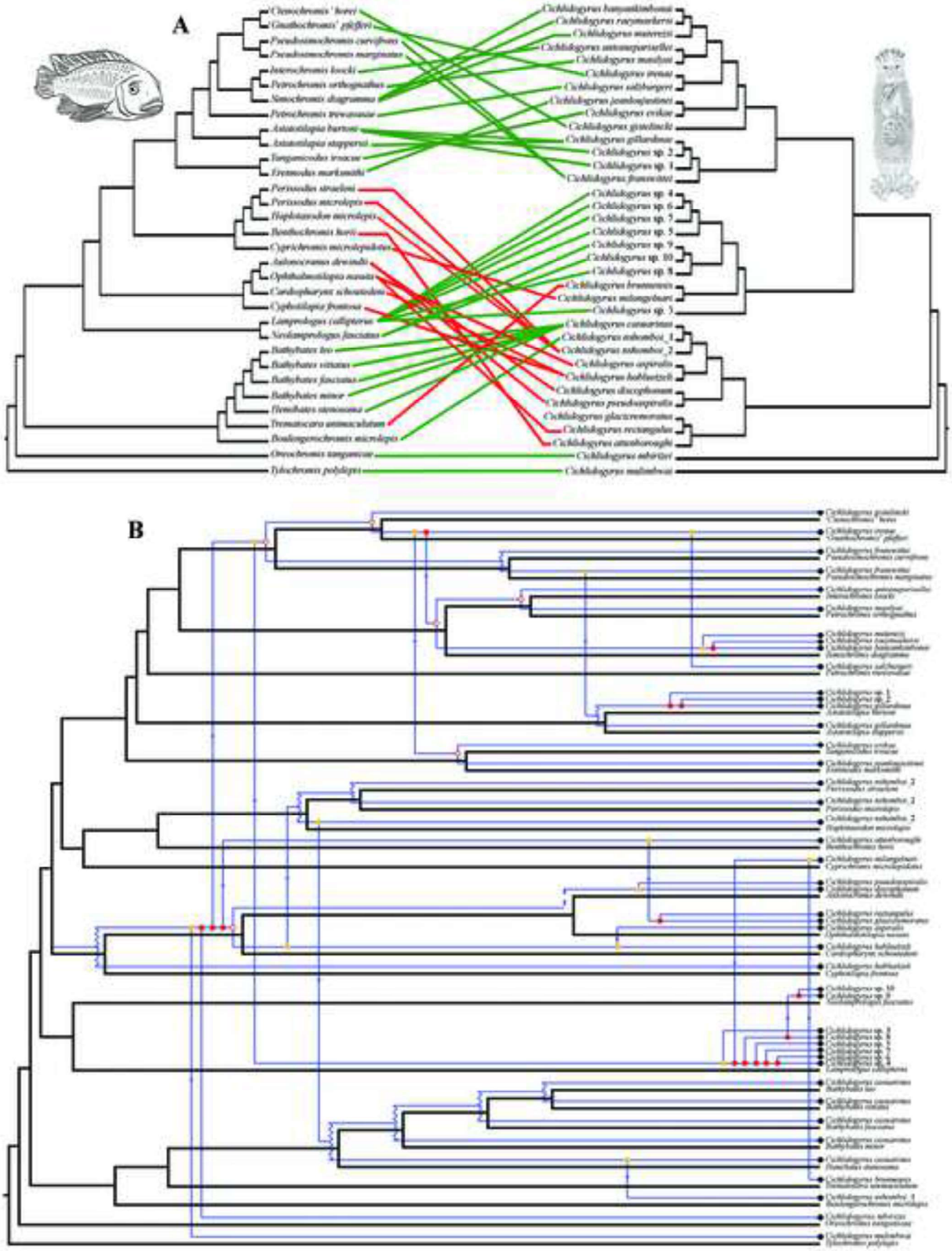
Cichlidogyrus irenae
Cichlidogyrus salzburgeri
Cichlidogyrus jeanloujustinei
Cichlidogyrus evikae
Cichlidogyrus gistelincki
Cichlidogyrus gillardinae
Cichlidogyrus sp. 1
Cichlidogyrus sp. 2
Cichlidogyrus franswittei
Cichlidogyrus sp. 4
Cichlidogyrus sp. 6
Cichlidogyrus sp. 7
Cichlidogyrus sp. 5
Cichlidogyrus sp. 9
Cichlidogyrus sp. 10
Cichlidogyrus sp. 8
Cichlidogyrus brunnensis
Cichlidogyrus milangelnari
Cichlidogyrus sp. 3
Cichlidogyrus casuarinus
Cichlidogyrus nshomboi_1
Cichlidogyrus nshomboi_2
Cichlidogyrus aspiralis
Cichlidogyrus habluetzeli
Cichlidogyrus discophonum
Cichlidogyrus pseudospiralis
Cichlidogyrus glacicremoratus
Cichlidogyrus rectangulus
Cichlidogyrus attenboroughi
Cichlidogyrus mbirizei
Cichlidogyrus pouyaudi
Cichlidogyrus mulimbwai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'Gnathochromis' pfefferi
Pseudosimochromis curvifrons
Pseudosimochromis marginatus
Interochromis loocki
Petrochromis orthognathus
Simochromis diagramma
Petrochromis trewavasae
Astatotilapia burtoni
Astatotilapia stappersi
Tanganicodus irsacae
Eretmodus marksmithi
Perissodus straeleni
Perissodus microlepis
Haplotaxodon microlepis
Benthochromis horii
Cyprichromis microlepidotus
Aulonocranus dewindti
Ophthalmotilapia nasuta
Cardiopharynx schoutedeni
Cyphotilapia frontosa
Lamprologus callipterus
Neolamprologus fasciatus
Bathybates leo
Bathybates vittatus
Bathybates fasciatus
Bathybates minor
Hemibates stenosoma
Trematocara unimaculatum
Boulengerochromis microlepis
Oreochromis tanganicae
Tylochromis polylepis
Cichlidogyrus banyankimbonai
Cichlidogyrus raeymaekersi
Cichlidogyrus muterezii
Cichlidogyrus antoinepariselle
Cichlidogyrus makasai
Cichlidogyrus irenae
Cichlidogyrus salzburgeri
Cichlidogyrus pseudozambezensis
Cichlidogyrus evikae
Cichlidogyrus gistelincki
Cichlidogyrus gillardinae
Cichlidogyrus sp. 2
Cichlidogyrus sp. 1
Cichlidogyrus franswittei
Cichlidogyrus sp. 4
Cichlidogyrus sp. 6
Cichlidogyrus sp. 7
Cichlidogyrus sp. 5
Cichlidogyrus sp. 9
Cichlidogyrus sp. 10
Cichlidogyrus sp. 8
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Cichlidogyrus milangelnari
Cichlidogyrus sp. 3
Cichlidogyrus casuarinus
Cichlidogyrus nshomboi_1
Cichlidogyrus nshomboi_2
Cichlidogyrus aspiralis
Cichlidogyrus habluetzeli
Cichlidogyrus discophonum
Cichlidogyrus pseudoaspiralis
Cichlidogyrus glacicremoratus
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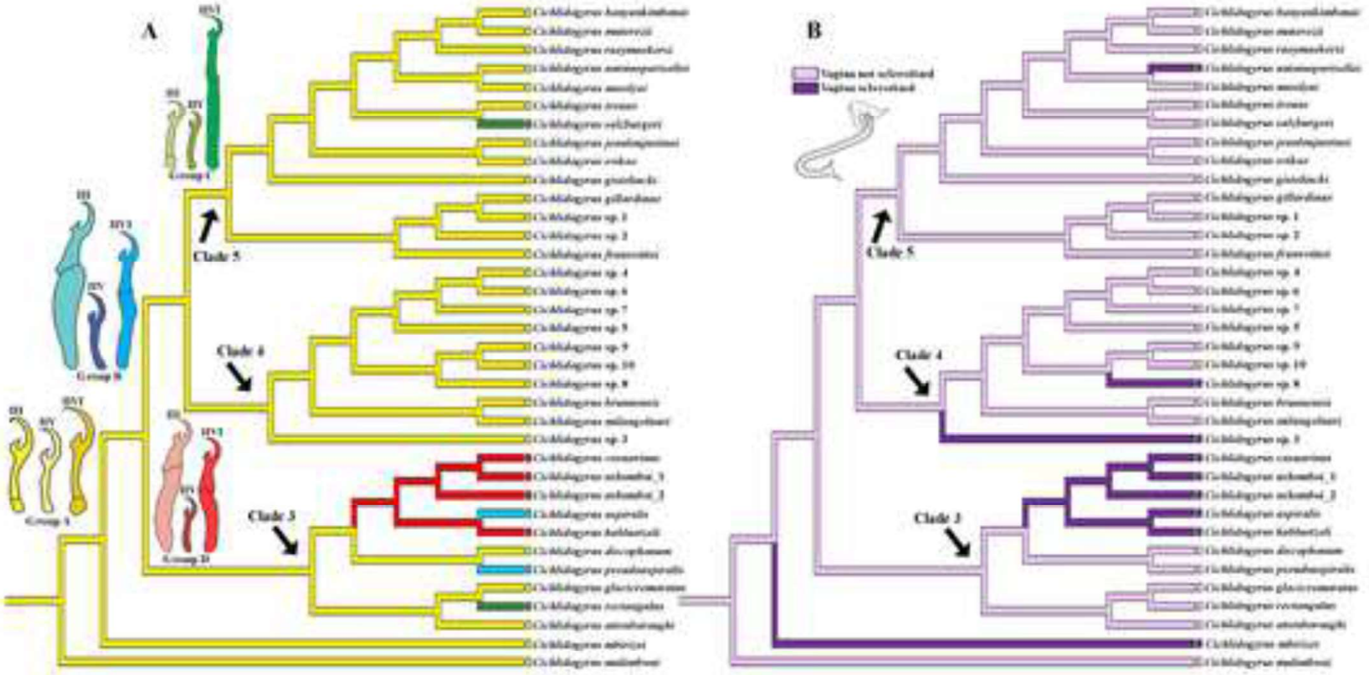
Cichlidogyrus attenboroughi
Cichlidogyrus mbirizei
Cichlidogyrus mulimbwai
B

Figure4


A
Clade 5
Clade 4
Clade 3
B
Vagina not sclerotised
Vagina sclerotised
Clade 5
Clade 4
Clade 3