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# Morphological and genetic divergence in a gill monogenean parasitizing distant cichlid lineages of Lake Tanganyika: *Cichlidogyrus nshomboi* (Monogenea: Dactylogyridae) from representatives of Boulengerochromini and Perissodini

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## ABSTRACT

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



## KEYWORDS

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

## Introduction

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

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

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

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

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

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

A recent field survey revealed the presence of specimens of *Cichlidogyrus* that could be identified as *C. nshomboi* based on morphological criteria on all examined cichlid species belonging to Perissodini. This was unexpected as Perissodini and Boulengerochromini are only distantly related: *B. microlepis* represents the earliest diverging lineage of the East African radiation, in which Perissodini are deeply nested (Ronco et al. 2021). Additionally, members of these tribes also have profoundly different ecologies. *Boulengerochromis microlepis* is a substrate-brooder that ranks amongst the world's largest cichlids. It is a highly mobile top-predator that occupies the lake's bentho-pelagic habitat ( Meyer et al. 2015; Konings 2019). Perissodini contains nine species that are all mouth-brooders, but that belong to two groups with different ecologies (Koblmüller et al. 2007, Takahashi et al. 2007). One of these contains the two species of *Haplotaxodon* Boulenger, 1906: *Haplotaxodon microlepis* Boulenger, 1906 and *Haplotaxodon trifasciatus* Takahashi & Nakaya, 1999 (considered synonyms by Konings (2019)). These are open-water predators that feed on juveniles of pelagic fishes and on zooplankton. The second group consists of six representatives of *Perissodus* Boulenger, 1898 and of *Xenochromis hecqui* Boulenger, 1899. These species have a lepidophagous, or scale-eating, diet, a spectacular feeding mode that is not found in other Lake Tanganyika tribes. It should be noted, however, that the generic position of *Xenochromis hecqui* Boulenger, 1899 is debated and that it has, at least, a less strict lepidophagous diet than the other members of the group

(Takahashi et al. 2007, Konings 2019). Most members of this scale-eating group occur in deeper waters, and little is known about their ecology and behaviour. *Perissodus microlepis* Boulenger, 1898 and, to a lesser degree, *Perissodus straeleni* (Poll, 1948), however, are found in shallower waters (Konings 2019). These sister species are relatively recent offshoots of the perissodine radiation. The two species differ in hunting technique with *P. microlepis* being a highly mobile scale-eating cichlid, and *P. straeleni* behaving more as an ambush predator (Yanagisawa et al. 1990).

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

We hypothesize herein that a) a single species of *Cichlidogyrus* infects boulengerochromine and perissodine cichlids. Additionally, we hypothesize that b) the genetic and morphological differentiation between monogeneans mirror the phylogenetic and ecological distinctness of the hosts, and c) that larger differences are

found between monogeneans infecting heterospecific host populations than between monogeneans infecting conspecific host populations.

## Materials and Methods

### Study area and cichlid hosts

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

### Monogenean isolation and identification

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

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

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

**Genetic characterization**

We sequenced and amplified three nuclear and one mitochondrial marker for the examined monogeneans, following Kmentová et al. (2016). The domains C1-D2 of the partial 28S rDNA region were sequenced using universal primers (Hassouna et al. 1984). As this marker is highly conservative and commonly used for species recognition in flatworms (Vanhove et al. 2013, Rahmouni et al., 2020), we used it to confirm the conspecificity of our specimens. We further obtained sequences of the partial 18S, the entire ITS1 rDNA using the primers S1-IR8 (Šimková et al. 2003), as well as the partial mitochondrial COI gene for a selection of samples using ASmit1 and Schisto3 (Littlewood et al. 1997), with ASmit2 as internal primers for the nested PCR (Plaisance et al. 2008). Sequence data was checked manually for sequencing errors, edited using Sequencher® v.5.0 (Gene Codes Corporation, Ann Arbor, MI USA), and aligned using ClustalW (Thompson et al. 1994) implemented in MEGA X (Kumar et al. 2018). The sequence fragments from the nuclear genes were subjected to NCBI BLAST (Altschul et al. 1990) in order to compare them with all previously submitted sequences of *Cichlidogyrus*. The CLC Sequence Viewer v. 8.0 (CLC Bio-Qiagen, 2016) was used to visualize the nucleotide substitutions in the nuclear

and the mitochondrial alignments. Nucleotide sequences were deposited in GenBank under accession numbers: (available after acceptance). Uncorrected *p*-distances were calculated between specimens and groups using MEGA X. For the sequences of COI, DnaSP v. 5.10.01 (Librado and Rozas 2009) was used to study genetic diversity by calculating the number of polymorphic sites, nucleotide diversity, number of haplotypes, sequence conservation, and average number of nucleotide differences.

**Geomorphometric analyses and size of the anchors**

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

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

normality tests indicated that the conditions of normality were not met. Statistica v. 13.5 (TIBCO Inc., 2018) was used to visualize the distribution of the anchors size.

**Morphometrics of the male copulatory organ**

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

**Results**

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

**Genetic divergence of the nuclear and mitochondrial genes**

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

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

**Geomorphometry of dorsal and ventral anchors**

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

**Shape and size of the dorsal anchors (DA)**

The PCA of landmark data on the DA is visualized in Fig. 2. The three most informative PCs together accounted for 77.70% of the total variance. The first PC explained 60.21% of the total variance and fully separated

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

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

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

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

perissodine cichlids. Within the perissodine hosts, specimens of *C. nshomboi* clustered following a geographical pattern. The groups of northern *P. microlepis* branched off first and monogeneans of both *P. microlepis* and *P. straeleni* in the central (western) part of Lake Tanganyika were separated from the groups of *C. nshomboi* parasitizing *P. microlepis* in the southern (eastern) part of the lake.

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

**Shape and size of the ventral anchors (VA)**

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

In the CVA performed on the VA dataset, the first two CVs axes explained 96.89% of the variance. These two axes fully separated the parasite specimens from the two tribes and from the two main geographical groupings identified above (Fig. 4e). The shape changes along CV1 (60.98%) and CV2 (35.94%) mostly corresponded to those obtained with DA. The first CV corresponded to a change in the length of the outer root and the curvature of the shaft, whereas CV2 corresponded with a change in the length of the point, the curvature of the shaft and the thickness of the base (Fig. 4e).

The pairwise Procrustes distances and permutation tests for the VA dataset are shown in Table 2. As for the DA, the highest distances were found between the groups of *C. nshomboi* parasitizing boulengerochromine and those parasitizing perissodine hosts, followed by those between parasites from perissodines from northern and central/southern parts of the lake (Table 2). Permutation test conducted on the VA dataset of *C. nshomboi* revealed similar patterns of shape variation at tribal and intraspecies levels as obtained with the DA, except for additional significant differences in the shape of the VA between groups parasitizing *P. microlepis* from northern and central localities (NB3 vs. CB1), and between those from *P. straeleni* and one of the northern groups on *P. microlepis* (NB2 vs. CB2). In the NJ tree constructed with the Mahalanobis distances of the VA (Table S1), similar well-supported clusters (≥ 95%) were obtained, to those based on the DA dataset. Hence similar tribe-specific and geographical patterns were found (Fig. 3a).

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

**Morphometrical intraspecific variability of the male copulatory organ**

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

**Discussion**

We studied monogenean flatworms parasitizing the gills of cichlids belonging to two phylogenetically distant Tanganyika tribes: Boulengerochromini represented by the predator *B. microlepis* and Perissodini represented by the planctivorous *H. microlepis* and the scale eaters *P. microlepis* and *P. straeleni*. We hypothesised (a) that a single species of *Cichlidogyrus* infected boulengerochromine and perissodine cichlids. All studied cichlid hosts indeed carried a single monogenean species that we could identify as *C. nshomboi*. The conspecificity was

383 confirmed based on the morphology of the haptoral and reproductive organs and by sequence identity in 18S and
384 28S rDNA. The MCO, commonly used to delineate species in Monogenea (Mendlová et al. 2012; Pouyaud et al.
385 2006; Van Steenberge et al. 2015), was highly similar between specimens parasitizing different host species. The
386 most variable part of the ITS region (ITS1), showed only limited differentiation (1.1%), as 1% is suggested as a
387 cut-off between inter- and intra-specific variation for the entire ITS (including its less variable parts) (Zietara &
388 Lumme 2003). Previously, *C. nshomboi* was only known from *B. microlepis* from the central-western part of Lake
389 Tanganyika (Muterezi Bukinga et al. 2012). This study hence represents the first record of a species of
390 *Cichlidogyrus* parasitizing members of the endemic Lake Tanganyika tribe Perissodini.

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

407 The second scenario is that the presence of *C. nshomboi* on two distinct Tanganyikan cichlid tribes is due
408 to a past host-switching event. Although the probability of host-switching decreases with increasing phylogenetic
409 distance between donor and recipient hosts (Charleston and Robertson 2002), host-switches in monogeneans have
410 been documented between distantly related hosts, including across fish families (Benovics et al. 2018; Huyse et
411 al. 2003; Šimková et al. 2017; Ziętara and Lumme 2002). In *Cichlidogyrus*, such a distant host switch was
412 evidenced in *C. amieti* Birgi & Euzet, 1983, which expanded its range from cichlids to nothobranchiids (Birgi and

413 Euzet 1983; Messu Mandeng et al., 2015). Moreover, the evolutionary history and phylogenetic isolation of *B.*
414 *microlepis* could have facilitated a distant host switch. Although different species of *Cichlidogyrus* can coexist on
415 a single cichlid host, a range expansion of *C. nshomboi* could have been facilitated by the extinction of the original
416 *Cichlidogyrus* fauna of *B. microlepis*. Parasite extinctions were reported from the Lake Tanganyika *Cichlidogyrus*-
417 cichlid system (Vanhove et al. 2015). Mitochondrial genealogies of *B. microlepis* suggested a population
418 expansion following a decline at around 60 kya (Koblmüller et al. 2015). If this species' numbers dropped below
419 the persistence threshold of *Cichlidogyrus* (Gubbins et al. 2015), *B. microlepis* could have undergone parasite
420 extinction. Such an event could also have been caused by stochasticity in host availability, driven by the high
421 fecundity and mobility of *B. microlepis*. Without closely-related species available, this would have facilitated a
422 colonisation by a *Cichlidogyrus* species infecting a distantly related lineage, such as the Perissodini.

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

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

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

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

The morphological differentiation between groups of *C. nshomboi* parasitizing different populations of *P. microlepis* can be linked to population genetic structure of the host. *Perissodus microlepis* occurs along the rocky littoral zone, which has a patchy distribution along the lake's shoreline (Koblmüller et al. 2009). Population genetic studies on southern populations of *P. microlepis* showed that habitat fragmentation by environmental barriers restricted gene flow in this species, resulting in low levels of within-population genetic diversity

(Koblmüller et al. 2009). Geographically structured cichlid species often contain distinct colour morphs in Lake Tanganyika. Although Konings (2019) did not report geographical variants in *P. microlepis*, Yanagisawa et al. (1990) observed differences in the coloration of the anal fin in distinct populations of *P. straeleni*. They interpreted this as an adaptation to their preys, facilitating the scale eater to approach it. Unfortunately, no genetic data (COI) was available for *C. nshomboi* occurring on *P. microlepis* across this geographical gradient. Additionally, we did not study geographical differentiation on *C. nshomboi* infecting less-geographically structured species such as *B. microlepis* (Koblmüller et al. 2014) and the deep-water species of Perissodini.

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

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**Author contributions**

CR, MVS, MPMV and AŠ designed the study. MVS, MPMV and AŠ supervised the work. MPMV co-organised the fieldtrips, parasite collection, preparation of specimens and provided scientific background in the field. CR performed the lab work and data analysis, CR and MVS wrote 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 publicly available from the NCBI database or from the author upon request. The voucher specimens of monogeneans are housed in the Royal Museum for Central Africa (RMCA, Belgium) (see materials and methods for details on repositories and accession numbers).

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**Figures captions**

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

**Fig. 2** Geomorphometric analyses on the DA dataset of *C. nshomboi* specimens parasitizing boulengerochromine and perissodine hosts. Scatter plot showing the variation in shape of DA along PC1 and PC2 (**a**), and PC1 and PC3 (**b**). Wireframe graphs illustrating the shape changes from overall mean next to each PC,

828 with starting shapes (consensus) in black and target shapes (changes +0.1) in grey. Results from PCA and
829 multivariate regression of PC1 **(d)** and PC3 **(e)** against log centroid size (CS) **(c)**. The shape changes along PC1
830 ($r$ = -0.341, $p$ < 0.0001) and PC3 ($r$ = -0.044, $p$ < 0.0058) were correlated with anchor size. Scatter plot showing
831 the variation in shape along CV1 and CV2 (**e**). Wireframe graphs illustrating the shape changes from the overall
832 mean based on CVA are next to each PC, with starting shapes (consensus) in black and target shapes (changes
833 +0.7) in grey.

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

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

847 **Table captions**

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

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

857 *p*-values from permutation test with 10 000 randomizations (right of the diagonal) (significant *p*-values after Holm-
858 Bonferroni correction are indicated by '*'). The sampling sites are as in Table 1

859 **Table 3** Comparison of measurements of male copulatory organ (MCO) aspects among the studied
860 groups of *C. nshomboi* from Boulengerochromini and Perissodini. All measurements are given in micrometres as
861 the average ± standard deviation, the range in parentheses (min-max), and the number of specimens measured.
862 The sampling sites are as in Table 1.

Figure1

Click here to access/download;Figure;Fig. 1.TIFF

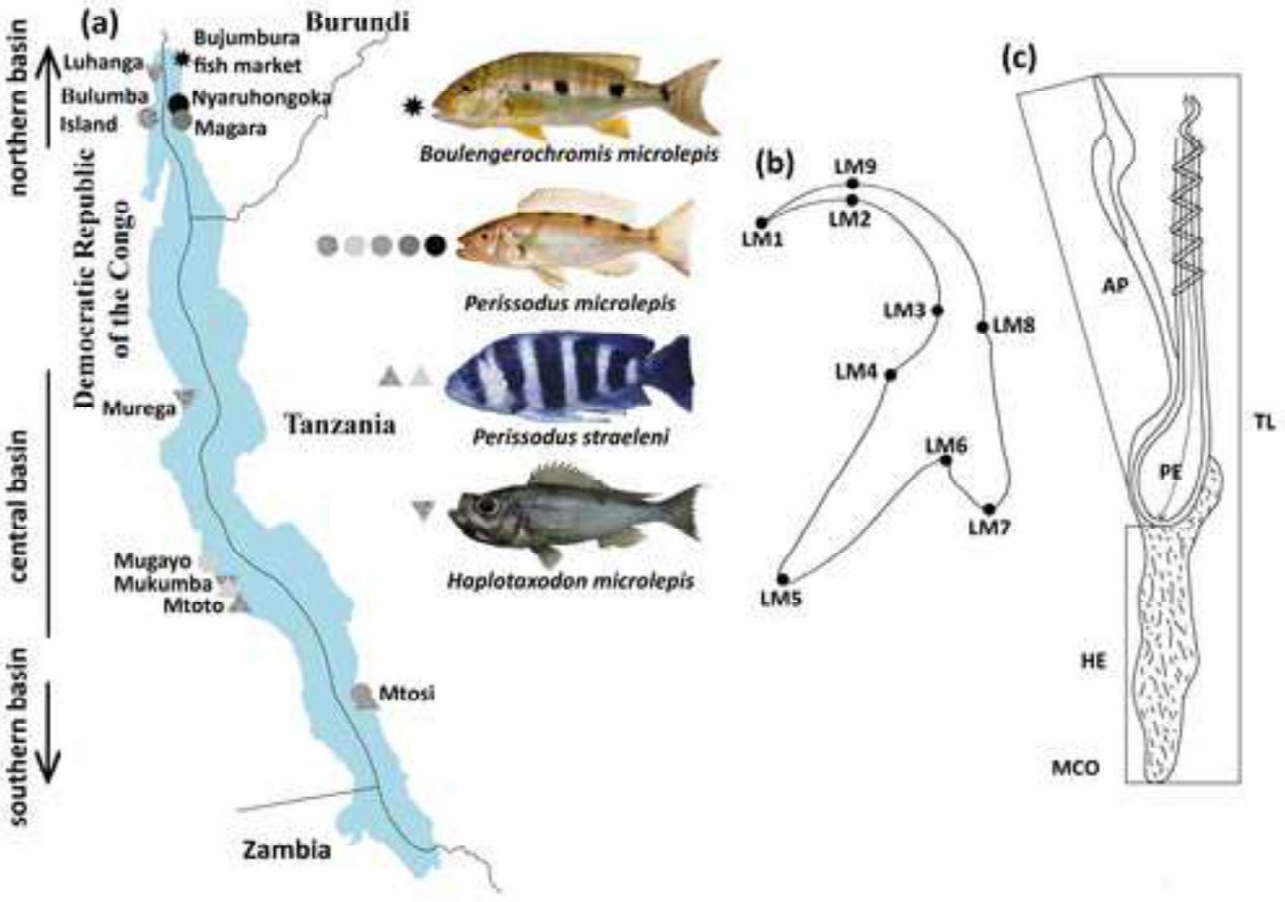

Figure2

Click here to access/download;Figure;Fig. 2.TIFF

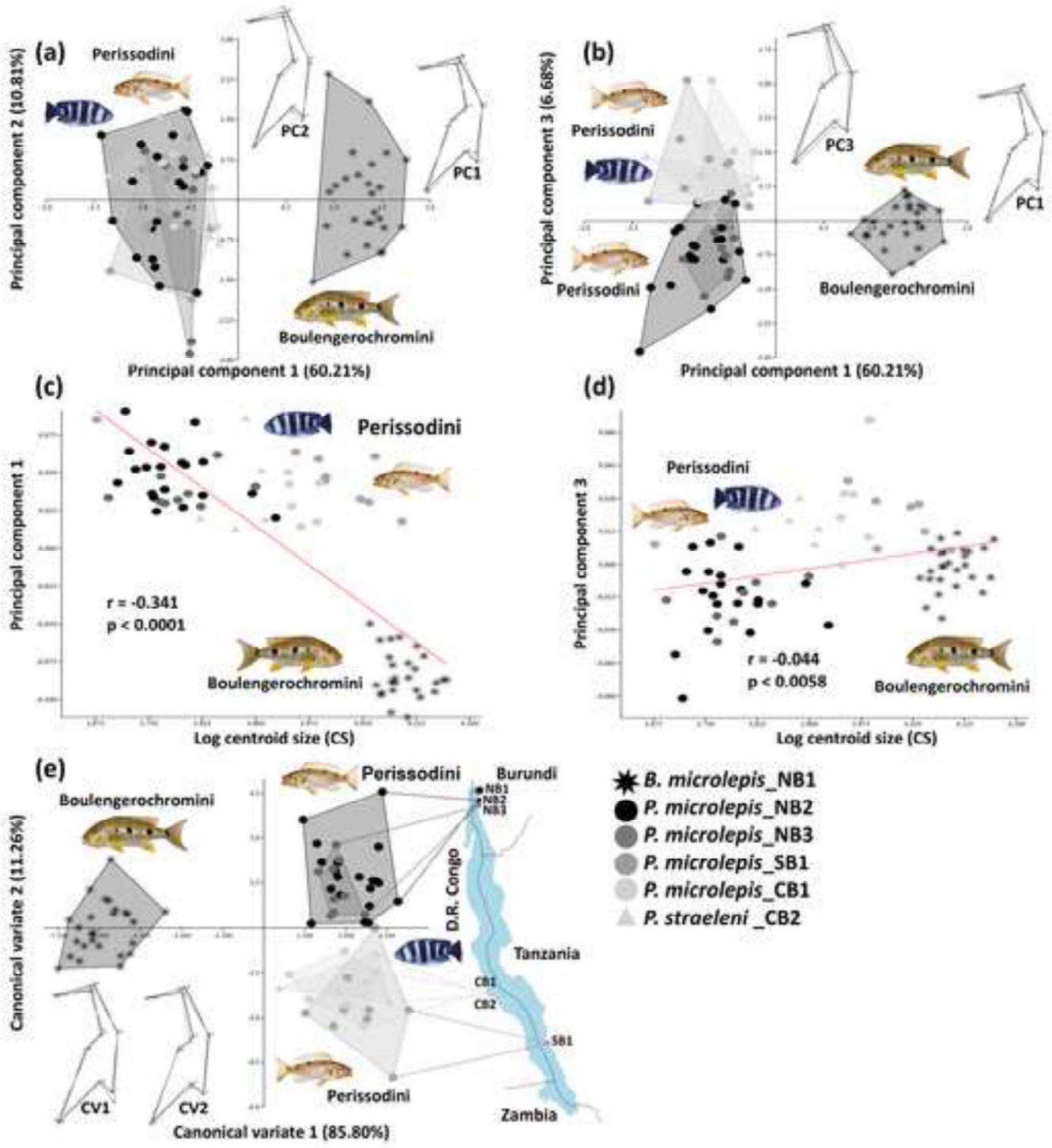

Figure3

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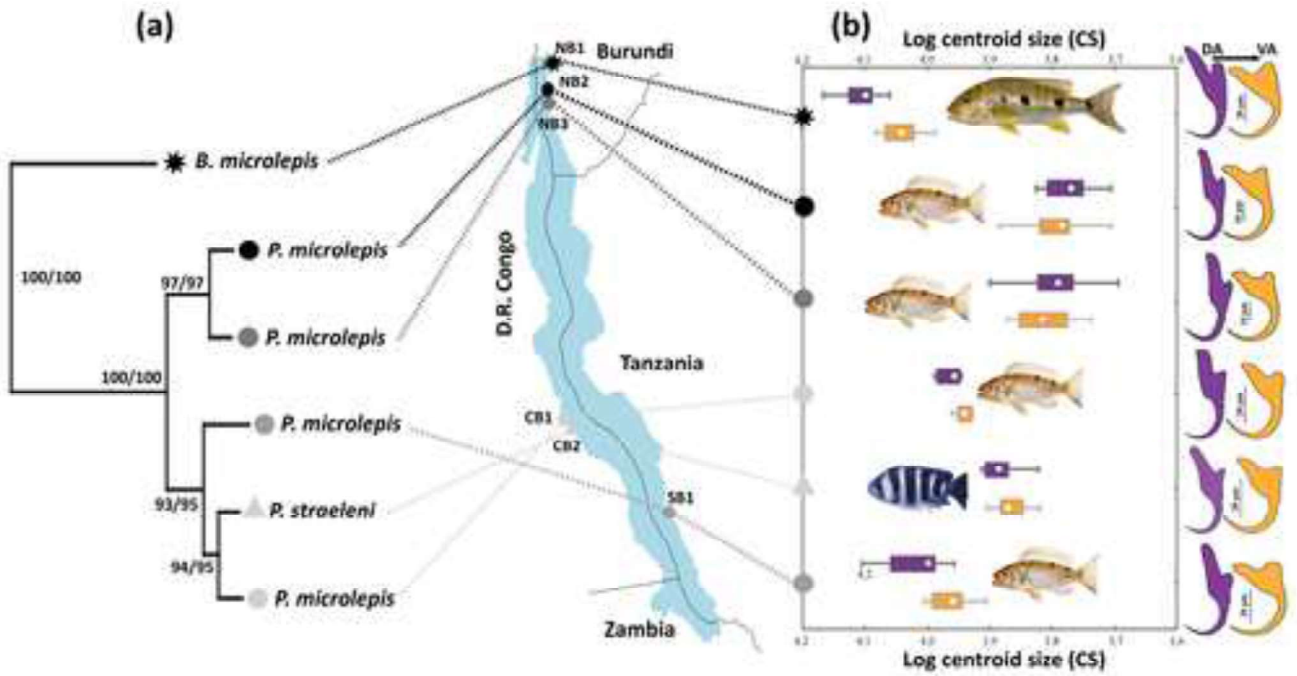

**Table 1** List of cichlid species with dates of sampling, localities in Lake Tanganyika and their abbreviations used in the study (see also Van Steenberge et al., 2011). Specimens of *Cichlidogyrus* parasitizing the cichlid species sampled from the localities highlighted in bold were targeted for the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

| Cichlid tribe | Cichlid species | *n* cichlids | Date | Locality of sampling | LT basin | Abbreviations | GPS Coordinates | *n* parasites | 18S-ITS1 rDNA | 28S rDNA | COI mitDNA | *n*DA | *n*VA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Boulengerochromini | *B. microlepis* | 04 | 05/09/2013 | **Bujumbura fish market** | **Northern** | *B. microlepis*_NB1 | 3°23'S, 29°22'E | **98** | OL675289-91 | OL688788-90 | OL675241-47 | **26** | **26** |
| Perissodini | *P. microlepis* | 05 | 09/09/2013 | **Nyaruhongoka** | **Northern** | *P. microlepis*_NB2 | 3°41′S, 29°20′E | **39** | OL675292-94 | OL688791-93 | OL675248-51 | **20** | **20** |
| | | 03 | 09/09/2013 | **Magara** | **Northern** | *P. microlepis*_NB3 | 3°44′S, 29°19′E | **9** | OL675295-97 | OL688794-96 | - | **9** | **9** |
| | | 11 | 24/04/2008 | **Mtosi** | **Southern** | *P. microlepis*_SB1 | 7°35′S, 30°38′E | **15** | OL675298-300 | OL688797-800 | - | **9** | **9** |
| | | 01 | 11/05/2010 | **Mugayo** | **Central** | *P. microlepis*_CB1 | 6°46′S, 29°33′E | **6** | - | - | - | **6** | 7 |
| | | 06 | 25/03/2010 | Bulumba Island | Northern | - | 3°46′S, 29°07′E | 2 | - | OL688801-04 | - | - | - |
| | *P. straeleni* | 07 | 14/05/2010 | **Mukumba** | **Central** | *P. straeleni*_CB2 | 6°56′S, 29°42′E | **7** | OL675301-02 | OL688805-08 | - | **5** | **5** |
| | | 02 | 15/04/2010 | Mtoto | Central | - | 6°58′S, 29°43′E | 2 | OL675303 | OL688809 | - | 2 | 2 |
| | | 01 | 24/04/2008 | Mtosi | Southern | - | 7°35′S, 30°38′E | 2 | - | - | - | 2 | 2 |
| | *H. microlepis* | 02 | 24/04/2010 | Murega | Central | - | 5°38′S, 29°23′E | - | - | - | - | - | - |
| | | 02 | 1997 | Makumba | Central | - | 6°56′S, 29°42′E | - | - | - | - | - | - |
| | | 03 | 1957 | Luhanga | Northern | - | 3°31′S, 29°08′E | 3 | - | - | - | 3 | 3 |

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

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

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

| Cichlid Tribe | Cichlid species | Locality | MCO total straight length (TL) | Copulatory tube curved length (PE) | Heel straight length (HE) | Accessory piece straight length (AP) |
|---|---|---|---|---|---|---|
| Boulengerochromini | *B. microlepis* | NB1 | 49 ± 1.94 (46-53)$_{20}$ | 24 ± 1.22 (21-26)$_{20}$ | 24 ± 1.14 (22-26)$_{20}$ | 20 ± 1.86 (17-14)$_{20}$ |
| Perissodini | *P. microlepis* | NB2 | 48 ± 3.61 (43-53)$_{14}$ | 27 ± 0.82 (26-28)$_{14}$ | 19 ± 2.87 (14-24)$_{14}$ | 22 ± 1.38 (19-24)$_{14}$ |
| | | NB3 | 47 ± 1.87 (44-49)$_{8}$ | 26 ± 0.35 (26-27)$_{8}$ | 19 ± 0.99 (18-21)$_{8}$ | 21 ± 2.10 (17-24)$_{8}$ |
| | | CB1 | 49 ± 2.08 (46-51)$_{4}$ | 27 ± 2.50 (24-30)$_{4}$ | 19 ± 1.29 (17-20)$_{4}$ | 19 ± 2.50 (16-22)$_{4}$ |
| | | SB1 | 53 ± 1.81 (50-56)$_{7}$ | 28 ± 1.57 (25-30)$_{7}$ | 26 ± 0.89 (25-27)$_{7}$ | 18 ± 1.25 (16-20)$_{7}$ |
| | *P. straeleni* | CB2 | 53 ± 2.42 (48-54)$_{6}$ | 27 ± 2.50 (22-29)$_{6}$ | 25 ± 1.75 (24-28)$_{6}$ | 17 ± 2.13 (14-20)$_{6}$ |