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KMENTOVA, Nikol; Koblmuller, S; VAN STEENBERGE, Maarten; ARTOIS, Tom; Bukinga, FM; N'sibula, TM; Risasi, DM; Mulungula, PM; Gelnar, M & VANHOVE, Maarten (2020) Failure to diverge in African Great Lakes: The case of Dolicirroplectanum lacustre gen. nov. comb. nov. (Monogenea, Diplectanidae) infecting latid hosts. In: JOURNAL OF GREAT LAKES RESEARCH, 46 (5) , p. 1113 -1130.

DOI: 10.1016/j.jglr.2019.09.022
Handle: http://hdl.handle.net/1942/32629

Failure to diverge in African Great Lakes: the case of *Dolicirroplectanum lacustre* comb. nov. (Monogenea, Diplectanidae) infecting latid hosts

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Abstract

Speciation of fish in the African Great Lakes has been widely studied. Surprisingly, extensive speciation in parasites was only recently discovered in these biodiversity hotspots, notably in monogeneans (Platyhelminthes) from Lake Tanganyika. *Diplectanum* is a monogenean genus of which only a single species is known from the Great Lakes: *Diplectanum lacustre* (Diplectanidae) living on latid perches of Lake Albert. Despite their primary marine origin, latids have diversified in African freshwaters including several Great Lakes. In better-studied marine diplectanid species, incongruence between morphological and genetic differentiation was documented. As freshwater systems provide more opportunities for speciation than the marine realm, we ask whether diplectanids of *Lates* spp. of the Great Lakes underwent similar diversification as their hosts.

Fresh and museum specimens of five African latid species (*Lates angustifrons, L. mariae, L. microlepis, L. niloticus, L. stappersii*) were examined for the presence of monogenean gill parasites. Monogeneans were characterised morphologically via morphometrics of sclerotised structures and genetically using nuclear ribosomal and mitochondrial markers.

Continuous morphological variation was documented in these parasites. In addition, the genetic distance, based on the COI region, between parasites of geographically isolated host species did not reach the level typically associated with distinct diplectanid species. Therefore, a single species of a newly described genus, *Dolicirroplectanum lacustre* gen. nov. comb. nov. is suggested to infect latid species in the examined basins. We discuss this parasite's failure to diverge in the light of the congruence between the rate of molecular evolution in COI and host historical distribution.

Keywords: parasitic flatworm - *Lates* – DNA barcoding - evolutionary history - Nile perches

Introduction

African Great Lakes are known for their species rich flocks of cichlid fish, that are well established models in evolutionary biology (Salzburger, 2018). Remarkably, Lake Tanganyika is characterised by extraordinary diversity and high degrees of endemism not only of cichlids but also other fish families (Salzburger et al., 2014) as well as invertebrate taxa (Coulter, 1991) including parasitic flatworms (Pariselle et al., 2015). Monogeneans (Platyhelminthes) are mainly parasites of fish. They display a high level of host specificity believed to be connected with their direct life cycle (a single host needed) combined with the adaptive evolution of monogenean hardparts responsible for attachment (the haptor in the posterior part of the body) and reproduction (Poulin, 2002).

Parasite speciation mirroring host diversification was reported for monogeneans infecting tropheine cichlids in Lake Tanganyika (Vanhove et al., 2015). However, the history of monogenean interactions with their hosts does not always feature only co-speciation events. For example, in *Dactylogyrus* Diesing, 1850 infecting cyprinid fishes, diversification can be mainly explained by intrahost speciation (Šimková et al., 2004). In the case of *Cichlidogyrus casuarinus* Pariselle, Muterezi Bukinga & Vanhove, 2015, a monogenean infecting deepwater cichlids in Lake Tanganyika, no specificity or preference was detected towards its various well-diverged host species (Kmentová et al., 2016), a process called “failure to diverge” (Brooks, 1979).

Among the parasitic flatworms known to infect lates perches (Latidae) are diplectanids (Diplectanidae), a monogenean family with more than 250 species described worldwide, mainly from marine perciform fishes (Domingues and Boeger, 2008). The genus *Lates* L., 1758 consists of 11 species, seven of which inhabit African freshwaters, with the rest to be found in marine, brackish and freshwater habitats in the Indo-Pacific region (Otero, 2004). While seven diplectanid species from three different genera were documented from *Lates*

81 *calcarifer* (Bloch, 1790) from the Indo-Pacific region (Tingbao et al., 2006), only one species
82 was described from African *Lates* spp. so far: *Diplectanum lacustre* Thurston & Paperna,
83 1969 infecting *Lates niloticus* L. from Lake Volta and the Victoria Nile near Lake Albert
84 (Paperna and Thurston, 1969), and from near Cairo in Egypt (Ergens, 1981). The native range
85 of *L. niloticus* includes most major river basins and Great Lakes in the Nilo-Sudanic region
86 and large parts of the Congo basin (Paugy et al., 2003). Importantly, *L. niloticus* was
87 introduced to Lake Victoria for fisheries with a dramatic impact on the local environment
88 (Ogutu-Ohwayo, 1995). In Lake Tanganyika, four endemic latid fishes, *Lates angustifrons*
89 Boulenger, 1906, *Lates mariae* Steindachner, 1909, *Lates microlepis* Boulenger, 1898 and
90 *Lates stappersii* (Boulenger, 1914), with different habitat preferences, are present (Poll,
91 1953).
92 Small interspecific morphological differences and high levels of phenotypic plasticity render
93 the species status of some diplectanids questionable (Poisot et al., 2011; Schoelinck et al.,
94 2012; Wu et al., 2005) with unclear phylogenetic relationships within and between some of
95 the genera (Villar-Torres et al., 2019). In the 21st century, species delineation is often based
96 on a combination of morphological and molecular data (Schlick-Steiner et al., 2010).
97 Integrative techniques revealed problems in various taxonomic groups, especially in soft-
98 bodied organisms or lineages with heteromorphic life stages such as parasitic flatworms
99 (Georgieva et al., 2013; Rahmouni et al., 2017). Species identification using specific
100 molecular tags derived from the cytochrome *c* oxidase subunit 1 gene (COI) in the
101 mitochondrial DNA, known as DNA barcoding, was successfully implemented in many
102 taxonomic groups such as fishes (Hubert et al., 2008), mammals (Francis et al., 2010) and
103 lepidopteran insects (Hebert et al., 2003). However, this approach proved problematic in
104 many other taxa (DeSalle et al., 2005; Will and Rubinoff, 2004) including monogenean
105 flatworms (Vanhove et al., 2013). So far, little correlation between host specificity and

106 taxonomic diversification was found in diplectanid monogeneans (Desdevises et al., 2001;
107 Villar-Torres et al., 2019). However, the latter studies were conducted in a marine system
108 with no real geographic barrier between host species that even form mixed schools. As
109 freshwater systems provide more opportunities for speciation than the marine realm, and
110 given the age of Lakes Albert and Tanganyika, which are situated in different basins, these
111 lakes are a perfect study system to investigate diplectanid evolution under allopatry. We
112 hypothesize that diplectanid monogeneans infecting latids belong to different species in Lake
113 Albert and Lake Tanganyika. If so, is there a congruence between the level of morphological
114 and molecular diversification in diplectanid parasites infecting African of *Lates* spp.? Within
115 Lake Tanganyika, we ask whether diplectanids of *Lates* spp. underwent similar diversification
116 as their hosts, or whether they rather failed to diverge like the above-mentioned *C.*
117 *casuarinus*, a monogenean infecting bathybatine cichlids. These are, like latids, non-littoral
118 fishes, and this lack of parasite specificity is considered an adaptation of low host availability
119 outside of the littoral zone (Kmentová et al., 2016).
120

121 **Material & Methods**

122 **Sampling**

123 Fish samples of five latid species (*Lates angustifrons, L. mariae, L. microlepis, L. niloticus, L.*
124 *stappersii*) were examined in this study. Samples included specimens of all *Lates* species
125 from the ichthyology collection of the Royal Museum for Central Africa (RMCA) (Tervuren,
126 Belgium) and fresh specimens from recent field expeditions (2010, 2016, 2017 and 2018). At
127 Lake Albert, fresh specimens of *L. niloticus* were obtained from local fishermen (Nzunzu,
128 Uganda). For Lake Tanganyika, the four endemic latid species (*Lates angustifrons, L. mariae,*
129 *L. microlepis* and *L. stappersii*) were either caught with gill nets from the experimental
130 fishing unit of the Centre de Recherche en Hydrobiologia-Uvira (CRH) (Uvira, Democratic

131 Republic of the Congo) or obtained from local fish markets (see Table 1). To provide a
132 broader geographical range for morphological comparison, fish specimens of *L. niloticus*
133 from seven additional localities throughout the host's range were examined. In total, gills (one
134 side in the case of museum specimens) of 158 fish specimens from 20 localities in African
135 freshwaters (see Table 1) were examined following the standard protocol of Ergens & Lom
136 (Ergens and Lom, 1970). In the field, fresh monogenean specimens were either mounted on
137 slides using a solution of glycerine ammonium picrate (GAP) or using Hoyer's medium in the
138 case of ethanol-fixed specimens from Lake Albert and specimens retrieved from the museum
139 collection. Some of the individuals were cut in three parts with the anterior and posterior parts
140 mounted on slides for morphological characterisation and the rest preserved in 99% ethanol
141 for genetic analyses. To characterize internal anatomy, some specimens were stained using
142 the Carmine method described by Justine (2005) without the initial step of putting a live
143 parasite under a cover slip. Parasite identification and description were carried out using an
144 Olympus BX51 microscope equipped with a drawing tube and OLYMPUS KL 1500 LED
145 illumination. Specimens were compared with the holotype (MRAC MT.35572) and voucher
146 material (MRAC MT.35573) of *D. lacustre*. Drawings were edited with a graphics tablet
147 compatible with Adobe Illustrator CS6 16.0.0 and Adobe Photoshop CS6 13.0. Fish tissue
148 samples were deposited in the ichthyology collection of the RMCA under collection number
149 2016.20.P for Lake Tanganyika and 2016.036.P for Lake Albert. Parasite voucher specimens
150 are available from the invertebrate collection of the RMCA, the Iziko South African Museum
151 (SAMC), Cape Town, Republic of South Africa; the Muséum national d'Histoire naturelle
152 (MNHN), Paris, France; the Natural History Museum (NHMUK), London, United Kingdom;
153 and the Finnish Museum of Natural History (MZH), Helsinki, Finland.

154 **Morphometrics**

155 Measurements of sclerotised structures were taken at a magnification of 1000× (objective ×
156 100 immersion, ocular × 10) using an Olympus BX51 microscope with incorporated phase
157 contrast and the software Digital Image Analysis v4. In total, 29 parameters of the hardparts
158 of the haptoral and copulatory organs were measured for morphometric characterisation and a
159 detailed redescription (see Fig. 1). Terminology was based on Justine & Henry (2010). To
160 investigate the level of morphological differentiation (haptor morphology), raw measurements
161 were analysed by multivariate statistical techniques in R (R Core Team, 2013). Principal
162 component analyses (PCAs) were conducted with scaled variables on 17 morphological
163 characters of the haptor using the package adegenet (Jombart, 2008). Results of the PCA were
164 visualised with the packages ggplot2 (Wickham, 2009) and factoextra (Kassambara and
165 Mundt, 2017). To visualise the variance in the total size of the ventral anchor, a density plot
166 using uncorrected measurements was drawn using ggplot2 and factoextra. A Kruskall-Wallis
167 test of multiple comparison with Bonferroni's post-hoc correction via Dunn's test,
168 implemented in the package FSA (Ogle et al., 2019), respectively, was conducted to test the
169 relation of the host species and the catch locality to copulatory organ measurements,
170 respectively. The assumption of normality was tested by Shapiro-Wilk's W tests implemented
171 in stats. The assumption of homogeneous variance within sample groups was tested by
172 Levene's test in the R package lawstat (Gastwirth et al., 2017).

173 **Molecular characterisation**

174 Morphological characterisation was combined with genetic characterisation using tissue
175 samples of the central part of some of the parasite individuals collected from fresh fish
176 specimens from Lake Tanganyika and Lake Albert, as described above. No fresh material was
177 available from other locations. To genetically verify parasite species delineation, we used
178 three different nuclear sequence fragments, from the small and large ribosomal subunit gene
179 (18 and 28 rDNA) and the first internal transcribed spacer region (ITS-1). To assess

180 intraspecific genetic diversity, part of the mitochondrial COI gene was used. Whole genomic
181 DNA was extracted using the Qiagen Blood and Tissue Isolation Kit following the
182 manufacturer's instructions with some modifications (samples in ATL buffer (180 µl) with
183 protein kinase (20 µl) were kept in 1.5 ml Eppendorf tubes overnight at room temperature).
184 The DNA extract was concentrated to a volume of 80 µl in 1.5 ml Eppendorf tubes using a
185 vacuum centrifuge and stored at a temperature of -20 °C. Partial 18S rDNA and ITS-1 were
186 amplified using the S1 (5´-ATTCCGATAACGAACGAGACT-3´) (Sinnappah et al., 2001)
187 and Lig5.R (5´-GATACTCGAGCCGAGTGATCC-3´) (Blasco-Costa et al., 2012) primers.
188 Each reaction mix contained 1.5 unit of *Taq* polymerase, 1X buffer containing 0.1 mg/ml
189 bovine serum albumin (BSA), 1.5 mM $MgCl_2$, 200 mM dNTPs, 0.8 mM of each primer and 3
190 µl of isolated DNA (concentration was not measured) in a total reaction volume of 30 µl
191 under the following conditions: 2 min at 95 ºC, 39 cycles of 1 min at 95 ºC, 1 min at 55 ºC
192 and 1 min and 30 s at 72 ºC, and finally 10 min at 72 ºC. Primers C1 (5´-
193 ACCCGCTGAATTTAAGCAT-3´) and D2 (5´-TGGTCCGTGTTTCAAGAC-3´) (Hassouna
194 et al., 1984) were used for amplification of the partial 28S rDNA gene. Each PCR reaction
195 contained 1.5 unit of *Taq* polymerase, 1X buffer containing 0.1 mg/ml BSA, 1.5 mM $MgCl_2$,
196 200 mM dNTPs, 0.5 mM of each primer and 5 µl of isolated DNA (concentration was not
197 measured) in a total reaction volume of 30 µl under the following conditions: 2 min at 94 ºC,
198 39 cycles of 20 seconds at 94 ºC, 30 seconds at 58 ºC and 1 min and 30 s at 72 ºC, and finally
199 10 min at 72 ºC. Part of the mitochondrial COI gene was amplified using ASmit1 (5'-
200 TTTTTTGGGCATCCTGAGGTTTAT-3') combined with Schisto3 (5'-
201 TAATGCATMGGAAAAAAACA-3'), and with ASmit2 (5'-
202 TAAAGAAAGAACATAATGAAAATG-3') in case of nested PCR (Littlewood et al., 1997).
203 For both primer combinations, the amplification reaction contained 24 µl of PCR mix (one
204 unit of *Taq* polymerase, 1X buffer containing 2 mM $MgCl_2$, 0.1 mg/ml BSA, 0.2 mM dNTPs,

205 0.8 mM of each primer) with 1 µl of isolated DNA (concentration was not measured) in a
206 total reaction volume of 25 µl and was performed under the following conditions: initial
207 denaturation at 95°C for 5 min and then 40 cycles of 1 min at 94°C, 1 min at 50°C and 1 min
208 at 72°C, and final elongation for 7 min at 72°C. Amplification success was checked by
209 agarose gel electrophoresis and for positive samples, 2.5 µg of PCR product was
210 enzymatically cleaned up using 1 µl of ExoSAP-IT reagent under the following conditions: 15
211 min at 37 ºC and 15 min at 80 ºC. After cycle sequencing of purified PCR products using
212 BigDye v3.1, following the manufacturer's recommendations, fragments were cleaned up
213 using the BigDye XTerminator® Purification Kit and visualized on an ABI3130 capillary
214 sequencer. Electropherograms were visually inspected, corrected and sequences were aligned
215 using MUSCLE (Edgar, 2004) under default settings as implemented in MEGA v7 (Kumar et
216 al., 2016), together with selected previously published sequences of representatives of
217 Diplectanidae (see Table S1). The newly obtained haplotype sequences were deposited in
218 NCBI GenBank under the accession numbers MK937579-MK937581 (28S rDNA),
219 MK937574-MK937576 (18S+ITS-1 rDNA) and MK908145- MK908196 (COI mtDNA).
220

221 **Genetic distances and phylogeny**

222 The consistency of all alignments was checked and corrected under the "automated 1" option
223 in trimAL v1.2, which uses a heuristic search to find the best method for trimming the
224 alignment (Capella-Gutiérrez et al., 2009). As there is a lack of available ITS sequences of
225 diplectanid species, phylogenetic analyses were based on two regions: 18S and 28S rDNA.
226 These two regions were analysed separately because of the lack of species for which both
227 regions are available. Topali v2.5 (Milne et al., 2004) was used to identify the best fitting
228 model of molecular evolution based on the Bayesian information criterion (28S rDNA: GTR
229 + Γ, gamma shape parameter of 0.461; 18S rDNA: K2P + Γ, gamma shape parameter of

230 0.130). For each gene, pairwise distances were calculated using both the most appropriate
231 evolutionary model and, to compare with previous studies, uncorrected pairwise distances.
232 The number of haplotypes and polymorphic sites, haplotype diversity and nucleotide diversity
233 were calculated using ARLEQUIN v3.5 (Excoffier and Lischer, 2010). Phylogenetic analyses
234 were carried out using maximum likelihood (ML) and Bayesian inference (BI) in RAxML v8
235 (Stamatakis, 2014) and MrBayes v3.2.0 (Ronquist et al., 2012), respectively. A ML tree was
236 inferred using RAxML's standard tree search algorithm and bootstrap support was calculated
237 using the option with an automated number of replicates to obtain stable support values under
238 the frequency stopping criterion (Stamatakis, 2014). Bayesian inference was based on two
239 independent runs (100,000,000 generations, sampled every 1,000th generation following a
240 burn-in of 10%). Parameter convergence and run stationarity were assessed in Tracer v1.6
241 (http://beast.bio.ed.ac.uk). As Dactylogyridae and Diplectanidae were shown to be sister taxa
242 (Šimková et al., 2003), *Dactylogyrus extensus* (Mueller and Van Cleave, 1932) (sequence
243 from: Šimková, Matějusová, & Cunningham, 2006) together with *Cichlidogyrus*
244 *attenboroughi* Kmentová, Gelnar & Vanhove, 2016 (sequence from: Kmentová et al. (2018)
245 in the case of 28S rDNA region were selected as outgroup for phylogenetic inference.
246 Phylogenetic trees were edited in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/)
247 and Adobe Photoshop CS6. Phylogenetic relationships among COI haplotypes were inferred
248 by means of a Median Joining network (Bandelt et al., 1999) in PopART 1.7104 (Leigh and
249 Bryant, 2015).
250
251 **Results**
252 A single diplectanid species, morphologically identified as *Diplectanum lacustre* was
253 recorded from three of the four species of *Lates* from Lake Tanganyika (*L. angustifrons, L.*
254 *mariae, L. microlepis*) and from *L. niloticus* from Lakes Albert, Kossou, Nasser and Victoria,

from the Taja River in Sierra Leone and from the Bahr-Sara, mouth of the Mandoul River in Tchad. In total, 473 parasite specimens were collected (for more details see Table 1 and Fig. 2). Based on morphological characterisation and phylogenetic reconstruction (see Figs. 7&8), a new genus *Dolicirroplectanum* gen. nov. is described with *Dolicirroplectanum lacustre* comb. nov. as the type species. The internal anatomy is characterised, including the sclerotised vagina, prostatic reservoir and seminal vesicle, which were absent in the original description of *D. lacustre* comb. nov. Measurements of the parasite's internal organs and sclerotised haptoral and copulatory structures are presented in Table 2.

Taxonomy and species redescription

***Dolicirroplectanum* gen. nov. Kmentová, Gelnar & Vanhove (Fig. 3 - 5)**

Family: Diplectanidae Monticelli, 1903

Genus: *Dolicirroplectanum* gen. nov.

Type species: *Dolicirroplectanum lacustre* (Thurston & Paperna, 1969)

Type host: *Lates niloticus* L. (Latidae)

Type locality: Lake Volta, Ghana; Lake Albert, Uganda

Site: Gills

Additional hosts: *L. angustifrons, L. mariae, L. microlepis*

Other species: *Dolicirroplectanum penangi* comb. nov. for *Diplectanum penangi* Liang & Leong, 1991 (original designation)

Material examined: type material: MRAC MT. 35572, vouchers: MNHN HEL744-47 (4 specimens), USNPC 180-A 3-7; MRAC. MT. 38206-10, 38913-39058 (243 specimens), MZH 10067-71 (6 specimens), SAMC-A089971-72 (6 specimens), NHMUK 2018.4.13.4-13.7 (8 specimens)

Zoobank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN) (International Commission on Zoological Nomenclature, 2012), details of the genus have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:209675D6-2EBE-4E37-84CB-DA59994F7B2. The LSID for the new genus *Dolicirroplectanum* is urn:lsid:zoobank.org:act:89BFF3C5-271B-4482-98E0-AC667AA6611D.

Etymology: The genus name derives from Latin and refers to the barrel shape of the male copulatory organ, noticeably wider than in other diplectanid genera.

Diagnosis: Tegument smooth. Genital pore opening posterior to male copulatory organ (MCO). Genital atrium sclerotised. MCO wide, robust, composed of two nested tubes. Prostatic reservoir simple. Seminal vesicle sinistral. Accessory copulatory organ absent. Squamodiscs ventral, dorsal; rows of bone-shaped rodlets with open rings. Superficial root of ventral anchor reduced. Parasites of perciform fishes (*Lates* spp.). Vagina sclerotised or muscular.

Description:

Multiple pairs of head organs, two pairs of eye-spots. No tegument scales were observed. *Dolicirroplectanum* gen. nov. is characterised by two pairs of dorsal and ventral anchors with a regularly curved shaft point, a large and wide ventral bar and two dorsal bars. Dorsal anchors smaller than ventral ones and without developed outer root. 14 marginal hooklets of similar size and relatively small compared to other haptoral structures. Two squamodiscs, ventral and dorsal, formed by concentric open rows of bone-shaped rodlets of similar width in all rows.

Intestinal bifurcation follows pharynx, oesophagus absent. Caeca simple, terminate blindly. Testis spherical, intercaecal. Vas deferens emerges from anterior part of testis, enlarges into seminal vesicle. Seminal vesicle single in the middle region of body, transforms into elongated

duct connected with sclerotized part of copulatory organ. Prostatic reservoir simple. Slightly sclerotized MCO composed of two straight tubes, one inside the other, almost as wide as long. Ovary intercaecal, pre-testicular, encircles right caecum. Oviduct passes medially to oötype, surrounded by Mehlis' gland, oötype short, enters into uterus. Uterus sinistral. Vaginal atrium sclerotised or muscular.

Discussion: Species of *Dolicirroplectanum* gen. nov. can be distinguished by the combination of: 1) presence of a robust barrel-shaped MCO formed by two narrow nested tubes, almost as wide as long, 2) absence of an accessory piece, 3) squamodiscs composed of bone-shaped rodlets forming open rings, 4) superficial roots of ventral anchor reduced, 5) a simple prostatic reservoir not separated into zones, 6) seminal vesicle as an expansion of vas deferens, 7) ovary intercaecal, pre-testicular, encircles right caecum and 8) a lack of tegumental scales. The status of *Dolicirroplectanum* gen. nov. is supported by its placement outside of the clade including *Diplectanum aequans* (Wagener, 1857), the type species of *Diplectanum* (Figs. 7&8). Particularly, *Dolicirroplectanum* gen. nov. differs from other diplectanids including *D. aequans* by the short but wide sclerotised part of the MCO. In contrast to *D. aequans*, a simple prostatic reservoir is present. Conversely, a prostatic reservoir separated into three zones is one of the specific characters for *Diplectanum* sensu stricto mentioned in Domingues & Boeger (2009). *Diplectanum penangi* has all the diagnostic features attributed to *Dolicirroplectanum* gen. nov. The position within the genus was supported by its position in a phylogenetic reconstruction, clustering with *Dolicirroplectanum lacustre* comb. nov. (Figs. 6&7). The holotype of *D. penangi* comb. nov. could not be verified as the specimen was not provided by Lee Kong Chian Natural History Museum in Singapore and as the digital pictures we received were taken at insufficient magnification/resolution. Therefore, voucher material deposited in the National Museum of Natural History in Washington and the National Museum of Natural History in Paris was checked instead, and the two nested copulatory tubes and simple prostatic reservoir

were found to be present in *D. penangi* comb. nov. together with other characteristics mentioned in its original description (Fig. 5).

Redescription

Family: Diplectanidae Monticelli, 1903

Genus: *Dolicirroplectanum*

Dolicirroplectanum lacustre comb. nov. (Thurston & Paperna, 1969)

Synonyms: *Diplectanum lacustre*

Zoobank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN) (International Commission on Zoological Nomenclature, 2012), details of the species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:209675D6-2EBE-4E37-84CB-DA59994F7B2. The LSID for the new name *Dolicirroplectanum lacustre* is urn:lsid:zoobank.org:act:423241C3-777D-4F86-A70F-02B4D74F9E66.

Figures: 3, 44

Material examined: holotype: MRAC MT. 35572, paratype: MRAC MT. 35573

Vouchers: MRAC. MT. 38206-10, 38913-39058 (243 specimens), MZH 10067-71 (6 specimens), MNHN HEL744-47 (4 specimens), SAMC-A089971-72 (6 specimens), NHMUK 2018.4.13.4-13.7 (8 specimens)

Type host: *Lates niloticus* L. (Latidae)

Type locality: Lake Volta, Ghana; Lake Albert, Uganda

Site: Gills

Additional hosts: *L. angustifrons, L. mariae, L. microlepis*

Additional localities: Bahr-Sara, Tchad (08°56'N-17°58'E); Kisumu, Lake Victoria (00°06'S-34°45'E), Lake Kossou, Egypt (07°10'N-05°20'E), Lake Nasser, Egypt (24°05'N-33°00'E), Luxor market, Egypt (25°42'N 32°38'E), Njala, riv. Taja, Sierra Leone (08°06'N-12°04'E), Lake Albert – Nyawiega (01°28'N-30°56'E); Nzunzu (1°19'N, 30°72'E); Lake Tanganyika – Crock Island (8°42'S-31°07'E), Katukula (8°35'S-31°10'E), Mpulungu (8°46'S-31°07'E); Rumonge (3°97'S-29°43'E); Sumbu Bay (8°31'S-30°29'E); Bujumbura (3°23'S-29°22'E); Ilagala (5°12'S-29°50'E); Kilomoni (4°20′S, 29°09′E); Mulembwe (6°07′S, 29°16′E); Nyanza (4°20'S-29°35'E); Edith Bay (6°30'S-29°55'E); Uvira (3°22′ S 29°08′E)

Infection parameters: 4 of 8 *Lates angustifrons* infected with 1 – 15 specimens, 15 of 23 *L. mariae* infected with 1 – 18 specimens, 21 of 31 *L. microlepis* infected with 1 – 53 specimens. 1 of 1 *L. niloticus* from Bahr-Sara infected with 2 specimens, 2 of 3 *L. niloticus* from Kisumu (Lake Victoria) infected with 1-7 specimens, 4 of 5 *L. niloticus* from Lake Kossou infected with 5-9 specimens, 1 of 5 *L. niloticus* from Lake Nasser infected with 1 specimen, 1 of 3 *L. niloticus* from Nyawiega (Lake Albert) infected with 2 specimens, 5 of 11 *L. niloticus* from Nzuzu (Lake Albert) infected with 2-10 specimens, 1 of 1 *L. niloticus* from Nzuzu (Lake Albert) infected with 2 specimens.

Diagnosis: *Dolicirroplectanum lacustre* comb. nov. is a monogenean infecting gills of freshwater African latid species distinguished from its congener by the width of the outer root of the ventral anchor. The copulatory tube is oriented anteriorly.

Description: Tegument thin, smooth. Three pairs of head organs, two pairs of eye-spots, the posterior ones larger and closer together. Two squamodiscs, ventral squamodisc larger than dorsal, both consist of 9-12 concentric rows of bone-shaped rodlets, the two distal rows of which are composed of only rudimentary rodlets. Two pairs of anchors, rudiment of inner root and wide base of outer root in dorsal anchor. Marginal hooklets (14) of similar size. Ventral bar tapering towards extremities with terminal auricles. Dorsal bar broadening towards the centre

375 of the haptor area. Testis post-ovarial, thin vas deferens along the dextral intestinal caecum.
376 Single seminal vesicle in the middle of the body. Simple prostatic reservoir. MCO robust and
377 formed by two nested tubes. Copulatory tube oriented anteriorly. Ovary looping around the left
378 intestinal caecum towards the oviduct, surrounded by Mehlis' glands located near oötype.
379 Uterus simple tube towards vagina. Vagina is formed by a complex of sclerotized structures
380 consisting of an elongated primary canal followed by a secondary tube opening into an anterior
381 duct; duct continues into distal sclerotized part ending in blade-shaped structure. Orientation of
382 sclerotized vagina with blade-shaped end always anterior. Sclerotised vagina can be absent.
383 Vitellaria dense, located around outer wall of intestinal caeca.

384 **Discussion:** *Dolicirroplectanum lacustre* comb. nov. resembles its congener
385 *Dolicirroplectanum penangi* comb. nov. infecting *Lates calcarifer* in Asia. The type species of
386 the genus can be easily distinguished from *D. penangi* comb. nov. by the comparative
387 morphology of the anchors, especially the thinner outer root in *D. penangi* comb. nov. (see Fig.
388 5). Contrary to *D. lacustre* comb. nov., a sclerotised vagina was not observed in *D. penangi*
389 comb. nov. (Liang and Leong, 1991). Our findings are based only on a combination of the
390 original description of *D. penangi* comb. nov. and voucher material deposited in the National
391 Museum of Natural History in Washington and the National Museum of Natural History in
392 Paris as the Lee Kong Chian Natural History Museum in Singapore refused to provide the
393 holotype material.

394 **Morphometric variation**

395 Morphological variation was visualized based on a PCA performed on 17 standardised
396 haptoral morphometric parameters from 148 individuals. The first PC explained 48.4 % of the
397 variation in the data, the second one 10.1 %. Results show a high level of variability in
398 specimens of *D. lacustre* comb. nov. infecting *L. niloticus* and a continuous size gradient not

399 related to the locality of origin with an intermediate position of specimens from Lake Kossou
400 and Lake Victoria along the first axis. Moreover, individuals collected in the Taja River seem
401 to be separated from the others. Interestingly, two morphotypes were retrieved from different
402 fish specimens in Lake Albert (Lake Albert1 and Lake Albert2). Therefore, the morphology
403 of *D. lacustre* comb. nov. does not seem to be influenced by neither geographical nor host
404 species origin (Fig. 8A). Moreover, two specimens from Lake Albert (belonging to Lake
405 Albert2) were, based on the haptoral sclerotised structures and MCO, more similar to those
406 collected outside the lake (see Table 2). The position of specimens in the scatterplot was
407 mainly influenced by the size of dorsal anchors, maximum width of the dorsal bar and length
408 of both squamodiscs. However, almost all parameters were correlated with the first axis.
409 Other PCs did not show a clearer separation. The length of the dorsal anchor was shown to be
410 related to the combination of host species and geographic origin, in a gradient, with two
411 morphotypes recognised in Lake Albert (Lake Albert1 and Lake Albert2), as visualised in a
412 density plot (Fig. 8B).

413 MCO parameters from 91 individuals of *D. lacustre* comb. nov. were compared. Significantly
414 wider and longer copulatory organs were observed in specimens collected from *L. niloticus*
415 (n=31), than in those collected from *L. microlepis* (n=38) (Bonferroni's post-hoc correction,
416 MCO length $Z_{2,87}$=-6.48, P<0.001, MCO width $Z_{2,89}$=-6.74, P<0.001) and *L. mariae* (n=22)
417 (Bonferroni's post-hoc correction, MCO length $Z_{2,87}$=-4.98, P<0.001, MCO width $Z_{2,89}$=-4.25,
418 P<0.001). The influence of geographical origin was tested only for samples from these three
419 host species from Lake Albert and Lake Tanganyika as there was an insufficient number of
420 high-quality specimens from other localities and *L. angustifrons*, respectively. In both
421 parameters of the MCO, a significantly larger size was observed in specimens from Lake
422 Albert, morphotype Lake Albert1 (Bonferroni's post-hoc correction, MCO length – $Z_{1,89}$ =
423 6.61, P <0.001, MCO width – $Z_{1,87}$ = 6.41, P <0.001).

424 Apart from these size differences, the variable presence of a sclerotised vagina was
425 documented (see Table 2), also including data from two previous records of the species
426 (Ergens, 1981; Thurston and Paperna, 1969).

427 **Genetic characterisation and phylogeography**

428 Uncorrected p-distances between *D. lacustre* comb. nov. collected from Lake Tanganyika and
429 Lake Albert, respectively, varied among the amplified regions from 0.5% in 18S rDNA (441
430 base pairs (bp)), 1.1% in 28S rDNA (810 bp) to 9% in ITS-1 rDNA (478 bp) and 9.0 – 10.2%
431 in COI mtDNA (412 bp). In previous studies, the ability to align ITS-1 sequences was used as
432 a criterion for diplectanid species delineation (Poisot et al., 2011; Wu et al., 2007). No
433 intralacustrine variability in rDNA regions was detected. Sequences of the ITS-1 region of all
434 populations of *D. lacustre* comb. nov. in our study were alignable and included 19 indels. For
435 comparison with the threshold of 14.5% difference in the COI region to distinguish intra- and
436 interspecific diversity proposed for diplectanids by Vanhove et al. (2013), genetic distances
437 were also calculated using the K2P model (Kimura, 1980), under which they amounted to 9.6
438 – 10.7%. Intralacustrine variation in COI was higher in Lake Albert than in Lake Tanganyika
439 (Table 3). The haplotype network showed two distinct haplogroups, corresponding to the two
440 lakes (Fig. 9). Identical COI haplotypes were shared among individuals of *D. lacustre* comb.
441 nov. collected from *L. mariae* originating from the central subbasin (Mulembwe) and *L.*
442 *microlepis* collected from the northern and southern subbasins of Lake Tanganyika (Uvira and
443 Mpulungu).

444 **Phylogeny**

445 Phylogenetic inference at the family level (Diplectanidae) was based on two separate
446 alignments of the 28S and 18S nuclear rDNA with 33 and 15 taxa, respectively (Table S1).
447 The alignment of 28S rDNA and 18S rDNA totalled 803 and 482 bp, respectively.

448 Phylogenetic analyses of 28S rDNA placed the haplotypes of *Dolicirroplectanum lacustre*
449 comb. nov. in a monophyletic clade sister to *Dolicirroplectanum penangi* comb. nov.
450 collected from *Lates calcarifer* in Asia (Fig. 6). The tree obtained from the 18S rDNA
451 fragment placed *D. lacustre* comb. nov. in a poorly resolved clade with species of
452 *Pseudorhabdosynochus* Yamaguti, 1958 and *Echinoplectanum* Justine and Euzet (2006) (Fig.
453 7). ML and BI produced the same topologies. In both phylogenetic trees, the previous notion
454 of *Diplectanum* appeared polyphyletic with the type species, *Diplectanum aequans*, placed
455 outside the clade including species of *Dolicirroplectanum* gen. nov., hence supporting the
456 erection of a new genus.

457 **Discussion**

458 The main aim of this study was to examine the level of diversification in diplectanid parasites
459 infecting latid hosts in two of the African Great Lakes, Lakes Albert and Tanganyika.
460 Moreover, museum specimens from throughout the host's range were added to provide a
461 broader geographical range for morphological comparison. Morphological and molecular
462 characterisation identified a single species in both lakes, reassigned to *Dolicirroplectanum*
463 gen. nov. Despite the persistent geographic separation between Lakes Albert and Tanganyika
464 for 9 MYA (Cohen et al., 1993), and the speciation of the hosts, their respective populations
465 of *D. lacustre* comb. nov. have not reached the level of morphological and genetic
466 differentiation typically associated with distinct species. Hence, we conclude that this is an
467 example of a lineage that failed to speciate.

468 **Diplectanid species infecting latid fishes in Africa – molecular and morphological**
469 **perspectives**

470 The monophyly of *Diplectanum* was already rejected in previous studies (Chotnipat et al.,
471 2015; Villar-Torres et al., 2019) with *Dolicirroplectanum lacustre* comb. nov. being classified

472 outside of *Diplectanum* sensu stricto (Chotnipat et al., 2015; Domingues and Boeger, 2008).
473 The phylogenetic reconstructions based on ribosomal regions place *D. lacustre* comb. nov. in
474 a separate lineage together with *D. penangi* comb. nov. infecting an Asian latid species, *L.*
475 *calcarifer*, but outside the clade that includes *D. aequans*, the type species of *Diplectanum*.
476 This, combined with a detailed morphological characterisation, leads us to propose the new
477 genus *Dolicirroplectanum* gen. nov., now including *D. lacustre* comb. nov. and *D. penangi*
478 comb. nov. Overall, the phylogenetic position of other diplectanid genera corresponds with
479 the study by Villar-Torres et al. (2019). The genetic distance between *D. lacustre* comb. nov.
480 from Lake Tanganyika and Lake Albert, and *D. penangi* comb. nov., is 7.9% based on the
481 28S rDNA fragment. This is comparable to the situation in *Laticola latesi* (Tripathi, 1957)
482 and *L. paralatesi* (Nagibina, 1976), which infect *L. calcarifer* in Hainan province, China
483 (Tingbao et al., 2006). However, these diplectanid species occur sympatrically, infecting a
484 single host species, whereas there is no contact between *L. calcarifer* and the species of *Lates*
485 from Lakes Albert and Tanganyika.

486 Interestingly, copulatory tube width and length differ between most of the parasite individuals
487 collected from *L. niloticus* from Lake Albert and three of Lake Tanganyika's species, *L.*
488 *angustifrons*, *L. microlepis* and *L. mariae,* respectively. Differences in the MCO may be the
489 basis for species delineation in diplectanids (e.g. in *Echinoplectanum*: Sigura & Justine,
490 2008). However, the mean values of individuals of *L. niloticus* from other localities (Lake
491 Kossou, Lake Victoria) do not differ from Lake Tanganyika's specimens. Moreover, two
492 specimens from Lake Albert (belonging to Lake Albert2) are, based on the haptoral
493 sclerotised structures and the MCO, more similar to those collected outside this lake (see
494 Table 2). A complex pattern of morphological variation emerging from the other populations
495 of *D. lacustre* comb. nov., with a lot of overlapping features between host species and
496 localities (Fig. 8 and Table 2), does not suggest the existence of different species. The

497 intermediate position of some specimens, particularly from Lake Albert (referred to as Lake
498 Albert2), prevents a clear correlation between parasite morphotype, host species identity
499 and/or geographic origin. Future genetic characterisation of such morphotypes is needed to
500 address diversification of *D. lacustre* comb. nov. in detail. Internal anatomy is documented
501 only in fresh specimens from Lake Albert and Lake Tanganyika, with high levels of
502 intralacustrine variation and without structural differences in organisation between these
503 lakes.

504 Moreover, the impossibility to align ITS-1 rDNA sequences is generally considered as an
505 indicator for diplectanid species delineation (Wu et al., 2005a; Poisot et al., 2011). Since
506 haplotypes from the two populations of *D. lacustre* comb. nov. in our study are alignable, the
507 hypothesis of a single species is supported. Also, as the model-corrected genetic distance of
508 10.7% over the COI fragment does not reach the "best-compromise threshold" (Meier et al.,
509 2006) for barcoding of 14.5% proposed by Vanhove et al. (2013) for diplectanids infecting
510 Indo-Pacific groupers, we consider all specimens in our study as conspecific and belonging to
511 *D. lacustre* comb. nov. Its records therefore increased from four to ten areas (see Table 1 and
512 taxonomic part of the result section).

513 Based on differences in the size of haptoral structures and the split ends of the internal tube of
514 the MCO (see Table 2 and Fig. 4), specimens from Taja River might be considered as
515 belonging to a different species. This could be explained by the long separation between the
516 Upper Guinean and Nil ichthyofaunal provinces (Roberts, 1975). However, more samples and
517 molecular data from Taja River are needed to confirm the identity of the species of
518 *Dolicirroplectanum* infecting *L. niloticus* in the Upper Guinean province.

519 **Host range of the diplectanid monogeneans infecting lates perches in Lake Tanganyika**

520 Based on our results, the host species list of *D. lacustre* comb. nov. was extended with three
521 of the four latid species from Lake Tanganyika. No monogeneans were found on *L. stappersii*.
522 A potential reason could be its different life style compared to other latid species in the lake.
523 In contrast to its congeners, *L. stappersii* is truly pelagic throughout its life, usually not
524 moving into inshore waters (Mannini et al., 1999; Mulimbwa and Mannini, 1993). Short-lived
525 and slow swimming monogenean larvae (oncomiracidia) are assumed to infect fish hosts in
526 littoral habitats, typically synchronised with their hosts' period of reproduction (Whittington
527 et al., 1999). Therefore, there is less chance for parasite infection in *L. stappersii* compared to
528 other latid species (see Rohde, 1980; Rohde et al., 1995). Moreover, there is no sign of
529 species diversification in Lake Tanganyika as we found haplotypes shared between latid hosts
530 and between subbasins.

531 The African Great Lakes are highly biodiverse areas with a remarkable species richness and a
532 high level of endemism (Salzburger et al., 2014). Parasite diversification linked with host
533 speciation was recently discovered in monogeneans belonging to *Cichlidogyrus* Paperna,
534 1960 infecting littoral cichlids of Lake Tanganyika (Vanhove et al., 2015). Interestingly, also
535 in Lake Tanganyika's pelagic zone, the same pattern as in *D. lacustre* comb. nov., apparently
536 without host preference or host-related speciation processes, was observed in *Cichlidogyrus*
537 *casuarinus*, a parasite of bathybatine cichlids (Kmentová et al., 2016). However, the
538 haplotype and nucleotide diversity in the COI region are remarkably lower in *D. lacustre*
539 comb. nov. (0.517 and 0.001 compared to 0.987 and 0.0205, respectively). Host species
540 hybridisation might explain the more generalist life style of certain monogeneans due to an
541 influence of host genetics on the susceptibility to infection, host specificity, and parasite
542 speciation (Šimková et al., 2013; Vanhove et al., 2011). However, there are no reports of
543 hybridisation among latid species (Otero, 2004). Moreover, a lack of host-related speciation in
544 diplectanids was observed in *Pseudorhabdosynochus cyanopodus* Sigura & Justine, 2008

545 infecting two deep-sea grouper species in New Caledonia (Schoelinck et al., 2012) with a
546 maximum intraspecific distance of 1.2% in the COI region compared to 0.7% in *D. lacustre*
547 comb. nov. in Lake Tanganyika. Our results therefore correspond with previous studies in
548 marine and freshwater habitats where decreased host specificity in pelagic ecosystems was
549 proposed to increase the chance of finding a host if host species exhibit low population
550 densities (Kmentová et al., 2016; Rohde, 1980; Schoelinck et al., 2012).

551 Despite the generally high degree of endemism of macrofauna in Lake Tanganyika (Coulter,
552 1991; Salzburger et al., 2014; Snoeks, 2000), this might not be reflected in all
553 microinvertebrate taxa. *Dolicirroplectanum lacustre* comb. nov. and some other monogenean
554 species are found to naturally occur both within and outside Lake Tanganyika. *Gyrodactylus*
555 *sturmbaueri* Vanhove, Snoeks, Volckaert & Huyse, 2011 was described from *Simochromis*
556 *diagramma* (Günther, 1894), but also parasitizes on *Pseudocrenilabrus philander* (Weber,
557 1897) in Lake Kariba and the River Nwanedi (Zahradníčková et al., 2016). *Cichlidogyrus*
558 *mbirizei* Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle 2012, *C. halli* (Price and
559 Kirk 1967) and *Scutogyrus gravivaginus* (Paperna and Thurston 1969) are known from an
560 endemic tilapia, *Oreochromis tanganicae* (Günther, 1894), but were also reported from other
561 species of *Oreochromis* Günther, 1889 and other cichlids in Africa (Douëllou, 1993; Pariselle
562 and Euzet, 2009) as well as cage-cultured tilapia species in Asia (Lim et al., 2016; Mohd
563 Agos et al., 2016). This highlights the ability of some monogenean species to survive in a
564 wide range of environments and host species (Huyse et al., 2006).

565

566 **Rate of molecular evolution of *Dolicirroplectanum lacustre* comb. nov. and its**
567 **implications**

568 For want of paleontological data, substitution rates in parasitic flatworms are typically
569 estimated using host fossils or calibrated with paleogeographical events and assuming that

570 parasite speciation follows that of the hosts (Meinilä et al., 2004). With a mean distance of
571 10% between the Lake Tanganyika and Lake Albert populations, the substitution rate in our
572 412 bp COI mtDNA region, using the end of rifting in the eastern African Rift Valley 9 MYA
573 (Cohen et al., 1993) for the age of the most recent common ancestor of their hosts, is
574 estimated at 0.5%/MY.

575 Often, molecular evolution of parasites is considered and was proven to be faster than within
576 the homologous loci of their hosts (Hafner et al., 1994; Huyse et al., 2005). Surprisingly, *D.*
577 *lacustre* comb. nov. appears to have a slower rate of molecular evolution in its mitochondrial
578 DNA than most fish taxa (1-4% in cytochrome *b*) (Bermingham et al., 1997; He and Chen,
579 2007; Muss et al., 2001). However, preliminary molecular data of African latids show even
580 less difference over the COI region (4.5-6% uncorrected p-distance between Lake Tanganyika
581 and Lake Albert, own unpublished data) than their monogenean parasite. Therefore, the
582 widespread hypothesis of a faster evolutionary rate of parasites compared to hosts, based on
583 their shorter generation time (Cable and Harris, 2002; Thomas et al., 2010) does hold in the
584 case of *D. lacustre* comb. nov. The link between the rate of morphological and molecular
585 evolution varies among different taxa (Omland, 1997) with studies showing either rate
586 decoupling (Poisot et al., 2011) or rate correlation. However, it seems that in the case of *D.*
587 *lacustre* comb. nov., there is a correlation between a slow rate of molecular evolution in the
588 COI gene, which is a structural coding marker known to be under balancing selection (Wu et
589 al., 1997), and failure to diverge seen in the lack of speciation.

590 There are three possible scenarios explaining the observed situation in *D. lacustre* comb. nov.
591 First, the rate of evolution of latids and their parasites is slower in comparison to other fish
592 (Bermingham et al., 1997; Muss et al., 2001) and other monogenean taxa. Indeed, the
593 mutation rate of *D. lacustre* comb. nov. seems to be much lower than the 13.7 – 20.3% per
594 million years estimated for *Gyrodactylus* von Nordmann, 1832 by Meinilä et al. (2004). This

595 can be explained by their different life history, as diplectanids lack the asexual reproduction
596 that has also led to a high species richness promoted by host switches and peripatric
597 speciation processes in *Gyrodactylus* (Boeger et al., 2003). The failure to diverge of *D.*
598 *lacustre* comb. nov. in Lake Tanganyika then corresponds with the hypothesis suggesting that
599 a lower rate of molecular evolution resulted in low diversification.

600 Secondly, the invasion of the studied latid lineage could be more recent than the lakes'
601 formation, like in the case of the cichlid genera *Tylochromis* Steindachner, 1895 and
602 *Oreochromis* (Klett and Meyer, 2002; Koch et al., 2007). This could explain the low level of
603 genetic intralacustrine variation of *D. lacustre* comb. nov. reported in Lake Tanganyika (0.7%
604 of uncorrected p-distance in COI) which contrasts with greater genetic variation seen in the
605 host species (2-3% of uncorrected p-distance in COI, unpublished data). However, low
606 intralacustrine genetic diversity in *D. lacustre* comb. nov. could also be caused by bottleneck
607 events that have reduced the genetic variation present in the system.

608 A third possible scenario involves a latid origin in the proto-Tanganyikan region with more
609 recent admixture of populations via lacustrine and riverine connections resulting in the
610 polyphyly of latid species in Lake Tanganyika (suggested by Otero (2004) based on
611 morphological data), as was documented for haplochromine cichlids in the lake (Meyer et al.,
612 2015; Salzburger et al., 2005). Therefore, molecular and morphological similarity of *D.*
613 *lacustre* comb. nov. in nowadays geographically isolated areas could be the result of recent
614 and maybe multiple episodes of gene flow. In any case, phylogenetic reconstruction combined
615 with the latids' fossil record is needed to discern between the above-mentioned hypotheses.

616 **Conclusions**

617 Diplectanid parasites occur primarily in marine environments. Discussion has arisen about the
618 incongruence between their morphological species delineation and the level of molecular

differentiation. In our study, we focused on a unique allopatric situation of diplectanid parasites infecting latid species inhabiting freshwater lakes to study this incongruence. Based on morphological examination, a single diplectanid species was recorded from three of the four species of *Lates* from Lake Tanganyika (*L. angustifrons, L. mariae, L. microlepis*) and from *L. niloticus* from Lakes Albert, Kossou, Nasser and Victoria, from the Taja River in Sierra Leone and from Bahr-Sara in Tchad. Thus, similar to another monogenean species infecting pelagic host species of the cichlid tribe Bathybatini in Lake Tanganyika, this parasite on African *Lates* apparently failed to diverge. Results of phylogenetic reconstruction combined with detailed morphological characterisation led us to propose *Dolicirroplectanum* gen. nov. with *D. lacustre* comb. nov. as type species. Despite a persistent geographic barrier between Lake Albert and Lake Tanganyika and speciation in the hosts, their respective populations of *D. lacustre* comb. nov. infecting these lakes' latid fishes have not reached species-level distinction. We suggest a link between the lack of morphological differentiation between the parasite populations in both lakes, and the low rate of molecular evolution of the mitochondrial COI gene, estimated at 0.5%/MY (assuming *Lates* from Lakes Albert and Tanganyika diverged 9 MYA). As alternatives, scenarios proposing either more recent invasion of the latid lineage into Lake Tanganyika or recent gene flow among the latid lineages in Lakes Albert and Tanganyika could explain the apparently slow rate of the hosts' molecular evolution and lack of parasite differentiation. Therefore, detailed studies of host phylogeography, dated using the fossil record, are needed to discern between these scenarios. Although species-level differentiation can be expected in the future under persisting separation between the lakes, the question about the existence of genetically intermediate populations of *D. lacustre* comb nov. remains.

Acknowledgments

The authors would like to thank: Ludmila Raisingerová, Chahrazed Rahmouni, Mulongaibalu Mbalassa, Vercus Lumami Kapepula, Joseph Mbirize Ndalozibwa, Jos Snoeks and Christian Sturmbauer for help with obtaining or identifying samples. Fieldwork was carried out with the approval of the competent local authorities under mission statement 031/MINRST/CRH-U/2016 and an export permit from the CRH-Uvira and from the National Fish and Fisheries Research Institute (Nafirri, Jinja, Uganda). We also thank Tine Huyse, Nathan Vranken, Emmanuel Vreven, Miguël Parrent (RMCA), Filip Volckaert (KU Leuven) and people in the parasitological group, Masaryk University, Brno and Research Centre of Hydrobiology, Uvira for their hospitality and the RMCA for providing access to the collections.

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Funding

Research was supported by the Czech Science Foundation (GBP505/12/G112 – ECIP) and GA19-13573S), Belgian Science Policy (BELSPO), EMBRC Belgium - FWO project GOH3817N as well as by a joint program between the Austrian agency for international mobility and cooperation in education, science and research and the Ministry of Education, Youth and Sports (project number 8J18AT007), the Austrian Agency for International Cooperation in Education and Research (OEAD; project number CZ 08/2018; to SK) and the Systematics Research Fund (SRF; to SK).

967 Table 1: An overview of host species examined for monogenean parasites with locality and

968 country.

Host species	Locality (geographic coordinates, year)	Locality – subbasins (Danley et al., 2012) or country	Number of fish specimens examined (accession number in RMCA)	Number of infected fish specimens	Number of monogenean individuals*
		Lake Tanganyika			
L. angustifrons	Mpulungu (08°46'S-31°07'E, 27.7.1967)	The southern subbasin	1 (MRAC 190480)	1	2
	Mpulungu (12.4.2018)	The southern subbasin	4(-)	1	15
	Rumonge (03°58'S-29°25'E, 30.6.1967)	The northern subbasin	2 (MRAC 94069.0052-53)	2	3
	Sumbu Bay (08°31'S-30°29'E, 31.3.1947)	The southern subbasin	1 (MRAC 90850)	1	1
L. mariae	Bujumbura (03°23'S-29°22'E, 5.5.1947)	The northern subbasin	5 (MRAC 90908-912)	5	14
	Ilagala (05°12'S-29°50'E, 20.8.1993)	The northern subbasin	3 (MRAC 93152.0318-20)	2	10
	Kilomoni (04°20′S, 29°09′E, 12.8.2016)	The northern subbasin	2 (-)	1	1
	Mpulungu (27.7.1967)	The southern subbasin	2 (MRAC 190493-94)	1	3
	Mpulungu (16.4.2018)	The southern subbasin	7 (-)	0	0
	Mulembwe (06°07′S 29°16′E, 9.4.2010)	The central subbasin	7 (-)	3	19
	Nyanza (04°20'S-29°35'E, 1.1.1997)	The northern subbasin	1 (MRAC 53738)	1	23
	Rumonge (30.6.1994)	The southern subbasin	1 (MRAC 94069.0067)	0	0
	Sumbu Bay (31.3.1947)	The southern subbasin	2 (MRAC 90878-79)	2	22
L. microlepis	Bujumbura (4.5.1947)	The northern subbasin	5 (MRAC 90805-9)	5	24
	Crock Island (08°42'S-31°07'E, 16.4.2018)	The southern subbasin	8 (-)	2	13
	Edith Bay (06°30'S-29°55'E, 30.5.1947)	The southern subbasin	3 (MRAC 90833-35)	5	10
	Katukula (08°35'S-31°10'E, 14.4.2018)	The southern subbasin	5 (-)	4	22
	Moba Bay (30.12.1995)	The central subbasin	2 (MRAC 90725-6)	0	0
	Mpulungu (13.4. – 17.4. 2018)	The southern subbasin	13 (-)	6	29
	Nyanza Lac (1.1.1937)	The northern subbasin	11 (MRAC 53698-703; 53725-29)	11	181
	Sumbu Bay (9.4.1995)	The southern subbasin	3 (MRAC 95096.1192,98,99)	1	1
	Uvira (03°22′ S 29°09′E, 12.8.2016)	The northern subbasin	7 (-)	2	17
L. stappersii	Karala (05°33'S-29°28'E, 10.4.1947)	The northern subbasin	1 (MRAC P90928)	0	0
	Kasasa (08°31'S-30°42'E, 6.9.1967)	The southern subbasin	3 (MRAC 190126;35-6)	0	0
	Mpulungu (12.4.2008)	The southern subbasin	3 (-)	0	0
	Mpulungu (6.4.2018)	The southern subbasin	3 (-)	0	0

	Uvira (12.8.2016)	The northern subbasin	28 (-)	0	0
		Other localities			
L. niloticus	Bahr-Sara (08°56'N-17°58'E, 1. -. 31.3.1965)	Tchad	1 (MRAC 154006)	1	2
	Kisumu, Lake Victoria (00°06'S-34°45'E, 17.12.1991)	Kenya	3 (MRAC 91104.37-39)	2	8
	Kossou (07°10'N-05°20'E, 17.12.1973)	Ivory Coast	5 (MRAC 74014.328-29; 2755-56)	4	22
	Lake Nasser (24°05'N-33°00'E, 26.2. – 11.3.1984)	Egypt	3 (MRAC 84006.0116-18)	0	0
	Lake Nasser (1.9.-30.9.1983)	Egypt	2 (MRAC 83030.0114-15)	1	1
	Luxor market (25°42'N 32°38'E, 24.11.2000)	Egypt	1 (MRAC 190480)	0	0
	Njala, riv. Taja (08°06'N-12°04'e, 12.4.1969)	Sierra Leone	5 (MRAC 73010.7057-61)	1	8
	Nyawiega, Lake Albert (01°28'N-30°56'E, 21.11.-6.12.1989)	Uganda	3 (MRAC 89059.0279)	1	2
	Nzunzu, Lake Albert (01°19'N-30°72'E, 5.4.-6.4.2017)	Uganda	1 (MRAC 2016.036.P)	1	2
	Nzunzu, Lake Albert (5.4.-6.4.2017)	Uganda	11 (MRAC 2016.036.P)	2	18

* Only one gill arch examined in the case of specimens retrieved from the RMCA

973 Table 2: Comparison of measurements performed on haptoral and genital hardparts of *Dolicirroplectanum lacustre* comb. nov. reported in Thurston
974 and Paperna (1969) from Lake Volta, in Ergens (1981) from Cairo and in this study with host species and locality (a – mean value±standard
975 deviation, b – range).

Parameters (µm)	*L. niloticus*, Lake Volta	*L. niloticus*, Cairo	*L. angustifrons*, Lake Tanganyika	*L. mariae*, Lake Tanganyika	*L. microlepis*, Lake Tanganyika
Total length	650-1000	-	544,2 (n=1)	522,0±35,4[a] (487,4 - 587,3)[b]; n=11	675,0±66,9 (588,1 - 813,1); n=13
Total width	150-250	-	188,7 (n=1)	178,8±39,5 (115,5 - 243,3); n=11	183,2±15,1 (4,7 - 204,1); n=13
Ventral anchor					
Length to notch	19-20	20-22 (n=3)	20,4 (19,5 - 21,3); n=7	19,3±1,4 (16,3 - 24,4); n=25	18,7±0,9 (16,4 - 21,2); n=61
Total length	70-80	53-57 (n=3)	43,2 (41,4 - 44,1; n=7)	42,6±2,0 (41,1 - 47,2); n=28	43,2±2,4 (40,5 - 48,3); n=62
Length to inner root	-	-	22,8 (20,9 - 24,8); n=7	22,6±1,6 (18,3 - 26,9); n=27	22,2±1,1 (18,7 - 24,9); n=52
Inner root length	10-20	10-12 (n=3)	8,7 (7,2 - 9,8); n=7	9,8±0,8 (7,7 - 11,2); n=28	9,2±1,1 (6,1 - 11,4); n=54
Outer root length	40-60	32-35 (n=3)	22,6 (20,1 - 24,4); n=7	23,7± (19,1 - 27,9); n=24	24,8±2,6 (19,2 - 30,1); n=52
Point length	5-7	9-10 (n=3)	8,2 (7,3 - 9,5); n=6	7,2±1,1 (5,4 - 9,2); n=25	7,0±1,1 (4,9 - 9,0); n=45
Dorsal anchor					
Total length	40-50	44-47 (n=3)	35,3 (32,6 - 36,6); n=6	32,3±2,0 (28,4 - 35,6); n=25	33,4±2,1 (26,0 - 38,6); n=47
Point length	-	9-10 (n=3)	7,4 (6,9 - 8,0); n=6	6,3±0,9 (4,7 - 8,1); n=13	6,0±0,9 (4,1 - 8,2); n=31
Ventral bar					
Straight length	50-60	38-44 (n=3)	44,8 (40,7 - 51,5); n=4	50,8±6,4 (39,2 - 63,3); n=30	46,6±4,5 (36,4 - 55,5); n=58
Maximum width	-	8-11 (n=3)	8,2 (7,6 - 9,2); n=6	11,4±2,1 (7,2 - 15,3); n=27	9,3±1,7 (6,1 - 13,2); n=52
Dorsal bar					
Straight length	35-40	34-38 (n=3)	31,5 (29,9 - 33,5); n=6	37,6±3,9 (29,6 - 46,1); n=29	32,8±3,5 (24,3 - 39,5); n=60
Maximum width	-	9-12 (n=3)	8,9 (7,7 - 9,8); n=5	7,2±1,4 (5,1 - 9,6); n=28	6,5±0,9 (4 - 9,0); n=59
Ventral squamodisc					
Length	30-40	38-44 (n=3)	41,6 (35,2 - 48,6); n=4	38,4±7,6 (32,3 - 57,4); n=15	38,3±6,5 (26,7 - 57,2); n=22
Width	50-70	38-51 (n=3)	47,8 (46,2 - 50,0); n=4	41,9±7,3 (29,8 - 60,4); n=16	42,5±4,4 (33,3 - 53,1); n=22
Dorsal squamodisc					
Length	-	38-44 (n=3)	35,9 (33,6 - 39,6); n=4	34,8±4,8 (29,5 - 43,4); n=6	34,9±5,8 (21,3 - 42,9); n=16
Width	-	38-51 (n=3)	42,9 (41,5 - 45,0); n=4	38,0±7,9 (33,4 - 57,2); n=8	40,4±4,3 (30,0 - 46,2); n=16
Hook			10,5 (9,5 - 11,3); n=4	10,4±0,6 (9,4 - 11,8); n=27	10,0±0,7 (7,9 - 11,7); n=49
Copulatory tube straight length	21-23	-	27,6 (24,5 - 29,2); n=3	33,9±5,2 (24,1 - 43,6); n=20	32,3±3,9 (21,4 - 41,5); n=38

Copulatory tube width	-	-	20,6 (19,6 - 21,3); n=3	20,6±4,0 (13,5 - 29,1); n=22	33,4±2,1 (26,0 - 38,6); n=47
Vagina	Not reported	Not reported	Reported in 0 out of 5 available specimens	Reported in 2 out of 24 available specimens	Reported in 0 out of 38 available specimens
Total length	-	-	-	22,1±0,8 (21,5 - 22,6); n=2	-
Tube length	-	-	-	5±0,6 (4,6 - 5,4); n=2	-
Point length	-	-	-	6±0,3 (5,8 - 6,2); n=2	-
Eyes spots					
Smaller pair distance	-	-	44,2 (40,0 - 48,4); n=2	37,3±6,3 (30,0 - 50,0); n=14	-
Larger pair distance	-	-	42,6 (42,2 - 43,0); n=2	32,9±5,1 (26,8 - 46,7); n=14	-
Pharynx length	-	-	-	34,3±9,9 (22,9 - 53,0); n=8	-
Testes					
Length	-	-	-	94,3 (n=1)	-
Width	-	-	-	42,7±13,9 (29,7 - 78,1); n=13	-
Ovary width	-	-	-	31,0±10,9 (31,0 - 64,6); n=14	-

Parameters (µm)	***L. niloticus*, Lake Albert1**	***L. niloticus*, Lake Albert2**	***L. niloticus*, Lake Victoria**	***L. niloticus*, river Taja**	***L. niloticus*, Lake Kossou**
Total length	736,5±115,3[a] (644,1 - 931,3)[b]; n=5	553,1±52,1(497,2 - 600,3); n=3	537,9±34,8 (509,2 - 576,6); n=3	662,1±113,8 (510,9 - 787,0); n=5	574,7±22,2 (550,3 - 593,7); n=3
Total width	282,0±57,6 (191,4 - 333,9); n=5	182,8±46,9 (151,7 - 236,7); n=3	220,5±11,4 (210,6 - 233,0); n=3	185,0±56,4 (141,7 - 277,8); n=5	217,3±13,6 (208,3 - 233,0); n=3
Ventral anchor					
Length to notch	21,1±1,1 (19,3 - 23,1); n=18	19,3±1,2 (18,2 - 20,6); n=3	20,5±0,6 (19,7 - 21,1); n=4	17,1±0,7 (16,2 - 18,1); n=6	20,9±1,0 (19,5 - 23,1); n=10
Total length	53,4±2,9 (50,0 - 66,4); n=18	42,1±2,1 (40,2 - 44,0); n=3	43,1±1,4 (42,7 - 45,8); n=5	34,4±1,2 (32,8 - 36,5); n=6	43,9±1,8 (40,5 - 45,9); n=10
Length to inner root	24,7±1,9 (21,0 - 29,4)	23,8±0,8 (22,9 - 24,3); n=3	24,9±1,4 (23,3 - 25,8); n=3	21,0±1,1 (19,6 - 22,5); n=5	23,7±1,2 (21,4 - 25,2); n=8
Inner root length	11,3±0,7 (10 -12,9); n=18	10,7±1,0 (9,8 - 11,8); n=3	10,5±0,9 (9,5 - 11,5)	8,9±0,7 (8,1 - 9,7); n=6	10,0±0,9 (8,0 - 11,1); n=10
Outer root length	32,5±3,3 (29,7 - 43,2); n=18	23,3±1,6 (22,0 - 25,0); n=3	24,1±1,5 (22,7 - 25,7); n=3	17,5±13,4 (15,6 - 19,4); n=6	23,5±1,3 ((21,5 - 25,7); n=10
Point length	7,9±1,1 (6 - 9,4); n=18	8,5±0,7 (7,9 - 9,3); n=3	9,5±0,9 (8,8 - 10,7)	7,4±1,1 (5,6 - 8,3); n=5	8,0±1,3 (5,4 - 9,6); n=9
Dorsal anchor					
Total length	47,3±4,0 (42,7 - 60,2); n=17	39,0±1,0 (37,6 - 39,9); n=3	40,7±0,4 (40,2 - 41,0); n=3	28,1±1,0 (27,0 - 29,6); n=6	39,9±1,8 (36,7 - 42,4); n=10
Point length	7,9±1,0 (5,9 - 9,2); n=12	7,7±1,2 (6,8 - 8,5); n=2	8,9±0,6 (8,6 - 9,6); n=3	6,6±1,4 (4,8 - 8,6); n=5	7,8±0,6 (7,3 - 8,5); n=3
Ventral bar					
Straight length	72,6±4,8 (61,5 - 78,8); n=16	59,1±11,5 (52,2 - 72,4); n=3	45,8±6,4 (36,5 - 50,0); n=4	40,0±0,9 (39,2 - 40,5); n=2	50,1±1,6 (47,8 - 52,3); n=7
Maximum width	18,5±1,9 (13,5 - 21,0); n=11	15,3±0,4 (15,0 - 15,6); n=2	15,3 (n=1)	10,2±2,7 (8,3 - 12,1); n=2	13,2±1,4 (11,2 - 14,7); n=7

Dorsal bar					
Straight length	55,4±3,2 (47,6 - 59,9); n=17	41,0±2,1 (39,5 - 42,4); n=2	39,0±2,8 (36,7 - 42,1); n=3	30,0±2,9 (26,1 - 33,5); n=6	37,4±2,5 (32,7 - 40,6); n=10
Maximum width	18,4±2,3 (13,5 - 22,0); n=15	16,5±2,7 (13,8 - 19,6); n=4	7,4±0,5 (6,9 - 7,8); n=3	6,3±2,1 (4,3 - 9,8); n=5	9,3±2,7 (6,5 - 13,9); n=8
Ventral squamodisc					
Length	64,1±7,2 (51,8 - 80,4); n=14	38,4 (n=1)	35,0±2,8 (32,4 - 39,6); n=5	32,1±5,7 (27,9 - 38,6); n=3	34,6±4,4 (29,8 - 38,6); n=3
Width	78,3±13,0 (62,5 - 118,4); n=14	44,0 (n=1)	51,8±3,5 (48,1 - 55,9); n=5	36,4±6,4 (31,5 - 43,7); n=3	50,9±3,0 (47,5 - 52,9); n=3
Dorsal squamodisc					
Length	61,4±11,1 (45,4 - 85,6); n=11	39,0±1,8 (36,9 - 40,1); n=3	32,0±2,4 (29,6 - 35,7); n=5	24,3±2,8 (21,3 - 26,8); n=3	35,3±4,3 (30,3 - 40,1); n=4
Width	63,9±6,3 (49,9 - 71,2); n=11	48,8±10,5 (42,3 - 60,9); n=3	42,9±3,5 (38,8 - 46,9); n=5	24,9±0,8 (24,2 - 25,8); n=3	41,9±2,4 (38,4 - 43,5); n=4
Hook	11,2±0,8 (10,3 - 14,0); n=18	10,4±1,0 (9,3 - 11,0); n=3	11,4± (11,0 - 12,2); n=4	9,7±0,7 (8,4 - 10,3); n=6	11,3±0,9 (10,1 - 13,2); n=11
Copulatory tube straight length	58,3±8,8 (44,5 - 83,1); n=18	44,3±3,8 (40,8 - 49,5); n=4	43,7±11,8 (36,1 - 64,6); n=5	27,3±2,9 (24,2 - 31,2); n=4	35,5±4,8 (29,2 - 42,0); n=6
Copulatory tube width	36,2±4,3 (27,2 - 44,5); n=18	27,6±5,3 (21,0 - 33,2); n=4	26,1± (24,5 - 28,0); n=5	26,1±3,6 (21,2 - 30); n=4	22,1±2,3 (18,2 - 24,5); n=6
Vagina	Reported in 18 out of 18 available specimens	Reported in 4 out of 4 available specimens	Reported in 1 out of 6 available specimens	Reported in 0 out of 6 available specimens	Reported in 3 out of 11 available specimens
Total length	41,1±4,5 (34,0 - 47,3); n=14	35,7±5,6 (30,1 - 41,3); n=3	-	-	-
Tube length	7,8±1,1 (4,8 - 9,3); n=14	6,9±1,4 (5,3 - 7,9); n=3	6,7 (n=1)	-	-
Point length	7,0±0,7 (6,0 - 8,5); n=16	8,0±1,1 (6,7 - 8,7); n=3	8.9 (n=1)	-	-
Eyes spots					
Smaller pair distance	56,7±10,2 (42,8 - 80,4); n=16	-	-	-	-
Larger pair distance	47,2±8,5 (34,9 - 64,6); n=17	-	-	-	-
Pharynx length	56,3±11,0 (39,9 - 73,2); n=10	-	-	-	-
Testes		-	-		-
Length	102,1±46,7 (57,9 - 150,9); n=3	-	-	-	-
Width	70,3±16,2 (50,5 - 103,2); n=9	-	-	-	-
Ovary width	72,7±11,1 (50,7 - 88,9); n=9	-	-	-	-

982 Table 3: Genetic intraspecific variability indices in a 412 bp portion of COI mtDNA region of *Dolicirroplectanum lacustre* comb. nov.

	Maximum uncorrected p-distance (number of individuals)	**Nucleotide diversity**	**Haplotype diversity**	**Number of polymorphic sites**
Lake Albert	1.2% (14)	0.0036+/-0.0026	0.8022+/-0.0936	6
Lake Tanganyika	0.7% (38)	0.0019+/-0.0016	0.5747+/-0.0713	4

983

Figure captions

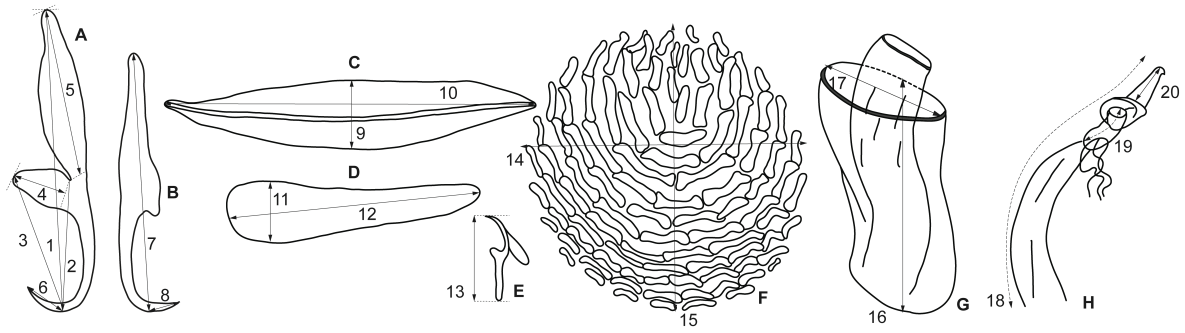


Figure 1: Measurements for sclerotized structures of haptor and reproductive organs of *Dolicirroplectanum lacustre* comb. nov. A Ventral Anchor: 1—Total length, 2—Length to notch, 3—Length to inner root, 4—Inner root length, 5—Outer root length, 6—Point length; B Dorsal anchor: 7—Total length, 8—Point length; C Ventral bar: 9—Straight length 10—Maximum width; D Dorsal bar: 11—Straight length, 12—Maximum width; E Hook: 13—Hook length; F Squamodisc: 14—Squamodisc length, 15—Squamodisc width; G Male copulatory organ: 16—Copulatory tube length, 17—Copulatory tube width; H Vagina: 18—Total length, 19—Tube length, 20—Point length.

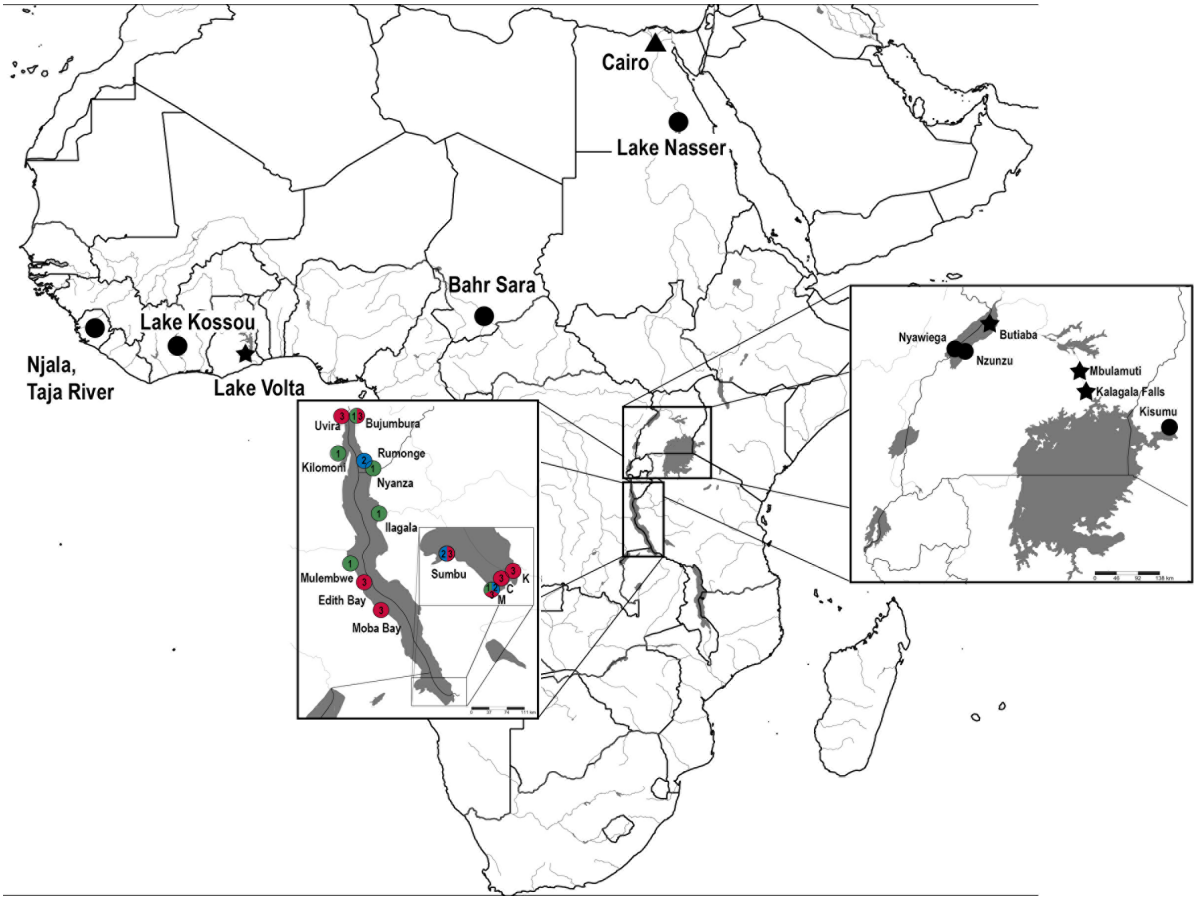
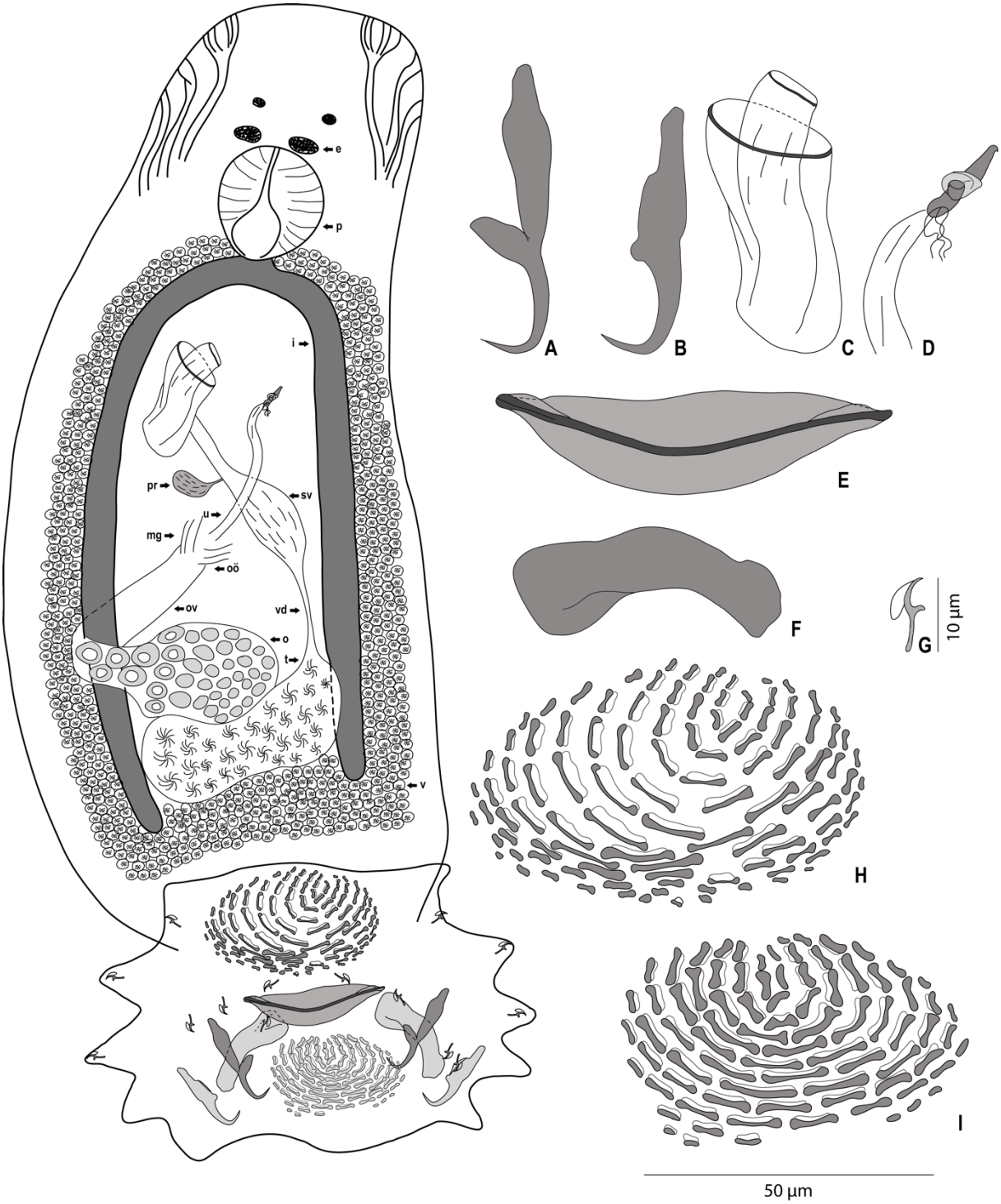


Figure 2: Localities with confirmed presence of *Dolicirroplectanum lacustre* comb. nov. Star – localities sampled for the original description of *D. lacustre* by Thurston & Paperna, 1969, triangle – locality documented by Ergens, 1981, circle – localities sampled in this study. M – Mpulungu, C – Crocodile Island, K – Katukula. Colours denote host species: black – *Lates niloticus*, blue (number 2) – *L. angustifrons*, green (number 1) – *L. mariae*, red (number 3)– *L. microlepis*. Map created using SimpleMappr software v7.0.0. (available at http://www.simplemappr.net. Accessed February 25, 2018).



1002

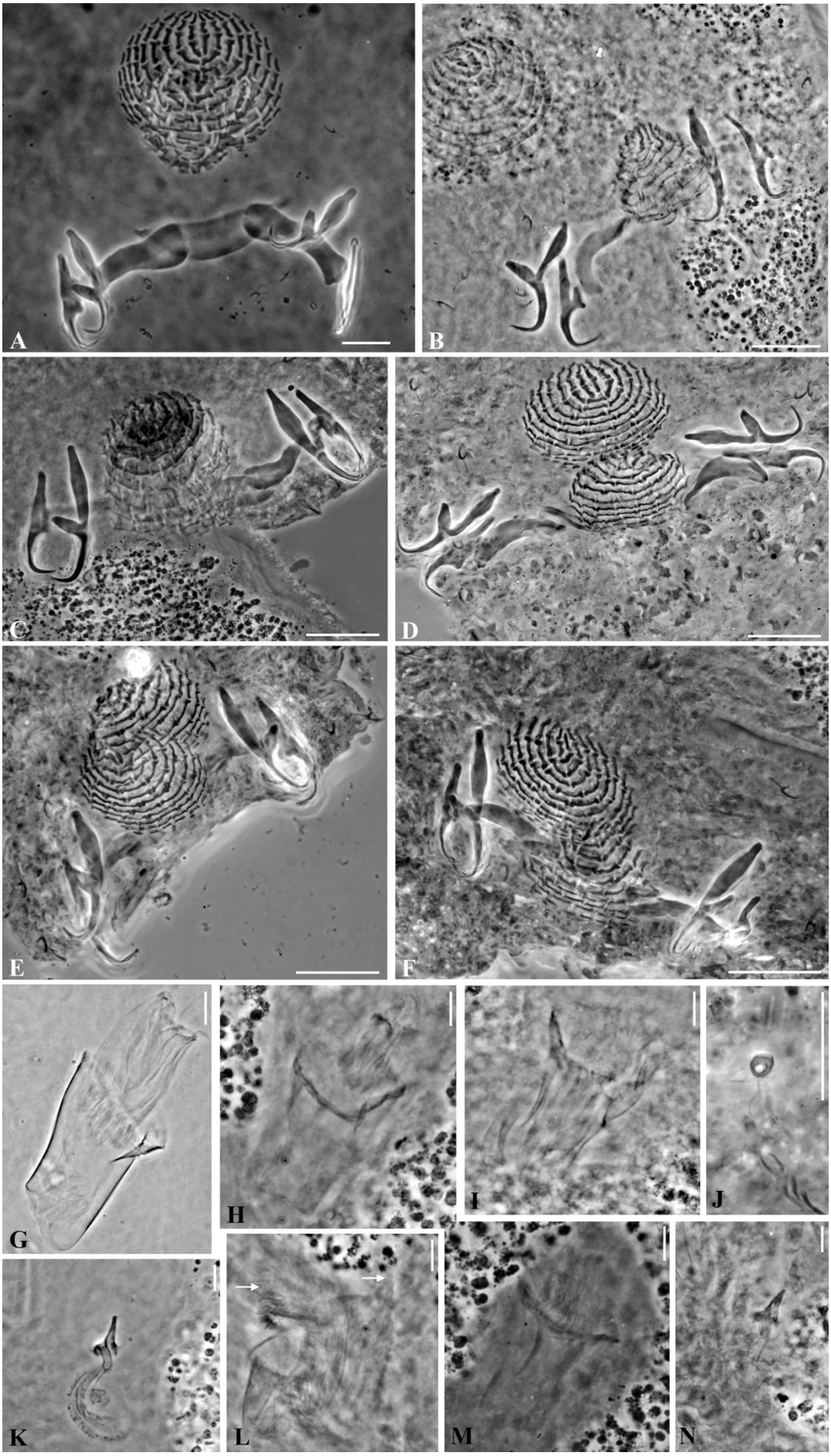
1003 Figure 3: *Dolicirroplectanum lacustre* comb. nov. collected from *Lates niloticus* in Lake Albert.

1004 Specimen drawn from the ventral view. e, eye spots; i, intestine; mg, Mehlis' glands; o, ovary;

1005 oö, oötype; ov, oviduct; p, pharynx; pr, prostatic reservoir; sv, seminal vesicle; t, testes; u,

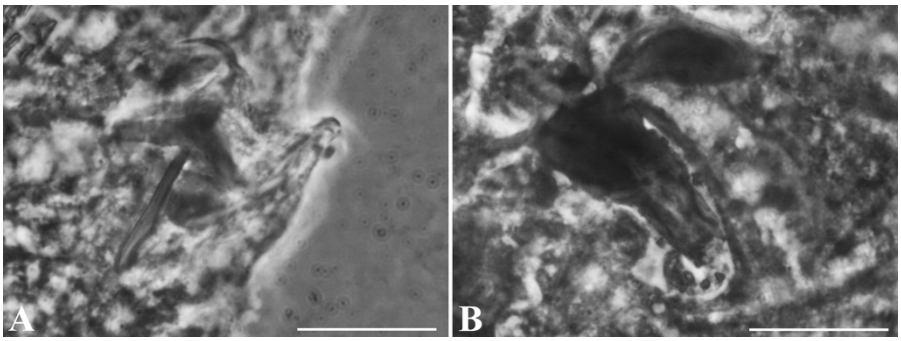
1006 uterus; v, vittelaria; vd, vas deferens, A, ventral anchor; B, dorsal anchor; C, male copulatory

1007 organ; D, vagina, E, ventral bar; F, dorsal bar; G, hook; H, ventral squamodisc; I, dorsal
1008 squamodisc.



1009

1010 Figure 4: Haptoral and male genital sclerotised structures of *Dolicirroplectanum lacustre* comb.
1011 nov. from different host species and localities collected in this study (scale bars A-F: 25 µm;
1012 G-N: 10 µm). A) Opisthaptor, *L. niloticus* in Lake Albert B) Opisthaptor, *L. niloticus* in Taja
1013 River C) Opisthaptor, *L. microlepis* in Lake Tanganyika D) Opisthaptor, *L. niloticus* in Lake
1014 Victoria E) Opisthaptor, *L. niloticus* in Lake Kossou F) Opisthaptor, *L. mariae* in Lake
1015 Tanganyika G) Male copulatory organ, *L. niloticus* in Lake Albert H) Male copulatory organ,
1016 *L. mariae* in Lake Tanganyika I) Male copulatory organ, *L. niloticus* in Lake Kossou J)
1017 Sclerotised vagina, *L. mariae* in Lake Tanganyika K) Sclerotised vagina, *L. niloticus* in Lake
1018 Albert L) Male copulatory organ, *L. niloticus* in Taja River M) Male copulatory organ, *L.*
1019 *microlepis* N) Sclerotised vagina, *L. niloticus* in Lake Victoria. Pictures were stacked.

1020 

1021 Figure 5: Sclerotised structures of *Dolicirroplectanum penangi* comb. nov.. A) Ventral anchor
1022 (MNHN HEL1086), Hainan, China B) Male copulatory organ (USNPC 180-A 6), Zhanjiang,
1023 China. Scale bar: 20 µm; several layers in the picture were combined.

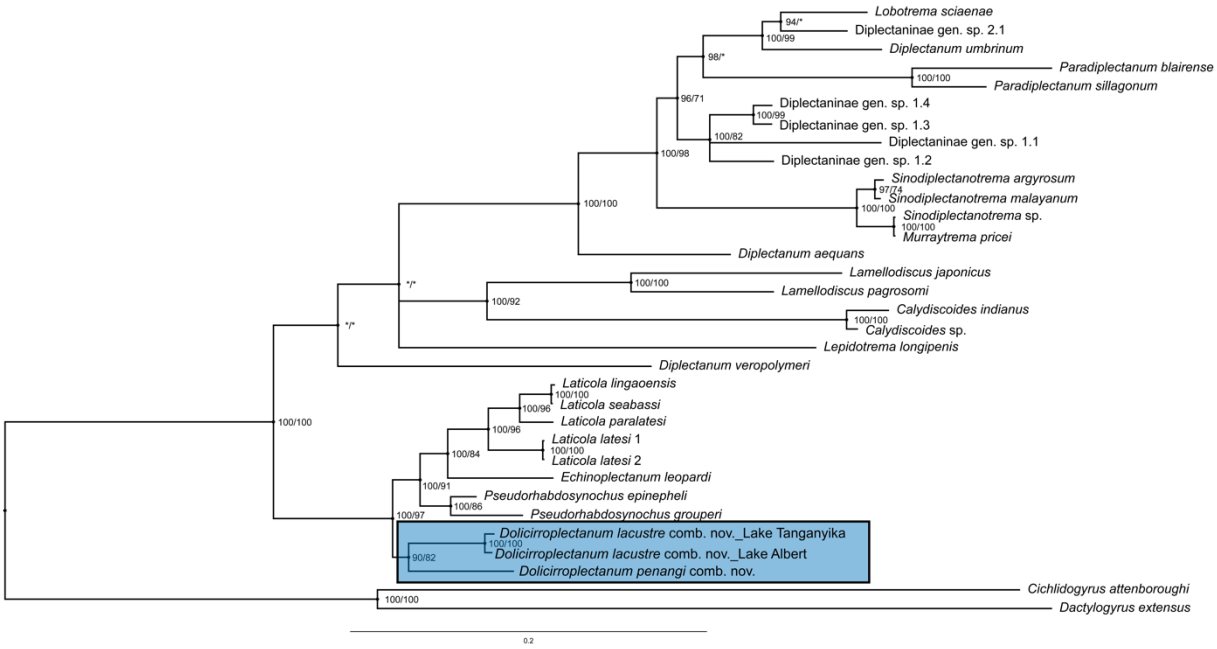


Figure 6: Bayesian inference phylogram based on 28S fragments from 33 haplotypes of different diplectanid species. Posterior probabilities for Bayesian inference (before slashes) and bootstrap percentages for maximum likelihood (behind slashes) are shown. The values lower than 90 of posterior probability and 80 for maximum likelihood are marked with an asterisk. The clade containing two lineages of *Dolicirroplectanum lacustre* comb. nov. is boxed. The scale-bar indicates the expected number of substitutions per site.

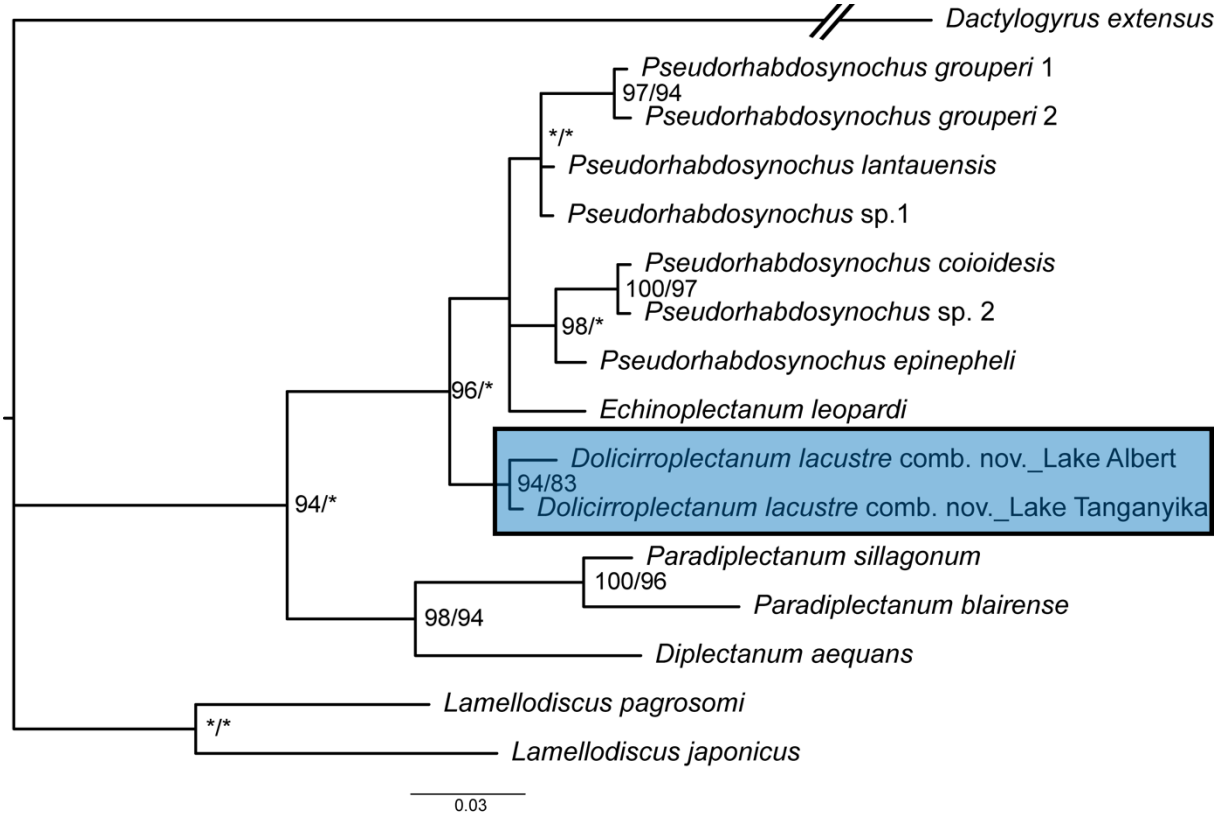


Figure 7: Bayesian inference phylogram based on 18S fragments from 15 haplotypes of different diplectanid species. Posterior probabilities for Bayesian inference (before slashes) and bootstrap percentages for maximum likelihood (behind slashes) are shown. The values lower than 90 of posterior probability and 80 for maximum likelihood are marked with an asterisk. The clade containing two lineages of *Dolicirroplectanum lacustre* comb. nov. is boxed. The scale-bar indicates the expected number of substitutions per site.

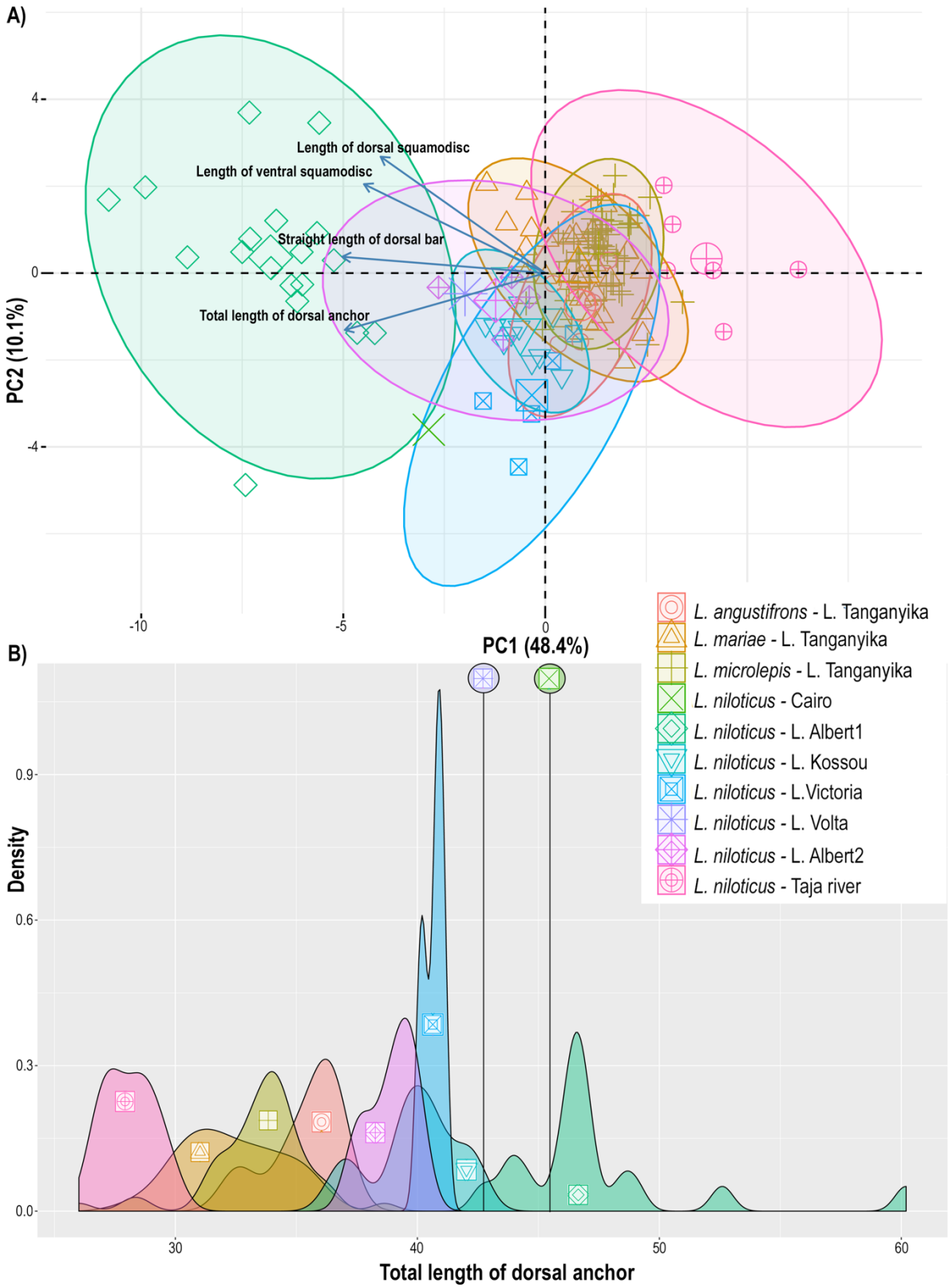
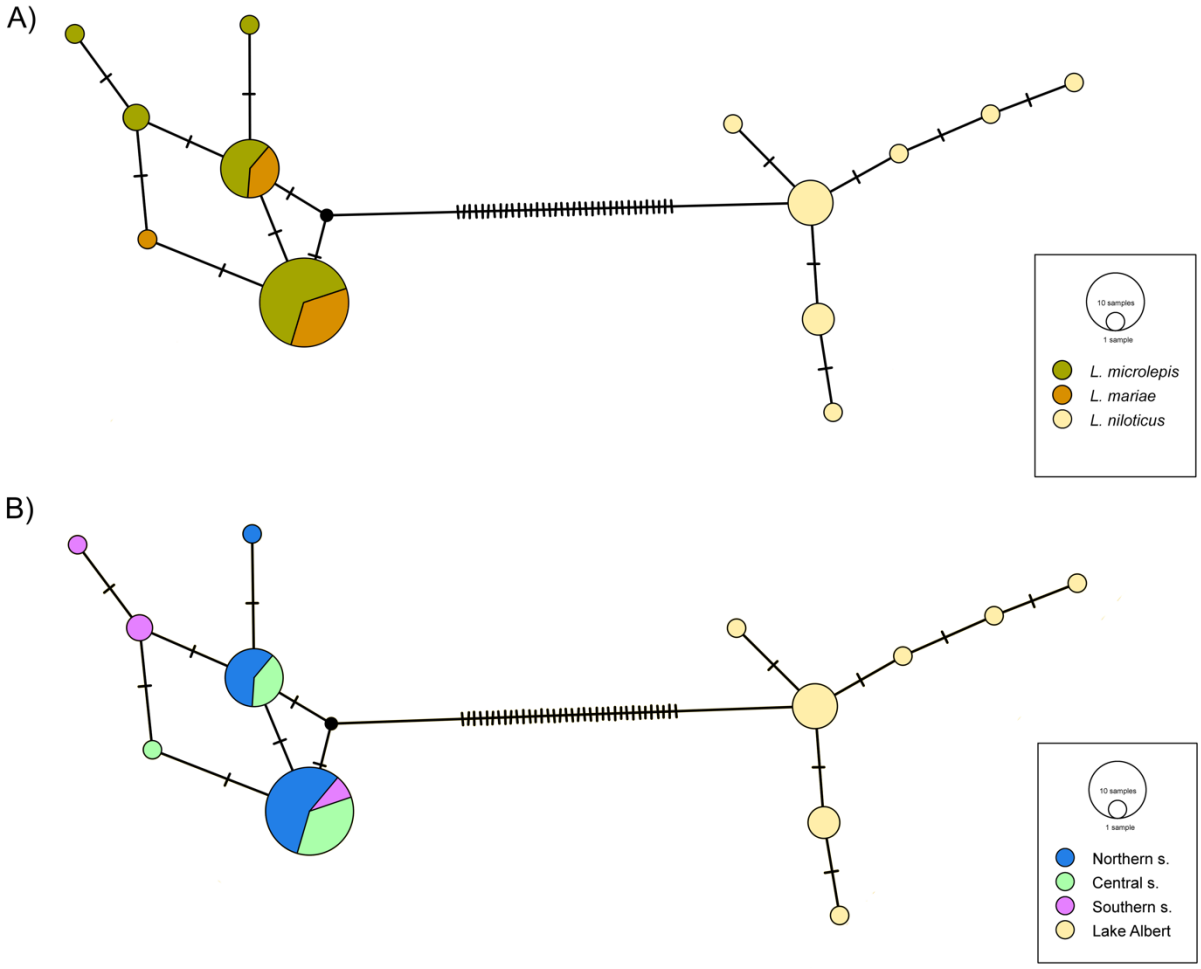


Figure 8: Morphometric variability of haptoral structures of *Dolicirroplectanum lacustre* comb. nov. A) biplot of PCA (first two axes) based on measurements of haptoral sclerotized structures.

1041 B) Density plots depicting the total size of the dorsal anchor. Colours and signs denote host

1042 species and locality of specimens.



1043

1044 Figure 9: Haplotype network of *Dolicirroplectanum lacustre* comb. nov. COI sequences (n =

1045 52). The circles represent different haplotypes with the size proportional to the number of

1046 individuals sharing this haplotype. Haplotypes are connected with lines, indicating the number

1047 of substitutions between haplotypes. Colours correspond to A) the host species and B)

1048 geographic origin (subbasins in Lake Tanganyika).