

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

Peer-reviewed author version

KMENTOVA, Nikol; Koblmuller, S; VAN STEENBERGE, Maarten; ARTOIS, Tom;  
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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>

1 **Failure to diverge in African Great Lakes: the case of**

2 ***Dolicirroplectanum lacustre* comb. nov. (Monogenea,**

3 **Diplectanidae) infecting latid hosts**

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26 **Abstract**

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

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

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

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

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56 **Introduction**

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

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

75 Among the parasitic flatworms known to infect lates perches (Latesidae) are diplectanids  
76 (Diplectanidae), a monogenean family with more than 250 species described worldwide,  
77 mainly from marine perciform fishes (Domingues and Boeger, 2008). The genus *Lates* L.,  
78 1758 consists of 11 species, seven of which inhabit African freshwaters, with the rest to be  
79 found in marine, brackish and freshwater habitats in the Indo-Pacific region (Otero, 2004).  
80 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 21<sup>st</sup> 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 haptor 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 Kruskal-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<sub>2</sub>, 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 °C, 39 cycles of 1 min at 95 °C, 1 min at 55 °C  
192 and 1 min and 30 s at 72 °C, and finally 10 min at 72 °C. 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<sub>2</sub>,  
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 °C,  
198 39 cycles of 20 seconds at 94 °C, 30 seconds at 58 °C and 1 min and 30 s at 72 °C, and finally  
199 10 min at 72 °C. Part of the mitochondrial COI gene was amplified using ASmit1 (5'-  
200 TTTTTTGGGCATCCTGAGGTTTAT-3') combined with Schisto3 (5'-  
201 TAATGCATMGGAAAAAACA-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<sub>2</sub>, 0.1 mg/ml BSA, 0.2 mM dNTPs,

205 0.8 mM of each primer) with 1  $\mu$ l of isolated DNA (concentration was not measured) in a  
206 total reaction volume of 25  $\mu$ l 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  $\mu$ g of PCR product was  
210 enzymatically cleaned up using 1  $\mu$ l of ExoSAP-IT reagent under the following conditions: 15  
211 min at 37 °C and 15 min at 80 °C. 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 +  $\Gamma$ , gamma shape parameter of 0.461; 18S rDNA: K2P +  $\Gamma$ , 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 1,000<sup>th</sup> 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, Matějusková, & 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,

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

### 263 **Taxonomy and species redescription**

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

265 **Family:** Diplectanidae Monticelli, 1903

266 **Genus:** *Dolicirroplectanum* gen. nov.

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

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

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

270 **Site:** Gills

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

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

274 **Material examined:** type material: MRAC MT. 35572, vouchers: MNHN HEL744-47 (4  
275 specimens), USNPC 180-A 3-7; MRAC. MT. 38206-10, 38913-39058 (243 specimens), MZH  
276 10067-71 (6 specimens), SAMC-A089971-72 (6 specimens), NHMUK 2018.4.13.4-13.7 (8  
277 specimens)

278 **Zoobank registration:** To comply with the regulations set out in article 8.5 of the amended  
279 2012 version of the International Code of Zoological Nomenclature (ICZN) (International  
280 Commission on Zoological Nomenclature, 2012), details of the genus have been submitted to  
281 ZooBank. The Life Science Identifier (LSID) of the article is  
282 urn:lsid:zoobank.org:pub:209675D6-2EBE-4E37-84CB-DA59994F7B2. The LSID for the  
283 new genus *Dolicirroplectanum* is urn:lsid:zoobank.org:act:89BFF3C5-271B-4482-98E0-  
284 AC667AA6611D.

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

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

292 **Description:**

293 Multiple pairs of head organs, two pairs of eye-spots. No tegument scales were observed.  
294 *Dolicirroplectanum* gen. nov. is characterised by two pairs of dorsal and ventral anchors with  
295 a regularly curved shaft point, a large and wide ventral bar and two dorsal bars. Dorsal anchors  
296 smaller than ventral ones and without developed outer root. 14 marginal hooklets of similar size  
297 and relatively small compared to other haptor structures. Two squamodiscs, ventral and  
298 dorsal, formed by concentric open rows of bone-shaped rodlets of similar width in all rows.  
299 Intestinal bifurcation follows pharynx, oesophagus absent. Caeca simple, terminate blindly.  
300 Testis spherical, intercaecal. Vas deferens emerges from anterior part of testis, enlarges into  
301 seminal vesicle. Seminal vesicle single in the middle region of body, transforms into elongated

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

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

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

329 **Redescription**

330 **Family:** Diplectanidae Monticelli, 1903

331 **Genus:** *Dolicirroplectanum*

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

333 **Synonyms:** *Diplectanum lacustre*

334 **Zoobank registration:** To comply with the regulations set out in article 8.5 of the amended  
335 2012 version of the International Code of Zoological Nomenclature (ICZN) (International  
336 Commission on Zoological Nomenclature, 2012), details of the species have been submitted to  
337 ZooBank. The Life Science Identifier (LSID) of the article is  
338 urn:lsid:zoobank.org:pub:209675D6-2EBE-4E37-84CB-DA59994F7B2. The LSID for the  
339 new name *Dolicirroplectanum lacustre* is urn:lsid:zoobank.org:act:423241C3-777D-4F86-  
340 A70F-02B4D74F9E66.

341 **Figures:** 3, 44

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

343 **Vouchers:** MRAC. MT. 38206-10, 38913-39058 (243 specimens), MZH 10067-71 (6  
344 specimens), MNHN HEL744-47 (4 specimens), SAMC-A089971-72 (6 specimens), NHMUK  
345 2018.4.13.4-13.7 (8 specimens)

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

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

348 **Site:** Gills

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

350 **Additional localities:** Bahr-Sara, Tchad (08°56'N-17°58'E); Kisumu, Lake Victoria (00°06'S-  
351 34°45'E), Lake Kossou, Egypt (07°10'N-05°20'E), Lake Nasser, Egypt (24°05'N-33°00'E),  
352 Luxor market, Egypt (25°42'N 32°38'E), Njala, riv. Taja, Sierra Leone (08°06'N-12°04'E), Lake  
353 Albert – Nyawiega (01°28'N-30°56'E); Nzunzu (1°19'N, 30°72'E); Lake Tanganyika – Crock  
354 Island (8°42'S-31°07'E), Katukula (8°35'S-31°10'E), Mpulungu (8°46'S-31°07'E); Rumonge  
355 (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-  
356 29°50'E); Kilomoni (4°20'S, 29°09'E); Mulembwe (6°07'S, 29°16'E); Nyanza (4°20'S-  
357 29°35'E); Edith Bay (6°30'S-29°55'E); Uvira (3°22' S 29°08'E)

358 Infection parameters: 4 of 8 *Lates angustifrons* infected with 1 – 15 specimens, 15 of 23 *L.*  
359 *mariae* infected with 1 – 18 specimens, 21 of 31 *L. microlepis* infected with 1 – 53 specimens.  
360 1 of 1 *L. niloticus* from Bahr-Sara infected with 2 specimens, 2 of 3 *L. niloticus* from Kisumu  
361 (Lake Victoria) infected with 1-7 specimens, 4 of 5 *L. niloticus* from Lake Kossou infected with  
362 5-9 specimens, 1 of 5 *L. niloticus* from Lake Nasser infected with 1 specimen, 1 of 3 *L. niloticus*  
363 from Nyawiega (Lake Albert) infected with 2 specimens, 5 of 11 *L. niloticus* from Nzuzu (Lake  
364 Albert) infected with 2-10 specimens, 1 of 1 *L. niloticus* from Nzuzu (Lake Albert) infected  
365 with 2 specimens.

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

369 **Description:** Tegument thin, smooth. Three pairs of head organs, two pairs of eye-spots, the  
370 posterior ones larger and closer together. Two squamodiscs, ventral squamodisc larger than  
371 dorsal, both consist of 9-12 concentric rows of bone-shaped rodlets, the two distal rows of  
372 which are composed of only rudimentary rodlets. Two pairs of anchors, rudiment of inner root  
373 and wide base of outer root in dorsal anchor. Marginal hooklets (14) of similar size. Ventral bar  
374 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 haptor 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 (Chotnirat 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 haptor 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 tanganyicae* (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

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

## 642 **Acknowledgments**

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

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## 959 **Funding**

960 Research was supported by the Czech Science Foundation (GBP505/12/G112 – ECIP) and  
961 GA19-13573S), Belgian Science Policy (BELSPO), EMBRC Belgium - FWO project  
962 GOH3817N as well as by a joint program between the Austrian agency for international  
963 mobility and cooperation in education, science and research and the Ministry of Education,  
964 Youth and Sports (project number 8J18AT007), the Austrian Agency for International  
965 Cooperation in Education and Research (OEAD; project number CZ 08/2018; to SK) and the  
966 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*
<b>Lake Tanganyika</b>					
<i>L. angustifrons</i>	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
<i>L. mariae</i>	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
	Bujumbura (4.5.1947)	The northern subbasin	5 (MRAC 90805-9)	5	24
	<i>L. microlepis</i>	Crock Island (08°42'S-31°07'E, 16.4.2018)	The southern subbasin	8 (-)	2
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
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
<b>Other localities</b>					
<b><i>L. niloticus</i></b>	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

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

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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)	<i>L. niloticus</i> , Lake Volta	<i>L. niloticus</i> , Cairo	<i>L. angustifrons</i> , Lake Tanganyika	<i>L. mariae</i> , Lake Tanganyika	<i>L. microlepis</i> , Lake Tanganyika
<b>Total length</b>	650-1000	-	544,2 (n=1)	522,0±35,4 <sup>a</sup> (487,4 - 587,3) <sup>b</sup> ; n=11	675,0±66,9 (588,1 - 813,1); n=13
<b>Total width</b>	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
<b>Ventral anchor</b>					
<b>Length to notch</b>	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
<b>Total length</b>	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
<b>Length to inner root</b>	-	-	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
<b>Inner root length</b>	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
<b>Outer root length</b>	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
<b>Point length</b>	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
<b>Dorsal anchor</b>					
<b>Total length</b>	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
<b>Point length</b>	-	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
<b>Ventral bar</b>					
<b>Straight length</b>	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
<b>Maximum width</b>	-	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
<b>Dorsal bar</b>					
<b>Straight length</b>	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
<b>Maximum width</b>	-	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
<b>Ventral squamodisc</b>					
<b>Length</b>	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
<b>Width</b>	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
<b>Dorsal squamodisc</b>					
<b>Length</b>	-	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
<b>Width</b>	-	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
<b>Hook</b>					
<b>Copulatory tube straight length</b>	21-23	-	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
			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

<b>Copulatory tube width</b>	-	-	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
<b>Vagina</b>	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
<b>Total length</b>	-	-	-	22,1±0,8 (21,5 - 22,6); n=2	-
<b>Tube length</b>	-	-	-	5±0,6 (4,6 - 5,4); n=2	-
<b>Point length</b>	-	-	-	6±0,3 (5,8 - 6,2); n=2	-
<b>Eyes spots</b>					
<b>Smaller pair distance</b>	-	-	44,2 (40,0 - 48,4); n=2	37,3±6,3 (30,0 - 50,0); n=14	-
<b>Larger pair distance</b>	-	-	42,6 (42,2 - 43,0); n=2	32,9±5,1 (26,8 - 46,7); n=14	-
<b>Pharynx length</b>	-	-	-	34,3±9,9 (22,9 - 53,0); n=8	-
<b>Testes</b>					
<b>Length</b>	-	-	-	94,3 (n=1)	-
<b>Width</b>	-	-	-	42,7±13,9 (29,7 - 78,1); n=13	-
<b>Ovary width</b>	-	-	-	31,0±10,9 (31,0 - 64,6); n=14	-

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<b>Parameters (µm)</b>	<b><i>L. niloticus</i>, Lake Albert1</b>	<b><i>L. niloticus</i>, Lake Albert2</b>	<b><i>L. niloticus</i>, Lake Victoria</b>	<b><i>L. niloticus</i>, river Taja</b>	<b><i>L. niloticus</i>, Lake Kossou</b>
<b>Total length</b>	736,5±115,3 <sup>a</sup> (644,1 - 931,3) <sup>b</sup> ; 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
<b>Total width</b>	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
<b>Ventral anchor</b>					
<b>Length to notch</b>	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
<b>Total length</b>	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
<b>Length to inner root</b>	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
<b>Inner root length</b>	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
<b>Outer root length</b>	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
<b>Point length</b>	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
<b>Dorsal anchor</b>					
<b>Total length</b>	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
<b>Point length</b>	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
<b>Ventral bar</b>					
<b>Straight length</b>	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
<b>Maximum width</b>	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

<b>Dorsal bar</b>					
<b>Straight length</b>	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
<b>Maximum width</b>	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
<b>Ventral squamodisc</b>					
<b>Length</b>	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
<b>Width</b>	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
<b>Dorsal squamodisc</b>					
<b>Length</b>	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
<b>Width</b>	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
<b>Hook</b>	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
<b>Copulatory tube straight length</b>	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
<b>Copulatory tube width</b>	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
<b>Vagina</b>	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
<b>Total length</b>	41,1±4,5 (34,0 - 47,3); n=14	35,7±5,6 (30,1 - 41,3); n=3	-	-	-
<b>Tube length</b>	7,8±1,1 (4,8 - 9,3); n=14	6,9±1,4 (5,3 - 7,9); n=3	6,7 (n=1)	-	-
<b>Point length</b>	7,0±0,7 (6,0 - 8,5); n=16	8,0±1,1 (6,7 - 8,7); n=3	8,9 (n=1)	-	-
<b>Eyes spots</b>					
<b>Smaller pair distance</b>	56,7±10,2 (42,8 - 80,4); n=16	-	-	-	-
<b>Larger pair distance</b>	47,2±8,5 (34,9 - 64,6); n=17	-	-	-	-
<b>Pharynx length</b>	56,3±11,0 (39,9 - 73,2); n=10	-	-	-	-
<b>Testes</b>					
<b>Length</b>	102,1±46,7 (57,9 - 150,9); n=3	-	-	-	-
<b>Width</b>	70,3±16,2 (50,5 - 103,2); n=9	-	-	-	-
<b>Ovary width</b>	72,7±11,1 (50,7 - 88,9); n=9	-	-	-	-

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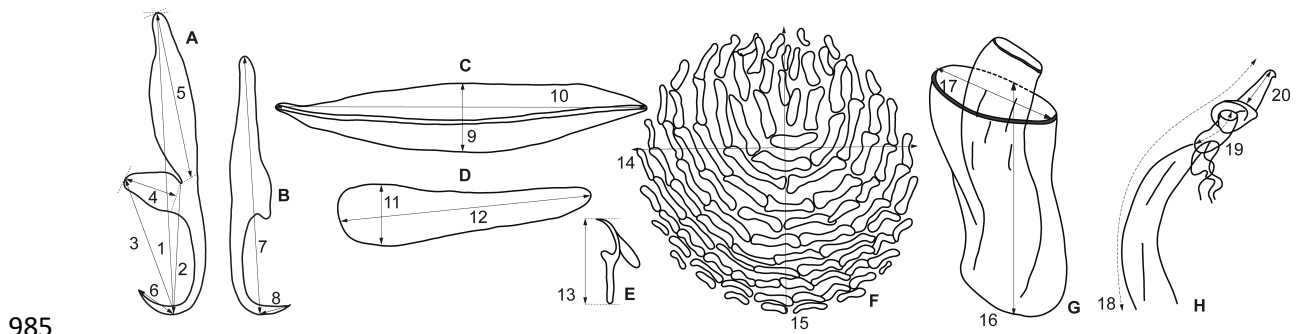


982 Table 3: Genetic intraspecific variability indices in a 412 bp portion of COI mtDNA region of *Dolicirroplectanum lacustre* comb. nov.

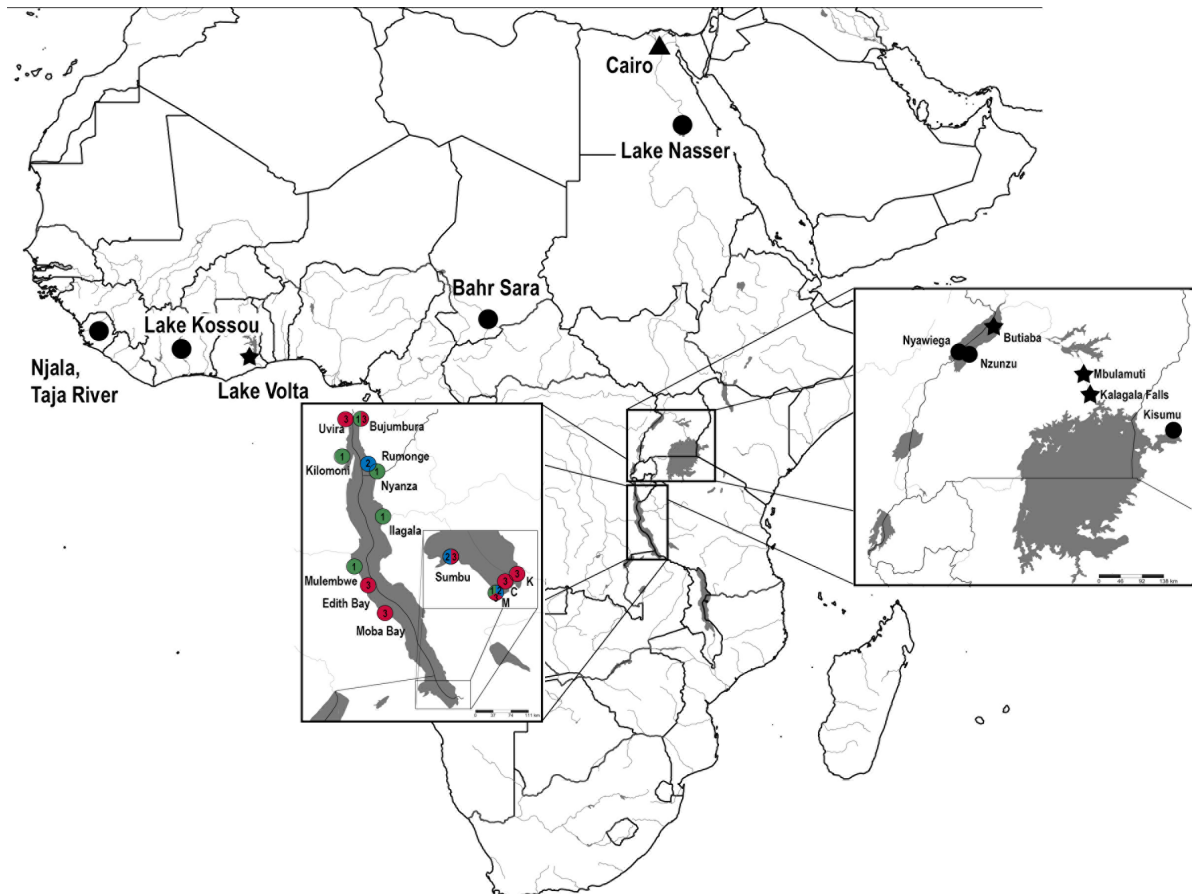
	Maximum uncorrected p-distance (number of individuals)	Nucleotide diversity	Haplotype diversity	Number of polymorphic sites
Lake Albert	1.2% (14)	0.0036+/-0.0026	0.8022+/-0.0936	6
Lake Tanganyika	0.7% (38)	0.0019+/-0.0016	0.5747+/-0.0713	4

983

984 **Figure captions**

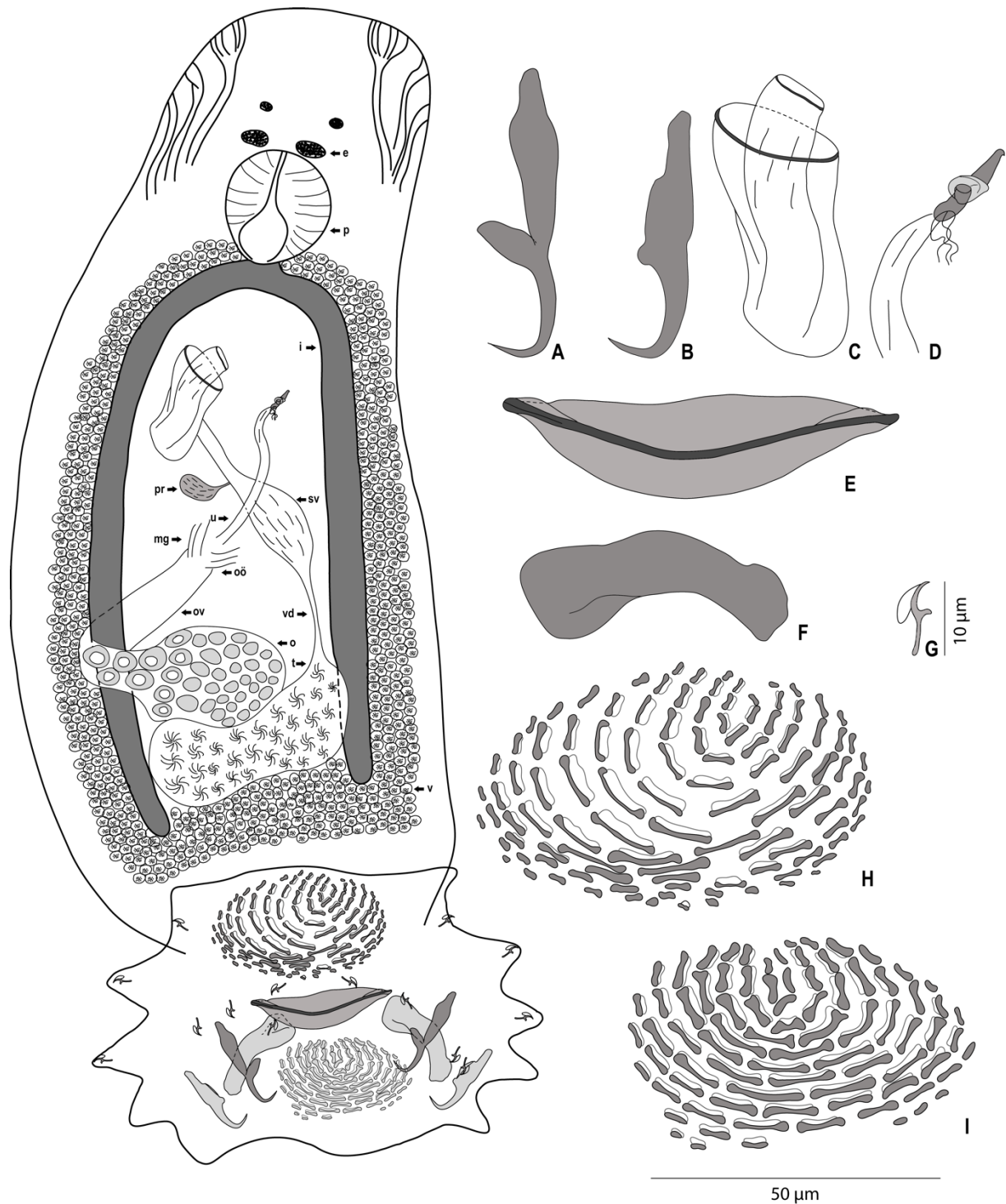


986 Figure 1: Measurements for sclerotized structures of haptor and reproductive organs of  
987 *Dolocirroplectanum lacustre* comb. nov. A Ventral Anchor: 1—Total length, 2—Length to  
988 notch, 3—Length to inner root, 4—Inner root length, 5—Outer root length, 6—Point length;  
989 B Dorsal anchor: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



994

995 Figure 2: Localities with confirmed presence of *Dolocirroplectanum lacustre* comb. nov. Star  
 996 – localities sampled for the original description of *D. lacustre* by Thurston & Paperna, 1969,  
 997 triangle – locality documented by Ergens, 1981, circle – localities sampled in this study. M –  
 998 Mpulungu, C – Crocodile Island, K – Katukula. Colours denote host species: black – *Lates*  
 999 *niloticus*, blue (number 2) – *L. angustifrons*, green (number 1) – *L. mariae*, red (number 3)–  
 1000 *L. microlepis*. Map created using SimpleMappr software v7.0.0. (available at  
 1001 <http://www.simplemappr.net>. Accessed February 25, 2018).



1002

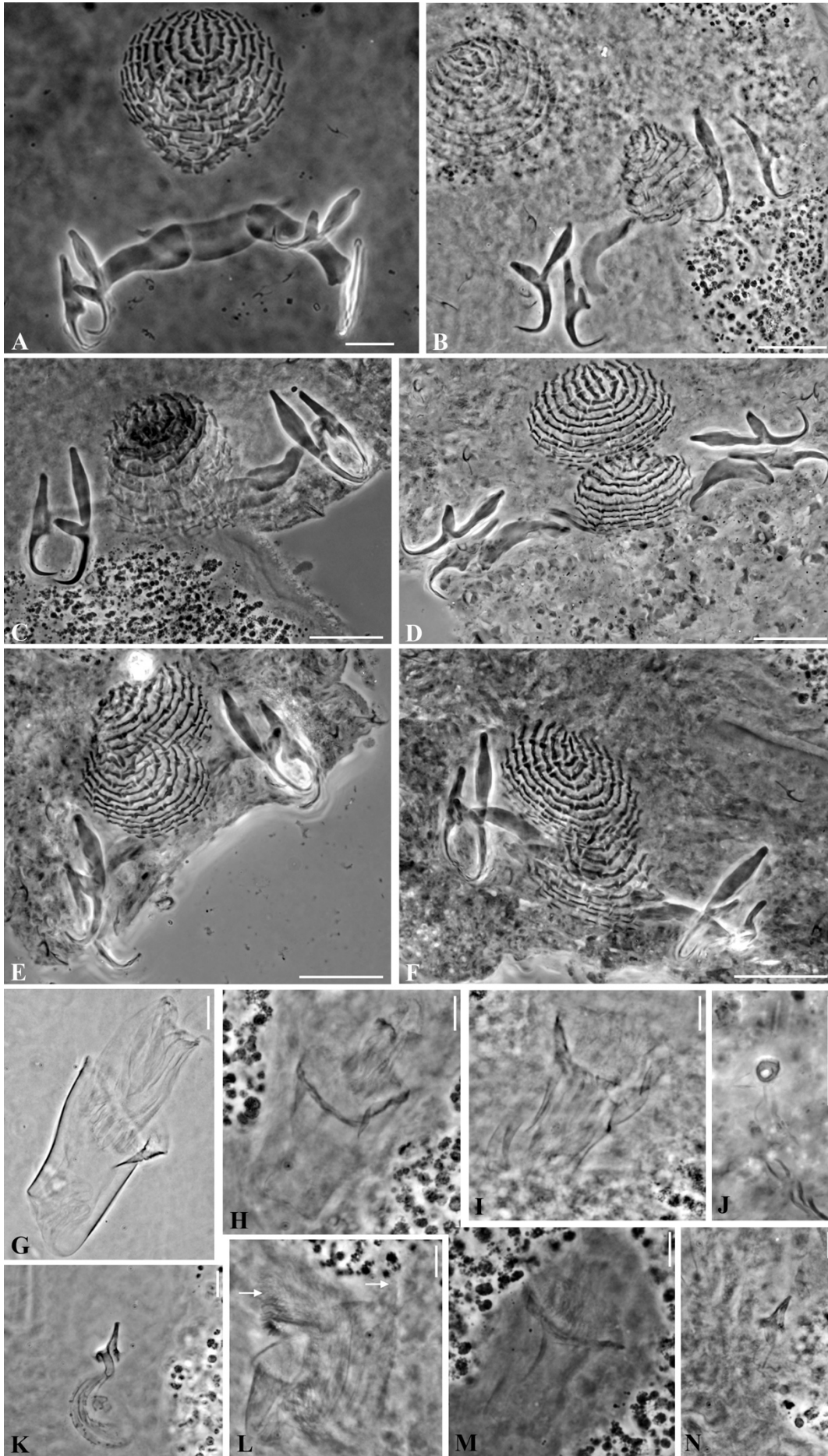
1003 Figure 3: *Dolicirroplectanum lacustre* comb. nov. collected from *Lates niloticus* in Lake Albert.

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

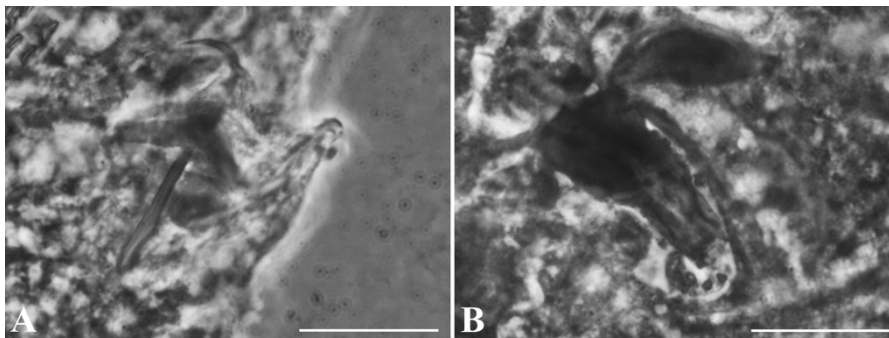
1005 oö, oötype; ov, oviduct; p, pharynx; pr, prostatic reservoir; sv, seminal vesicle; t, testes; u,

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

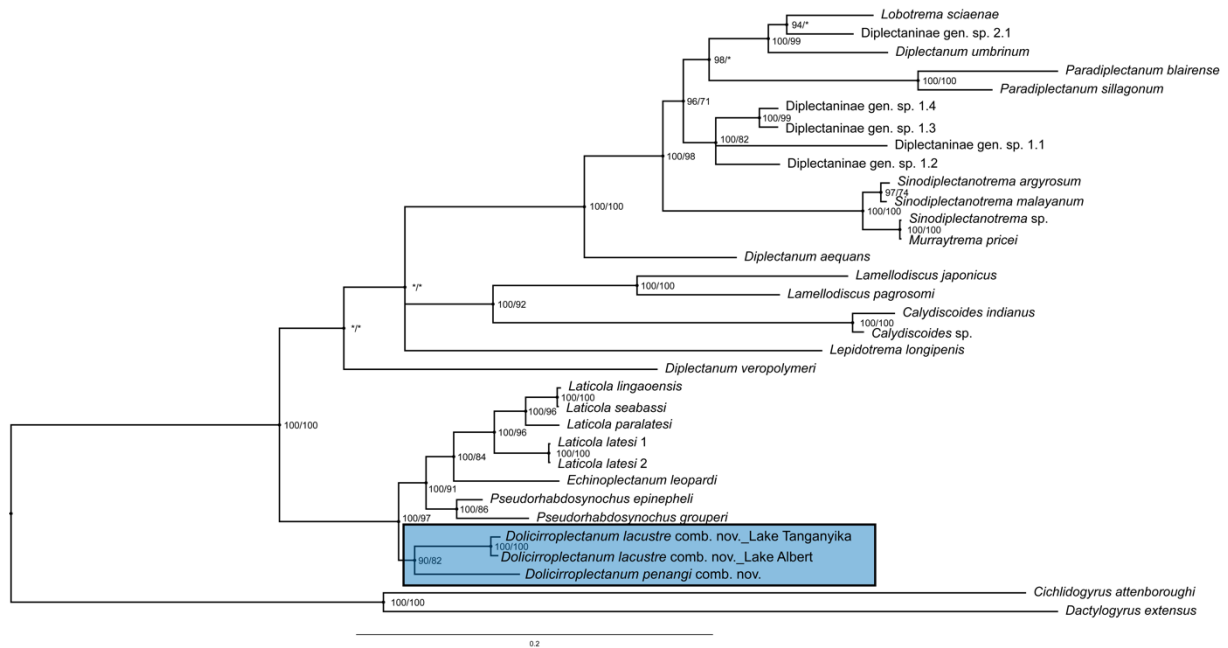
- 1007 organ; D, vagina, E, ventral bar; F, dorsal bar; G, hook; H, ventral squamodisc; I, dorsal  
1008 squamodisc.



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.



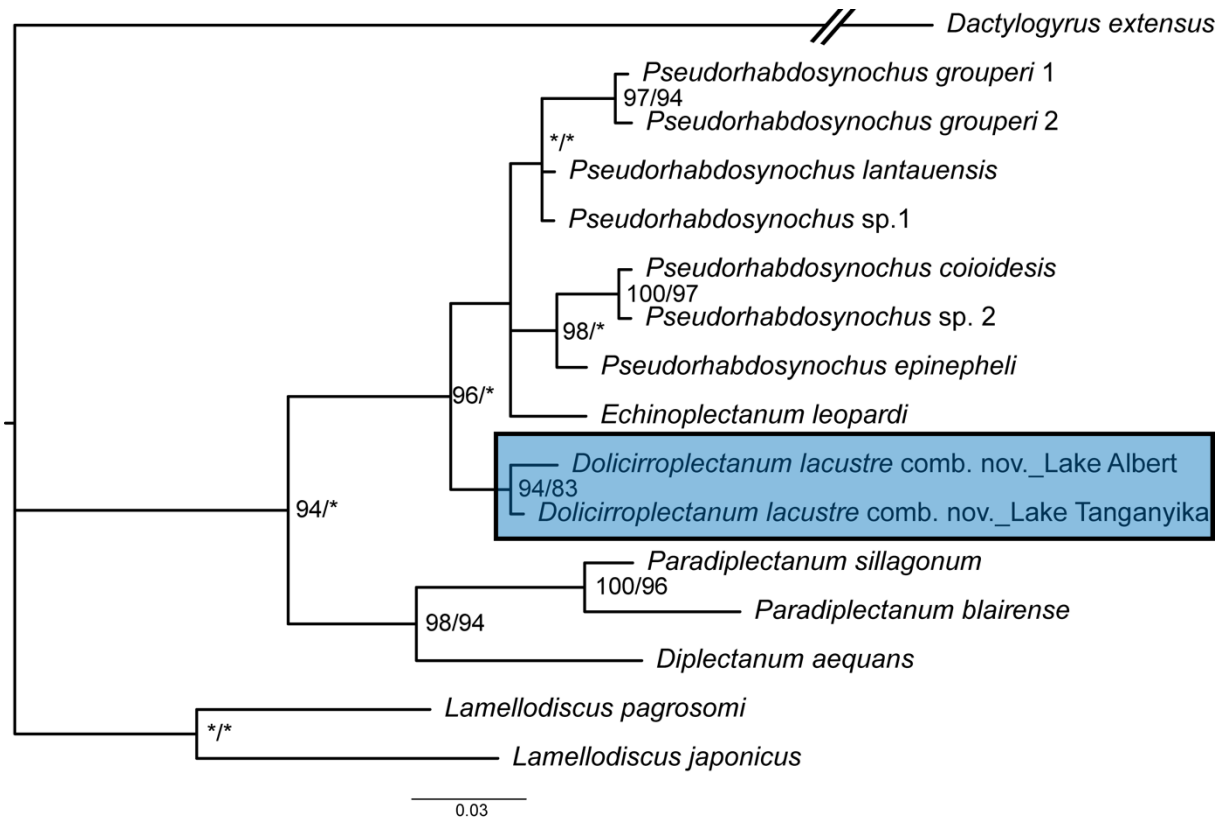
1020  
1021 Figure 5: Sclerotised structures of *Dolicirroplectanum penangi* comb. nov.. A) Ventral anchor  
1022 (MNHN HEL1086), Hainan, China B) Male copulatory organ (USNPC 180-A 6), Zhanjiang,  
1023 China. Scale bar: 20 µm; several layers in the picture were combined.



1024

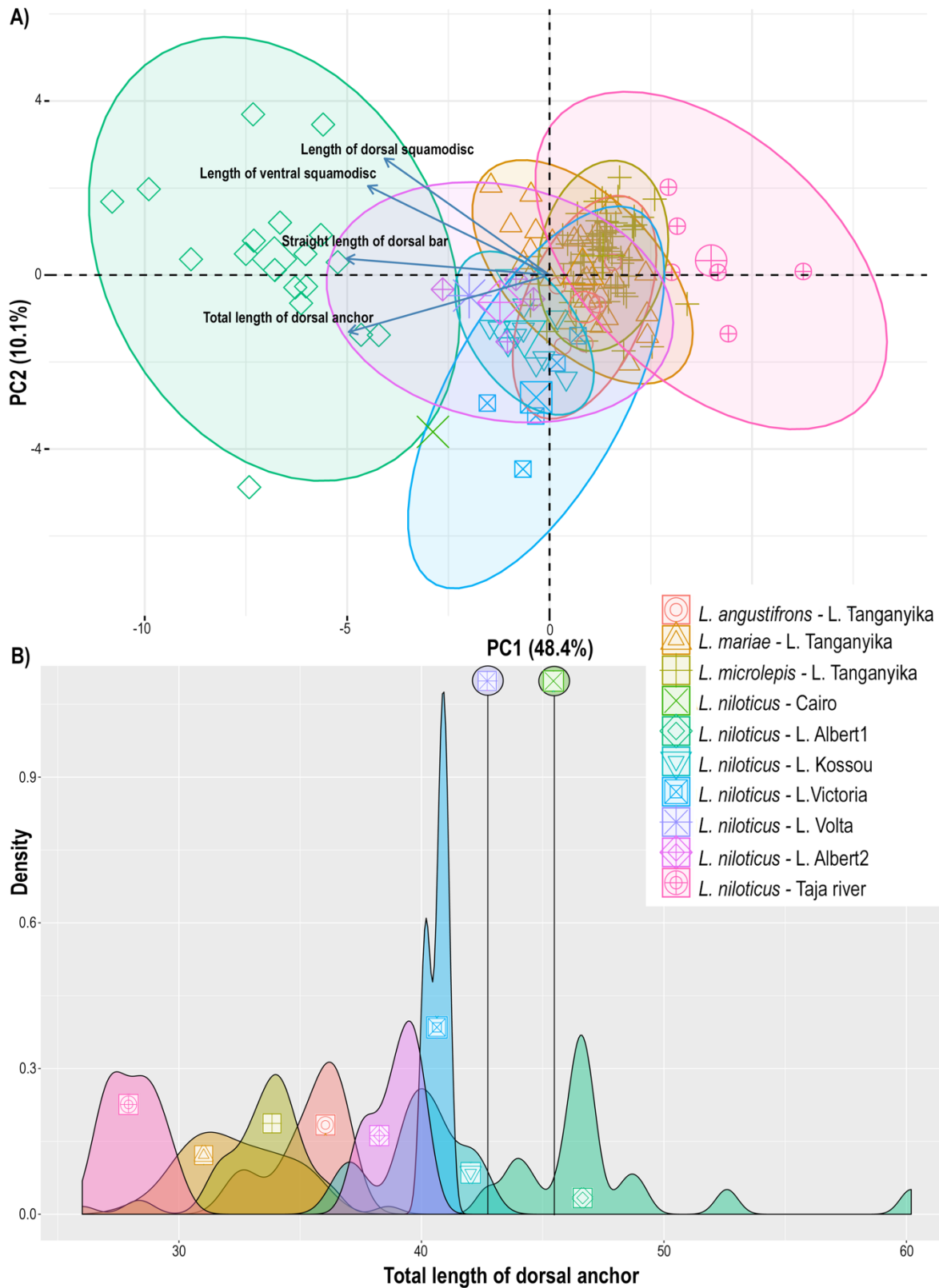
1025 Figure 6: Bayesian inference phylogram based on 28S fragments from 33 haplotypes of  
 1026 different diplectanid species. Posterior probabilities for Bayesian inference (before slashes)  
 1027 and bootstrap percentages for maximum likelihood (behind slashes) are shown. The values  
 1028 lower than 90 of posterior probability and 80 for maximum likelihood are marked with an  
 1029 asterisk. The clade containing two lineages of *Dolicirroplectanum lacustre* comb. nov. is  
 1030 boxed. The scale-bar indicates the expected number of substitutions per site.





1031

1032 Figure 7: Bayesian inference phylogram based on 18S fragments from 15 haplotypes of  
 1033 different diplectanid species. Posterior probabilities for Bayesian inference (before slashes)  
 1034 and bootstrap percentages for maximum likelihood (behind slashes) are shown. The values  
 1035 lower than 90 of posterior probability and 80 for maximum likelihood are marked with an  
 1036 asterisk. The clade containing two lineages of *Dolicirroplectanum lacustre* comb. nov. is  
 1037 boxed. The scale-bar indicates the expected number of substitutions per site.

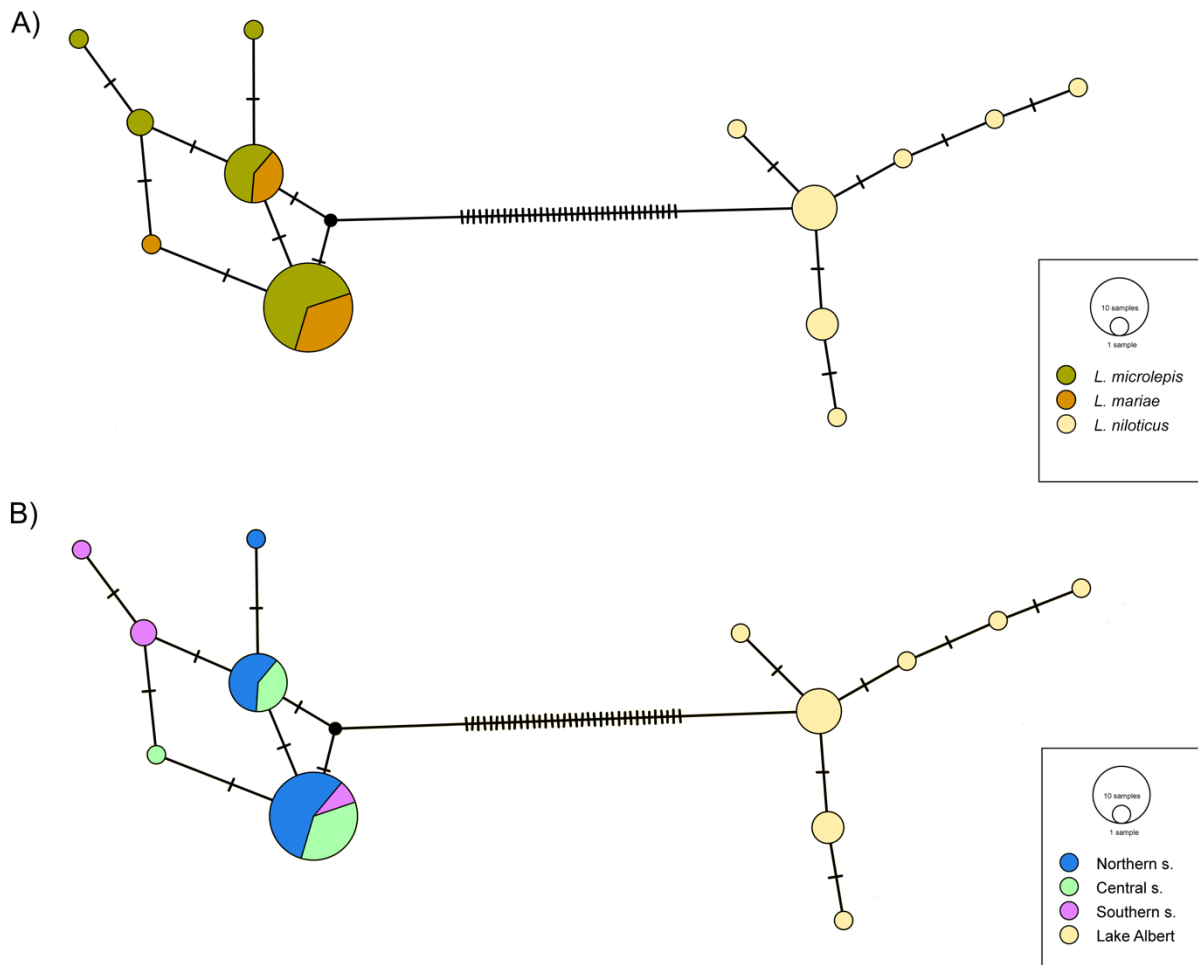


1038

1039 Figure 8: Morphometric variability of haptoral structures of *Dolocirroleptanum lacustre* comb.

1040 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 *Dolocirroplectanum 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).