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1 **Diversification dynamics of freshwater bivalves (Unionidae: Parreysiinae: Coelaturini)**
2 **indicate historic hydrographic connections throughout the East African Rift System**

3

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24 **Abstract**

25 Invertebrates are exceptionally diverse, but many are in decline because of anthropogenic
26 changes to their habitat. This situation is particularly problematic for taxa that are not well
27 monitored or taxonomically poorly understood, because the lack of knowledge hampers
28 conservation. Despite their important functional role in freshwater ecosystems, African bivalves
29 of the family Unionidae remain poorly studied compared to their highly threatened relatives in
30 Europe, the U. S. A. and Canada. To resolve relationships and to study diversification dynamics
31 in space and time, we performed time-calibrated phylogenetic studies and biogeographical
32 modeling on the unionids from the East African Rift System and surroundings, including
33 representatives of all currently recognized Afrotropical genera except for *Brazzaea* (and *Unio*
34 from southern Africa). Our analyses indicate that all sampled Afrotropical unionids belong to the
35 tribe Coelaturini (subfamily Parreysiinae), as does the genus *Moncetia* from Lake Tanganyika,
36 which is currently attributed to the family Iridinidae. Colonization of Africa from Eurasia by
37 Parreysiinae occurred ~17 Ma ago, and the subsequent diversification of Coelaturini in Africa
38 continued at a steady pace, although net diversification decreased over time as more niches and
39 ecoregions became occupied. Clades in Coelaturini largely reflect drainage basins, with the
40 oldest lineages and highest regional diversity occurring in Lake Tanganyika, followed by the
41 Congo Basin watershed in general. The species assemblage of Lake Tanganyika reflects multiple
42 independent events of colonization and intralacustrine diversification since the Late Miocene or

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43 Early Pliocene. The clades of other regions, including that containing the species from Lake
44 Malawi, are comparatively young. Biogeographical analyses indicate that the colonization
45 history was mainly driven by cladogenesis in sympatry, whereas few anagenetic events
46 contributed to the modern distribution of Coelaturini. Ancestral range estimations demonstrate
47 that Coelaturini originated in the Victoria and/or Tanganyika ecoregions, and that the Congo
48 Basin played an essential role in the colonization of Africa by Coelaturini.

49

50 **Keywords:** Freshwater mussels, *Coelatura*, Africa, systematics, phylogeography, biogeographic
51 modeling



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52 **1. Introduction**

53 Invertebrates are exceptionally diverse and central to the functioning of many ecosystems
54 (Wilson 1987; Eisenhauer et al. 2019), but substantial parts of their biodiversity remain poorly
55 studied. Many invertebrates across ecosystems are vulnerable to anthropological stress and two
56 thirds of the monitored populations show a mean abundance decline of 45% since the 16th
57 century (Dirzo et al. 2014). This decline is especially problematic for those taxa for which little
58 systematic knowledge exists because lack of data hampers the implementation of conservation
59 policies, which makes poorly known biodiversity vulnerable to irretrievable loss.

60 Freshwater bivalves of the family Unionidae, which contains at least 620 extant species (Bogan
61 and Roe 2008; Graf and Cummings 2019), are among the most endangered of freshwater biota
62 (Lydeard et al. 2004; Lopes-Lima et al. 2017) as a result of the continued anthropogenic
63 degradation of their ecosystems (Pereira et al. 2014). Unionids have complex life histories that
64 include parental care and larval parasitism (Barnhart et al. 2008) and they perform many
65 valuable ecosystem functions (Strayer et al. 1994; Lopes-Lima et al. 2017; Vaughn 2018).

66 Important spatial bias occurs, however, in knowledge on unionid biodiversity: A long research
67 tradition exists on the ecology (Ortmann 1920; Dillon 2000) and systematics (Simpson 1900;
68 Ortmann 1920; Haas 1969; Graf and Cummings 2007; Lopes-Lima et al. 2017) of the unionids
69 of the U. S. A. and Europe, whereas knowledge on Asian Unionidae has started to develop only
70 much more recently (Brandt 1974; Bolotov et al. 2017; Bolotov et al. 2018; Pfeiffer et al. 2018;
71 Wu et al. 2018; Zieritz et al. 2018). Knowledge on Unionidae of sub-Saharan Africa remains
72 much more restricted in that populations are not monitored and insights into their species



73 richness and taxonomy are still primarily based on shell morphology and distributional data
74 (Mandahl-Barth 1988; Daget 1998; Scholz and Glaubrecht 2004; Graf and Cummings 2011;
75 Seddon et al. 2011). Due to a scarcity of phylogenetic studies, the taxonomic validity and
76 phylogenetic position of many Afrotropical genera is uncertain (Graf and Cummings 2007),
77 although hypothetical systematic reclassifications have been proposed (Lopes-Lima et al. 2017;
78 Graf and Cummings 2019).
79 Nevertheless, the combined efforts of previous studies have helped delineate the deeper
80 phylogenetic relationships among Unionidae and the placement of some African taxa. Whelan et
81 al. (2011) recovered Unionidae as monophyletic and retrieved the African genera *Coelatura*,
82 *Prisodontopsis* and *Nitia* as a well-supported clade, the tribe Coelaturini, which is sister to the
83 Asian Indochinellini, as has later been confirmed by Bolotov et al. (2018). These tribes belong to
84 the subfamily Parreysiinae (Bolotov et al. 2017; Lopes-Lima et al. 2017), which is sister to the
85 Unioninae (Pfeiffer et al. 2019). Coelaturini supposedly contains 38 nominal species that belong
86 to 8 genera (Graf and Cummings 2007, 2019): *Brazzaea* Bourguignat, 1885, *Coelatura* Conrad,
87 1853, *Grandidieria* Bourguignat, 1885, *Mweruella* Haas, 1936, *Nitia* Pallary, 1924, *Nyassunio*
88 Haas, 1936, *Prisodontopsis* Tomlin, 1928 and *Pseudospatha* Simpson, 1900, of which
89 *Mweruella* and *Prisodontopsis* may have to be synonymized (Graf et al. 2014). However, the
90 potential attribution of *Brazzaea*, *Grandidieria*, *Nyassunio* and *Pseudospatha* to Coelaturini has
91 not been substantiated. Indeed, some authors (Kat 1987) have suggested that, among others,
92 *Brazzaea* and *Pseudospatha* would be more closely related to *Unio* (*Cafferia*) *caffer*, a unionid



93 from southern Africa belonging to the subfamily Unioninae. This hypothesis remains to be
94 tested, however.

95 The endangered state of Unionidae worldwide combined with the poor knowledge on the
96 biodiversity and systematics of African unionids and the lack of conservation studies on African
97 populations is alarming, especially considering the large-scale ecosystem change observed in
98 many African freshwater systems (Thieme et al. 2010; Darwall et al. 2011). Here, we aim to fill
99 the existing knowledge gap by testing the monophyly of Coelaturini with extended taxon
100 sampling and by evaluating phylogenetic support for the identification system of Mandahl-Barth
101 (1988) based on the morphological-geographical recognition of species. We reconstruct
102 phylogenetic relationships of Unionidae occurring throughout Central and East Africa, with a
103 focus on the East African Rift System (EARS). The EARS contains ~90 % of the species
104 diversity of Afrotropical unionids and representatives of all currently recognized genera
105 (Mandahl-Barth 1988; Daget 1998; Graf and Cummings 2007). Finally, we examine
106 diversification dynamics in Coelaturini in their spatial-temporal context via fossil-based time-
107 calibration and ancestral range estimations to reconstruct major colonization pathways among
108 African freshwater ecoregions. We also compare biogeographical patterns in Coelaturini to those
109 of their fish hosts.

110

111 **2. Material and methods**

112 ***2.1. Material and taxonomy sampling***



113 The material used for this study was obtained from the Systematics and Biodiversity Collection
114 of the Justus Liebig University (UGSB), the Natural History Museum London (NHMUK) and
115 the Museum d’Histoire Naturelle de Genève (MHNG), together with data from previous studies
116 (Whelan et al. 2011; Graf et al. 2014; Pfeiffer and Graf 2015; Bolotov et al. 2017) and almost a
117 decade of fieldwork (between 2006 and 2015). We obtained samples from all 8 genera that
118 supposedly belong to Coelaturini—in this respect *Unio caffer*, which is widely distributed in
119 southern Africa, was not included in our study (see Whelan et al. 2011). For *Brazzaea* only
120 historic material was available, however, and because the extracted DNA was of very poor
121 quantity and quality, this taxon is not further considered here. Unfortunately, sequencing of
122 specimens from West Africa housed at the MHNG resulted in similar issues. Material yielding
123 good quality DNA and genetic sequences is enlisted in Table S1. It includes specimens from all
124 major lakes of the EARS that are currently inhabited by Afrotropical unionids and from, among
125 others, the Congo River and several of its tributaries, the Nile, including the Victoria Nile, the
126 Zambezi, the Okavango, and the Cunene.

127 We used an extensive set of Asian Parreysinae from previous studies (Whelan et al. 2011; Graf
128 et al. 2014; Pfeiffer and Graf 2015; Bolotov et al. 2017) as outgroup, including specimens of
129 *Indonaia* Prashad, 1918, *Radiatula* Simpson, 1900, *Indochinella* Bolotov, Pfeiffer, Vikhrev &
130 Konopleva, 2018 and *Parreysia tavoyensis* (Gould, 1843) (see Table S1).

131

132 ***2.2. DNA extraction and sequencing***



133 Genomic DNA was extracted from ~1 mm³ of muscle or mantle tissue using a CTAB protocol
134 (Wilke et al. 2006). Four molecular markers were sequenced, i.e. fragments of the mitochondrial
135 cytochrome c oxidase subunit I (COI) and the large ribosomal subunit (16S) complemented with
136 fragments of the nuclear histone 3 (H3) and the large ribosomal subunit (28S). We used the
137 following primers: LCO1490 and HCO2198 (COI; Folmer et al. 1994); 16sar-L and 16sbr-H
138 (16S; Palumbi et al. 2002); D23F and D4RB (28S; Park and Ó Foighil 2000); H3F and H3R (H3;
139 Colgan et al. 1998). DNA vouchers for newly sequenced material (~ 240 specimens) were
140 deposited at the UGSB; data on other specimens was retrieved from NCBI GenBank (Table S1).
141 PCR cycling condition are specified in Table S2. Bidirectional DNA sequencing was performed
142 on a 16-capillary 3730xl Genetic Analyzer (Applied Biosystems). New sequences were deposited
143 in GenBank (Table S1).

144

145 **2.3. Phylogenetic inference**

146 All four gene fragments were aligned individually using the online version of MAFFT v. 7
147 (Kato et al. 2017). Subsequently, we reduced the dataset to unique haplotypes and examined
148 substitutional saturation (Xia et al. 2003; Xia and Lemey 2009) for each gene fragment with
149 DAMBE v. 6.4.101 (Xia 2017). Fragments of the protein-coding genes (COI: 660 bp; H3: 328
150 bp) were translated to amino acid sequences with MESQUITE v. 3.51 (Maddison and Maddison
151 2018) to evaluate the presence of stop codons. Fragments of the ribosomal genes (16S, 28S)
152 were analyzed with GBlocks v. 0.91b (Castresana 2000; Talavera and Castresana 2007) after
153 alignment to exclude hypervariable sections from the aligned sequences. We allowed gap



154 positions within the final blocks, but we excluded sections with multiple contiguous non-
155 conserved positions. These criteria reduced the length of the alignment from 541 bp to 453 bp
156 and from 928 bp to 407 bp for 16S and 28S, respectively.

157 All gene fragments were concatenated using MESQUITE and unique haplotypes were
158 recognized with DAMBE. Subsequently, we used Partition Finder v. 2.1.1 (Lanfear et al. 2016)
159 and a Bayesian information criterion (BIC) to infer the best-fit partitioning scheme and
160 substitution models (Table S3). Phylogenetic analyses on this concatenated dataset were
161 performed using Bayesian inference (BI) and maximum likelihood (ML) on the CIPRES Science
162 Gateway v. 3.3 (Miller et al. 2010). ML was performed with RAxML-HPC BlackBox v. 8.2.12
163 (Stamatakis 2014) using the GTR + Γ substitution model applied to the partitions indicated in
164 Table S3, simultaneous best-tree search and bootstrapping with recommended stop rule. BI was
165 performed using MrBayes v. 3.2.3 (Ronquist and Huelsenbeck 2003) with the partitions and
166 substitution models indicated in Table S3. Two independent analyses were performed, each by
167 running four chains simultaneously for 30,000,000 Markov chain Monte Carlo (MCMC)
168 generations, sampling each 1000th tree. We examined whether stationarity of MCMC runs was
169 reached in the Bayesian analyses using Tracer v. 1.7.1 (Rambaut et al. 2018). The resulting trees
170 were visualized with FigTree v. 1.4.3 (Rambaut 2009).

171

172 ***2.4. Estimation of divergence times***

173 We estimated divergence times with BEAST v. 1.10.2 (Suchard et al 2018; Drummond et al
174 2012) on the CIPRES Science Gateway. We conducted three independent runs, with substitution

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175 and clock models unlinked among the gene fragments, an uncorrelated lognormal relaxed-clock
176 model (Drummond et al. 2006), a Yule speciation process for the tree prior (Yule 1925;
177 Gernhard 2008), 80,000,000 MCMC generations, and sampling each 1,000th tree. Initially, best-
178 fit substitution models as identified by PartitionFinder were implemented (see Table S3),
179 however, because of potential overparameterization of complex substitution models a stationary
180 state was not reached in preliminary runs. Therefore, we repeated the analyses with the simpler
181 HKY model, which also performed consistently well in model comparisons. Site heterogeneity
182 and invariant site parameters (+ Γ , + I, + Γ + I) were set to the best performing variant.
183 Divergence times were estimated with three fossil calibration points, which we established based
184 on the guidelines of Parham et al. (2012; see supplementary text for more details): The oldest
185 fossil of *Nitia* was used to calibrate the MRCA of the *Nitia* clade (lognormal prior with 7.5 ± 1.3
186 Ma [mean \pm SD; 95% confidence interval (CI): 5.3-10.4 Ma]), the oldest fossil of *Coelatura* cf.
187 *hauttecoeuri* was used to calibrate the MRCA of the *C. hauttecoeuri*/*C. bakeri*/*C. aegyptiaca*
188 clade (lognormal prior with 5.2 ± 0.5 Ma [mean \pm SD; 95% CI: 4.3-6.3 Ma]), whereas the oldest
189 fossil of *Coelatura* was used to calibrate the MRCA of *Coelatura sensu lato* (lognormal prior
190 with 10.0 ± 1.6 Ma [mean \pm SD; 95% CI: 7.2-13.5 Ma]) (Van Damme and Pickford 2010).
191 The results of the three independent runs were examined in Tracer after discarding 8,000,000
192 runs (10%) as burn-in. In individual runs, the ESS values of some parameters indicated a
193 potential lack of convergence, but upon combining the results of all three runs, parameter values
194 consistently showed ESS values >200 indicating proper mixing of the MCMC. From the 80,000
195 trees obtained per run 10 % was discarded as burn-in after which the remaining trees were



196 subsampled in LogCombiner of the BEAST package to yield 36,000 trees per run. For each of
197 these subsampled tree files we then identified the maximum clade credibility (MCC) tree with
198 TreeAnnotator (BEAST package). These three MCC trees allowed us to evaluate the robustness
199 of the outcome (topology and node ages) of our analyses for independent runs. The tree with the
200 highest log clade credibility was selected as the best supported tree for subsequent analyses.

201

202 **2.5. Species delimitation**

203 To enable biogeographical analyses, we first pruned the gene tree into a species tree using the
204 `prune_specimens_to_species` function of the package BioGeoBEARS v. 1.1.2. (Matzke 2014,
205 2018) in R v. 3.4.3 (R Core Team 2018) after removing the outgroup from the phylogeny.
206 Pruning requires an indication of species entities, which we approximated with operational
207 taxonomic units (OTUs). These OTUs were defined based on the following information: First,
208 we used three automated species recognition methods, i.e. automated barcode gap discovery
209 (ABGD) (Puillandre et al. 2012), the Poisson tree processes (PTP) model (Zhang et al. 2013) and
210 the generalized mixed Yule-coalescent (GMYC) approach (Fujisawa and Barraclough 2013).

211 ABGD is a fast single-locus method that was performed on the web server

212 (<http://wwabi.snv.jussieu.fr/public/abgd/abgdweb.html>) using our COI alignment, because of its
213 exceptional suitability for species identification, delimitation and discovery in Metazoa (Hebert
214 et al. 2003). We used default parameters, except for relative gap width, which was set to 1.0. The
215 other two methods are tree-based. We used the BEAST MCC tree for the PTP analysis, which
216 was ran with BI and ML on the bPTP server (<https://species.h-its.org/ptp>) using 500,000 MCMC



217 generations (other parameters as default) after removing the outgroup taxon *P. tavoyensis*.
218 Finally, GMYC was performed on the GMYC server (<https://species.h-its.org/gmyc>) with the
219 BEAST MCC tree and a single threshold. Subsequently, the proposed OTUs were compared
220 between methods, after which we related them to the morphology-based taxonomy using the
221 relevant literature (e.g. Mandahl-Barth 1988) and comparison with museum records and the
222 MUSSELP database (<http://mussel-project.uwsp.edu/fmuotwaolcb/index.html>).

223

224 **2.6. Diversification dynamics**

225 We used LogCombiner to sample 1,000 trees from the BEAST posterior distribution. For each of
226 these trees we removed the outgroup and reconstructed the corresponding OTU-based tree. The
227 posterior distribution of OTU-based trees was examined with DensiTree v. 2.2.5 (Bouckaert and
228 Heled 2014). Subsequently, we constructed lineage-through-time (LTT) plots and their 95%
229 confidence interval with functions of the R packages ape v. 5.0 (Paradis et al. 2004) and phytools
230 v. 0.6-44 (Revell 2012). We also extracted parameters for birth-death (BD) and pure-birth (PB)
231 models from our dataset accounting for incomplete sampling, which we used to simulate 100
232 trees under each model. We used these simulations to establish 95% confidence intervals on the
233 LTTs for the BD and PB models to compare with the empirical dataset. Additionally, we tested
234 for rate changes in the diversification dynamics of Coelaturini since their MRCA using functions
235 from the R package TreePar v. 3.3 (Stadler 2015), and with a Bayesian analysis of
236 macroevolutionary mixtures (BAMM) in BAMM v. 2.5.0 (Rabosky et al. 2013; Rabosky et al.
237 2017). Priors for speciation and extinction rates (i.e. λ and μ , respectively) in the BAMM



238 analysis were defined using the setBAMMpriors function of the R package BAMMtools v. 2.1.6
239 (Rabosky et al. 2014) and the expected number of shifts was set to 1.0. The analysis was
240 conducted with 10,000,000 generations, sampled each 10,000 generations, and with a burn-in of
241 10%. The sampling fraction was defined as the number of OTUs divided by the total number of
242 nominal valid species ($26/38 = 68.4\%$).

243

244 ***2.7. Ancestral range estimation***

245 We examined how Coelaturini were distributed over the African continent with a phylogenetic
246 approach to biogeography based on reticulate models as implemented in BioGeoBEARS.
247 Specifically, we fit six models, i.e. DEC, DEC+J, DIVALIKE, DIVALIKE+J, BayAreaLIKE,
248 BayAreaLIKE+J (Massana et al. 2015; Matzke 2018). DEC is the dispersal-extinction-
249 cladogenesis model of Ree and Smith (2008) as implemented in the software package Lagrange.
250 DIVALIKE is a model that represents a likelihood interpretation of parsimony-based dispersal-
251 vicariance analyses (Ronquist 1997). The BayAreaLIKE model is a simplified likelihood
252 interpretation of Bayesian analyses of biogeography including many areas, as implemented in the
253 program BayArea (Landis et al. 2013). This last model is similar to DEC, but it assumes that no
254 particular events are associated with cladogenesis. Comparison of DEC and BayAreaLIKE
255 therefore indicates the importance of the cladogenesis model on biogeographical estimations for
256 a dataset. Finally, these three models were fit with and without the parameter J, which represents
257 the frequency of founder-event jump dispersal, which is a consequence of founder-event
258 speciation. Founder-event speciation is a crucial process of dispersal, at least in island systems



259 (Matzke 2014), to which the East African lakes may belong (Salzburger et al. 2014). After
260 analyzing these six models we compared the model fit using a corrected Akaike information
261 criterion (AICc). Biogeographical modeling was performed for OTU-trees based on the BEAST
262 MCC tree and two ultrametricized versions of the MrBayes consensus tree. The latter two trees
263 were: 1) constructed from the MrBayes consensus tree with functions of phytools, and 2) by
264 sampling a tree from the BEAST posterior distribution that has an identical topology to the
265 MrBayes consensus tree. To examine the role of anagenetic and cladogenetic biogeographical
266 events in shaping the diversity and distribution of Coelaturini, we additionally performed
267 biogeographical stochastic mapping (BSM; see Dupin et al. 2017). This analysis was performed
268 in BioGeoBEARS with 1,000 replicates and parameters of the best-fit biogeographical model
269 used as priors.

270 We used African freshwater ecoregions that were established based on the composition and
271 distribution of the freshwater fish fauna (Abell et al. 2008) as geographic units for our analyses.
272 These ecoregions have been used to describe patterns in unionid diversity before, which is
273 significantly correlated with species richness in fish (Graf and Cummings 2011). For practical
274 purposes, however, some ecoregions were modified for this study, because for some ecoregions
275 no bivalve records were available, but more importantly some bivalve OTUs were widely
276 distributed (covering up to 5 ecoregions), which made biogeographical analyses computationally
277 intensive. In all these cases, it was possible to combine several small fish ecoregions into a
278 broader unionid ecoregion without losing information, e.g. by combining subzones within the
279 Okavango, Congo and Nile watersheds. Finally, we defined a total of 12 geographic areas and



280 we used the occurrence information of specimens to define the distribution of OTUs throughout
281 these ecoregions.

282

283 **3. Results**

284 **3.1. Phylogenetic inference**

285 Examination of the sequence alignments for each gene fragment revealed no evidence of
286 substitutional saturation. The concatenated dataset contained 1,848 bp and 205 unique
287 haplotypes. Phylogenetic reconstruction with RAxML and MrBayes resulted in highly congruent
288 topologies and an overall strongly supported backbone (Fig. 1). These analyses indicated that the
289 seven included genera belong to Coelaturini, which was recovered as a highly supported,
290 monophyletic taxon (BPP=1.00; BS=100). Additionally, our analyses indicate that *Moncetia*,
291 supposedly an iridiniid (see 4.1. Evolutionary and taxonomic implications), also belongs to
292 Coelaturini, and that it is part of the clade including *Pseudospatha tanganyikense*. The
293 Tanganyikan genera *Grandidieria* (BPP=1.00; BS=100) and *Pseudospatha* + *Moncetia*
294 (BPP=1.0; BS=100) are monophyletic, genetically distinct and have basal positions in
295 Coelaturini. Contrasting topologies, however, were recovered in the MrBayes + RAxML trees
296 versus the BEAST MCC tree: In the MrBayes consensus tree and the RAxML tree, *Grandidieria*
297 is sister to all other Coelaturini, whereas it is sister to *Pseudospatha* in the BEAST MCC tree. In
298 each case a related node is weakly supported (BPP= 0.93; BS = 67 versus BPP = 0.66), however.
299 The genus *Nitia* is also monophyletic and highly supported (BPP=1.00; BS=100) and was
300 recovered as the sister group to *Coelatura sensu lato* in the Bayesian analysis (BPP=0.99),



301 whereas in the RAxML analysis it was recovered (albeit very poorly supported; BS < 50) as the
302 sister group to the highly supported clade (BPP=1.00, BS=97) of *Coelatura* from the Nile
303 watershed, including among others Lakes Albert, Edward and Victoria. We consider this latter
304 grouping in the RAxML analysis uninformative given the low associated BS values. *Coelatura*
305 *sensu lato* contains all other Coelaturini, including the genera *Mweruella*, *Prisodontopsis*, and
306 *Nyassunio*, which renders the current definition of the widespread *Coelatura* paraphyletic.
307 Within *Coelatura sensu lato*, as for Coelaturini in general, the spatial distribution of clades
308 coincides well with watershed boundaries, with various deeply divergent clades occurring in the
309 Congo watershed (Fig. 1), which are all highly supported (BPP=1.00; BS=94-100). Three of
310 these clades occur directly in the Congo Basin, whereas the fourth occurs mainly in Lake
311 Mweru, although it has also colonized the Luvua River in the Upper Congo Basin. Finally, the
312 fifth clade occurs in Lake Tanganyika and surroundings, indicating that Lake Tanganyika has
313 been colonized at least twice by Coelaturini. The other two recovered groups are the clade
314 geographically distributed throughout the Okavango and Zambezi (BPP= 1.00; BS=95) and the
315 clade including taxa from the Luapula River and Lake Malawi (BPP= 1.00; BS=82). Analysis of
316 our genetic data did not recover *Nyassunio* from Lake Malawi as monophyletic. Instead these
317 specimens form a polytomy with *Coelatura* from Zambia and the Luapula River. This polytomy
318 is one of three major polytomies in the current phylogeny, the others representing *Coelatura*
319 from the Nile drainage and those from the Upper Zambezi. At least two of these polytomies
320 supposedly contain several species.

321



322 **3.2. Molecular-clock analyses**

323 The fossil-calibrated BEAST phylogeny (Fig. 2) overall produced the same topology to that of
324 RAxML and MrBayes, apart from the issue of *Grandidieria* and *Pseudospatha* mentioned above
325 (compare Figs. 1 and 2) and the uncertain position of *Nitia* in the RAxML analysis. Based on this
326 calibration, Coelaturini would have diverged from Indochinellini ~23 Ma (95 % highest
327 probability density (HPD): 15.25-31.95 Ma), with a MRCA of Coelaturini originating ~17 Ma
328 (95% HPD: 11.98-23.28 Ma). The diversification between *Grandidieria* and *Pseudospatha*
329 occurred ~14 Ma (95 % HPD: 8.61-19.57 Ma). Multiple cladogenetic events in the Congo Basin
330 occurred in the Middle to Late Miocene (95% HPD: 7.42-12.56, 6.63-11.72, 5.54-10.46).
331 Diversification within clades from Lake Tanganyika ensued since the Miocene-Pliocene
332 transition (95% HPD: 2.64-9.75, 3.89-11.20, 2.99-8.07 Ma), whereas lineage diversification
333 within the Okavango-Zambezi region, the Lake Mweru clade and the Lake Malawi clade
334 occurred simultaneously at ~3 Ma (95 % HPD: 1.68-4.98, 1.50-4.89, 1.74-4.52 Ma).

335

336 **3.3. Species delimitation**

337 Although the OTUs proposed by our automated species recognition methods were not identical
338 the results display a high level of congruence (Table S4). ABGD and PTP suggested 11-48
339 OTUs in the ingroup, but the best-fit ABGD estimation (31 OTUs) was very similar to the ML
340 estimation of the PTP method (29 OTUs) and to the GMYC solution (32 OTUs). This similarity
341 highlights the overall robustness of these methods for our dataset, and it allowed us to synthesize
342 these results in a single hypothesis of OTUs (Table S4). This synthetic hypothesis includes 26



343 OTUs, because some proposed OTUs divided well-supported clades into hypothetically
344 reciprocally monophyletic subclades that have limited BPP or BS support. Acknowledging that
345 the results of OTU methods are often conflicting, we performed subsequent analyses with 24 to
346 27 OTUs to investigate the robustness of downstream analyses. These analyses indicated highly
347 similar results under various hypothetical OTU assignments. In what follows OTUs are indicated
348 with species names, although these names reflect morphological similarity in the absence of
349 molecular data on topotypical specimens for many of the studied species.

350

351 ***3.4. Analyses of diversification dynamics***

352 Examination of 1,000 OTU-based trees (Fig. 3) indicates a limited effect of topological changes
353 among gene trees on the construction of the OTU-based tree, which consolidates the robustness
354 of downstream OTU-based analyses. The LTT plot (Fig. 3) indicates a steady increase in
355 diversity since the MRCA of Coelaturini. This accumulation closely follows the expected
356 diversity under pure-birth and birth-death models, which are almost identical for our dataset.
357 Over the last 5 Ma, lineages accumulated somewhat faster in the empirical dataset compared to
358 the pure-birth and birth-death simulations, although their 95% CIs continue to overlap broadly.
359 We observed evidence for a single shift in diversification rates along the phylogeny of
360 Coelaturini ($p = 0.002$) in TreePar, i.e. a decrease in the number of lineages around 3 Ma ago.
361 The BAMM analyses are generally congruent with this result and indicate that speciation rates
362 gradually decreased over time (Fig. 4B). The extinction rate is very low, and almost constant,
363 resulting in a ~2-fold decrease in net diversification rates over time.



364

365 **3.5. Biogeographical analyses**

366 Analyzing biogeographical models for our OTU-based trees indicated a substantially better fit
367 for the DEC model than for DIVALIKE and BayAreaLIKE (AICc weight for DEC and DEC+J =
368 0.86-0.99, Table 1), regardless of the topology or the number of OTUs considered. This result
369 implies that changes upon cladogenesis are important for the estimation of historic biogeography
370 in Coelaturini. Biogeographical analyses using the BEAST topology gave highest support for the
371 DEC model (AICc weight = 0.57), whereas it was DEC+J for the MrBayes topology (AICc
372 weight = 0.67). In each case, the other variant of the DEC model received considerable support
373 too (AICc weight = 0.29-0.33). For the BEAST topology, the difference between both models is
374 not significant (one-tailed χ^2 : $p = 0.270$), whereas for the MrBayes analysis the support of
375 DEC+J over DEC trends towards significance (one-tailed χ^2 : $p = 0.046$). As such the importance
376 of jump dispersal upon founder-event speciation in Coelaturini remains ambiguous, with a low
377 jump-dispersal parameter for DEC+J models (Table 1). The extinction parameter was also low,
378 which is consistent with low extinction rates in the BAMM analysis (Fig. 4B), and which may
379 explain why LTT plots under pure-birth and birth-dead models are very similar for this empirical
380 dataset (Fig. 3; see 3.4. Analyses of diversification dynamics). Ancestral range estimations under
381 DEC and DEC+J models are highly similar, regardless of the topology; we report DEC here.
382 Stochastic mapping indicated that few anagenetic events have occurred: dispersal was
383 reconstructed for 7 out of 50 branches (Fig. 4C). The majority of cladogenetic events ($n = 14-16$
384 out of 25) occurred in sympatry, with fewer events relating to subset sympatry ($n = 5-7$) and

19



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385 vicariance (n = 4). The MRCA of Coelaturini occurred in the ecoregions of Lakes Tanganyika,
386 Victoria, or less likely, that of the Central Congo River. Subsequently, within *Coelatura sensu*
387 *lato*, colonization occurred from the northern part of the East African Rift throughout the Nile
388 watershed, and gradually southward towards the ecoregions of the Okavango + Zambezi, Mweru
389 + Upper Lualaba + Lake Malawi.

390

391 **4. Discussion**

392 ***4.1. Evolutionary and taxonomic implications***

393 This first rift-wide analysis of Coelaturini has several evolutionary and taxonomic implications.
394 First, it indicates that *Moncetia*, a monotypic genus from Lake Tanganyika currently attributed to
395 the family Iridinidae (Leloup 1950; Mandahl-Barth 1988; Daget 1998; Graf and Cummings
396 2007), is a unionid. This affiliation was suggested before by Kat (1987), although he did not
397 substantiate it with data. Kondo (1984) attributed *Moncetia* to Mutelidae [= Iridinidae], but
398 reported it to have larval glochidia, which are considered to be an apomorphy of Unionidae +
399 Margaritiferidae + Hyriidae (Parodiz and Bonetto 1963; Graf and Cummings 2006), whereas
400 Iridinidae produce lasidium larvae. It remains unclear, however, how Kondo (1984) attributed
401 glochidia to *Moncetia*, because he reported to have collected them directly from fish hosts and
402 two other glochidium-bearing bivalve genera, i.e. *Pseudospatha* and *Coelatura*, co-occur with
403 *Moncetia* in Lake Tanganyika. Beyond revealing that *Moncetia* belongs to Coelaturini and thus
404 that it should have glochidium larvae indeed, we additionally show that it belongs to the same
405 clade as *Pseudospatha*. Interestingly, Leloup (1950) considered *Moncetia* and *Pseudospatha* to



406 be morphologically continuous, although he placed both in Iridinidae. Studies with faster
407 evolving markers and more comprehensive geographic sampling of Lake Tanganyika are
408 required to examine how *Pseudospatha* and *Moncetia* are related to each other. Our results also
409 call for an in-depth comparative study of the reproductive biology of Coelaturini altogether,
410 especially of its representatives from Lake Tanganyika, given that *Grandidieria* has secondarily
411 lost its parasitic larval stage (Leloup 1950; Kondo 1990), and that both *Grandidieria* and
412 *Moncetia* were reported to brood their eggs within the inner demibranchs of their gills only
413 (Kondo 1984, 1990). Such endobranchy has to our knowledge not been observed in other
414 Unionidae, which are known to brood eggs in all four demibranchs (tetrageny) or the outer pair
415 of demibranchs only (ectobranchy) (Graf and Cummings 2006; Wu et al. 2018). Endobranchy is
416 the common condition in Hyriioidea (Hyriidae) and Etherioidea (Etheriidae + Mycetopodidae +
417 Iridinidae; Graf and Cummings 2006). In any case, our results and those of Whelan et al. (2011)
418 jointly disprove the previous hypothesis that *Pseudospatha*, *Moncetia* and *Prisodontopsis* are
419 more closely related to the South African *Unio caffer* than to *Coelatura* (Kat 1987).
420 Interestingly, the widespread genus *Coelatura* belongs to the crown-group of Coelaturini rather
421 than the stem, implying that its morphological traits, such as its general habitus, larval and
422 brooding morphology, the position of the umbo and umbonal sculpture, may be derived rather
423 than ancestral for Coelaturini. This finding increases the likelihood that Late Cenozoic African
424 fossils with seemingly atypical features, such as *Pseudobovaria* with its strongly anteriorly
425 projected umbo (see Van Damme and Pickford 2010), in fact represent Coelaturini.



426 Taxonomic revision of *Coelatura sensu lato* is thus required, but beyond the scope of the current
427 paper. Conceivable strategies are either to resurrect/erect genus names for individual clades, or
428 alternatively, to lump *Coelatura*, *Mweruella*, *Prisodontopsis*, *Nyassunio* and many deeply
429 divergent lineages into *Coelatura*, formalizing the concept of *Coelatura sensu lato* as used here.
430 Ideally, the taxonomic decision would be informed further with complementary studies on
431 morphology, anatomy and life-history to ensure that taxa are readily diagnosable. Beyond the
432 need to resolve the paraphyly of *Coelatura sensu lato*, a striking result of our analysis is that
433 multiple nominal species that are morphologically distinct and that occupy distinct regions of
434 watersheds are recovered in large polytomies. One example is the clade of the Nile watershed,
435 which includes specimens of *C. aegyptiaca* (Nile), *C. bakeri* (Lake Albert), *C. stuhlmanni* (Lake
436 Edward), *C. hauttecoeuri*, *C. alluaudi* (Lake Victoria), and possibly other species from Lake
437 Victoria that were not sampled (*C. cridlandi*). A second example is the clade including the
438 Coelaturini of Lake Malawi, which contains *C. choziensis* (Lake Bangweulu, Upper Congo), *C.*
439 *luapulaensis* (Upper Congo), *C. mossambicensis* (Lake Malawi and Lower Zambezi), *C.*
440 *hypsiprymna* (Lake Malawi) and *N. nyassaensis* (Lake Malawi). A similar issue was observed in
441 gastropods (Viviparidae) from the Nile drainage: morphologically distinct nominal *Bellamyia*
442 species that occupy separate geographic regions were not recovered as separate entities in
443 phylogenetic analyses with a limited number of gene fragments (Schultheiß et al. 2014).
444 However, subsequent analysis with 15 microsatellites recovered several of these nominal species
445 as distinct molecular groups of which each occupies a geographically separated, though
446 hydrographically interconnected region within the Nile watershed (Van Bocxlaer et al.



447 unpublished data). Whereas our phylogenetic analyses of Coelaturini resolve relationships
448 between taxa from distinct watersheds to large extent, our markers did not fully resolve the
449 relationships of nominal species within drainage basins, except perhaps among multiple deeply
450 separated species in the Congo Basin. Population genomic analyses will be required to examine
451 the reality of previously proposed species entities. This current lack of resolution is one of the
452 main reasons why we currently refrain from revising *Coelatura sensu lato*. It also implies that
453 some of our OTUs may comprise multiple, cryptic species. As a result, we used our robust
454 phylogenetic backbone to reconstruct biogeographical patterns and processes among but not
455 within watersheds.

456

457 **4.2. Diversification dynamics within Coelaturini**

458 Basal cladogenetic events within Coelaturini arose between taxa that are currently living in Lake
459 Tanganyika. The MRCA of the clade containing *Grandidieria*, *Pseudospatha* and *Moncetia* is
460 ~14 Ma (95% HPD: 8.61-19.57 Ma), which is broadly consistent with age estimates for the
461 formation of Lake Tanganyika (~ 9 - 12 Ma; Cohen et al. 1993). Whether biodiversity within
462 Lake Tanganyika has resulted from intralacustrine radiation versus independent colonizations
463 has been the subject of considerable debate for freshwater gastropods (West and Michel 2000;
464 Wilson et al. 2004). Our analyses indicate that at least two independent colonization events of
465 the lake occurred by Coelaturini, followed by considerable intralacustrine diversification.
466 Intralacustrine diversification is also observed in other Tanganyikan taxa, such as the spiny eel
467 *Mastacembelus* (Brown et al. 2010) and the cichlid *Tropheus* (Baric et al. 2003).



468 The diversification of Coelaturini throughout the EARS resulted in a steady accumulation of
469 lineages over time, with an apparent, but non-significant increase ~5 Ma. Assuming that it is not
470 an artifact related to the species delimitation methods, this increase may reflect 1) Pliocene-
471 Pleistocene climate change (Cane and Molnar 2001), or 2) the creation of new ecological
472 opportunities after the extinction of several unionid lineages in sub-Saharan Africa, most notably
473 *Pseudobovaria*, *Pseudodiplodon* and *Gautieraia* (Van Damme and Pickford 2010). Overall,
474 however, a decrease in net diversification rates over time is observed in the BAMM analyses,
475 which suggests rapid diversification of Coelaturini upon colonization of Africa, with a
476 subsequent deceleration of diversification as niches and ecoregions become progressively
477 occupied. In-depth examination of molecular diversity in several drainage basins, including the
478 Lake Victoria Basin, the Okavango-Zambezi region and the Lake Malawi Basin, will be required
479 to reconstruct the dynamics of species diversity in Coelaturini in more detail.

480 Another aspect that needs clarification is that our analyses of diversification dynamics and our
481 best-fit biogeographical models all reconstruct very low levels of extinction. Whereas this
482 finding is perhaps not surprising for recently diversifying clades (see e.g. Condamine et al.
483 2015), several Afrotropical unionid genera have gone extinct since the Middle Miocene (Van
484 Damme and Pickford 2010). Further studies are required to examine whether the reconstructed
485 level of extinction is congruent with the paleontological record.

486

487 **4.3. Historical biogeography**



488 Our phylogenetic framework suggests that patterns of colonization and continent-wide
489 diversification dynamics in Africa are similar for Coelaturini and several fish families (Rüber et
490 al. 2006; Brown et al. 2010). This finding is not surprising given the general correlation between
491 African bivalve and fish diversity (Graf and Cummings 2011), and the fact that most Coelaturini
492 have a fish-parasitizing larval stage (which also represents the phase of greatest mobility in their
493 life cycle). As such, Parreysiinae probably colonized Africa attached to a fish host, like other
494 parasites did (Pariselle 2003), and this colonization by Parreysiinae from Eurasia is thus expected
495 to reflect the patterns of their freshwater fish hosts.

496 *4.3.1. Colonization of Africa*

497 Our fossil-calibrated phylogeny indicates that the MRCA of Coelaturini dates to ~17 Ma (95%
498 HPD: 11.98-23.28 Ma), which suggests that the colonization of Africa from Eurasia by
499 Parreysiinae likely occurred in the Early or Middle Miocene. This estimate is consistent with
500 geological evidence for the closure of the Tethys seaway ~18-20 Ma (Okay et al. 2010), and with
501 the first evidence of mammal exchanges between both continents (Rögl 1999; Harzhauser et al.
502 2007; Harzhauser and Piller 2007). Additionally, rifting in southwestern Ethiopia seems to have
503 occurred around the same time (~18 Ma; Ebinger et al. 2000; Pik et al. 2008), although much of
504 the eastern branch of the EARS was established ~20 Ma (Pickford 1982; Chorowicz 2005).
505 These events have formed a corridor that has facilitated the colonization of Africa from Eurasia
506 by freshwater biota, as evidenced by the fish families Mastacembelidae (Brown et al. 2010),
507 Anabantidae (Rüber et al. 2006), Clariidae (Agnese and Teugels 2005), Bagridae and Cyprinidae
508 (Stewart and Murray 2017) and viviparid gastropods (Schultheiß et al. 2014; Sil et al. 2019).



509 Despite the congruence with geological and other biogeographical data, our estimated timeframe
510 for the colonization of Africa by Parreysiinae is substantially younger than the Paleocene-Eocene
511 age estimates of Bolotov et al. (2017). These latter authors used a calibration strategy based on
512 the assignment of Paleogene Asian fossils to modern genera and fossil constraints on outgroup
513 taxa, which has been criticized before (Pfeiffer et al. 2018). Early Cenozoic estimates render the
514 absence of Coelaturini in the Oligocene and Early to Middle Miocene fossil record of the East
515 African Rift problematic (Kat 1987).

516 The route by which Parreysiinae colonized Africa from Eurasia remains equivocal. Colonization
517 probably occurred via the Arabian Peninsula and the Bab al-Mandab Strait towards the Awash
518 River in Ethiopia. Miocene deposits along this river contain freshwater fish fossils whose origins
519 were in Asia, such as *Labeo*, *Barbus*, *Labeobarbus*, *Bagrus* and *Clarias*, which corroborates this
520 hypothesis (Stewart and Murray 2017), but the associated freshwater mollusk fauna is currently
521 poorly surveyed. The alternative hypothesis is that the colonization of Africa from Asia took
522 place via a northern route through the Sinai Peninsula and Northern Egypt, as is suggested for
523 some terrestrial vertebrates (e.g. Pook et al. 2009; Gilbert et al. 2014). This colonization route
524 has not been substantiated for freshwater biota because during much of the Middle and Late
525 Miocene the Sinai Peninsula was occupied by marine waters (Westcott et al. 2000; Van Damme
526 and Van Bocxlaer 2009; Tsigenopoulos et al. 2010; Stewart and Murray 2017). The large-scale
527 colonization of Africa from Eurasia in the Miocene, and early dispersal within Africa, seem to
528 have coincided with the Middle Miocene Climatic Optimum (~15-17 Ma). This period was warm



529 with high precipitation (Zachos et al. 2001) that would have enhanced hydrographic connectivity
530 in Africa, and therefore dispersal and diversification of aquatic biota (Day et al. 2017).

531

532 4.3.2. Dispersal throughout the EARS

533 We outline hydrographic connections in the Late Miocene and Early Pliocene in Fig. 5 together
534 with a synthesis of the implications of our biogeographical analyses. Our biogeographical model
535 suggests that the MRCA of Coelaturini occurred within the ecoregions of Lake Tanganyika,
536 Victoria and (less likely) the Upper Congo. Regardless of the route that Parreysiinae used to
537 colonize Africa from Eurasia, it is unlikely that Lake Tanganyika was reached without passing
538 through areas of the Lake Victoria ecoregion first, implying that the MRCA of Coelaturini
539 probably occurred in central East Africa and then gave rise to separate lineages that colonized
540 the Tanganyika and Victoria regions ~14 Ma (as also supported by subset sympatry in our BSM).
541 Early occupation of the Lake Victoria area is congruent with the fossil record, as the oldest
542 fossils of Coelaturini occur in this ecoregion (i.e. Baringo Basin and Albertine Basin; De Groeve
543 2005; Van Damme and Pickford 2010), although these fossils are Late Miocene and thus
544 substantially younger than ~14 Ma. The alternative scenario is that Africa was primarily
545 colonized via the Lake Tanganyika ecoregion and subsequently via the Lake Victoria and Congo
546 ecoregions. The fossil record and BSM provide less evidence for this scenario, but rapid
547 colonization of the Lake Tanganyika ecoregion after migration to Africa is also recorded for
548 Viviparidae: The currently monotypic Tanganyikan viviparid genus *Neothauma* diverged rapidly



549 from other, more widespread African viviparid lineages (Schultheiß et al. 2014). However, the
550 fossil viviparid record indicates that several extinct species of *Neothauma* occupied the Lake
551 Victoria ecoregion in the Miocene (Van Damme and Pickford 1999; Salzburger et al. 2014).
552 *Neothauma tanganyicense*, and perhaps some of the Tanganyikan Coelaturini, may thus be
553 relicts in Lake Tanganyika from earlier more widespread taxa, suggesting that the lake had a
554 refugial function at a continental scale. Faunal exchange between the Lake Victoria and Lake
555 Tanganyika ecoregions could have occurred via the proto-Rusizi River, a precursor of the current
556 Rusizi, or alternatively via the Malagarasi River (Danley et al. 2012).

557 Our biogeographical analyses further suggest that Coelaturini colonized the Congo River from
558 the larger Lake Victoria ecoregion, which includes the developing Ethiopian rift. During the
559 Early and Middle Miocene the proto-Aruwimi River was diverted towards the Nile, which could
560 have allowed the colonization of Nilotic ecoregions and the Upper Congo from the larger Lake
561 Victoria area (Flügel et al. 2015). In the Late Miocene, the waters of the Lake Victoria ecoregion
562 drained towards the Albertine Basin, and then via a precursor of the Aruwimi River into the
563 Congo River (Van Damme and Pickford 1999). At that time, rifting resulted in the development
564 of Palaeolake Obweruka (Van Damme and Pickford 1999), which may have provided some
565 habitats in which Coelaturini persisted locally. The Late Miocene-Early Pliocene *Coelatura* cf.
566 *stanleyvillensis* from the Albertine Basin displays morphological features that are similar to those
567 of taxa currently inhabiting the Congo Basin (Van Damme and Pickford 2010). As rifting
568 proceeded, the hydrographic connectivity of the Congo and Victoria ecoregions may have
569 decreased, facilitating allopatric speciation in *Coelatura* ~10 Ma. Around the same time,



570 viviparid gastropods occurring in the Congo and Victoria ecoregions became isolated as well
571 (Schultheiß et al. 2014). Other evidence for aquatic colonization from the Victoria ecoregion
572 towards the Congo is observed in the haplochromine cichlid “Yaekama”, which is distributed in
573 the northeastern part of the Congo River System near Kisangani, but groups with the Lake
574 Victoria superflocks (Schwartz et al. 2012). Furthermore, the distribution of the poeciliid genus
575 *Hypsopanchax* also points to the connectivity of the Victoria ecoregion with the Congo Basin
576 drainage via a precursor of the Aruwimi (Poll and Lambert 1965; Roller et al. 2010; Bragança
577 and Costa 2019).

578 Subsequently, several diversification events occurred in Coelaturini within the Congo Basin ~8-
579 10 Ma, and some of the descendant lineages (*C. cf. leopoldvillensis*1 & 2, *C. cf. gabonensis*1 &
580 2) are currently sympatric. The potential biogeographical setting for the accumulation of
581 diversity in this hotspot is currently uncertain, but diversification caused by the hydrographic
582 separation of regions within the Congo Basin is conceivable. Alternatively, it is possible that
583 these lineages did not originate in the Congo Basin itself, but that they derive from independent
584 colonization events from separate source regions as in the spiny eel *Mastacembelus* (Day et al.
585 2017) and the fish fauna of the Upper Luapula River (Van Steenberge et al. 2014).

586 *Coelatura* colonized Lake Tanganyika from the Congo Basin relatively late ~5-9 Ma, while
587 *Grandidieria* and *Pseudospatha* + *Moncetia* already inhabited the lake. This pathway implies the
588 colonization of the Upper Congo waters (Lualaba and Lukuga Rivers) by *Coelatura* from the
589 proto-Aruwimi and Lower Congo. Fossil evidence that would substantiate the pathway and time
590 of this colonization of Lake Tanganyika has not been discovered yet. However, various fish taxa



591 of Lake Tanganyika have relatives in the Congo River basin, e.g. Lamprologini and *Lacustricola*
592 (Bragança and Costa 2019), and the Malagarasi River, a major tributary of Lake Tanganyika that
593 probably existed prior to rifting (Cohen et al. 1997), is also inhabited by a fish fauna that is
594 similar to that of the Congo Basin (Goodier et al. 2011; Kullander and Roberts 2011). A similar
595 distribution is also observed for the gastropod *Potadomoides* (West et al. 2003). As our
596 biogeographical reconstruction indicates, *Coelatura* also colonized the Malagarasi River and
597 coastal East Africa from Lake Tanganyika later.

598 Between ~6-8 Ma, *Coelatura* also dispersed from the Congo catchment to the Okavango region.
599 This colonization could have occurred by a connection from the Lower Congo system to the
600 headwaters of the Upper Zambezi via the Kasai River (Veatch 1935; Stankiewics and de Wit
601 2006; Moore et al. 2007). Viviparid gastropods have been hypothesized to have used this route to
602 colonize the Okavango region from the Congo River ~ 6.3 Ma (Schultheiß et al. 2014), and the
603 route has been invoked for various fish taxa (Bell-Cross 1966), such as catfish (Day et al. 2009),
604 tigerfish (Goodier et al. 2011), and cichlids (Schwartzter et al. 2012). The BSM analysis suggests
605 that parts of Zambia including the areas of Lake Bangweulu and Lake Mweru were colonized
606 simultaneously to the Okavango region by Coelaturini, followed by vicariance ~4.5-6.0 Ma. The
607 Mweru ecoregion was probably colonized from the Okavango ecoregion via the Chambeshi
608 River, which may have drained eastward into the Rufiji system in the Pliocene (Stankiewics and
609 de Wit 2006). Although the Chambeshi River is currently confluent with the Luapula, it
610 represents the former upper reaches of the Kafue, a major Upper Zambezi affluent (Moore and
611 Larkin 2001; Goudie 2005; Stankiewics and de Wit 2006) and a connection between the



612 Chambeshi and the Kafue probably existed until the early Pleistocene (0.78-2.58 Ma) (Cotterill
613 and de Wit 2011). A biogeographical pattern similar to that of Coelaturini was also recovered by
614 Schultheiß et al. (2014) for viviparids of their ‘clade II’, which were broadly distributed
615 throughout Zambia, and for the fishes of the Upper Luapula area (Van Steenberge et al. 2014).
616 Colonization of the Malawi Basin by Coelaturini is substantiated by fossils of ~2.5 Ma from
617 Paleolake Chiwondo (Schrenk et al. 1995; Van Damme and Gautier 2013). The colonization of
618 the coastal East African ecoregion may have occurred through the Malawi Basin, ~0.8-1.2 Ma
619 ago via the proto-Ruhuhu River, which would have been the outlet of Lake Malawi at that time
620 (Ivory et al. 2016). Finally, a recolonization of the Congo River occurred from Lake Mweru
621 ~1.85-3.04 Ma by *Coelatura briarti*, which may have taken place via the Luvua River, a
622 tributary of the Lualaba River.

623 As mentioned before, the proto-Aruwimi River was diverted towards the Nile during the Early
624 and Middle Miocene, which could have allowed an early colonization of Nilotic ecoregions
625 (Flügel et al. 2015). Currently, we have no evidence of such a colonization for Coelaturini and
626 the extant Nilotic fauna results from Early Pliocene dispersal, which is congruent with the fossil
627 record (see Van Damme and Van Bocxlaer 2009). Several fish taxa from Early Pliocene deposits
628 (lower Tinde Member) of Paleolake Manonga in Northern Tanzania have Nilotic affinities,
629 indicating connectivity of the southern Lake Victoria ecoregion to Nilotic ecoregions at that
630 time, either via the Albertine Basin, the Omo-Turkana Basin or both (Stewart 1997). Coelaturini
631 have not been recovered from the deposits of Paleolake Manonga (Van Damme and Gautier
632 1997), however, the Early Pliocene fossils of *C. aegyptiaca* and *C. bakeri* from the Omo-



633 Turkana Basin substantiate faunal exchange between the Lake Victoria ecoregion and Nilotic
634 ecoregions at that time (Van Bocxlaer 2011; supplementary text on fossil calibrations).

635 In summary, knowledge on the historic hydrographic connections throughout the EARS and
636 surroundings and our results on distribution patterns in Coelaturini are highly congruent. Many
637 particularities of the biogeography of Coelaturini are furthermore substantiated by similar
638 patterns in other freshwater invertebrates and various fish taxa. A striking result of our study is
639 the ancient colonization of the Lake Tanganyika ecoregion, where various genera of Coelaturini
640 appear to have evolved, persisted and coexisted. Our analyses furthermore suggest that each of
641 the lineages colonizing the Tanganyika ecoregion underwent diversification in the Late Miocene-
642 Early Pliocene. For major clades of Coelaturini elsewhere, population genetic analyses will be
643 required to study biodiversity patterns within drainage basins, and the evolutionary processes
644 that underpin this diversification.

645

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665

666 **Competing interests**

667 The authors have no competing interests to declare.

668

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1058 **Tables**

1059 **Table 1.** Comparison of the six biogeographical models that were fit to our OTU phylogeny with
 1060 their log-likelihood (LnL), number of parameters (NumPar), parameter values (d =
 1061 diversification, e = extinction and j = jump-dispersal) and relative AICc support with weights
 1062 (AICc_wt). The best-fit model is highlighted in bold.

Model	LnL	NumPar	d	e	j	AICc	AICc_wt
BEAST MCC tree							
DEC	-67.51	2	0.0058	1.00E-12	-	139.5	0.5700
DEC+J	-66.9	3	0.0050	1.00E-12	0.0077	140.9	0.2900
DIVALIKE	-69.69	2	0.0070	1.00E-12	-	143.9	0.0640
DIVALIKE+J	-68.28	3	0.0055	1.00E-12	0.0076	143.6	0.0730
BAYAREALIKE	-82.14	2	0.0073	8.60E-02	-	168.8	0.0000
BAYAREALIKE+J	-73.4	3	0.0045	1.00E-07	0.0150	153.9	0.0004
MrBayes consensus tree							
DEC	-72.95	2	0.1400	2.00E-01	-	150.4	0.3300
DEC+J	-70.97	3	0.1200	1.00E-12	0.0094	149.0	0.6700
DIVALIKE	-81.46	2	0.2000	8.10E-01	-	167.4	0.0001
DIVALIKE+J	-77.31	3	0.1300	1.00E-12	0.0090	161.7	0.0012
BAYAREALIKE	-86.86	2	0.2100	2.02E+00	-	178.2	0.0000
BAYAREALIKE+J	-87.07	3	0.3100	3.24E+00	0.0040	181.2	0.0000

1063

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1066 **Figures**

1067 **Fig. 1.** Phylogenetic relationships in Coelaturini reconstructed with Bayesian inference
1068 (MrBayes) using data from four gene fragments (COI, 16S rRNA, H3 and 28S rRNA). As the
1069 maximum likelihood reconstruction (RAxML) is very similar we indicate Bayesian posterior
1070 probabilities and bootstrap support values for relevant branches. Colored symbols for the 11
1071 ingroup clades are used in fig. 4A. Representative taxa are displayed in the inset: a)
1072 *Pseudospatha tanganyicensis*, b) *Grandidieria burtoni*, c) *Prisodontopsis aviculaeformis*, d)
1073 *Mweruella mweruensis*, e) *Coelatura aegyptiaca*, f) *Nitia acuminata*, g) *Nyassunio nyassaensis*,
1074 h) *Moncetia anceyi*.

1075

1076 **Fig. 2.** Fossil-calibrated MCC tree reconstructed with BEAST. Posterior probabilities (BPP) are
1077 indicated with colored circles, node bars denote 95% HPD intervals of node ages and asterisks
1078 indicate the nodes that were used for fossil calibration. *Parreysia* was included in the analysis
1079 but removed from the tree to increase readability for the ingroup.

1080

1081 **Fig. 3.** OTU-based trees reconstructed from 1,000 trees that were randomly sampled from the
1082 posterior distribution of BEAST. The inset indicates how lineages accumulated through time (in
1083 blue), which compares well to pure-birth and birth-death models for this dataset (in red). A
1084 decrease in diversification rates was observed ~ 3 Ma ago in TreePar.

1085



1086 **Fig. 4.** Diversification and biogeographical analyses. A) The distribution of Coelaturini across
1087 Africa is highlighted in transparent purple, together with the sampled localities; the boundaries of
1088 freshwater ecoregions are marked in grey; sampling localities are indicated with the clade-
1089 specific color-coded symbols of Fig. 1 to reconstruct the geographic distribution of the 11 clades
1090 of Coelaturini from Figs 1 & 2. B) Speciation and extinction rates as inferred from the BAMM
1091 analysis. C) OTU-based phylogeny with ancestral range estimations derived from the DEC
1092 model and the results of biogeographical stochastic mapping. Dashed branches indicate
1093 anagenetic dispersal events, whereas letters at nodes indicate cladogenetic events of s = subset
1094 sympatry, y = sympatry, and v = vicariance.

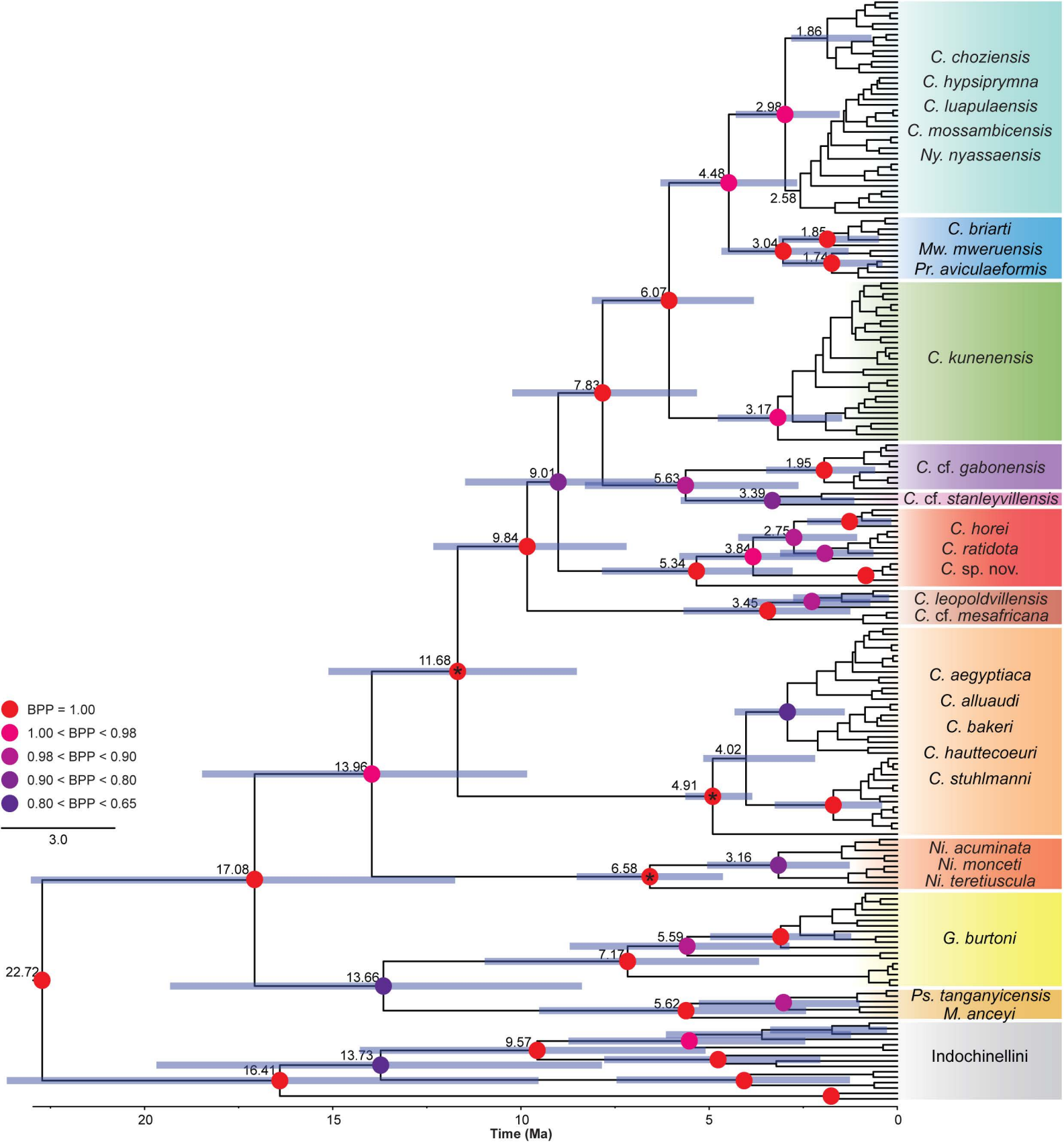
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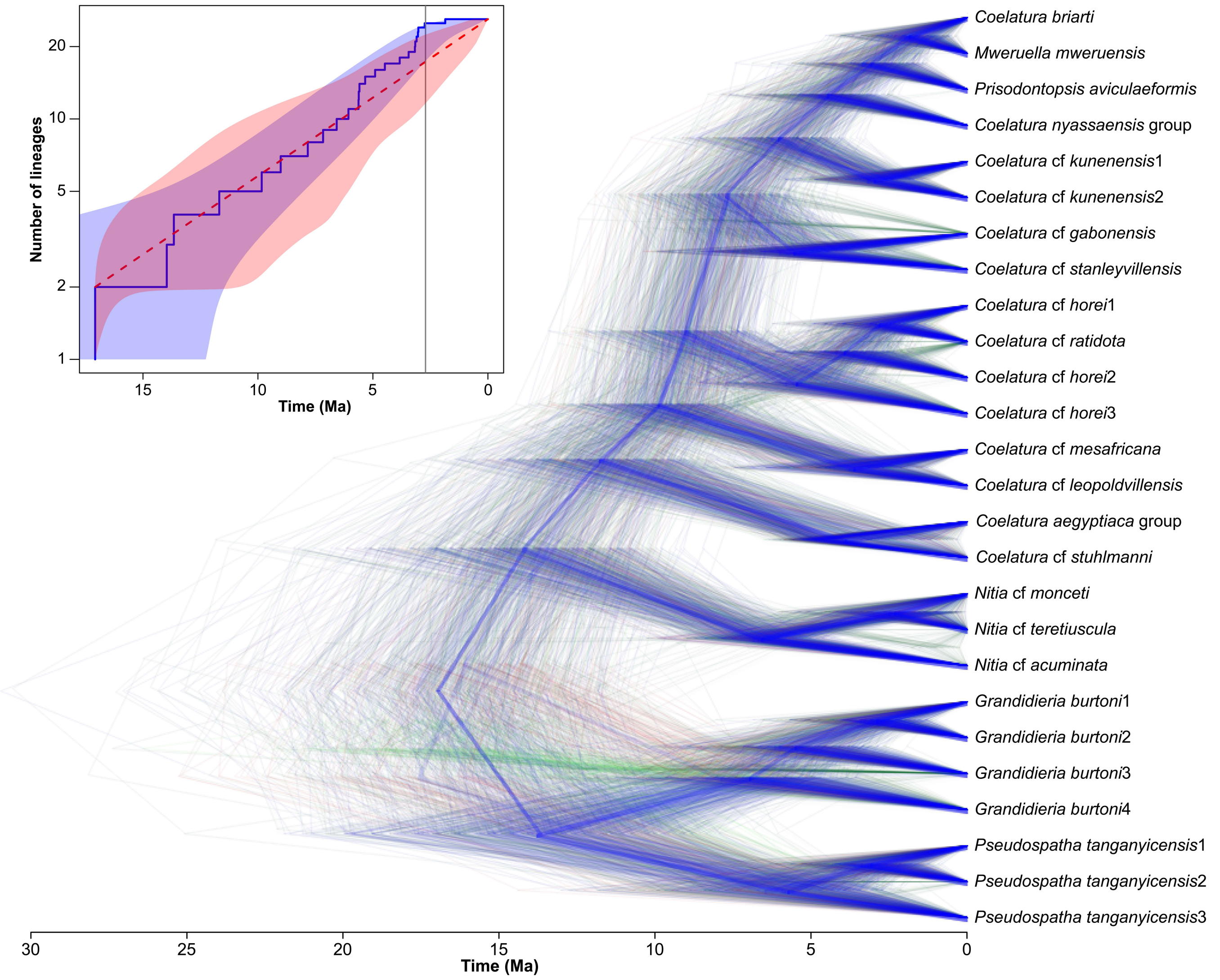
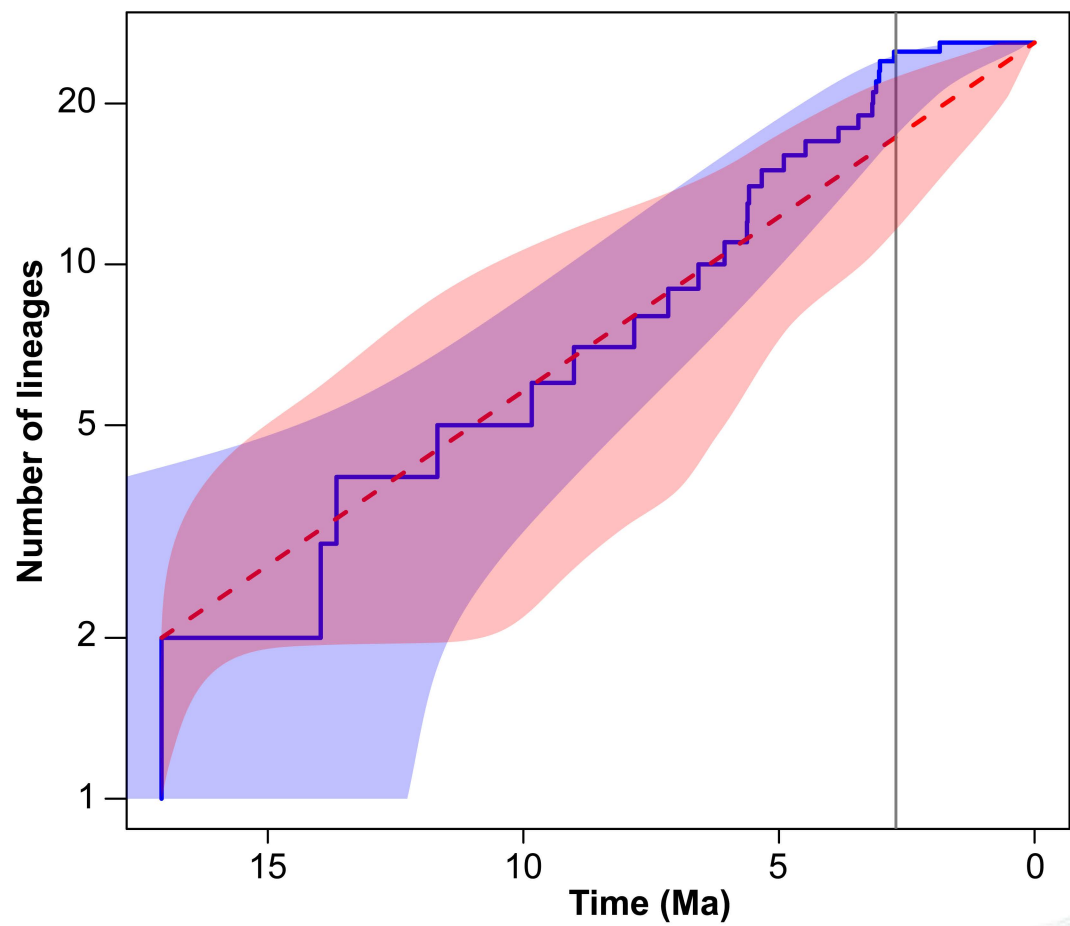
1096 **Fig. 5.** Reconstruction of hydrographic connections throughout the East African Rift System
1097 (EARS) during A) the Late Miocene (~10 Ma) and B) Early Pliocene (~4 Ma), with arrows
1098 representing colonization patterns of Coelaturini during these (sub-)epochs. In the Early to
1099 Middle Miocene Parreysiinae colonized the EARS from Eurasia. The MRCA of Coelaturini
1100 subsequently colonized the Victoria and Tanganyika ecoregions (black arrows). A secondary
1101 colonization of the Tanganyika ecoregion occurred via the Congo, from where the Okavango
1102 was also colonized (grey arrows). During the Pliocene, Coelaturini were established in three
1103 faunal provinces directly within the East African Rift (bioregions S = Upper Congo, T =
1104 Tanganyika, and V = Victoria; indicated in bold). The Nile was colonized from the larger
1105 Victoria ecoregion, whereas the Mweru and Malawi regions from the Okavango via the Upper
1106 Zambezi (black arrows). Finally, in the Pleistocene, a recolonization of the Congo headwaters

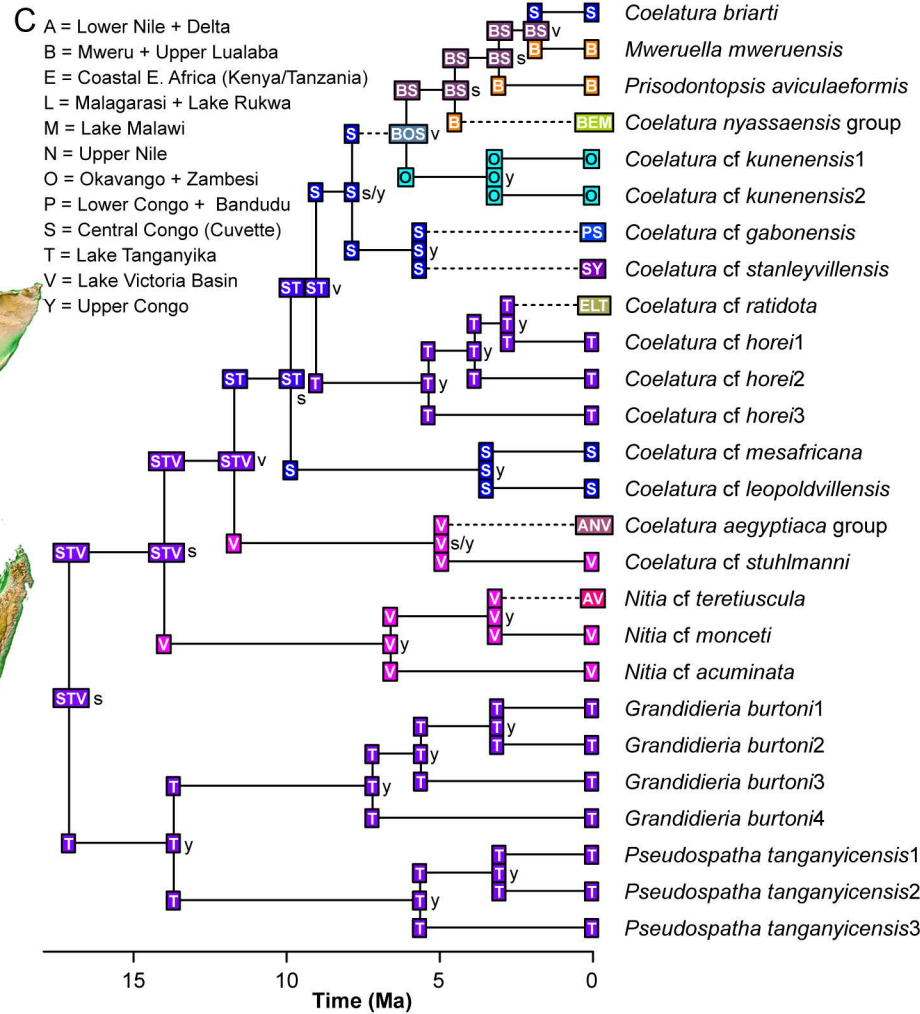
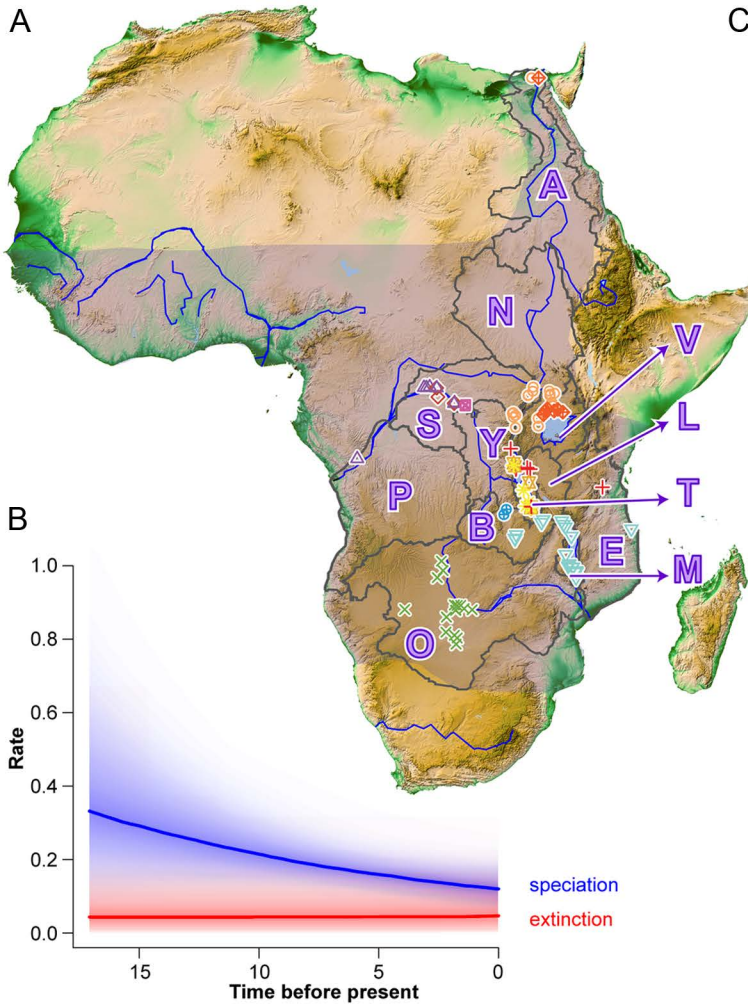


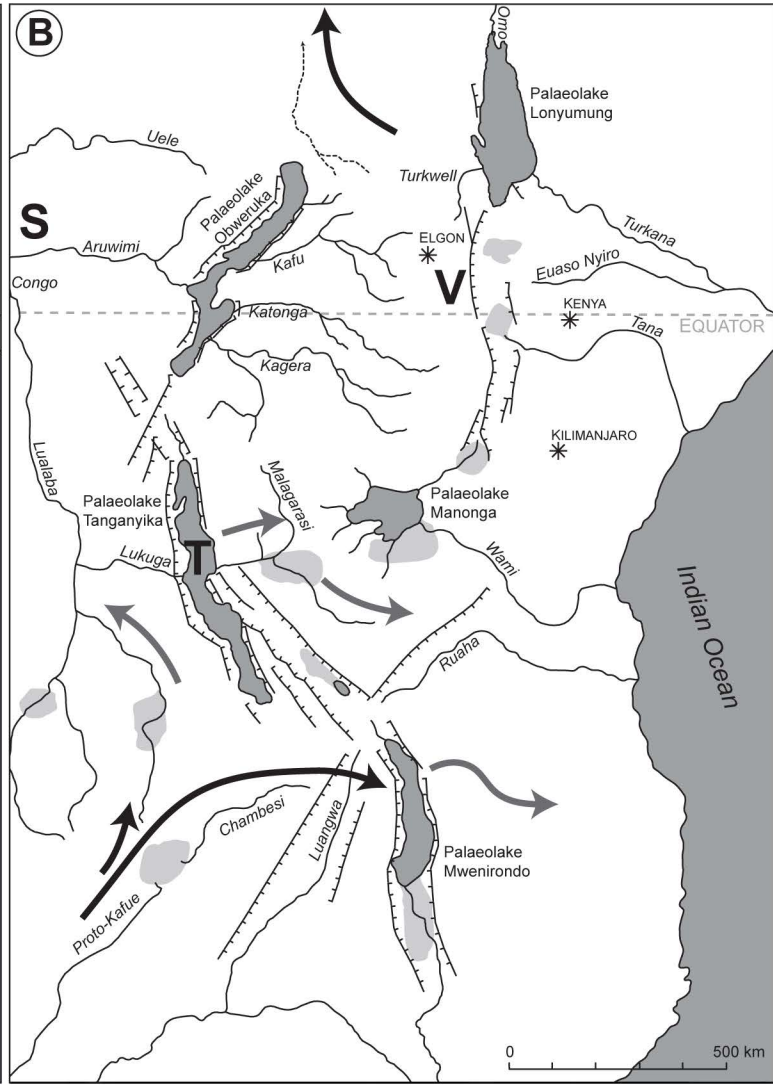
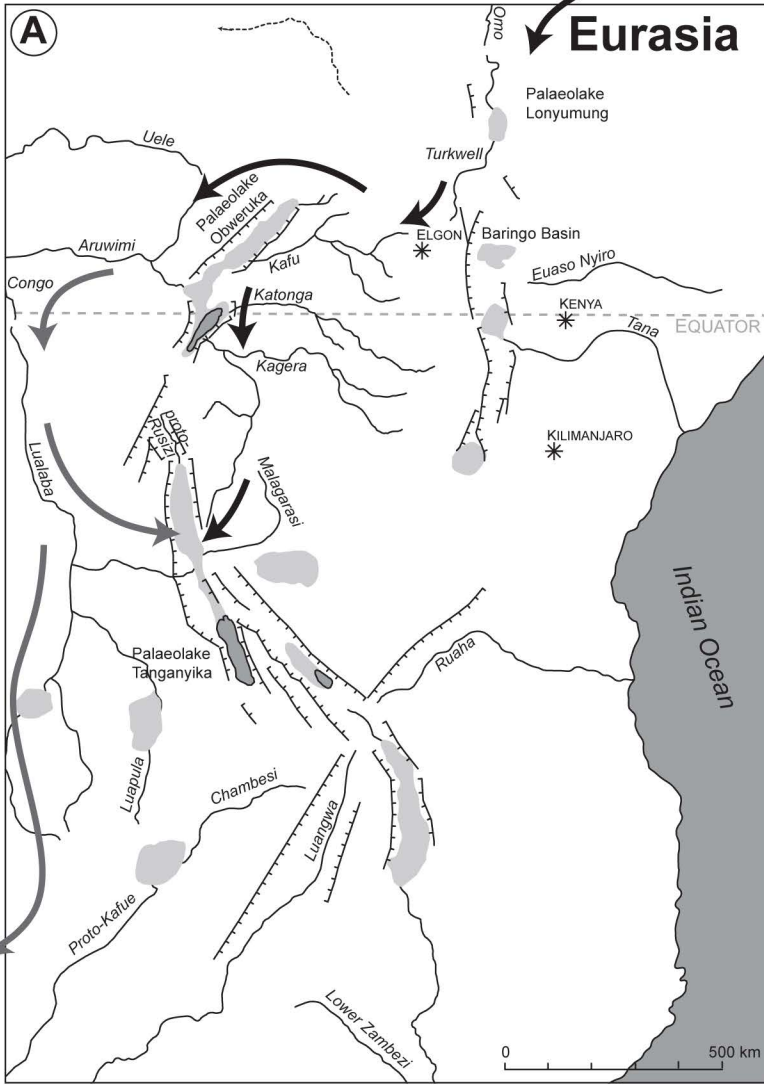
1107 from Lake Mweru and dispersal to the Malagarasi and Eastern coastal Africa occurred from
1108 Lakes Tanganyika and Malawi, respectively (grey arrows). Paleolakes are indicated in dark grey
1109 whereas wetlands in light grey.











Supplementary material for:

**Diversification dynamics of freshwater bivalves (Unionidae: Parreysiinae: Coelaturini)
indicate historic hydrographic connections throughout the East African Rift System
Ortiz-Sepulveda et al.**

1 **Fossil calibrations**

2 **Constraining the most recent common ancestor (MRCA) of *Nitia***

3 Specimens of *Nitia* cf. *monceti* are reported by Van Damme and Pickford (2010) from the
4 Nkondo Member of the Nkondo Formation in the Albertine Basin. They attribute these
5 specimens to mollusk association G3a of Paleolake Obweruka, which is estimated to have
6 occurred 5.5-7.5 Ma. The assignment of these absolute ages is based on mammalian
7 biochronological inference (Pickford et al. 1993; Van Damme and Pickford 2010). *Nitia* is not
8 found in older mollusk associations from the Albertine Rift, nor in Middle Miocene deposits at
9 Rusinga Island (Kat 1987) or in the Turkana Grits (Boschetto 1988).

10 Assignment of the fossils to *Nitia* is based on their antero-dorsally elongated shells that bear a
11 thin, straight hinge with compressed cardinal teeth – a set of characteristics that does not exist in
12 other Coelaturini. Our phylogenetic analyses retrieved the three included *Nitia* species as a
13 monophyletic clade (Fig. 1) suggesting that their morphological distinctness reflects common
14 descent. Whereas the fossil specimens strongly resemble extant *Nitia monceti* from Lake
15 Victoria, we cannot exclude that these similarities are convergent. As such, we decided to use the
16 fossils to constrain the age of the MRCA of *Nitia*, rather than the split of *N. monceti* from its
17 sister taxon.

18 Based on the proposed ages of mollusk association G3a, we consider 5.5 Ma as the minimum age
19 for the appearance of *Nitia*, so we used a lognormal prior with a mean of 7.5 Ma and a 95%
20 confidence interval (CI) spanning 5.3-10.4 Ma to constrain the age of the MRCA of *Nitia*.

21

22

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23 **Constraining the MRCA of the clade of *Coelatura aegyptiaca*, *C. bakeri* and *C. hauttecoeuri***

24 The morphologically-variable species of *Coelatura* from the Nile catchment, namely *C.*
25 *aegyptiaca*, *C. alluaudi*, *C. bakeri*, *C. hauttecoeuri*, and *C. stuhlmanni* were recovered as a clade.
26 In this catchment a variety of fossil outcrops spanning the Neogene period occur, but as Kat
27 (1987) already remarked, *Coelatura* is conspicuously absent from Early and Middle Miocene
28 sediments. The earliest specimens that are assignable to *C. hauttecoeuri*, *C. bakeri* and *C.*
29 *aegyptiaca* and, therefore, to the abovementioned recovered phylogenetic clade, are of Late
30 Miocene and Early Pliocene age. The oldest specimens of *C. hauttecoeuri* are found at Mosionin
31 and Tabarin in the Tugen Hills, and they derive from sediments that belong to the lower Pliocene
32 Mabaget Formation, which was previously considered to be part of the Chemeron Formation (De
33 Groeve 2005; Van Damme and Pickford 2010). Several tuffs of the Chemeron Formation have
34 been dated (Deino et al. 2002), on the basis of which the Mabaget Formation is general
35 considered to be 5.3-4.5 Ma (Roche et al. 2013). Other fossils of *C. cf. hauttecoeuri* have been
36 recovered from the Sinda and Nyamavi Beds in the Albertine Basin (Van Damme and Pickford
37 2010), but as this material derives from mollusk association G5, it is substantially younger (3.5-
38 2.5 Ma). The earliest fossils of *C. bakeri* and *C. aegyptiaca* were recovered from sediments that
39 were deposited in Paleolake Lonyumung in the Turkana Basin. These taxa were recovered from
40 multiple Early Pliocene formations, i.e. the Basal Member of the Shungura Formation (~3.9 Ma),
41 the Kanapoi Formation (4.10-4.15 Ma) and the Nachukui Formation (~4.0Ma; Van Bocxlaer
42 2011, 2020).

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43 Assignment of these fossils to morphospecies has been performed based on general shell
44 morphology (including geometric morphometrics) and differences in umbonal sculpture as
45 indicated in Van Bocxlaer (2011).

46 We here specifically used the *C. hauttecoeuri* specimens from the Tugen Hills with a hard
47 minimum age of 4.5 Ma to calibrate the MRCA of the clade of *Coelatura aegyptiaca*, *C. bakeri*
48 and *C. hauttecoeuri*. To do so, we used a lognormal prior with a mean of 5.2 Ma and a 95% CI
49 of 4.3-6.3 Ma.

50

51 **Constraining the MRCA of *Coelatura sensu lato***

52 The oldest fossil that can be unambiguously assigned to the genus *Coelatura* is *Coelatura* cf.
53 *stanleyvillensis* from the Oluka Formation of the Albertine Basin (Van Damme and Pickford
54 2010). The material has a trapezoid outline in lateral view, with low beaks that bear a zigzag
55 ornamentation if not corroded. As such it is distinct from all more basal Coelaturini. *Coelatura*
56 cf. *stanleyvillensis* occurs in mollusk associations G2b and G3.

57 In some sections of their report Van Damme and Pickford (2010, p. 37) suggested the earliest
58 specimens to have an age of ~5.5 to 5.0 Ma, however, this age probably refers to younger
59 specimens from the Lower Nyaburogo Formation. Indeed, the authors elsewhere report the
60 abovementioned mollusk associations to range between ~8.5 and 4.5 Ma (Van Damme and
61 Pickford 2010, Fig. 2 and Table 2). Therefore, the minimum age of mollusk association G2b, 7.5
62 Ma, can be considered to represent a hard minimum age for the first appearance of *Coelatura* in
63 the fossil record. Here, we used a lognormal prior with a mean age of 10.0 Ma and a 95%
64 confidence interval of 7.2-13.5 Ma to calibrate the age of the MRCA of *Coelatura sensu lato*.

Supplementary material for:

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109 **Table S1.** Specimens used for this study with their collection code, DNA voucher number, species
110 identification, locality information and accession numbers from NCBI GenBank. Letter codes have been
111 added to accession numbers that share the same haplotype for a specific gene fragment. Collection data:
112 ANSP = Academy of Natural Sciences of Drexel University; NHMUK = Natural History Museum,
113 London; RMBH = Russian Museum of Biodiversity Hotspots; UA = University of Alabama Museum of
114 Natural History; UGSB = Systematics and Biodiversity Collection of the Justus-Liebig University,
115 Giessen; UMMZ = University of Michigan Museum of Zoology; some individuals carry codes consisting
116 of the country code, the year of collecting and sampling number.

Collection code	DNA voucher	Species	Country/Waterbody	GenBank accession numbers				Reference
				16S	28S	COI	H3	
UGSB220	6926	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218152	MT218018/C	MT216486/p	MT183444/II	This study
UGSB222	6927	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218153	MT218019/C	MT216487/o	MT183445	This study
UGSB256	6929	<i>C. bakeri</i>	Uganda; Lake Albert	MT218154/bb	MT218020/P	MT216488	MT183446/JJ	This study
UGSB256	6930	<i>C. bakeri</i>	Uganda; Lake Albert	MT218155	MT218021	MT216489	MT183447/JJ	This study
UGSB273	6931	<i>C. bakeri</i>	Uganda; Lake Albert	MT218156	MT218022	MT216490	MT183448	This study
UGSB273	6932	<i>C. bakeri</i>	Uganda; Lake Albert	MT218157/bb	MT218023/Q	MT216491	n.a.	This study
UGSB108	6936	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218158/rr	MT218024/C	MT216492	MT183449/KK	This study
UGSB197	6938	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218159/aa	MT218025/C	MT216493/p	MT183450	This study
UGSB186	6943	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218160/aa	MT218026/C	MT216494	MT183451	This study
UGSB186	6944	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218161	MT218027/C	MT216495	MT183452/II	This study
UGSB186	6946	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218162	MT218028/C	MT216496/q	MT183453	This study
UGSB222	6947	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218163	MT218029/C	MT216497	MT183454/AA	This study
UGSB222	6948	<i>N. nyassaensis</i>	Malawi; Lake Malawi	MT218164/rr	MT218030/C	MT216498	MT183455/AA	This study
UGSB5689	13006	<i>C. kunenensis</i>	Botswana; Chobe River	n.a.	MT218031/E	n.a.	MT183341/HH	This study
UGSB5689	13007	<i>C. kunenensis</i>	Botswana; Chobe River	MT218165	MT218032/E	n.a.	MT183342/FF	This study
UGSB16303	15140	<i>C. choziensis bangweolica</i>	Zambia; Lake Bangweulu	n.a.	MT218033/C	MT216345/f	MT183343/FF	This study

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UGSB5662	15142	<i>C. choziensis bangweolica</i>	Zambia; Lake Bangweulu	n.a.	MT218034/C	MT216346	MT183344/II	This study
UGSB16305	15143	<i>C. choziensis</i>	Zambia; Lake Kampolombo	n.a.	MT218035/C	MT216347	n.a.	This study
UGSB5665	15144	<i>C. choziensis</i>	Zambia; Lake Kampolombo	n.a.	MT218036/C	MT216348	n.a.	This study
UGSB16306	15145	<i>C. kunenensis</i>	Namibia; Zambezi River	n.a.	MT218037/E	MT216349/n	MT183345/FF	This study
UGSB16307	15146	<i>C. kunenensis</i>	Namibia; Zambezi River	n.a.	MT218038/K	MT216350	MT183346	This study
UGSB16308	15147	<i>C. kunenensis</i>	Namibia; Zambezi River	n.a.	MT218039/E	MT229885/n	MT231525/FF	This study
UGSB16313	15152	<i>C. kunenensis</i>	Botswana; Chobe River	n.a.	MT218040/E	MT216351	MT183347/FF	This study
UGSB16316	15155	<i>C. kunenensis</i>	Botswana; Chobe River	MT218166	MT218041/E	MT216352	MT183348/FF	This study
UGSB5275	21548	<i>Nitia monceti</i>	Uganda; Lake Victoria	MT218167/zz	MT218042/F	MT216353	MT183349/BB	This study
UGSB5305	21549	<i>C. hauttecoeuri kyogae</i>	Uganda; Lake Kyoga	MT218168	MT218043/P	MT216354/g	MT183350/JJ	This study
UGSB4846	21550	<i>Nitia teretiuscula</i>	Uganda; Lake Victoria	MT218169	MT218044	n.a.	MT183351/BB	This study
UGSB5407	21551	<i>C. hauttecoeuri</i>	Uganda; Lake Victoria	MT218170	MT218045/Q	MT216355	MT183352	This study
UGSB5298	21552	<i>C. alluaudi</i>	Uganda; Lake Victoria	MT218171	MT218046/P	MT216356/g	MT183353/JJ	This study
UGSB5597	21555	<i>C. stuhlmanni</i>	DR Congo; Lake Edward	MT218172/yy	MT218047	MT216357	MT183354/JJ	This study
UGSB5414	21556	<i>C. bakeri</i>	Uganda; Lake Albert	MT218173/xx	MT218048/Q	MT216358	MT183355	This study
UGSB5318	21557	<i>Nitia monceti</i>	Uganda; Lake Victoria	MT218174/zz	MT218049	MT216359	MT183356/BB	This study
UGSB5490	21558	<i>C. hauttecoeuri</i>	Uganda; Lake Victoria	MT218175/xx	MT218050/Q	MT216360/g	MT183357/JJ	This study
UGSB0020	21559	<i>Nyassunio nyassensis</i>	Malawi; Lake Malawi	MT218176/ww	MT218051/C	n.a.	MT183358/FF	This study
UGSB15538	21568	<i>C. mossambicensis</i> aff.	Malawi; Shire River	MT218177	MT218052/C	MT216361	n.a.	This study
UGSB15539	21569	<i>C. hypsiprymna</i>	Malawi; Shire River	MT218178	MT218053/C	MT216362	MT183359/AA	This study
UGSB15540	21570	<i>C. hypsiprymna</i>	Malawi; Shire River	MT218179	MT218054/C	MT216363	MT183360/II	This study
UGSB4891	21571	<i>Nitia monceti</i>	Kenya; Lake Victoria	MT218180/zz	MT218055/F	n.a.	MT183361/BB	This study
UGSB15541	21572	<i>C. alluaudi</i>	Kenya; Lake Victoria	MT218181	MT218056/P	MT216364	MT183362/JJ	This study
UGSB15558	21576	<i>C. stuhlmanni</i> aff.	Rwanda; Lake Burera	MT218182	MT218057	MT216365/g	MT183363/JJ	This study
UGSB5163	21578	<i>C. hauttecoeuri</i>	Uganda; Nile River	MT218183/xx	MT218058/Q	MT216366/g	MT183364	This study
UGSB15560	21579	<i>C. hauttecoeuri</i>	Uganda; Victoria Nile	MT218184/xx	n.a.	MT216367/e	n.a.	This study
UGSB15561	21580	<i>C. hauttecoeuri</i>	Uganda; Victoria Nile	MT218185/xx	MT218059/P	MT216368/d	n.a.	This study
UGSB15562	21581	<i>C. hauttecoeuri</i>	Uganda; Victoria Nile	MT218186/xx	n.a.	MT216369	n.a.	This study

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UGSB15564	21583	<i>C. hauttecoeuri kyogae</i>	Uganda; Lake Kyoga	MT218187/vv	MT218060/P	MT216370/e	n.a.	This study
UGSB15565	21584	<i>C. hauttecoeuri kyogae</i>	Uganda; Lake Kyoga	MT218188/xx	n.a.	MT216371/g	n.a.	This study
UGSB15566	21585	<i>C. hauttecoeuri kyogae</i>	Uganda; Lake Kyoga	MT218189/xx	n.a.	MT229886/g	n.a.	This study
UGSB15567	21586	<i>C. hauttecoeuri kyogae</i>	Uganda; Lake Kyoga	MT218190/xx	n.a.	MT229887/g	n.a.	This study
UGSB15568	21587	<i>C. hauttecoeuri kyogae</i>	Uganda; Lake Kyoga	MT218191/xx	n.a.	MT216372	n.a.	This study
UGSB15569	21588	<i>C. stuhlmanni</i>	Uganda; Lake Edward	MT218192/yy	MT218061	MT216373	n.a.	This study
UGSB15570	21589	<i>C. stuhlmanni</i>	Uganda; Lake Edward	MT218193/yy	MT218062/P	MT216374/g	MT183365	This study
UGSB15571	21590	<i>Nitia acuminata</i>	Uganda; Lake Victoria	MT218194/zz	MT218063/F	MT216375	n.a.	This study
UGSB15572	21591	<i>Nitia monceti</i>	Uganda; Lake Victoria	MT218195	MT218064/F	MT216376	n.a.	This study
UGSB15573	21592	<i>C. hauttecoeuri</i>	Uganda; Lake Victoria	MT218196/xx	MT218065/P	MT216377/e	MT183366/JJ	This study
UGSB15574	21593	<i>C. hauttecoeuri</i>	Uganda; Lake Victoria	MT218197/xx	MT218066	MT216378	MT183367/JJ	This study
UGSB5521	21594	<i>C. alluaudi</i>	Uganda; Lake Victoria	MT218198/yy	MT218067/P	MT216379	MT183368/JJ	This study
UGSB5543	21595	<i>C. hauttecoeuri</i>	Uganda; Lake Victoria	MT218199/yy	n.a.	MT216380/e	MT183369/JJ	This study
UGSB15575	21596	<i>Nitia monceti</i>	Uganda; Lake Victoria	MT218200/zz	MT218068/F	MT216381	MT183370/BB	This study
UGSB5556	21598	<i>C. hauttecoeuri</i>	Uganda; Lake Victoria	MT218201/xx	MT218069/Q	MT216382/e	MT183371	This study
UGSB15577	21599	<i>C. hauttecoeuri kyogae</i>	Uganda; Lake Kyoga	MT218202/vv	MT218070/P	MT216383/e	MT183372/JJ	This study
UGSB15578	21600	<i>C. hauttecoeuri kyogae</i>	Uganda; Lake Kyoga	MT218203/xx	MT218071/P	MT216384/g	MT183373/JJ	This study
UGSB15579	21601	<i>C. kunenensis</i>	Zambia; Zambezi River	MT218204/uu	MT218072/E	MT216385	MT183374/FF	This study
UGSB15580	21602	<i>C. kunenensis</i>	Zambia; Zambezi River	MT218205/uu	MT218073/K	MT216386	MT183375	This study
UGSB15581	21603	<i>C. kunenensis</i>	Zambia; Zambezi River	MT218206/uu	MT218074/E	MT216387/c	MT183376/FF	This study
UGSB15582	21604	<i>C. kunenensis</i>	Zambia; Zambezi River	MT218207/uu	MT218075/E	MT216388	MT183377/FF	This study
UGSB15583	21605	<i>C. kunenensis</i>	Zambia; Luanginga River	MT218208/uu	n.a.	MT216389	n.a.	This study
UGSB15584	21606	<i>C. kunenensis</i>	Zambia; Zambezi River	MT218209	MT218076/E	MT216390	MT183378	This study
UGSB15585	21607	<i>C. horei</i>	Burundi; Lake Tanganyika	MT218210	MT218077	MT216391	MT183379	This study
UGSB15867	21852	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218211/tt	MT218078	MT216392/q	n.a.	This study
UGSB15868	21853	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218212/tt	MT218079/C	MT216393	n.a.	This study
UGSB0377	21854	<i>C. stuhlmanni</i>	DR Congo; Lake Edward	MT218213/yy	n.a.	MT216394/g	MT183380/JJ	This study
UGSB15869	21855	<i>C. hypsiprymna</i>	Malawi; Shire River	MT218214/ww	MT218080/C	MT216395/o	MT183381/KK	This study

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UGSB15870	21856	<i>C. mossambicensis</i> aff.	Malawi; Shire River	MT218215/tt	MT218081/C	MT216396/i	MT183382	This study
UGSB15871	21857	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218216/ww	MT218082/C	MT216397	MT183383/FF	This study
UGSB15872	21858	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218217/tt	MT218083/C	MT216398/q	MT183384/AA	This study
UGSB1284	21859	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218218/ww	MT218084/C	MT216399	MT183385/II	This study
UGSB15873	21860	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218219/ww	MT218085	MT216400	MT183386	This study
UGSB15874	21861	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218220/tt	MT218086	MT216401/q	MT183387/II	This study
UGSB15876	21863	<i>C. hauttecoeurii</i> aff.	Kenya; Lake Victoria	MT218221/yy	MT218087/P	MT216402/d	MT183388/JJ	This study
UGSB15877	21864	<i>C. stuhlmanni</i>	Uganda; Lake Edward	MT218222/yy	MT218088	MT216403/g	MT183389/JJ	This study
UGSB5585	21865	<i>C. stuhlmanni</i>	DR Congo; Lake Edward	MT218223/yy	MT218089	MT216404/g	MT183390/JJ	This study
UGSB15878	21866	<i>C. hauttecoeurii</i> aff.	Tanzania; Lake Victoria	MT218224/xx	MT218090/P	MT216405/g	n.a.	This study
UGSB15880	21868	<i>C. horei</i>	Tanzania; Lake Tanganyika	MT218225	MT218091/G	MT216406	MT183391/HH	This study
UGSB15881	21869	<i>C. horei</i>	Tanzania; Lake Tanganyika	MT218226/ss	MT218092/G	MT216407	MT183392/HH	This study
UGSB16265	22108	<i>Nyassunio nyassaensis</i>	Malawi; Lake Malawi	MT218227/rr	MT218093/C	MT216408	MT183393/II	This study
UGSB16266	22109	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218228/qq	MT218094/C	MT216409/q	MT183394	This study
UGSB16267	22110	<i>C. stuhlmanni</i>	DR Congo; Lake Edward	MT218229	MT218095	MT216410/e	MT183395/JJ	This study
UGSB16268	22111	<i>C. stuhlmanni</i>	DR Congo Semliki River	MT218230	MT218096/P	MT216411/g	MT183396/JJ	This study
UGSB16269	22112	<i>C. stuhlmanni</i>	DR Congo; Semliki River	MT218231	MT218097	MT216412/g	n.a.	This study
DCR-KM10-134	22113	<i>C. briarti</i>	DR Congo; Lomami River	MT218232	MT218098	MT216413	MT183397	This study
DCR-KM10-134	22114	<i>C. gabonensis</i>	DR Congo; Lomami River	MT218233/pp	MT218099/L	MT216414	MT183398	This study
DCR-KM10-134	22115	<i>C. cf. mesafricana</i>	DR Congo; Lomami River	MT218234	MT218100/H	MT216415/j	MT183399/CC	This study
DCR09-005	22116	<i>C. gabonensis</i>	DR Congo; Congo River	MT218235/pp	MT218101/L	MT216416	MT183400	This study
DCR09-005	22117	<i>C. gabonensis</i>	DR Congo; Congo River	MT218236/oo	MT218102/L	MT216417/k	MT183401	This study
DRC09-008	22118	<i>C. gabonensis</i>	DR Congo; Congo River	MT218237/pp	MT218103/L	MT216418/k	n.a.	This study
DRC09-008	22119	<i>C. gabonensis</i> aff.	DR Congo; Congo River	MT218238	MT218104/H	MT216419/j	MT183402/CC	This study
DRC10-061	22120	<i>C. leopoldvillensis</i>	DR Congo; Congo River	MT218239/nn	MT218105/I	MT216420	MT183403	This study
MLW10-109	22121	<i>Nyassunio nyassensis</i>	Malawi; Lake Malawi	MT218240	MT218106/C	MT216421/q	MT183404	This study
MLW10-109	22122	<i>C. hypsiprymna</i>	Malawi; Lake Malawi	MT218241	n.a.	MT216422	n.a.	This study
DRC09-023	22123	<i>C. gabonensis</i>	DR Congo; Congo River	MT218242/oo	MT218107/I	MT216423/h	MT183405	This study

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DRC09-023	22124	<i>C. gabonensis</i>	DR Congo; Congo River	MT218243/pp	MT218108/I	MT216424/h	MT183406/DD	This study
DRC09-017	22125	<i>C. leopoldvillensis</i>	DR Congo; Congo River	MT218244	MT218109/I	MT216425	n.a.	This study
DRC09-016	22126	<i>C. gabonensis</i>	DR Congo; Congo River	MT218245/pp	MT218110/L	MT216426/k	MT183407/FF	This study
DCR10-057	22127	<i>C. leopoldvillensis</i>	DR Congo; Lomami River	MT218246	MT218111/I	MT216427	MT183408/DD	This study
MLW10-108	22129	<i>C. hypsiprymna</i>	Malawi; Shire River	MT218247/qq	MT218112/C	MT216428	MT183409	This study
MLW10-108	22130	<i>C. hypsiprymna</i>	Malawi; Shire River	MT218248/qq	MT218113/C	MT216429/i	MT183410/KK	This study
DRC-KM10-011	22131	<i>C. cf. mesafricana</i>	DR Congo; Aruwimi River	MT218249	MT218114/H	MT216430/j	MT183411	This study
DCR09-035	22132	<i>C. cf. stanleyvillensis</i>	DR Congo; Tshopo River	MT218250	MT218115/I	MT216431	MT183412/FF	This study
DRC10-077	22133	<i>C. cf. leopoldvillensis</i>	DR Congo; Congo River	MT218251/nn	MT218116/I	MT216432	MT183413	This study
DRC10-077	22134	<i>C. gabonensis</i>	DR Congo; Congo River	n.a.	MT218117/I	MT216433	MT183414	This study
UGSB16281	22209	<i>C. kunenensis</i>	Botswana; Boteti River	MT218252/mm	MT218118/J	MT216434/n	MT183415/FF	This study
UGSB16282	22210	<i>C. kunenensis</i>	Botswana; Boteti River	MT218253/ll	MT218119/E	MT216435/n	MT183416/FF	This study
UGSB16283	22211	<i>C. kunenensis</i>	Botswana; Thamalakane River	MT218254	MT218120/E	MT216436/n	MT183417/FF	This study
UGSB16284	22212	<i>C. kunenensis</i>	Botswana; Boteti River	MT218255/mm	MT218121/E	MT216437/n	MT183418/HH	This study
UGSB16285	22213	<i>C. kunenensis</i>	Botswana; Boteti River	MT218256/ll	MT218122/K	MT216438/n	MT183419/HH	This study
UGSB16286	22214	<i>C. kunenensis</i>	Botswana; Thamalakane River	MT218257/mm	MT218123/J	MT216439	n.a.	This study
UGSB16287	22215	<i>C. kunenensis</i>	Botswana; Boteti River	MT218258/mm	MT218124/E	MT216440/n	MT183420/FF	This study
UGSB16288	22216	<i>C. kunenensis</i>	Botswana; Boteti River	MT218259/mm	MT218125/K	MT216441/n	MT183421/FF	This study
UGSB16289	22217	<i>C. kunenensis</i>	Angola/Namibia; Cubango River	MT218260/ll	MT218126/E	MT216442	n.a.	This study
UGSB16290	22218	<i>C. kunenensis</i>	Angola/Namibia; Cubango River	MT218261/ll	MT218127/E	MT216443	MT183422	This study
UGSB17584	22222	<i>C. gabonensis</i>	DR Congo; Congo River	MT218262/pp	MT218128/L	MT229888/k	n.a.	This study
ZAM15-010 UGSB17585	22856	<i>Pseudospatha tanganyicensis</i>	Tanzania; Lake Tanganyika	MT218263/kk	MT218129	MT216444	MT183423/EE	This study
ZAM15-030 UGSB17586	22857	<i>Pseudospatha tanganyicensis</i>	Zambia; Lake Tanganyika	MT218264/jj	n.a.	MT216445/l	n.a.	This study
ZAM15-030 UGSB17587	22858	<i>Pseudospatha tanganyicensis</i>	Zambia; Lake Tanganyika	MT218265/jj	n.a.	MT229889/l	n.a.	This study
ZAM15-013 UGSB17588	22859	<i>C. horei</i>	Zambia; Lake Tanganyika	MT218266/ii	n.a.	MT216446	n.a.	This study
ZAM15-043 UGSB17589	22860	<i>Grandidieria burtoni</i>	Zambia; Lake Tanganyika	MT218267/hh	MT218130/M	MT216447	MT183424/EE	This study
ZAM15-047 UGSB17590	22861	<i>Grandidieria burtoni</i>	Zambia; Lake Tanganyika	MT218268	n.a.	MT216448	n.a.	This study

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NHMUK 20191054 UGSB17561	22862	<i>Pseudospatha tanganyicensis</i>	Tanzania; Lake Tanganyika	MT218269/gg	MT218131	MT216449	MT183425/DD	This study
NHMUK 20191055 UGSB17592	22863	<i>Pseudospatha tanganyicensis</i>	Tanzania; Lake Tanganyika	MT218270/gg	n.a.	MT216450	n.a.	This study
NHMUK 20191056 UGSB17593	22864	<i>Moncetia anceyi</i>	Tanzania; Lake Tanganyika	MT218271/kk	MT218132	MT216451	MT183426/GG	This study
NHMUK 20191057 UGSB17594	22865	<i>Pseudospatha tanganyicensis</i>	Tanzania; Kigoma; Lake Tanganyika	MT218272	MT218133	MT216452	MT183427/GG	This study
ZAM15-012 UGSB17595	22866	<i>C. horei</i>	Zambia; Lake Tanganyika	MT218273/ff	n.a.	MT229890	n.a.	This study
ZAM15-011 UGSB17597	22868	<i>C. horei</i>	Tanzania; Lake Tanganyika	MT218274/ii	n.a.	MT216453	n.a.	This study
ZAM15-011 UGSB15881	22869	<i>Grandidieria burtoni</i>	Tanzania; Lake Tanganyika	MT218275	MT218134/M	MT216454/m	MT183428/EE	This study
ZAM15-011 UGSB17599	22870	<i>Grandidieria burtoni</i>	Tanzania; Lake Tanganyika	MT218276	n.a.	MT216455	n.a.	This study
ZAM15-007 UGSB17600	22871	<i>C. horei</i>	Tanzania; Lake Tanganyika	MT218277/ff	n.a.	MT216456	n.a.	This study
ZAM15-001 UGSB17602	22873	<i>Grandidieria burtoni</i>	Zambia; Lake Tanganyika	MT218278	n.a.	MT216457	n.a.	This study
ZAM15-030 UGSB17606	22877	<i>Grandidieria burtoni</i>	Zambia; Lake Tanganyika	MT218279	n.a.	MT216458/m	n.a.	This study
ZAM15-034 UGSB17608	22879	<i>Grandidieria burtoni</i>	Zambia; Lake Tanganyika	MT218280	n.a.	MT216459	n.a.	This study
ZAM15-025 UGSB17609	22880	<i>Grandidieria burtoni</i>	Zambia; Lake Tanganyika	MT218281/hh	MT218135/M	MT216460	MT183429/EE	This study
ZAM15-045 UGSB17610	22881	<i>Grandidieria burtoni</i>	Zambia; Lake Tanganyika	MT218282	n.a.	MT216461	n.a.	This study
TAN15-004 UGSB17611	22884	<i>Grandidieria burtoni</i>	Tanzania; Lake Tanganyika	MT218283	MT218136	MT216462/a	MT183430/GG	This study
TAN15-004 UGSB17612	22885	<i>Grandidieria burtoni</i>	Tanzania; Lake Tanganyika	MT218284	n.a.	MT216463/a	n.a.	This study
TAN15-001 UGSB17613	22886	<i>Grandidieria burtoni</i>	Tanzania; Lake Tanganyika	MT218285/ee	n.a.	MT216464	n.a.	This study
TAN15-001 UGSB17614	22887	<i>Grandidieria burtoni</i>	Tanzania; Lake Tanganyika	MT218286/ee	n.a.	MT216465	n.a.	This study
UGSB17615	22888	<i>C. cf. kunenensis</i>	Botswana; Lake Liambezi	MT218287	n.a.	MT216466	n.a.	This study
UGSB17616	22889	<i>C. kunenensis</i>	Namibia; Zambezi River	MT218288	MT218137/E	MT216467	MT183431	This study
UGSB17617	22890	<i>C. kunenensis</i>	Botswana; Lake Liambezi	MT218289/uu	n.a.	MT216468	n.a.	This study
UGSB17619	22892	<i>C. kunenensis</i>	Botswana; Lake Liambezi	MT218290/uu	MT218138/E	MT229891/c	MT231526/FF	This study
UGSB17620	22893	<i>C. kunenensis</i>	Botswana; Lake Selinda	MT218291/dd	MT218139	MT216469	MT183432	This study
UGSB17621	22894	<i>C. kunenensis</i>	Botswana; Lake Selinda	MT218292/dd	MT218140	MT216470	MT183433	This study
NHMUK 20191058 UGSB17622	22895	<i>C. sp. nov.</i>	Tanzania; Malagarasi River	MT218293	MT218141/N	MT216471/r	MT183434/HH	This study
NHMUK 20191059 UGSB17623	22896	<i>C. sp. nov.</i>	Tanzania; Malagarasi River	MT218294/ss	n.a.	MT216472/r	n.a.	This study

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NHMUK 20191060 UGSB17624	22897	<i>C. sp. nov.</i>	Tanzania; Malagarasi River	MT218295/ss	MT218142/N	n.a.	MT183435/HH	This study
NHMUK 20191061 UGSB17625	22898	<i>C. sp. nov.</i>	Tanzania; Malagarasi River Delta	MT218296/ss	MT218143	MT216473	MT183436	This study
NHMUK 20191062 UGSB17626	22899	<i>C. sp. nov.</i>	Tanzania; Malagarasi River Delta	MT218297	MT218144/N	n.a.	MT183437/HH	This study
NHMUK 20191063 UGSB17629	22902	<i>C. sp. nov.</i>	Tanzania; Lake Nyamagoma	MT218298/ss	MT218145	MT216474	MT183438	This study
NHMUK 20191087 UGSB17632	22925	<i>Grandidieria burtoni</i>	Tanzania; Lake Tanganyika	MT218299/cc	MT218146/O	MT216475/b	n.a.	This study
NHMUK 20191088 UGSB17633	22926	<i>Grandidieria burtoni</i>	Tanzania; Lake Tanganyika	MT218300/cc	MT218147	MT216476/b	MT183439	This study
NHMUK 20191064 UGSB17637	22930	<i>Grandidieria burtoni</i>	Tanzania; Lake Tanganyika	MT218301/cc	MT218148/O	MT216477/b	MT183440/GG	This study
NHMUK 20191089 UGSB17638	22931	<i>Grandidieria burtoni</i>	Tanzania; Lake Tanganyika	MT218302	MT218149	MT216478/b	MT183441/GG	This study
NHMUK 20191065 UGSB17639	22932	<i>Grandidieria burtoni</i>	Tanzania; Lake Tanganyika	MT218303/cc	n.a.	MT216479/b	n.a.	This study
UGSB17434	22933	<i>C. cf. mossambicensis</i>	Tanzania; Ruvuma branch	MT218304	n.a.	MT216480	n.a.	This study
UGSB17435	22934	<i>C. hypsiprymna</i>	Tanzania; Lake Malawi	MT218305	n.a.	MT216481	n.a.	This study
UGSB17457	22935	<i>C. horei</i>	Tanzania; Lake Tanganyika	MT218306/ss	n.a.	MT216482	n.a.	This study
UGSB17458	22938	<i>C. ratidota</i>	Tanzania; Morogoro	n.a.	MT218150/N	MT216483	MT183442/HH	This study
UGSB17643	22940	<i>C. cf. stanleyvillensis</i>	DR Congo; Kibibi River	MT218307	MT218151	MT216484	MT183443	This study
UGSB17645	22942	<i>C. cf. stanleyvillensis</i>	DR Congo; Congo River	MT218308	n.a.	MT216485	n.a.	This study
ANSP416304	Caegyplbi1	<i>C. aegyptiaca</i>	Egypt; Nile river	n.a.	JN243871/B	JN243893	n.a.	Graf et al. (2014)
ANSP416304	Caegyplbi2	<i>C. aegyptiaca</i>	Egypt; Nile river	n.a.	_____/B	KJ081162	n.a.	Graf et al. (2014)
ANSP416304	Caegyplbid	<i>C. aegyptiaca</i>	Egypt; Nile river	n.a.	JN243870/B	JN243892	n.a.	Whelan et al. (2011)
ANSP416276	CchozieZM	<i>C. choziensis</i>	Zambia; Lake Bangweulu	n.a.	KJ081186	KJ081167	n.a.	Graf et al. (2014)
ANSP416294	CgabonenCO	<i>C. gabonensis</i>	DR Congo; Congo River	n.a.	JN243873	JN243895	n.a.	Whelan et al. (2011)
ANSP419411	CkunenenZM	<i>C. kunenensis</i>	Zambia; Zambezi River	n.a.	KJ081184	KJ081164	n.a.	Graf et al. (2014)
ANSP419411	CkuneneZM1	<i>C. kunenensis</i>	Zambia; Zambezi River	n.a.	KJ081185/E	KJ081165	n.a.	Graf et al. (2014)
ANSP419411	CkuneneZM2	<i>C. kunenensis</i>	Zambia; Zambezi River	n.a.	_____/E	KJ081166	n.a.	Graf et al. (2014)
ANSP416547	CluapulaZM	<i>C. luapulaensis</i>	Zambia; Kalungu River	n.a.	KJ081188/C	KJ081169	n.a.	Graf et al. (2014)
ANSP416547	CluapulZM1	<i>C. luapulaensis</i>	Zambia; Kalungu River	n.a.	_____/C	KJ081170	n.a.	Graf et al. (2014)

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ANSP416547	CluapulZM2	<i>C. luapulaensis</i>	Zambia; Kalungu River	n.a.	_____/C	KJ081171	n.a.	Graf et al. (2014)
ANSP416245	CluapulZM3	<i>C. luapulaensis</i>	Zambia; Chambeshi River	n.a.	_____/C	KJ081172	n.a.	Graf et al. (2014)
ANSP416245	CluapulZM4	<i>C. luapulaensis</i>	Zambia; Chambeshi River	n.a.	_____/C	KJ081173/f	n.a.	Graf et al. (2014)
RMBH biv_108_1	Indoand1	<i>Indonaia andersoniana</i>	Myanmar; Lake Indawgyi	KX865606	KX865709	KX865835	n.a.	Bolotov et al. (2017)
RMBH biv_263_1	Indoand2	<i>Indonaia andersoniana</i>	Myanmar; Nant Sa Yi River	MF352337	MF352397	MF352275	n.a.	Bolotov et al. (2017)
UMMZ304377	MmweruenZM	<i>Mweruella mweruensis</i>	Zambia; Lake Mweru	n.a.	KJ081189	KJ081174	n.a.	Graf et al. (2014)
UMMZ304377	MmwerueZM1	<i>Mweruella mweruensis</i>	Zambia; Lake Mweru	n.a.	KJ081190	KJ081175	n.a.	Graf et al. (2014)
UMMZ304377	MmwerueZM2	<i>Mweruella mweruensis</i>	Zambia; Lake Mweru	n.a.	KJ081191/D	KJ081176	n.a.	Graf et al. (2014)
UMMZ304378	Mmwerulbi2	<i>Mweruella mweruensis</i>	Zambia; Lake Mweru	n.a.	_____/D	KJ081178	n.a.	Graf et al. (2014)
UMMZ304378	Mmwerulbid	<i>Mweruella mweruensis</i>	Zambia; Lake Mweru	n.a.	_____/D	KJ081177	n.a.	Graf et al. (2014)
ANSP416305	NteretEgy1	<i>Nitia teretiuscula</i>	Egypt; Nile river	n.a.	_____/A	KJ081160	n.a.	Graf et al. (2014)
ANSP416305	NteretEgy2	<i>Nitia teretiuscula</i>	Egypt; Nile river	n.a.	_____/A	KJ081161	n.a.	Graf et al. (2014)
ANSP416305	NteretiEgy	<i>Nitia teretiuscula</i>	Egypt; Nile river	n.a.	JN243875/A	JN243897	n.a.	Whelan et al. (2011)
UA20739/ UMMZ304644	Oxypug1	<i>Indochinella aff. pugio</i>	Myanmar; Paukin Lake	KP795046	JN243879	JN243899	n.a.	Whelan et al. (2011); Pfeiffer and Graf (2015)
RMBH biv_258_2	Oxypug2	<i>Indochinella pugio</i>	Myanmar; Lake Nant Phar	MF352326	MF352389	MF352262	n.a.	Bolotov et al. (2017)
RMBH biv_147_3	Oxytav1	<i>Indochinella</i> sp. ‘Tavoy’	Myanmar; Tavoy River	KX865623	KX865724/T	KX865852	n.a.	Bolotov et al. (2017)
RMBH biv_148_15	Oxytav2	<i>Indochinella</i> sp. ‘Tavoy’	Myanmar; Tavoy River	KX865628	KX865729/T	KX865857	n.a.	Bolotov et al. (2017)
UMMZ304379	Paviculbi1	<i>Prisodontopsis aviculaeformis</i>	Zambia; Lake Mweru	n.a.	KJ081193	KJ081180	n.a.	Graf et al. (2014)
UMMZ304379	Paviculbi2	<i>Prisodontopsis aviculaeformis</i>	Zambia; Lake Mweru	n.a.	_____/D	KJ081181	n.a.	Graf et al. (2014)
UMMZ304379	Paviculbid	<i>Prisodontopsis aviculaeformis</i>	Zambia; Lake Mweru	n.a.	_____/D	KJ081179	n.a.	Graf et al. (2014)
ANSP416269	PaviculaZM	<i>Prisodontopsis aviculaeformis</i>	Zambia; Lake Mweru	n.a.	JN243874	JN243896	n.a.	Whelan et al. (2011)
ANSP416269	PaviculZM1	<i>Prisodontopsis aviculaeformis</i>	Zambia; Lake Mweru	n.a.	_____/D	KJ081182	n.a.	Graf et al. (2014)
ANSP416269	PaviculZM2	<i>Prisodontopsis aviculaeformis</i>	Zambia; Lake Mweru	n.a.	_____/D	KJ081183	n.a.	Graf et al. (2014)
n.a.	PtavoyeBur	<i>Parreysia tavoyensis</i>	Myanmar	n.a.	JN243877	JN243901	n.a.	Whelan et al. (2011)
UA20714/ UMMZ304645	Radbonn	<i>Radiatula cf. bonneaudii</i>	Myanmar; Irrawaddy River	KP795047	JN243878	JN243898	n.a.	Whelan et al. (2011); Pfeiffer and Graf (2015)

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RMBH biv_107_1	Radburn1	<i>Radiatula burmana</i>	Myanmar; Lake Indawgyi	KX865609/bbb	KX865710	KX865838	n.a.	Bolotov et al. (2017)
RMBH biv_107_3	Radburn2	<i>Radiatula burmana</i>	Myanmar; Lake Indawgyi	KX865611/bbb	KX865712/R	KX865840	n.a.	Bolotov et al. (2017)
RMBH biv_106_2	Radburn3	<i>Radiatula burmana</i>	Myanmar; Lake Indawgyi	KX865613	KX865715/R	KX865843	n.a.	Bolotov et al. (2017)
UMMZ304646	Radhum1	<i>Radiatula aff. humilis</i>	Cambodia; Mekong River Basin	KP795048	KP795006	KP795023	n.a.	Pfeiffer and Graf (2015)
RMBH biv_126_1	Radhum2	<i>Radiatula aff. humilis</i>	Thailand; Chi River	KX865615	KX865716	KX865844	n.a.	Bolotov et al. (2017)
RMBH biv_124_2	Radhum3	<i>Radiatula aff. humilis</i>	Thailand; Chi River	KX865622	KX865723	KX865851	n.a.	Bolotov et al. (2017)
RMBH biv_248_1	Radmou1	<i>Radiatula mouhoti</i>	Myanmar; Sittaung River	MF352305/aaa	MF352363/S	MF352234	n.a.	Bolotov et al. (2017)
RMBH biv_256	Radmou2	<i>Radiatula mouhoti</i>	Myanmar; Sittaung River	MF352321/aaa	MF352382/S	MF352257	n.a.	Bolotov et al. (2017)

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118 **Table S2.** Cycling conditions for polymerase chain reactions for the four studied gene fragments.

Gene	Start	#Cycles	Denaturation	Annealing	Extension	Final Extension
COI	95 °C, 1 min	35	95 °C, 30 s	52 °C, 30 s	72 °C, 30 s	72 °C, 3 min
16S	95 °C, 1 min	35	95 °C, 30 s	52 °C, 30 s	72 °C, 30 s	72 °C, 3 min
H3	95 °C, 1 min	35	95 °C, 30 s	52 °C, 30 s	72 °C, 30 s	72 °C, 3 min
28S	94 °C, 4 min	36	94 °C, 40 s	55 °C, 40 s	72 °C, 105 s	72 °C, 10 min

119

120 **Table S3.** Information on data partitions and substitution models inferred with PartitionFinder.

121 Unpartitioned substitution models for COI and H3 were HKY + I + Γ and SYM + I, respectively.

Fragment	Codons	Substitution model
16S	n.a.	GTR + I + Γ
28S	n.a.	GTR + Γ
COI	position 1	GTR + Γ
	position 2	F81 + I
	position 3	GTR + Γ
H3	position 1	SYM + I
	position 2	JC
	position 3	SYM + I

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123 **Table S4.** Comparison of the results of automated species recognition methods and the finally recognized

124 OTUs (n = 26). ABGD, the maximum-likelihood solution of PTP and GMYC are broadly consistent and

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125 recognize 31, 29 and 32 OTUs respectively. Individuals for which COI sequence data was lacking are
126 indicated with 'n.a.' for ABGD. Individuals are labelled with their DNA voucher code followed by an
127 abbreviation indicating country and waterbody (see Table S1 for full details).

DNA Voucher	ABGD	PTP	GMYC	Recognized OTUs
21555-DRC-LE	4	26	27	<i>Coelatura aegyptiaca</i> group
21583-UG-LK	4	26	27	<i>Coelatura aegyptiaca</i> group
21588-UG-LE	4	26	27	<i>Coelatura aegyptiaca</i> group
21589-UG-LE	4	26	27	<i>Coelatura aegyptiaca</i> group
21594-UG-LV	4	26	27	<i>Coelatura aegyptiaca</i> group
21595-UG-LV	4	26	27	<i>Coelatura aegyptiaca</i> group
21599-UG-LK	4	26	17	<i>Coelatura aegyptiaca</i> group
21854-DRC-LE	4	26	27	<i>Coelatura aegyptiaca</i> group
21863-KN-LV	4	26	27	<i>Coelatura aegyptiaca</i> group
21864-UG-LE	4	26	27	<i>Coelatura aegyptiaca</i> group
21865-DRC-LE	4	26	27	<i>Coelatura aegyptiaca</i> group
22110-DRC-LE	4	26	27	<i>Coelatura aegyptiaca</i> group
22111-DRC-SR	4	26	27	<i>Coelatura aegyptiaca</i> group
22112-DRC-SR	4	26	27	<i>Coelatura aegyptiaca</i> group
21549-UG-LK	4	27	28	<i>Coelatura aegyptiaca</i> group
21551-UG-LV	4	27	29	<i>Coelatura aegyptiaca</i> group
21552-UG-LV	4	27	28	<i>Coelatura aegyptiaca</i> group
21556-UG-LA	4	27	29	<i>Coelatura aegyptiaca</i> group
21558-UG-LV	4	27	29	<i>Coelatura aegyptiaca</i> group
21572-KN-LV	4	27	28	<i>Coelatura aegyptiaca</i> group
21578-UG-NR	4	27	29	<i>Coelatura aegyptiaca</i> group
21579-UG-VN	4	27	29	<i>Coelatura aegyptiaca</i> group
21580-UG-VN	4	27	28	<i>Coelatura aegyptiaca</i> group
21581-UG-VN	4	27	28	<i>Coelatura aegyptiaca</i> group
21584-UG-LK	4	27	28	<i>Coelatura aegyptiaca</i> group
21587-UG-LK	4	27	28	<i>Coelatura aegyptiaca</i> group
21592-UG-LV	4	27	28	<i>Coelatura aegyptiaca</i> group
21593-UG-LV	4	27	28	<i>Coelatura aegyptiaca</i> group
21598-UG-LV	4	27	29	<i>Coelatura aegyptiaca</i> group
21600-UG-LK	4	27	28	<i>Coelatura aegyptiaca</i> group
21866-TZ-LV	4	27	28	<i>Coelatura aegyptiaca</i> group
6929-UG-LA	4	27	28	<i>Coelatura aegyptiaca</i> group
6930-UG-LA	4	27	28	<i>Coelatura aegyptiaca</i> group
6931-UG-LA	4	27	28	<i>Coelatura aegyptiaca</i> group
6932-UG-LA	4	27	29	<i>Coelatura aegyptiaca</i> group

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CaegypIbi1-EG-NR	4	27	29	<i>Coelatura aegyptiaca</i> group
CaegypIbid-EG-NR	4	27	29	<i>Coelatura aegyptiaca</i> group
CaegypIbi2-EG-NR	13	27	29	<i>Coelatura aegyptiaca</i> group
22113-DCR-LR	1	22	9	<i>Coelatura briarti</i>
22114-DCR-LR	6	7	19	<i>Coelatura</i> cf. <i>gabonensis</i>
22116-DCR-CR	6	7	19	<i>Coelatura</i> cf. <i>gabonensis</i>
22117-DCR-CR	6	7	19	<i>Coelatura</i> cf. <i>gabonensis</i>
22118-DCR-CR	6	7	19	<i>Coelatura</i> cf. <i>gabonensis</i>
22123-DCR-CR	6	7	19	<i>Coelatura</i> cf. <i>gabonensis</i>
22124-DCR-CR	6	7	19	<i>Coelatura</i> cf. <i>gabonensis</i>
22126-DRC-CR	6	7	19	<i>Coelatura</i> cf. <i>gabonensis</i>
22134-DRC-CR	6	7	19	<i>Coelatura</i> cf. <i>gabonensis</i>
CgabonenCO-DRC-CR	6	7	19	<i>Coelatura</i> cf. <i>gabonensis</i>
22942-DRC-CR	8	8	18	<i>Coelatura</i> cf. <i>stanleyvillensis</i>
22940-DRC-CR	8	20	17	<i>Coelatura</i> cf. <i>stanleyvillensis</i>
22132-DRC-TR	8	21	17	<i>Coelatura</i> cf. <i>stanleyvillensis</i>
22895-TZ-MR	16	11	21	<i>Coelatura</i> cf. <i>ratidota</i>
22896-TZ-MR	16	11	21	<i>Coelatura</i> cf. <i>ratidota</i>
22902-TZ-LN	16	11	21	<i>Coelatura</i> cf. <i>ratidota</i>
22938-TZ-MO	17	11	21	<i>Coelatura</i> cf. <i>ratidota</i>
22897-TZ-MR	n.a.	11	21	<i>Coelatura</i> cf. <i>ratidota</i>
22899-TZ-MR	n.a.	11	21	<i>Coelatura</i> cf. <i>ratidota</i>
21868-TZ-LT	14	11	20	<i>Coelatura</i> cf. <i>horei</i> 1
21869-TZ-LT	14	11	20	<i>Coelatura</i> cf. <i>horei</i> 1
22898-TZ-MR	14	11	20	<i>Coelatura</i> cf. <i>horei</i> 1
22935-TZ-LT	14	11	20	<i>Coelatura</i> cf. <i>horei</i> 1
22859-ZM-LT	15	12	22	<i>Coelatura</i> cf. <i>horei</i> 2
22868-ZM-LT	15	12	22	<i>Coelatura</i> cf. <i>horei</i> 2
22871-ZM-LT	15	12	22	<i>Coelatura</i> cf. <i>horei</i> 2
22866-ZM-LT	n.a.	12	22	<i>Coelatura</i> cf. <i>horei</i> 2
21607-BR-LT	5	4	23	<i>Coelatura</i> cf. <i>horei</i> 3
21606-ZM-ZR	2	18	14	<i>Coelatura</i> cf. <i>kunenensis</i> 1
15145-NM-ZR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
15146-NM-ZR	2	19	15	<i>Coelatura</i> cf. <i>kunenensis</i> 2
15152-BT-CR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
15155-BT-CR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
21601-ZM-ZR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
21602-ZM-ZR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
21603-ZM-ZR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
21604-ZM-ZR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
21605-ZM-LR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
22209-BT-BR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2

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22210-BT-BR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
22211-BT-TR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
22212-BT-BR	2	19	15	<i>Coelatura</i> cf. <i>kunenensis</i> 2
22213-BT-BR	2	19	15	<i>Coelatura</i> cf. <i>kunenensis</i> 2
22214-BT-TR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
22215-BT-BR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
22216-BT-BR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
22217-AN/NM-CR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
22218-AN/NM-CR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
22888-BT-LL	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
22889-NM-ZR	2	19	15	<i>Coelatura</i> cf. <i>kunenensis</i> 2
22890-BT-LL	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
22893-BT-LS	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
22894-BT-LS	2	19	15	<i>Coelatura</i> cf. <i>kunenensis</i> 2
CkunenenZM-ZM-ZR	2	19	15	<i>Coelatura</i> cf. <i>kunenensis</i> 2
CkuneneZM1-ZM-ZR	2	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
CkuneneZM2-ZM-ZR	2	19	15	<i>Coelatura</i> cf. <i>kunenensis</i> 2
13006-BT-CR	n.a.	19	15	<i>Coelatura</i> cf. <i>kunenensis</i> 2
13007-BT-CR	n.a.	19	16	<i>Coelatura</i> cf. <i>kunenensis</i> 2
22115-DCR-LR	7	13	24	<i>Coelatura</i> cf. <i>mesafricana</i>
22119-DRC-CR	7	13	24	<i>Coelatura</i> cf. <i>mesafricana</i>
22131-DRC-CR	7	13	24	<i>Coelatura</i> cf. <i>mesafricana</i>
22120-DRC-CR	18	28	25	<i>Coelatura</i> cf. <i>leopoldvillensis</i>
22125-DRC-CR	19	29	25	<i>Coelatura</i> cf. <i>leopoldvillensis</i>
22127-DRC-LR	20	29	25	<i>Coelatura</i> cf. <i>leopoldvillensis</i>
22133-DRC-CR	21	29	25	<i>Coelatura</i> cf. <i>leopoldvillensis</i>
21576-RW-LB	4	6	26	<i>Coelatura</i> cf. <i>stuhlmanni</i>
15140-ZM-LB	1	16	11	<i>Coelatura nyassaensis</i> group
15142-ZM-LB	1	16	11	<i>Coelatura nyassaensis</i> group
15143-ZM-LK	1	16	11	<i>Coelatura nyassaensis</i> group
15144-ZM-LK	1	16	11	<i>Coelatura nyassaensis</i> group
21568-MW-SR	1	16	13	<i>Coelatura nyassaensis</i> group
21569-MW-SR	1	16	12	<i>Coelatura nyassaensis</i> group
21570-MW-SR	1	16	13	<i>Coelatura nyassaensis</i> group
21852-MW-LM	1	16	12	<i>Coelatura nyassaensis</i> group
21853-MW-LM	1	16	12	<i>Coelatura nyassaensis</i> group
21855-MW-LM	1	16	12	<i>Coelatura nyassaensis</i> group
21856-MW-LM	1	16	12	<i>Coelatura nyassaensis</i> group
21857-MW-LM	1	16	12	<i>Coelatura nyassaensis</i> group
21858-MW-LM	1	16	12	<i>Coelatura nyassaensis</i> group
21859-MW-LM	1	16	12	<i>Coelatura nyassaensis</i> group
21860-MW-LM	1	16	13	<i>Coelatura nyassaensis</i> group

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21861-MW-LM	1	16	12	<i>Coelatura nyassaensis</i> group
22108-MW-LM	1	16	13	<i>Coelatura nyassaensis</i> group
22109-MW-LM	1	16	12	<i>Coelatura nyassaensis</i> group
22121-MW-LM	1	16	12	<i>Coelatura nyassaensis</i> group
22122-MW-LM	1	16	13	<i>Coelatura nyassaensis</i> group
22129-MW-SR	1	16	12	<i>Coelatura nyassaensis</i> group
22130-MW-SR	1	16	12	<i>Coelatura nyassaensis</i> group
22933-TZ-RB	1	16	12	<i>Coelatura nyassaensis</i> group
22934-TZ-LM	1	16	11	<i>Coelatura nyassaensis</i> group
6926-MW-LM	1	16	11	<i>Coelatura nyassaensis</i> group
6927-MW-LM	1	16	12	<i>Coelatura nyassaensis</i> group
6936-MW-LM	1	16	12	<i>Coelatura nyassaensis</i> group
6938-MW-LM	1	16	11	<i>Coelatura nyassaensis</i> group
6943-MW-LM	1	16	11	<i>Coelatura nyassaensis</i> group
6944-MW-LM	1	16	12	<i>Coelatura nyassaensis</i> group
6946-MW-LM	1	16	12	<i>Coelatura nyassaensis</i> group
6947-MW-LM	1	16	12	<i>Coelatura nyassaensis</i> group
6948-MW-LM	1	16	12	<i>Coelatura nyassaensis</i> group
CchozieZM-ZM-LB	1	16	11	<i>Coelatura nyassaensis</i> group
CluapulaZM-ZM-KR	1	16	11	<i>Coelatura nyassaensis</i> group
CluapulZM1-ZM-KR	1	16	11	<i>Coelatura nyassaensis</i> group
CluapulZM2-ZM-KR	1	16	11	<i>Coelatura nyassaensis</i> group
CluapulZM3-ZM-CR	1	16	11	<i>Coelatura nyassaensis</i> group
CluapulZM4-ZM-CR	1	16	11	<i>Coelatura nyassaensis</i> group
21559-MW-LM	n.a.	16	12	<i>Coelatura nyassaensis</i> group
22860-ZM-LT	10	24	5	<i>Grandidieria burtoni</i> 1
22869-ZM-LT	10	24	5	<i>Grandidieria burtoni</i> 1
22870-ZM-LT	10	24	5	<i>Grandidieria burtoni</i> 1
22877-ZM-LT	10	24	5	<i>Grandidieria burtoni</i> 1
22873-ZM-LT	25	24	4	<i>Grandidieria burtoni</i> 1
22879-ZM-LT	26	24	5	<i>Grandidieria burtoni</i> 1
22880-ZM-LT	27	24	5	<i>Grandidieria burtoni</i> 1
22881-ZM-LT	28	24	5	<i>Grandidieria burtoni</i> 1
22886-TZ-LT	29	24	4	<i>Grandidieria burtoni</i> 1
22887-TZ-LT	30	24	4	<i>Grandidieria burtoni</i> 1
22861-ZM-LT	24	25	2	<i>Grandidieria burtoni</i> 2
22884-TZ-LT	11	5	3	<i>Grandidieria burtoni</i> 3
22885-TZ-LT	11	5	3	<i>Grandidieria burtoni</i> 3
22925-TZ-LT	31	2	1	<i>Grandidieria burtoni</i> 4
22926-TZ-LT	31	2	1	<i>Grandidieria burtoni</i> 4
22930-TZ-LT	31	2	1	<i>Grandidieria burtoni</i> 4
22931-TZ-LT	31	2	1	<i>Grandidieria burtoni</i> 4

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22932-TZ-LT	31	2	1	<i>Grandidieria burtoni</i> ⁴
MmweruenZM-ZM-LM	1	23	9	<i>Mweruella mweruensis</i>
MmwerueZM1-ZM-LM	1	23	9	<i>Mweruella mweruensis</i>
MmwerueZM2-ZM-LM	1	23	9	<i>Mweruella mweruensis</i>
Mmwerulbid-ZM-LM	1	23	9	<i>Mweruella mweruensis</i>
Paviculbi2-ZM-LM	1	23	9	<i>Mweruella mweruensis</i>
21590-UG-LV	4	1	32	<i>Nitia cf. acuminata</i>
21548-UG-LV	3	14	30	<i>Nitia cf. monceti</i>
21557-UG-LV	3	14	30	<i>Nitia cf. monceti</i>
21591-UG-LV	3	14	30	<i>Nitia cf. monceti</i>
21596-UG-LV	3	14	30	<i>Nitia cf. monceti</i>
21571-KN-LV	n.a.	14	30	<i>Nitia cf. monceti</i>
NteretEgy1-EG-NR	12	15	31	<i>Nitia cf. teretiuscula</i>
NteretEgy2-EG-NR	12	15	31	<i>Nitia cf. teretiuscula</i>
NteretiEgy-EG-NR	12	15	31	<i>Nitia cf. teretiuscula</i>
21550-UG-LV	n.a.	15	31	<i>Nitia cf. teretiuscula</i>
Mmwerulbi2-ZM-LM	1	17	10	<i>Prisodontopsis aviculaeformis</i>
Paviculbi1-ZM-LM	1	17	10	<i>Prisodontopsis aviculaeformis</i>
Paviculbid-ZM-LM	1	17	10	<i>Prisodontopsis aviculaeformis</i>
PavaculaZM-ZM-LM	1	17	10	<i>Prisodontopsis aviculaeformis</i>
PaviculZM1-ZM-LM	1	17	10	<i>Prisodontopsis aviculaeformis</i>
PaviculZM2-ZM-LM	1	17	10	<i>Prisodontopsis aviculaeformis</i>
22862-TZ-LT	23	9	6	<i>Pseudospatha tanganyicensis</i> ¹
22863-TZ-LT	23	9	6	<i>Pseudospatha tanganyicensis</i> ¹
22865-TZ-LT	23	9	6	<i>Pseudospatha tanganyicensis</i> ¹
22856-ZM-LT	9	10	7	<i>Pseudospatha tanganyicensis</i> ²
22864-TZ-LT	9	10	7	<i>Pseudospatha tanganyicensis</i> ²
22857-ZM-LT	22	3	8	<i>Pseudospatha tanganyicensis</i> ³