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4

–SHORT COMMUNICATION–

5

6 **Title:** First report of corkscrew gastropods of the genus *Bulinus* in Lake Malawi

7

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9

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16

17 **Abstract**

18 Open-coiled shells are rarely observed in gastropods, but they may occur at a relative frequency ranging from a
19 small percentage of specimens in a population up to being specific to populations or even species. The origin of
20 such corkscrew-like shells are poorly understood, but may include developmental anomalies, ecophenotypic
21 plasticity or genetic inheritance. Here, we report the first case of gastropods from Lake Malawi with corkscrew-
22 like shells, and we use comparative molecular studies with two mitochondrial gene fragments (COI and 16S rRNA)
23 to examine its origin. The specimens belong to the genus *Bulinus*, more specifically to one of five clades of *Bulinus*
24 recovered from the Malawi Basin. Beyond being seemingly specific to one of these *Bulinus* clades, network
25 analyses indicate low genetic differentiation from the other, regularly shaped specimens in this clade. Although
26 our results do not fully discard the possibility of corkscrew-like shells being genetically inherited or being caused
27 by growth disturbance, their restrictive occurrence in a few geographically isolated populations in sympatry with
28 specimens displaying a regular shell shape supports the hypothesis that the corkscrew-like shell shape in *Bulinus*
29 are caused by ecophenotypic plasticity. For now, it is unclear whether aspects of the physical environment, or
30 biotic interactions, such as a release from predation, caused the corkscrew-like morphotype. The phenomenon may

31 have been caused by the multiple environmental changes that Lake Malawi has been undergoing over recent
32 decades.

33

34 **Key Words:** ecophenotypic plasticity, ecological stress, shell-shape evolution, shell variability

35 Introduction

36 Gastropod species occupy an iconic place in the faunas of many ancient lakes, both because of their high levels of
37 endemism and their morphological disparity (see e.g. Brooks, 1950; Michel, 1994; Moore, 1898). Whether such
38 morphological disparity has evolved by adaption and to what extent disparity in gastropod shell shape correlates
39 with environmental conditions or the occupation of a diverse set of microhabitats are issues that are still highly
40 debated in evolutionary biology and palaeontology (e.g. Annandale, 1924; Geary et al., 2002; Gorthner, 1992;
41 Rasser and Covich, 2014; Van Bocxlaer, 2017; Vermeij and Covich, 1978). In short, gastropod shell shape figures
42 prominently in the discussion about diversification dynamics in ancient lakes (Albrecht et al., 2010; Glaubrecht,
43 2008; Salzburger et al., 2014; Van Bocxlaer et al., 2008, 2020), but the utility of certain morphological traits is
44 often poorly understood, especially for unique and unusual shell forms. Such phenotypes include, for example, the
45 extraordinarily large aperture of *Radix pinteri* (Lymnaeidae) endemic to ancient Lake Prespa (Albrecht et al.,
46 2008), long spines in *Tiphobia horei* (Paludomidae) endemic to Lake Tanganyika (Glaubrecht, 2008), strong ribs
47 in *Megalovalvata piligera nudicarinata* (Valvatidae) endemic to Lake Baikal (Egorov, 2006), a keel made up of
48 triangular segments in *Angulyagra (Acanthotropis) partelloi* (Viviparidae) endemic to Lake Lanao (Bartsch,
49 1909), or (partly) openly coiled shell in *Liobaicalia stiedae* (Amnicolidae), *Heleobia mirum* (Cochliopidae), and
50 *Gocea ohridana* (Hydrobiidae) endemic to lakes Baikal, Titicaca, and Ohrid, respectively (Hauffe et al., 2010;
51 Kroll et al., 2012; Sitnikova et al., 2001). Open-coiling is a rare condition in which successive whorls, which are
52 usually tightly coiled and overlapping, have become detached but are still coiled in a fairly uniform logarithmic
53 spiral. The phenomenon gives shells a corkscrew-like appearance, and although it is infrequent among freshwater
54 gastropods, such shell shapes appear in various taxa across marine, freshwater and terrestrial ecosystems around
55 the globe (Clements et al., 2008; Clewing et al., 2015; Rex & Boss, 1976). Sometimes corkscrew-like shells occur
56 in a small proportion of specimens of a population, but in other cases open-coiling defines the shell shape of entire
57 populations. Depending on the frequency of corkscrew-like shells the phenomenon is considered as a species-
58 specific trait or as ecophenotypic modifications occurring sporadically among certain populations of a single
59 species (Clewing et al., 2015; Table 1). By definition ecophenotypic changes are triggered by the environment,
60 related to abiotic factors (e.g. changes in water chemistry; Rasser et al., 2014), ecological/physiological stress
61 and/or biotic interactions (e.g. parasitism and predation; Zuykov et al., 2012).

62 Here, we report the first live-collected specimens of a freshwater gastropod from ancient Lake Malawi
63 (Lake Niassa/Nyasa) that displays a corkscrew-like shell shape. This meromictic lake is located at the southern
64 end of the western branch of the East African Rift System and of all African lakes it is in age (c. 5 Ma) and depth
65 (~700 m) only surpassed by Lake Tanganyika (Salzburger et al., 2014). The open-coiled specimens belong to the

66 genus *Bulinus*, which is widespread in Africa and the Middle East (Brown, 1994). This genus is of great medical
67 and veterinary interest because several of its species serve as intermediate hosts for blood flukes of the genus
68 *Schistosoma* (Trematoda). These parasites are responsible for schistosomiasis, a debilitating tropical disease
69 affecting humans and livestock (Brown, 1994). In Lake Malawi, four different *Bulinus* species occur, two of which
70 are endemic to the lake (Albrecht et al., 2018): *B. forskalii* (*B. forskalii* group), *B. globosus* (*B. africanus* group),
71 *B. nyassanus* (endemic; *B. tropicus/truncatus* complex), *B. succinoides* (endemic; *B. tropicus/truncatus* complex).
72 Of these, *B. globosus* and *B. nyassanus* are known to transmit *Schistosoma haematobium* (Madsen et al., 2001;
73 Marti et al., 1985) which causes human urinary schistosomiasis. This disease experienced an upsurge in incidence
74 around the shorelines of Lake Malawi over recent decades (Van Bocxlaer et al., 2014).

75 We here used genetic data based on two mitochondrial markers to examine whether the corkscrew-like
76 shells in *Bulinus* found in Lake Malawi may represent an ecophenotypic phenomenon or whether these individuals
77 are genetically divergent from their regular-shaped relatives and may thus represent a potential new species. This
78 question is also pertinent for other corkscrew-like specimens, such as those of *Heleobia mirum* from ancient Lake
79 Titicaca, for which it remains uncertain whether they represent a valid species or result from hybridization,
80 incomplete lineage sorting, ecophenotypic plasticity, or confused taxonomy (Kroll et al., 2012).

81 Given that few corkscrew *Bulinus* specimens were found, and that they occur in samples that also
82 contained tightly coiled specimens, we hypothesize that their morphology reflects a growth disturbance or
83 ecophenotypic plasticity. However, given that Lake Malawi is the only large lake on the African continent with
84 endemic *Bulinus* species, including one that has colonized deep waters (Mandahl-Barth, 1972), we cannot exclude
85 the possibility that the specimens represent a rare new species. We tested this hypothesis with phylogenetic and
86 phylogeographical studies of various *Bulinus* populations throughout the Malawi Basin.

87

88 **Material & Methods**

89 *Sampling*

90 Specimens of the freshwater gastropod genus *Bulinus* were collected in the Lake Malawi catchment in August
91 2006 by T. Geertz and R. Schultheiß, in October 2007 by T. Geertz and D. Simbeye, and in September 2014 by
92 B. Van Bocxlaer from a total of 16 localities (see Fig. 1), they were preserved in 80% ethanol. The hitherto
93 unknown specimens with a corkscrew-like shell (Fig. 2) were found during dredging at two close by locations
94 (sites MW06 and MW07, ~39 m depth; see Fig. 1) offshore Monkey Bay in the southern region of the Malawi
95 Basin. These specimens were photographed with a digital microscope system (KEYENCE VHX-2000;
96 KEYENCE Corp., Itasca, IL, USA).

97

98 *DNA isolation, PCR, and sequencing*

99 Genomic DNA was extracted from a total of 29 specimens (including 4 specimens with an aberrant shell morph)
100 using the CTAB protocol described by Wilke et al. (2006). We amplified two mitochondrial gene fragments, i.e.
101 cytochrome oxidase *c* subunit I (COI, 390 bp length) and large ribosomal subunit (16S, 344–444 bp length) using
102 the primers ASMIT1 and ASMIT2 (Stothard & Rollinson, 1997) and 16Sar and 16Sbr (Palumbi et al., 1991),
103 respectively. Polymerase chain reaction (PCR) was performed with the settings specified by Jørgensen et al.
104 (2007). PCR products were sequenced on an ABI 3730 XL sequencer at LGC Genomics (Berlin, Germany) using
105 the Big Dye Terminator Kit (Life Technologies). Vouchers (i.e., DNA, images, and shells) are stored in the
106 Systematics and Biodiversity collection of the Justus Liebig University, Giessen (UGSB; see Table 2 for details).

107

108 *Dataset compilation and DNA alignment*

109 For comparison, our molecular dataset (26 COI and 18 16S sequences) was completed with sequences from NCBI
110 GenBank (30 COI and 20 16S sequences; see Table 2). The final dataset consists of 57 specimens representing 21
111 nominal species (including the Lake Malawi endemics *B. nyassanus* and *B. succinoides*) and *Bulinus* spp. covering
112 the total distribution range of the genus (Africa, Madagascar, and the Middle East). The planorbid *Helisoma duryi*
113 was used as outgroup (COI: AY577513, 16S: AY577472).

114 The protein-coding COI gene fragment (390 bp) was aligned by eye using the sequence alignment editor
115 BioEdit version 7.2.5 (Hall, 1999). The non-coding 16S gene fragment was aligned using the default settings of
116 MAFFT as available at <https://www.ebi.ac.uk/Tools/msa/mafft> (Katoh and Standley, 2013) leaving a final
117 alignment of 460 bp. Both fragments were concatenated for the subsequent phylogenetic analysis resulting in a
118 final alignment length of 850 bp.

119

120 *Phylogenetic and phylogeographical analyses*

121 Phylogenetic relationships within *Bulinus* were reconstructed using Bayesian inference as implemented in
122 MrBayes version 3.2.2 (Ronquist et al., 2012). The settings were chosen as follows: number of MCMC generations
123 = 4,000,000; sample frequency = 100; burnin = 10,000 (25%); number of runs = 2. Partition-specific substitution
124 models were selected using jModelTest version 2.1.4 (Darrriba et al., 2012) based on the corrected Akaike
125 information criterion (AICc). Accordingly, HKY+I+ Γ was selected for the COI partition and GTR+I+ Γ for the
126 16S partition. The effective sample size (ESS) of the parameters for both runs were checked on Tracer version 1.5
127 (Rambaut and Drummond, 2007).

128 A statistical parsimony network analysis was performed on the COI dataset of the clade that contained
129 *Bulinus* specimens with a corkscrew-like shell (eight specimens in total) using TCS version 1.21 (Clement et al.,
130 2000) with a connection limit of 95%.

131

132 **Results & Discussion**

133 In total, six specimens from two localities at the southern part of Lake Malawi (MW06 and MW07, see Fig. 1)
134 displayed an aberrant corkscrew-like shell shape. Unfortunately, DNA was successfully amplified for only one
135 (MW06/1) out of the four specimens (see Fig. 2) from which DNA was extracted. No DNA was extracted from
136 the remaining two specimens because intact vouchers were required depending on the results of our study, i.e. in
137 case our analyses would indicate a new species.

138 Bayesian inference on our dataset of 57 specimens representing 21 nominal species resulted in high ESS
139 values (>3,000) for all major parameters, which indicated that our runs attained stationary and that the analysis
140 was robust. The resulting phylogeny reveals one highly-supported species complex (see Fig. 3A; A: *B.*
141 *truncatus/tropicus* complex, posterior probability, PP 0.95) and three moderately to highly-supported species
142 groups (see Fig. 3A, B: *B. africanus* group, PP 1.00; C: *B. forskalii* group, PP 0.90; and D: *B. reticulatus* group,
143 PP 0.93), which is consistent with previous molecular phylogenetic studies on *Bulinus* (Jørgensen et al., 2007;
144 Kane et al., 2008; Stothard et al., 2001; Tumwebaze et al., 2019). Accordingly, the *B. africanus* group forms a
145 highly-supported (PP 0.99) sister to the *B. forskalii* group, which together are sister to the *B. truncatus/tropicus*
146 complex (PP 0.95). The *B. reticulatus* group (here represented only by *B. wrighti*) is sister to all other
147 groups/complexes (PP 1.00).

148 Our phylogeny revealed the existence of five clades/lineages from Lake Malawi (clades I.–V.; PP ranging
149 from 0.67 up to 1.00; see Fig. 3A) and an additional *B. globosus* lineage from the Shire River (clade VI.; PP 0.53;
150 see Fig. 3A). Four of the Lake Malawi lineages cluster within the *B. truncatus/tropicus* complex and the other
151 lineage, i.e. *B. globosus* from Lake Malawi, clusters within the *B. africanus* group (see Fig. 3A). Interestingly, all
152 lineages within the *B. truncatus/tropicus* complex seem to be monophyletic (partly with weak support) and consist
153 of specimens only found in the lake, suggesting that these clades are potentially endemic to Lake Malawi.
154 Moreover, our analyses reveal some geographical structure. Specimens of clade I and II were only found in the
155 southern part of the lake (mainly around Monkey Bay) whereas clade III only in the north-western part (close to
156 the city Karonga, see Fig. 1). Clade IV consists of an isolated specimen from the north that appears distinct from
157 the well-supported clade III. However, based on the current dataset, we are not able to resolve the relationships
158 between these potentially endemic lineages and their sister groups due to the polyphyletic assemblage within the

159 *B. truncatus/tropicus* complex (see Fig. 3A), which also plagued earlier studies (Jørgensen et al., 2007; Stothard
160 et al., 2001). Hence, further studies using high-resolution genome-wide loci based on next-generation sequencing
161 techniques such RADseq (see e.g. Miura et al., 2019; Razkin et al., 2016) are required to better understand intra-
162 generic relationships.

163 The phylogeny further revealed that the corkscrew-like specimen clusters within clade I (in the *B.*
164 *truncatus/tropicus* complex). This clade is geographically constrained to the area of Monkey Bay, and it also
165 contains specimens of *B. nyassanus*. Further examination of this clade with a statistical parsimony network
166 analysis (see Fig. 3B), indicated that the specimen with corkscrew-like shell is separated from other specimens in
167 clade I with regular shells by five to eight mutational steps. Phylogenetic and network analyses of clade I therewith
168 indicate a short genetic distances between the specimen with corkscrew-like and those with regular shells, which
169 suggests that growth deformation or ecophenotypic plasticity may have led to the observed shell variability. If the
170 corkscrew-like shell morphology would be due to a general growth deformation, then we would expect to
171 sporadically find *Bulinus* individuals with corkscrew-like shells across *Bulinus* populations, however,
172 interestingly, all observed corkscrew-like *Bulinus* specimens have a very restricted geographical occurrence and
173 they all occupy deep waters. Therefore, our data are most congruent with the hypothesis of ecophenotypic
174 plasticity. Such plasticity may represent a possible response to habitat heterogeneity (Pigliucci, 2001; Schlichting
175 & Pigliucci, 1998) and it may even be a precursor to speciation (e.g. Pfennig et al., 2010). The cause of the observed
176 plasticity is currently unknown, but it may relate to changes in water chemistry (Arter, 1989; Dunithan et al.,
177 2012). Early embryonic exposure of *Biomphalaria glabrata* and *Physa acuta* to pharmaceutical chemicals has
178 been demonstrated to result in open-coiling (Baynes et al., 2019). Unfortunately, detailed data on water chemistry
179 and how it has changed over time are not available for the sampling sites examined. However, over the last 30
180 years, the human population density in Malawi has more than doubled and thus agricultural land use has
181 significantly increased and affected lakeshore habitats (Van Bocxlaer et al., 2014). The consequences for ancient
182 Lake Malawi are, among others, a gradual increase of sedimentation and eutrophication, especially in the south of
183 the lake (Otu et al., 2011) where the corkscrew-like *Bulinus* specimens were found. Thus, alterations in the benthic
184 ecosystem might explain ecophenotypic shell shape changes in *Bulinus*. However, given the limited frequency
185 with which corkscrew-like *Bulinus* specimens have been found, other factors such as ontogenetic changes cannot
186 be ruled out. Besides environmental parameters, parasites such as trematodes may be considered to have caused
187 shell alterations (Krist, 2000; McCarthy et al., 2003). Interestingly, the Lake Malawi endemic *B. nyassanus*, which
188 clusters within the same clade as the corkscrew-like *Bulinus*, is known to be the intermediate host for the urinary
189 blood fluke *Schistosoma haematobium* (Madsen et al., 2001). However, because the corkscrew-like *Bulinus*

190 specimens were not found near the shoreline and because no macroparasites were observed during the preparation,
191 the role of micro- and macroparasites remains uncertain.

192 The present study is based on very few specimens, and genetic data of a single specimen only, which is
193 placed in an incompletely resolved phylogeny. Additional specimens and other genetic markers will need to be
194 examined to verify the phylogenetic position of the corkscrew-like *Bulinus* specimens and whether they form a
195 genetically distinct clade.

196

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201

202 **Author contributions**

203 CC and CA conceived the study. CC analysed molecular data. CC, CA, and BVB interpreted the results. CC
204 drafted the initial manuscript. All authors have read and approved the final version of the manuscript.

205

206 **References**

- 207 Albrecht, C., Stelbrink, B., Clewing, C., 2019. Bulinidae P. Fischer & Cross, 1880. In: Lydeard, C. &
208 Cummings, K.S. (eds.) Freshwater mollusks of the world. Johns Hopkins University Press, Baltimore,
209 USA, 167–171.
- 210 Albrecht, C., Clewing, C., Kaunda, E., Mailosi, A., 2018. *Bulinus nyassanus*. The IUCN Red List of Threatened
211 Species 2018: e.T3318A120114450. Downloaded on 20 January 2020.
- 212 Albrecht, C., Clewing, C., Kahwa, D., Magombo, Z.L.K., Kaunda, E., Mailosi, A., 2018. *Bulinus succinoides*.
213 The IUCN Red List of Threatened Species 2018: e.T3320A122888722. Downloaded on 20 January 2020.
- 214 Albrecht, C., Clewing, C., Lange, C., 2018. *Bulinus globosus*. The IUCN Red List of Threatened Species 2018:
215 e.T99504682A120114163. Downloaded on 20 January 2020.
- 216 Albrecht, C., Clewing, C. & Lange, C., 2018. *Bulinus forskalii*. The IUCN Red List of Threatened Species 2018:
217 e.T165794A120113860. Downloaded on 20 January 2020.
- 218 Albrecht, C., Vogel, H., Hauffe, T., Wilke, T., 2010. Sediment core fossils in ancient Lake Ohrid: testing for
219 faunal change since the Last Interglacial. *Biogeosciences* 7, 3435–3446.
- 220 Albrecht, C., Wolff, C., Glöer, P., Wilke, T., 2008. Concurrent evolution of ancient sister lakes and sister

221 species: the freshwater gastropod genus *Radix* in lakes Ohrid and Prespa. *Hydrobiologia* 615, 157–167.

222 Annandale, N., 1924. The evolution of the shell-sculpture in fresh-water snails of the family Viviparidae. *Proc.*
223 *R. Soc. London B* 96, 60–76.

224 Arter, H.E., 1989. Effect of eutrophication on species composition and growth of freshwater mussels (Mollusca,
225 Unionidae) in Lake Hallwil (Aargau, Switzerland). *Aquat. Sci.* 51, 87–99.

226 Basch, P.E., 1968. A scalariform *Biomphalaria glabrata*. *Nautilus* 82, 21.

227 Bartsch, P., 1909. Notes on the Philippine pond snails of the genus *Vivipara*, with descriptions of new species.
228 *Proc. United States Natl. Museum* 37, 365–367.

229 Baynes, A., Montagut Pino, G., Duong, G. H., Lockyer, A. E., McDougall, C., Jobling, S., Routledge, E. J.,
230 2019. Early embryonic exposure of freshwater gastropods to pharmaceutical 5-alpha-reductase inhibitors
231 results in a surprising open-coiled “banana-shaped” shell. *Sci Rep* 9, 16439.

232 Boettger, C.R., 1949. Über das Auftreten einer erheblichen Schalenabweichung bei einer Posthornschnecke.
233 *Abh. Braunsch. Wiss. Ges.* 1, 1–7.

234 Brooks, J.L., 1950. Speciation in ancient lakes. *Q. Rev. Biol.* 25, 30–60.

235 Brown, D.S., 1994. Freshwater snails of Africa and their medical importance. Taylor and Francis, London, 608
236 pp.

237 Brown, D.S., Shaw, K.M., Southgate, V.R., Rollinson, D., 1986. *Bulinus guernei* (Mollusca: Gastropoda) of
238 West Africa: taxonomic status and role as host for schistosomes. *Zool. J. Linn. Soc.* 88, 59–90.

239 Burch, J.B., 1989. North American freshwater snails. Malacological Publications, Hamburg, Michigan, 365 pp.

240 Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. *Mol.*
241 *Ecol.* 9, 1657–1659.

242 Clements, R., Liew, T.-S., Vermeulen, J. J., Schilthuizen, M., 2008. Further twists in gastropod shell evolution.
243 *Biol. Lett.* 4, 179–182.

244 Clewing, C., Riedel, F., Wilke, T., Albrecht, C., 2015. Ecophenotypic plasticity leads to extraordinary gastropod
245 shells found on the “Roof of the World”. *Ecol. Evol.* 5, 2966–2979.

246 Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and
247 parallel computing. *Nat. Methods* 9, 772–772.

248 Dunithan, A., Jacquemin, S., Pyron, M., 2012. Morphology of *Elimia livescense* (Molluscs: Pleuroceridae) in
249 Indiana, USA covaries with environmental variation. *Am. Malacol. Bull.* 30, 127–133.

250 Egorov, R., 2006. Treasure of Russian Shells. Supplement 4. Illustrated Catalogue with Selected Keys of the
251 Recent Fresh- and Brackish-water Pectinibranch Molluscs (Gastropoda: Pectinibranchia) of Russia and

252 Adjacent Areas. Colus-Doverie, 88 pp.

253 Geary, D.H., Staley, A.W., Müller, P., Magyar, I., Spring, N., 2002. Iterative changes in Lake Pannon
254 *Melanopsis* reflect a recurrent theme in gastropod morphological evolution. *Paleobiology* 28, 208–221.

255 Glaubrecht, M., 2008. Adaptive radiation of thalassoid gastropods in Lake Tanganyika, East Africa: morphology
256 and systematization of a paludomid species flock in an ancient lake. *Zoosyst. Evol.* 84, 71–122.

257 Gorthner, A., 1992. Bau, Funktion und Evolution komplexer Gastropodenschalen in Langzeit-Seen. Mit einem
258 Beitrag zur Paläobiologie von *Gyraulus* “*multiformis*” im Steinheimer Becken. *Stuttgarter Beiträge zur*
259 *Naturkunde, Ser.B* 190, 1–173.

260 Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for
261 Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41, 95–98.

262 Hauffe, T., Eross, Z., Albrecht, C., Schreiber, K., 2010. *Gocea ohridana*. The IUCN Red List of Threatened
263 Species 2010: e.T155653A4815465. Downloaded on 20 January 2020.

264 Hirano, T., Saito, T., Tsunamoto, Y., Koseki, J., Ye, B., Do, V.T., Miura, O., Suyama, Y., Chiba, S., 2019.
265 Enigmatic incongruence between mtDNA and nDNA revealed by multi-locus phylogenomic analyses in
266 freshwater snails. *Sci. Rep.* 9, 6223.

267 Jørgensen, A., Jørgensen, L.V.G., Kristensen, T.K., Madsen, H., Stothard, J.R., 2007. Molecular phylogenetic
268 investigations of *Bulinus* (Gastropoda: Planorbidae) in Lake Malawi with comments in the topological
269 incongruence between DNA loci. *Zoologica Scripta* 36, 577–585.

270 Kane, R.A., Stothard, J.R., Emery, A.M., Rollinson, D., 2008. Molecular characterization of freshwater snails in
271 the genus *Bulinus*: a role for barcodes?. *Parasit Vectors* 1, 15.

272 Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in
273 performance and usability. *Mol. Biol. Evol.* 30, 772–780.

274 Krist, A.C., 2000. Effect of the digenean parasite *Proterometra macrostoma* on host morphology in the
275 freshwater snail *Elimia livescens*. *J. Parasitol.* 86, 262–267.

276 Kroll, O., Hershler, R., Albrecht, C., Terrazas, E.M., Apaza, R., Fuentealba, C., Wolff, C., Wilke, T., 2012. The
277 endemic gastropod fauna of Lake Titicaca: correlation between molecular evolution and hydrographic
278 history. *Ecol. Evol.* 2, 1517–1530.

279 Madsen, H., Bloch, P., Phiri, H., Kristensen, T.K., Furu, P., 2001. *Bulinus nyassanus* is an intermediate host for
280 *Schistosoma haematobium* in Lake Malawi. *Ann Trop Med Parasitol* 95, 353–360.

281 Mandahl-Barth, G., 1973. Descriptions of new species of African freshwater molluscs. *Proc. Malacol. Soc.*
282 London 40, 277–286.

283 Marti, H.P., Tanner, M., Degremont, A.A., Freyvogel, T.A., 1985. Studies on the ecology of *Bulinus globosus*,
284 the intermediate host of *Schistosoma haematobium* in the Ifakara area, Tanzania. *Acta Trop.* 42, 171–187.

285 McCarthy, H.O., Fitzpatrick, S.M., Irwin, S.W.S., 2003. Parasite alteration of host shape: a quantitative approach
286 to gigantism helps elucidate evolutionary advantages. *Parasitology* 128, 7–14.

287 Michel, E., 1994. Why snails radiate: a review of gastropod evolution in long-lived lakes, both recent and fossil.
288 In: Martens, K., Goddeeris, B., Coulter, G. (eds.), *Speciation in Ancient Lakes*. *Arch. Hydrobiol. Beih.*
289 *Ergebn. Limnol.* 44, Stuttgart, 285–317.

290 Moore, J.E.S., 1898. On the hypothesis that Lake Tanganyika represents an old Jurassic sea. *Q. J. Microsc. Sci.*
291 41, 303–321.

292 Otu, M.K., Ramlal, P., Wilkinson, P., Hall, R.I., Hecky, R.E., 2011. Paleolimnological evidence of the effects of
293 recent cultural eutrophication during the last 200 years in Lake Malawi, East Africa. *J. Great Lakes Res.*
294 37, 61–74.

295 Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G., 1991. The simple fool's
296 guide to PCR. University of Hawaii, Honolulu, 24 pp.

297 Pfennig, D.W., Wund, M.A., Snell-Rood, E.C., Cruickshank, T., Schlichting, C.D., Moczek, A.P., 2010.
298 Phenotypic plasticity's impacts on diversification and speciation. *Trends Ecol. Evol.* 25, 459–467.

299 Pigliucci, M., 2001. *Phenotypic plasticity: beyond nature and nurture*. Johns Hopkins Univ. Press, Baltimore, pp.
300 328.

301 Rambaut, A., Drummond, A.J., 2007. Tracer v. 1.5. Available from <http://tree.bio.ed.ac.uk/software/tracer>.

302 Rasser, M.W., 2014. Evolution in isolation: the *Gyraulus* species flock from Miocene Lake Steinheim revisited.
303 *Hydrobiologia* 739, 7–24.

304 Rasser, M.W., Covich, A.P., 2014. Predation on freshwater snails in Miocene Lake Steinheim: a trigger for
305 intralacustrine evolution? *Lethaia* 47, 524–532.

306 Razkin, O., Sonet, G., Breugelmans, K., Madeira, M.J., Gómez-Moliner, B.J., Backeljau, T., 2016. Species
307 limits, interspecific hybridization and phylogeny in the cryptic land snail complex *Pyramidula*: the power
308 of RADseq data. *Mol. Phylogenet. Evol.* 101, 267–278.

309 Rex, M. A., Boss, K. J., 1976. Open coiling in recent gastropods. *Malacologia* 15, 289–297.

310 Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard,
311 M.A., Huelsenbeck, J. P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice
312 across a large model space. *Syst. Biol.* 61, 539–542.

313 Salzburger, W., Van Bocxlaer, B., Cohen, A.S., 2014. Ecology and evolution of the African Great Lakes and

314 their faunas. *Annu. Rev. Ecol. Evol. Syst.* 45, 519–545.

315 Schlichting, C.D., Pigliucci, M., 1998. Phenotypic evolution. A reaction norm perspective. Sinauer Associates,
316 Sunderland, Massachusetts, 387 pp.

317 Sitnikova, P., Röpstorf, P., Riedel, F., 2001. Reproduction, duration of embryogenesis, egg capsules and
318 protoconchs of family Baicaliidae (Caenogastropoda) endemic to Lake Baikal. *Malacologia* 43, 59–85.

319 Stothard, J.R., Rollinson, D., 1997. Partial DNA sequences from the mitochondrial cytochrome oxidase subunit I
320 (COI) gene can differentiate the intermediate snail hosts *Bulinus globosus* and *B. nasutus* (Gastropoda:
321 Planorbidae). *J. Nat. Hist.* 31, 727–737.

322 Stothard, J.R., Brémond, P., Andriamaro, L., Sellin, B., Sellin, E., Rollinson, D., 2001. *Bulinus* species on
323 Madagascar: molecular evolution, genetic markers and compatibility with *Schistosoma haematobium*.
324 *Parasitology* 123, 261–275.

325 Tumwebaze, I., Clewing, C., Dusabe, M.C., Tumusiime, J., Kagoro-Rugunda, G., Hammoud, C., Albrecht, C.,
326 2019. Molecular identification of *Bulinus* spp. intermediate host snails of *Schistosoma* spp. in crater lakes
327 of western Uganda with implications for the transmission of the *Schistosoma haematobium* group
328 parasites. *Parasit. Vectors* 12, 565.

329 Van Bocxlaer, B., Albrecht, C., Stauffer Jr, J.R., 2014. Growing population and ecosystem change increase
330 human schistosomiasis around Lake Malawi. *Trends Parasitol.* 30, 217–220.

331 Van Bocxlaer, B., Ortiz-Sepulveda, C. M., Gurdebeke, P. R., Vekemans, X., 2020. Adaptive divergence is shell
332 morphology in an ongoing gastropod radiation from Lake Malawi. *BMC Evolutionary Biology* 20, e5.

333 Van Bocxlaer, B., 2017. Hierarchical structure of ecological and non-ecological processes of differentiation
334 shaped ongoing gastropod radiation in the Malawi Basin. *Proc. R. Soc. B* 284, 20171494.

335 Van Bocxlaer, B., Van Damme, D., Feibel, C.S., 2008. Gradual versus punctuated equilibrium evolution in the
336 Turkana Basin molluscs: evolutionary events or biological invasions? *Evolution* 62, 511–520.

337 Vermeij, G.J., Covich, A.P., 1978. Coevolution of freshwater gastropods and their predators. *Am. Nat.* 112, 833–
338 843.

339 Wilke, T., Davis, G.M., Qiu, D., Spear, R.C., 2006. Extreme mitochondrial sequence diversity in the
340 intermediate schistosomiasis host *Oncomelania hupensis robertsoni*: another case of ancestral
341 polymorphism? *Malacologia* 48, 143–157.

342 Zuykov, M., Vinarski, M., Pelletier, E., Demers, S., Harper, D.A.T., 2012. Shell malformations in seven species
343 of pond snail (Gastropoda, Lymnaeidae): analysis of large museum collections. *Zoosyst. Evol.* 88, 365–
344 368.

346 **Table 1.** Selected taxa of recent freshwater gastropods showing corkscrew-like shells. Modified from Clewing et
 347 al. (2015). *according to Albrecht et al. (2019).

Family	Species	Location	Reference (plus shell shape)
Ampularidae	<i>Pomacea canaliculata</i> ^B	inbred aquarium lineage	 B. Van Bocxlaer pers. obs.
Amnicolidae	<i>Liobaicalia stiedae</i> ^A	endemic to Lake Baikal, Russia	Sitnikova et al., 2001 
Bulinidae*	<i>Bulinus</i> sp.^B	Lake Malawi, Malawi (Monkey Bay)	 this study
Cochliopidae	<i>Heleobia mirum</i> ^A	endemic to Lake Titicaca, Bolivia/Peru	Kroll et al., 2012 
Hydrobiidae	<i>Gocea ohridana</i> ^A	endemic to Lake Ohrid, Macedonia/Albania	 Hauffe et al., 2010
Lymnaeidae	<i>Lymnaea stagnalis</i> ^B	former USSR, Germany, or USA	Zuykov et al., 2012 
Planorbidae	<i>Anisus leucostoma</i> ^B	Germany (laboratory)	 Boettger, 1949
	<i>Biomphalaria glabrata</i> ^B	Saint Lucia (laboratory)	Basch, 1968 
	<i>Gyraulus</i> sp. ^B	Lake Bangong system, Tibetan Plateau, China	 Clewing et al., 2015
Valvatidae	<i>Valvata lewis</i> ^B (morph <i>ontariensis</i>)	Lake Superior drainage, northwestern Ontario, Canada	Burch, 1989 

348 ^ACorkscrew-like shell = species-specific character.

349 ^BCorkscrew-like shell = sporadic intraspecific trait.

350 **Table 2.** List of studied specimens including taxon, specimen code, locality information, voucher number (stored
351 at the UGSB database), and GenBank accession numbers. Note that *B. guernei* is regarded as a synonym of *B.*
352 *truncatus* according to Brown et al. (1986). The outgroup taxon used for the phylogenetic analysis *Helisoma duryi*
353 (COI: AY577513, 16S: AY577472) is not listed here.

Species	Specimen code	Location	Coordinates	Voucher no.	GenBank accession no. COI	GenBank accession no. 16S
<i>Bulinus truncatus/tropicus</i> complex						
<i>B. guernei</i>	SN01.1	Senegal	N/A		EU076716	EU076725
<i>B. hexaploidus</i>	ET01.1	Ethiopia	N/A		EU076717	EU076723
<i>B. liratus</i>	MG01.1	Madagascar	N/A		AF369590	AY029543
<i>B. natalensis</i>	TZ01.1	Tanzania	N/A		EU076719	EU076730
<i>B. nyassanus</i>	MW01.1	Malawi, Lake Malawi	N/A		AY577510	AY577470
	MW02.1	Malawi, Lake Malawi, Monkey Bay	N/A		AM921838	-
<i>B. pemembraneus</i>	KE01.1	Kenya	N/A		EU076719	EU076724
<i>B. succinoides</i>	MW03.1	Malawi	N/A		EU076720	EU076729
<i>Bulinus</i> sp.	MW04.1	Malawi, Nkhotakota, Lake Malawi (shore)	-12.91953°N 34.30137°E	UGSB 22267	MT441583	-
	MW04.2	Malawi, Nkhotakota, Lake Malawi (shore)	-12.91953°N 34.30137°E	UGSB 22268	MT441582	MT396503
	MW05.1	Malawi, Karonga, Lake Malawi (shore)	-9.89302°N 33.92841°E	UGSB 22275	MT441580	MT396501
	MW06.1	Malawi, Monkey Bay, Lake Malawi (offshore, ~39 m depth)	-14.05566°N 34.92276°E	UGSB 22331	MT441578	MT396505
	MW07.1	Malawi, Monkey Bay, Lake Malawi (offshore)	-14.04809°N 34.92171°E	UGSB 22332	-	-
	MW07.2	Malawi, Monkey Bay, Lake Malawi (offshore)	-14.04809°N 34.92171°E	UGSB 23343	-	-
	MW07.3	Malawi, Monkey Bay, Lake Malawi (offshore)	-14.04809°N 34.92171°E	UGSB 23344	-	-
	MW08.1	Malawi, Karonga, Lake Malawi (offshore, ~11 m depth)	-9.95860°N 33.94851°E	UGSB 21855	MT441568	-
	MW09.1	Malawi, Karonga, Lake Malawi (offshore, ~8 to 16 m depth)	-9.95873°N 33.94894°E	UGSB 21858	MT441567	MT396492
	MW10.1	Malawi, Karonga, Lake Malawi (offshore, ~35 m depth)	-9.94955°N 33.95032°E	UGSB 21861	MT441579	MT396500
	MW11.1	Malawi, Monkey Bay, Lake Malawi (offshore, ~20 m depth)	-14.05723°N 34.91818°E	UGSB 21849	MT441566	-
	MW12.1	Malawi, Monkey Bay, Lake Malawi (offshore, ~6 to 10 m depth)	-14.05163°N 34.91516°E	UGSB 23345	MT441576	MT396499
	MW12.2	Malawi, Monkey Bay, Lake Malawi (offshore, ~6 to 10 m depth)	-14.05163°N 34.91516°E	UGSB 23346	MT441575	MT396498
	MW12.3	Malawi, Monkey Bay, Lake Malawi (offshore, ~6 to 10 m depth)	-14.05163°N 34.91516°E	UGSB 23347	MT441574	MT396497
MW12.4	Malawi, Monkey Bay, Lake Malawi (offshore, ~6 to 10 m depth)	-14.05163°N 34.91516°E	UGSB 23348	MT441573	MT396496	
MW13.1	Malawi, Chembe, Lake Malawi (offshore, ~2.5 m depth)	-14.03101°N 34.82889°E	UGSB 23353	MT441572	-	
MW13.2	Malawi, Chembe, Lake Malawi (offshore, ~2.5 m depth)	-14.03101°N 34.82889°E	UGSB 23354	MT441570	MT396493	
MW14.1	Malawi, Monkey Bay Lake Malawi, (shore)	-14.07023°N 34.91713°E	UGSB 23355	MT441569	-	
<i>B. truncatus</i>	CM01.1	Cameroon	N/A		AF369747	-
	IR01.1	Iran	N/A		EU076721	EU076728
	SD01.1	Sudan	N/A		AF369605	-
	MW15.1	Malawi	N/A		AF369604	AY029549
	CD01.1	DR Congo	N/A		-	EU076726

<i>B. tropicus</i>	KE02.1	Kenya	N/A		AF369602	-
	ZM01.1	Zambia	N/A		AF369603	-
	ZW01.1	Zimbabwe	N/A		EU076722	EU076728

***Bulinus africanus* group**

<i>B. globosus</i>	MW16.1	Malawi, Karonga, Lake Malawi (beach pool, ~50 m inland)	-9.89932°N 33.94370°E	UGSB 21857	MT441562	MT396488
	MW16.2	Malawi, Karonga, Lake Malawi (beach pool, ~50 m inland)	-9.89932°N 33.94370°E	UGSB 21856	MT441563	MT396489
	MW17.1	Malawi, Liwonde, Shire River	-15.05699°N 35.22272°E	UGSB 21845	MT441564	MT396490
	MW17.2	Malawi, Liwonde, Shire River	-15.05699°N 35.22272°E	UGSB 21846	MT441565	MT396491
	MW17.3	Malawi, Liwonde, Shire River	-15.05699°N 35.22272°E	UGSB 23338	MT441584	-
	MW18.1	Malawi, Karonga, North Rukuru River (estuary of Lake Malawi)	-9.90855°N 33.95027°E	UGSB 23349	MT441571	MT396495
	MW18.2	Malawi, Karonga, North Rukuru River (estuary of Lake Malawi)	-9.90855°N 33.95027°E	UGSB 23350	MT441587	MT396504
	MW19.1	Malawi, Karonga, Lake Malawi (beach pool, ~20 m inland)	-9.93023°N 33.94670°E	UGSB 23351	MT441585	MT396494
	MW19.2	Malawi, Karonga, Lake Malawi (beach pool, ~20 m inland)	-9.93023°N 33.94670°E	UGSB 23352	MT441586	-
	MW20.1	Malawi, Nkhata Bay, Lake Malawi (shore)	-11.59727°N 34.30258°E	UGSB 22256	MT441577	-
	MW05.2	Malawi, Karonga, Lake Malawi (shore)	-9.89302°N 33.92841°E	UGSB 22274	MT441581	MT396502
	TZ02.1	Tanzania	N/A		AF369595	AY029546
	NE01.1	Niger	N/A		AF369596	-
<i>B. africanus</i>	ZA01.1	South Africa	N/A		AF369601	-
<i>B. nasutus</i>	TZ03.1	Tanzania	N/A		AF369598	AY029547
<i>B. umbilicatus</i>	NE02.1	Niger	N/A		AF369600	-
<i>B. obtusispira</i>	MG02.1	Madagascar	N/A		AF369588	AY029542

***Bulinus forskalii* group**

<i>B. forskalii</i>	TZ04.1	Tanzania	N/A		AF369610	AY029550
	MG03.1	Madagascar	N/A		-	AY029545
<i>B. crystallinus</i>	AO01.1	Angola	N/A		AF369742	-
<i>B. bavayi</i>	MG04.1	Madagascar	N/A		AF369593	AY029544
<i>B. cernicus</i>	MU01.1	Mauritius	N/A		AF369613	-
<i>B. scalaris</i>	KE03.1	Kenya	N/A		AF369740	-
<i>B. senegalensis</i>	ML01.1	Mali	N/A		AF369745	-

***Bulinus reticulatus* group**

<i>B. wrighti</i>	OM01.1	Oman	N/A		AF369615	AY029552
	FR01.1	France, laboratory stock	N/A		AF369614	-

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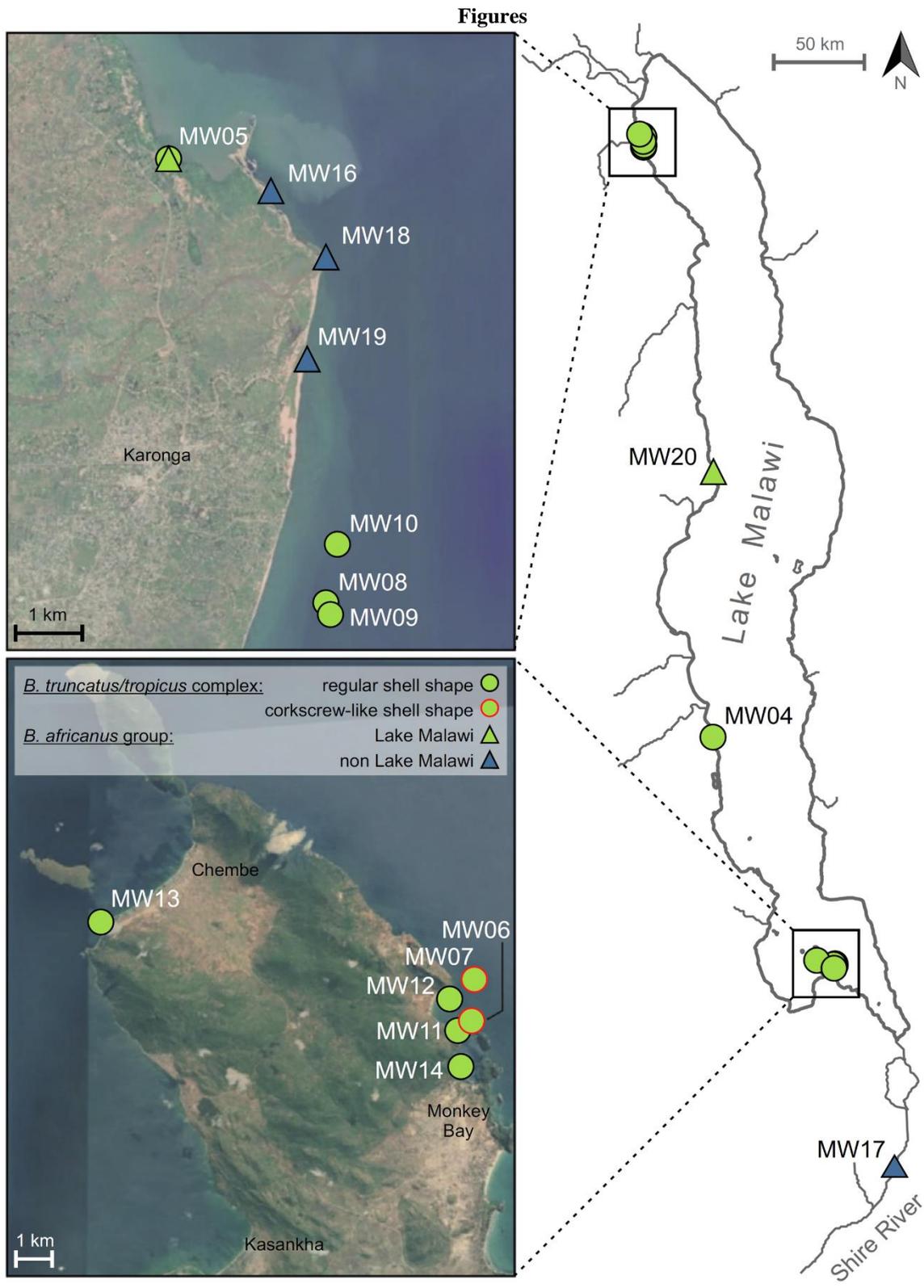


Figure 1. Sampling sites (with location codes, see Table 2) of *Bulinus* spp. at Lake Malawi and its catchment area. Detailed locality information is given in Table 2. Location symbols either denote specimens belonging to the *B. truncatus/tropicus* complex (circles) or to the *B. africanus* group (triangles). Symbol colours indicate whether the site is located within Lake Malawi (green) or outside the main water body (blue). The two locations where corkscrew-like specimens were observed are highlighted with a red frame.

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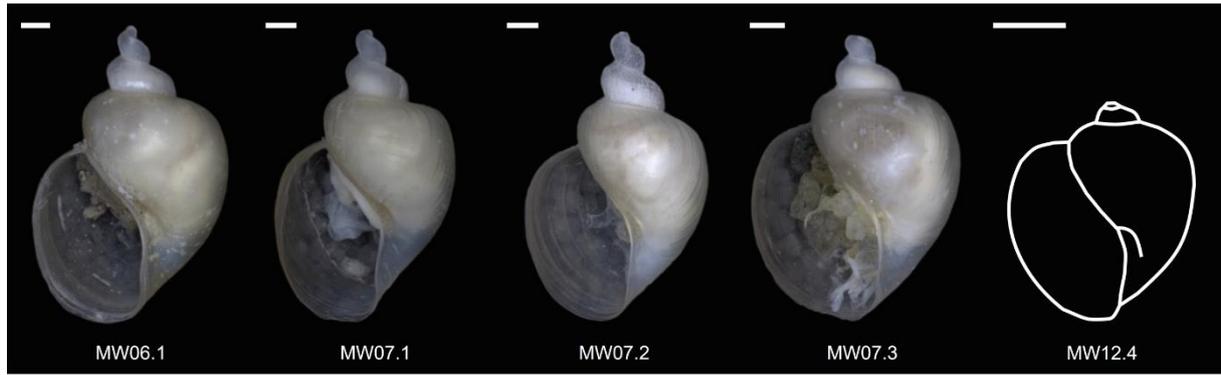
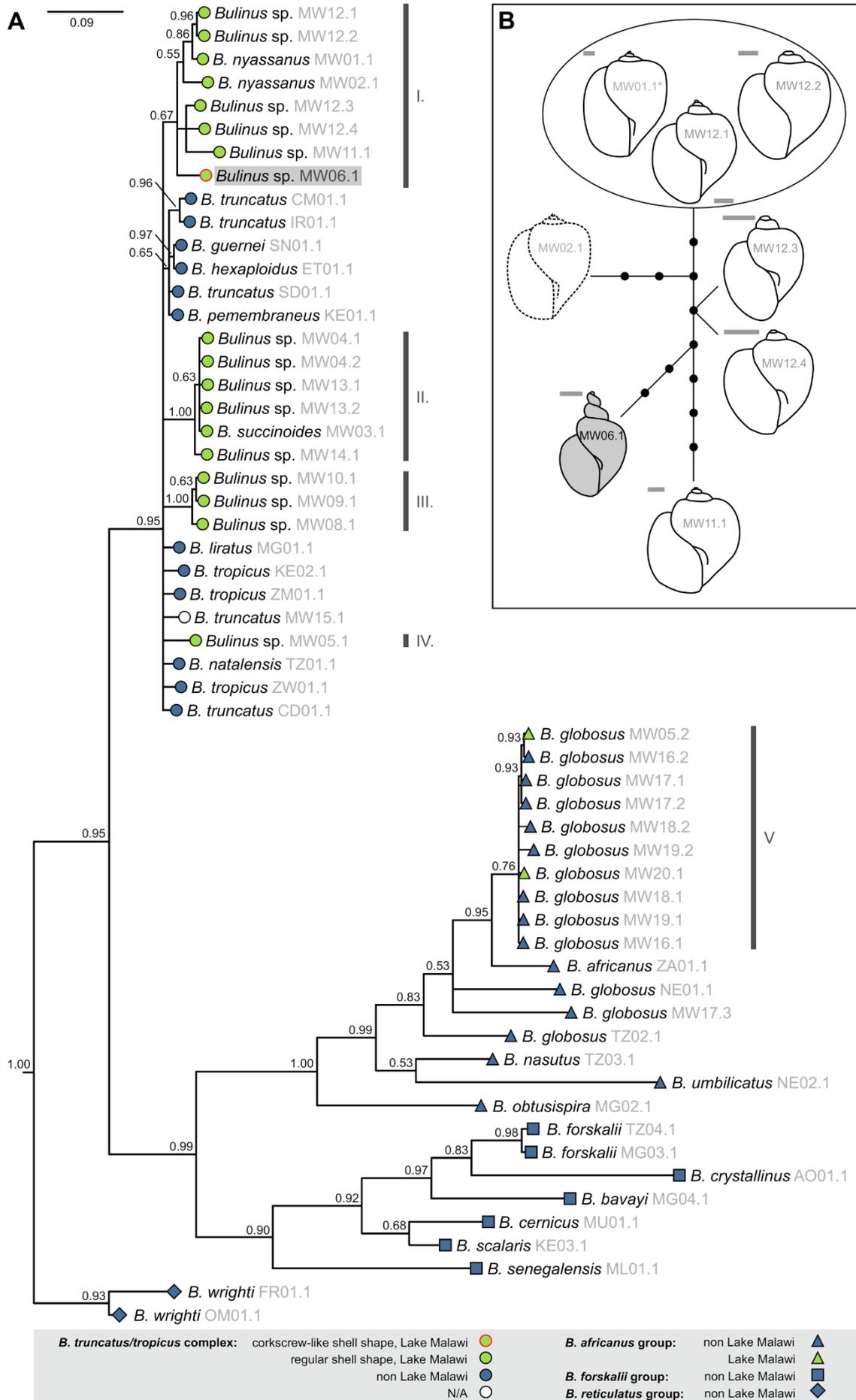


Figure 2. Specimens of *Bulinus* sp. from Lake Malawi with a corkscrew-like shell shape (specimens MW06.1, MW07.1–3). The line drawing indicates a specimen with a regular shell for comparison (MW12.4; from a site located nearby MW06 and MW07; see Fig. 1). Scale bar = 0.5 mm.

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450 **Figure 3.** (A) Phylogeny constructed with Bayesian inference of *Bulinus* spp. based on two mtDNA fragments
451 (COI and 16S). *Helisoma duryi* was used as outgroup but is not shown here. Specimens from Lake Malawi are
452 highlighted with green symbols, all non-Lake Malawi specimens with blue symbols (for the single specimen
453 marked with a white circle, detailed locality information are missing). While the symbols denote species groups
454 according to Stothard et al. (2001): circle – *B. tropicus/truncatus* complex; triangle – *B. africanus* group; square –
455 *B. forskalii* group; rhomb – *B. reticulatus* group. Clades/lineages composed of Lake Malawi specimens are
456 indicated by dark-grey bars (I–VI). Bayesian posterior probabilities (>0.50) are provided next to the nodes. (B)
457 Statistical parsimony network (connection limit: 95%) of clade I-*Bulinus* specimens from Lake Malawi.
458 Haplotypes are illustrated with their shell shape, and the corkscrew-like specimen is again highlighted in grey.
459 Note that the shell shape of MW02.1 is replicated from MW01.1, because the specimen was not imaged and
460 therefore it is illustrated with a dashed line. Scale bar = 0.5 mm.
461