

Small minor changes have been made in the final version (see marked MS below):

line 99: "gammarids" replaced by "crustaceans"
line 211: "through time" replaced by "over time"
line 217: "through time" replaced by "over time"
line 245: "does not show" replaced by "does not suggest"
line 247: "through time" replaced by "over time"
line 282: "showed" replaced by "suggests"
line 334: ", respectively" added at the end of the sentence
Table 1: "N/A" replaced by "-"
Table 2: "1 shift: 0.1" moved up one line

Best regards,

Björn Stelbrink

1 **Constant diversification rates of endemic gastropods in ancient Lake Ohrid:**

2 **Ecosystem resilience likely buffers environmental fluctuations**

3

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11

12 **Abstract**

13 Ancient lakes represent key ecosystems for endemic freshwater species. This high endemic
14 biodiversity has been shown to be mainly the result of intra-lacustrine diversification. Whereas the
15 principle role of this mode of diversification is generally acknowledged, actual diversification rates in
16 ancient lakes remain little understood. At least four modes are conceivable. Diversification rates may
17 be constant over time, they may fluctuate, rates may be higher in the initial phase of diversification, or
18 there may be a pronounced lag phase between colonization and subsequent diversification. As
19 understanding the tempo of diversification in ancient lake environments may help unveiling the
20 underlying processes that drive speciation and extinction, we here use the Balkan Lake Ohrid as a
21 model system and the largest species flock in the lake, the non-pyrgulinid Hydrobiidae, as a model
22 taxon to study changes in diversification rates over time together with the respective drivers.

23 Based on phylogenetic, molecular-clock, lineage-through-time plot and diversification-rate
24 analyses we found that this potentially monophyletic group is comparatively old and that it most likely
25 evolved with a constant diversification rate. Preliminary data of the SCOPSCO deep-drilling program
26 do indicate signatures of severe environmental/climatic perturbations in Lake Ohrid. However, so far
27 there is no evidence for the occurrence of catastrophic environmental events. We therefore propose
28 that the constant diversification rate observed in endemic gastropods has been caused by two factors:
29 i) a potential lack of catastrophic environmental events in Lake Ohrid and/or ii) a probably high
30 ecosystem resilience, buffering environmental changes. Parameters potentially contributing to the
31 lake's high ecosystem resilience are its distinct bathymetry, ongoing tectonic activities, and karst
32 hydrology.

33 The current study not only contributes to one of the overall goals of the SCOPSCO deep-
34 drilling program – inferring the driving forces for biotic evolution in Lake Ohrid. It might also enhance
35 our understanding of how ecosystem resilience, in general, may promote relative constant
36 diversification rates in isolated ecosystems. However, we encourage future studies testing hypotheses
37 about the lack of catastrophic events in Lake Ohrid. These studies should be based on high-resolution
38 data for the entire geological history of the lake, and potentially involving information from the
39 sediment fossil record, not only for gastropods but also for other groups with a high share of endemic
40 taxa.

41

42 **1 Introduction**

43 Ancient lakes represent key ecosystems for the world's endemic freshwater biodiversity (Brooks,
44 1950; Martens et al., 1994; Martens, 1997; Rossiter and Kawanabe, 2000). Two hypotheses have
45 been suggested for the underlying processes generating their often high levels of species richness.
46 Originally, ancient lakes were considered to be evolutionary refugia that accumulate immigrating
47 elements from extralimital areas during periods of environmental changes ('reservoir function').
48 Accordingly, distantly related 'relic' species may have colonized the lake at different times and
49 possibly from different geographic areas (e.g., Hauswald et al., 2008; Wilson et al., 2004). However,
50 with the advance of molecular techniques, several researchers noted that many endemic species are
51 considerably younger than the lake they inhabit. Hence, they suggested that the high endemic
52 biodiversity in ancient lakes is predominantly a result of intra-lacustrine diversification ('cradle
53 function') (e.g., Martens, 1997; Salzburger et al., 2005; Sherbakov, 1999).

54 Though the principle role of the cradle function is hardly disputed today, rates of
55 diversification in ancient lakes remain little understood (e.g., Cristescu et al., 2010; Martens et al.,
56 1994). As ancient lakes are considered to be comparatively stable systems (Martens, 1997), originally
57 diversification rates (i.e., speciation minus extinction rates) have been assumed to be constant over
58 time. However, in the past decades, several factors, typically related to environment change, have
59 been proposed to alter the tempo of diversification in species flocks. The most renowned theory,
60 punctuated equilibrium, suggests little net evolutionary change during periods of environmental
61 stability (Gould and Eldredge, 1977; but see e.g., Pennell et al., 2014; Van Bocxlaer et al., 2008). This
62 equilibrium might be 'punctuated' during phases of rapid environmental change. Another theory
63 suggests that diversification rates can be higher in the initial phase of diversification (particularly in
64 groups that diversify through an adaptive radiation) and may decline once niche spaces becomes
65 successively occupied (e.g., Purvis et al., 2009; Schluter, 2000). This may happen after a lake first
66 came into existence or after the occurrence of major environmental events such as volcanic ash
67 deposits, severe lake-level drops, and desiccation or salinization events (Cristescu et al., 2010; Kroll
68 et al., 2012; Salzburger et al., 2014). A forth theory proposes the opposite, i.e., the existence of a
69 pronounced lag phase between colonization and onset of diversification (e.g., Cristescu et al., 2010).

70 However, these scenarios have rarely been tested in ancient lake environments due to the
71 lack of appropriate candidate lakes and suitable model taxa. Criteria for a candidate lake would be a

72 long and continuous existence, providing sufficient time for repetitive cladogenesis, and a good
73 knowledge of its palaeo-limnological history, enabling a link between geological and biotic evolution.
74 The model taxon, in turn, should be monophyletic, permitting unbiased calculations of diversification
75 rates; species rich, thus providing sufficient power for evolutionary analyses; and reasonably old,
76 allowing for studying the effect of environmental changes on speciation rates over an extended period
77 of time.

78 Of the few ancient lakes in the world, even less fulfil the above criteria. Some lakes, though
79 being old, went through a series of major environmental events, and the respective endemic species
80 are often comparable young, as observed in Lake Malawi (e.g., Schultheiß et al., 2009, 2011) and
81 Lake Titicaca (Kroll et al., 2012). Other lakes such as Lake Baikal (e.g., Ivanov et al., 2013) and Lake
82 Tanganyika (e.g., Salzburger et al., 2014; Scholz et al., 2007) might be sufficiently old but lack a
83 continuous palaeo-limnological record. In fact, one of the very few ancient lakes enabling a link
84 between geological and biotic evolution throughout its existence, is the Balkan Lake Ohrid (Wagner et
85 al., 2014). It is the oldest freshwater lake in Europe and perhaps the most speciose in the world when
86 considering lake size (Albrecht and Wilke, 2008). Though the exact age of the lake remains
87 controversially discussed, biological data suggest an age of no older than 2–3 million years (Ma) (e.g.,
88 Albrecht et al., 2006; Trajanovski et al., 2010; Wysocka et al., 2013). Newer sedimentological and
89 seismological data obtained during the recently conducted SCOPSCO deep-drilling project in Lake
90 Ohrid revealed a minimum lake age (deep-water conditions) of c. 1.2 Ma (Wagner et al., 2014), and an
91 age of its oldest sediments of approximately 2.0 Ma (Lindhorst et al., 2015). This timeframe of 1.2–2.0
92 Ma for the origin of extant Lake Ohrid is also considered in the current study.

93 Besides its relatively well characterized limnological history, Lake Ohrid also harbors a high
94 number of endemic animal species. So far, at least 185 taxa have been described (Albrecht and
95 Wilke, 2008; Pešić, 2015; Stocchino et al., 2013; Wysocka et al., 2013). In addition, there is a rich
96 protist flora. Diatoms alone account for 789 taxa with 117 of them being endemic to the lake (Levkov
97 and Williams, 2012). Accordingly, a number of more than 300 endemic eukaryotic species for ancient
98 Lake Ohrid is conceivable. The majority of the animal groups form relatively old species flocks in
99 several higher taxa including crustaceans (Wysocka et al., 2008, 2013, 2014), leeches (Trajanovski et
100 al., 2010), and gastropods (Albrecht et al., 2006; Wilke et al., 2007, 2009). In fact, gastropods
101 represent the most speciose animal group in Lake Ohrid with 74 species described, 56 of which are

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103 endemic to the lake and its catchment (Albrecht and Wilke, 2008; Albrecht et al., 2009, 2014; Hauffe
104 et al., 2011; Radoman, 1985). The largest share of this diversity is held by snails of the family
105 Hydrobiidae (Caenogastropoda: Truncatelloidea), including 13 pyrgulinid and 27 other endemic
106 species (Radoman, 1983). The latter group comprises the nominal genera *Dolapia*, *Gocea*, *Lyhnia*,
107 *Ohrigocea*, *Ohridohauffenia*, *Ohridohoratia*, *Polinskiola*, *Pseudohoratia*, *Strugia*, and *Zaumia* (see Fig.
108 1). Pending a formal classification of this potentially monophyletic taxon, it is from hereon called the
109 'non-pyrgulinid Hydrobiidae'. Given that this group probably represents the largest species flock in
110 Lake Ohrid (see also Radoman, 1983), it appears to be a suitable candidate taxon to study speciation
111 processes in this ancient lake.

112 Therefore, the major goal of the present study is to test for changes in diversification rates
113 over time and to assess the underlying drivers. In order to achieve this objective, three specific goals
114 are addressed.

115 i) Based on molecular-clock analyses, we estimate the age of the most recent common ancestor
116 (MRCA) of this group as a baseline for our temporal studies.

117 ii) Utilizing lineage-through-time (LTT) plot and diversification-rate analyses, hereinafter we assess
118 changes in diversification rates over time.

119 iii) If deviations from a constant diversification model are inferred, we finally attempt to link
120 environmental and climatic fluctuations derived from the SCOPSCO program to these biotic
121 changes.

122

123 Given that Lake Ohrid has long been considered to be a relatively stable system with considerable
124 ecosystem resilience (*sensu* Stankovic, 1960), our working hypothesis is that there are no significant
125 changes in diversification rates over time in the lake's non-pyrgulinid Hydrobiidae.

126 The current study will complement palaeontological evidences for evolutionary processes
127 obtained from the SCOPSCO high-resolution sediment record and thus contribute to one of the overall
128 goals of the deep-drilling program – inferring the driving forces for biotic evolution in this fascinating
129 ancient lake. It might also enhance our general understanding of how environment change alters the
130 tempo of diversification in isolated ecosystems and how ecosystem stability may buffer such changes.

131

132 **2 Material and methods**

133 **2.1 Sampling**

134 Hydrobiid gastropods were collected during fieldtrips to Lake Ohrid and other waterbodies in the
135 Balkan region between 2003 and 2011 (Fig. 2; see Table 1 for details). The collection methods
136 followed those described in Schreiber et al. (2012) and included hand collecting, snorkeling, sieving
137 and dredging from small boats or the research vessel of the Hydrobiological Institute Ohrid. Samples
138 were preserved in 80% ethanol and determined in the laboratory to species level based on Radoman
139 (1983).

140

141 **2.2 DNA isolation, PCR amplification and DNA sequencing**

142 Genomic DNA was isolated from whole specimens using the CTAB protocol described in Wilke et al.
143 (2006). Voucher specimens and digital images were deposited in the Systematics and Biodiversity
144 Collection of the University of Giessen (UGSB). Fragments of the mitochondrial genes for cytochrome
145 oxidase c subunit I (COI) and large subunit rRNA (LSU rRNA or 16S rRNA) were amplified using the
146 universal primers LCO 1490 (Folmer et al., 1994) and COR722b (Wilke and Davis, 2000) as well as
147 16Sar-L and 16Sbr-H (Palumbi et al., 1991), respectively (for PCR conditions see Schreiber et al.,
148 2012). Subsequent Sanger sequencing was conducted either on a Long Read IR2 4200 sequencer
149 (LI-COR, Lincoln, NE, USA) using the Thermo Sequenase fluorescent labelled primer cycle
150 sequencing Kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) or on a ABI 3730 XL sequencer
151 (Life Technologies, Carlsbad, CA, USA) using the Big Dye Terminator Kit (Life Technologies,
152 Carlsbad, CA, USA). In total, we sequenced 65 specimens of 17 nominal species of the endemic non-
153 pyrgulinid Hydrobiidae. For comparison, we also analyzed 20 specimens of 15 closely-related species
154 occurring in lakes Prespa and Mikri Prespa as well as in surrounding waterbodies (Fig. 2; see Table 1
155 for locality details, UGSB collection numbers and GenBank accession numbers).

156

157 **2.3 Preliminary genetic analyses**

158 The protein-coding COI sequences were unambiguously aligned in BioEdit 7.0.9.0 (Hall, 1999),
159 resulting in a final alignment of 638 base pairs (bp). The 16S rRNA sequences were aligned using the
160 secondary structure model for the Hydrobiidae suggested by (Wilke et al., 2013), resulting in a final
161 alignment of 462 bp including gaps (an internal fragment of 45 bp was removed since no reliable
162 alignment could be achieved for this region).

163

164 **2.4 Phylogenetic inference and molecular clock analyses**

165 Prior to the phylogenetic analyses, our dataset of non-pyrgulinid Hydrobiidae was supplemented with
166 sequences of closely related species. We first performed searches with BLASTN 2.2.32 (Zhang et al.,
167 2000) against the NCBI nucleotide database as well as against the nucleotide database of the Wilke
168 lab, which contains DNA information for more than 300 hydrobiid species. A preliminary phylogenetic
169 analysis was conducted in order to identify the hydrobiid groups that are most closely related to our
170 endemic non-pyrgulinid Hydrobiidae. These preliminary analyses (details not shown) indicated that
171 endemic species from the sister Lake Prespa (Fig. 2) as well as from other Balkan waterbodies are the
172 closest relatives to the Ohrid taxa. These taxa were included in our final dataset (see Table 1).

173 The main phylogenetic analyses using Bayesian inference were performed in BEAST v.
174 1.8.0 (Drummond and Rambaut, 2007). Best-fit substitution models were estimated using jModelTest
175 0.1.1 (Posada, 2008) based on the Akaike information criterion. The models suggested for the COI
176 and 16S rRNA fragments were GTR+I+ Γ and GTR+I, respectively. We tested the COI dataset for
177 substitutional saturation using the test by Xia and Xie (2001) as implemented in DAMBE 5.0.23 (Xia
178 and Xie, 2001). The value for the proportion of invariant sites ($P_{inv} = 0.46$) was obtained from the
179 jModelTest output. The observed saturation was significantly lower than the critical values ($p < 0.001$),
180 suggesting that this partition can be used for further (molecular-clock) analyses. Note that we did not
181 test for saturation in the 16S dataset as, within the family Hydrobiidae, the 16S gene is more
182 conservative than the COI gene (Wilke et al., 2001, 2013).

183 We first ran two unconstrained analyses in BEAST, one under the strict-clock and one under
184 the relaxed-clock model using relative rates for both partitions. These initial runs, however, revealed
185 an extremely low effective sample size for the prior and posterior distributions suggesting that the runs
186 might have been over-parameterized due to the complex GTR+I+ Γ model (see e.g., Grummer et al.,
187 2014; Slager et al., 2014). Hence, the less complex HKY+I+ Γ and HKY+I models were used for the
188 final analyses for the COI and 16S rRNA partition, respectively.

189 For calibrating the molecular clock, an external trait-specific clock rate of $1.57 \pm 0.45\% \text{ Ma}^{-1}$
190 for the HKY+I+ Γ model was utilized for the COI portion of our dataset (Wilke et al., 2009). This rate
191 has been established for small, dioecious, subtropical or tropical Protostomia with a generation time of
192 approximately one year – all of these criteria apply to our non-pyrgulinid Hydrobiidae.

193 The final analyses (two strict-clock and two relaxed-clock runs) with a total of 85 sequences
194 were run for 100,000,000 generations each, sampling every 5,000 generations. The resulting log and
195 tree files for each strict-clock and relaxed-clock run were combined using LogCombiner v. 1.8.0
196 (BEAST) with a 50% burn-in. The maximum clade credibility (MCC) tree was identified based on the
197 posterior distribution (20,000 trees). Information from the post-burnin posterior distribution including
198 mean node ages and 95% highest posterior densities (HPD) was summarized using TreeAnnotator v.
199 1.8.0 (BEAST; no additional burn-in). A Bayes factor (BF) analysis of the likelihoods of both runs
200 (strict-clock vs. relaxed-clock model) was performed in Tracer 1.5 (Rambaut and Drummond, 2007);
201 1,000 bootstrap replicates) in order to determine the best-fitting clock model (see Newton and Raftery,
202 1994; Suchard et al., 2001).

203 The BF analysis, which compares the likelihoods of both BEAST runs (strict vs. relaxed clock
204 model), showed a decisive support for the relaxed-clock model with a BF of 45.61 ($\ln P_{\text{relaxed}} = -$
205 $5,213.40$ vs. $\ln P_{\text{strict}} = -5,318.42$).

206

207 **2.6 Lineage-through-time plot and diversification-rate analyses**

208 In a first explorative analysis, LTT plot analyses were conducted using the packages ape v. 3.3
209 (Paradis et al., 2004) and phytools v. 0.4-56 (Revell, 2012) for the R statistical environment 3.2.1 (R
210 Core Team, 2015) in order to examine whether major deviations from a constant diversification rate
211 can be directly observed over time. Thereby, all but the endemic non-pyrgulinid Hydrobiidae from Lake
212 Ohrid were pruned from the posterior distribution (20,000 trees) and the BEAST MCC tree. In a
213 second step, a LTT plot was generated for the MCC tree plus a 95% confidence interval based on the
214 posterior distribution.

215 However, generating LTT plots and detecting changes in the slope is an explorative
216 approach and might lead to misinterpretations (see Stadler, 2011). Therefore, potential shifts in
217 diversification rates over time were analyzed using the R package TreePar v. 3.3 (Stadler, 2015) by
218 testing a maximum of three shifts for ten trees randomly sampled from the posterior distribution. This
219 package implements a birth-death shift model (Stadler, 2011), which allows changes in speciation and
220 extinction rates along a phylogeny for a given timeframe and for pre-defined time intervals. Shifts were
221 analyzed along the pruned tree with default settings and a time interval of 0.1 Ma. The sampling
222 fraction was set to $17/27 = 0.63$ according to the actual number of species included divided by the

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225 number of nominal species described. Results (log likelihoods of different runs, i.e., constant
226 diversification rate vs. 1 shift, 1 shift vs. 2 shifts, and 2 shifts vs. 3 shifts) were compared by applying
227 likelihood ratio tests in order to examine whether shifts in rates explain the tree significantly better than
228 a constant diversification rate (indicated by p values > 0.95; see Stadler, 2011, 2015).

229

230 **3 Results**

231 **3.1 Phylogenetic inference and molecular clock analyses**

232 The relaxed-clock MCC tree (Fig. 3) shows that the endemic non-pyrgulinid Hydrobiidae from Lake
233 Ohrid likely form a monophyletic group (Bayesian posterior probability (BPP) = 0.87). Potential sister
234 to the Lake Ohrid group is a monophyletic group containing endemic non-pyrgulinid Hydrobiidae
235 species from its sister lakes, lakes Prespa and Mikri Prespa and their catchments. The split from a
236 MRCA for these two groups is supported by a BPP of 1.0. Closest relatives to the Ohrid/(Mikri) Prespa
237 group are other Balkan hydrobiids (BPP = 1.0) previously classified into the nominal subfamilies
238 Belgrandiellinae, Belgrandiinae, and Horatiinae (see Wilke et al., 2013).

239 The molecular-clock analyses indicates an age for the MRCA of the endemic non-pyrgulinid
240 Hydrobiidae from Lake Ohrid (i.e., the onset of diversification within this group) under the favored
241 relaxed-clock model of 1.75–3.76 Ma (95% HPD; see Fig. 3). Under the inferior strict-clock model, the
242 upper value is slightly lower with 1.75–2.68 Ma.

243

244 **3.2 Diversification-rate analysis**

245 The 95% LTT plot does not suggest major deviations from a constant diversification rate (Fig. 4A).
246 This finding is supported by the TreePar (birth-death shift) diversification-rate analysis. Accordingly,
247 the likelihood ratio tests suggest for eight out of ten random trees a constant rate over time. Only in
248 two of the random trees a single shift at an age of 0.1 Ma is detected (Fig. 4B and Table 2).

249

250 **4 Discussion**

251 **4.1 The Ohrid non-pyrgulinid Hydrobiidae: an old species flock**

252 Our phylogenetic analyses indicate that the non-pyrgulinid Hydrobiidae form a potential monophyletic,
253 speciose and endemic clade, and thus, by definition, represent a species flock (Greenwood et al.,

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256 1984; Schön and Martens, 2004). Therefore, *in situ* diversification appears to be a main process in this
257 group. This conclusion even holds under the assumption of a non-monophyly of the Ohrid taxa.

258 The age estimates obtained from the molecular-clock analyses revealed that the Lake Ohrid
259 flock potentially started to diversify before extant Lake Ohrid came into existence (i.e., in a pre-
260 lake/palaeo-lake phase or in other waterbodies of the Ohrid Graben system such as rivers or springs;
261 see Fig. 3 and Fig. 4A). In fact, karst springs have previously been proposed as potential ancestral
262 habitats for other invertebrate flocks inhabiting Lake Ohrid such as the pulmonate snail genus *Ancylus*
263 (Albrecht et al., 2006), leeches of the genus *Dina* (Trajanovski et al., 2010), and the isopod genus
264 *Proasellus* (Kilikowska et al., 2013). This may also be the case for the species flock studied here,
265 given that karst springs are the dominant habitat of its closest relatives outside lakes Ohrid and
266 Prespa. However, testing this hypothesis is beyond the scope of the current study and probably would
267 require a denser sampling, more precise limnological information about the early stage of Lake Ohrid,
268 and more specific approaches such as the reconstruction of ancestral waterbodies as well as better
269 calibration points for molecular clock analyses.

270

271 **4.2 The Ohrid non-pyrgulinid Hydrobiidae: constant rate of diversification over time**

272 As mentioned in the Introduction, at least four modes of tempo of speciation in ancient lake species
273 flocks are conceivable. Diversification rates may 1) be constant over time, 2) fluctuate ('punctuated
274 equilibrium'), 3) be higher in the initial phase of diversification, or 4) show a pronounced lag phase
275 between colonization and subsequent diversification.

276 As understanding the temporal frame of speciation in Lake Ohrid is of considerable
277 importance for reaching one of the main goals of the Ohrid scientific deep-drilling program – inferring
278 the driving forces for biotic evolution – the second specific goal of the current study aims at estimating
279 diversification rates over time. Given that Lake Ohrid has long been regarded as a relatively stable
280 system (Stankovic, 1960), our working hypothesis assumes no significant changes in diversification
281 rates over time in the lake's non-pyrgulinid Hydrobiidae.

282 Interestingly, our LTT plot suggests that the species flock most likely evolved with a constant
283 diversification rate. This finding is supported by our diversification-rate analysis, indicating a single
284 recent shift (drop of rates) at 0.1 Ma for two out of ten random trees (i.e., a timeframe in which
285 incomplete lineage sorting may play a role; Fig. 4B and Table 2). Therefore, our initial working

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287 hypothesis – a constant diversification rate – cannot be rejected. However, we cannot rule out a type II
288 error here due to poorly resolved phylogenetic trees or insufficient sampling size. As for the quality of
289 the phylogenetic tree used for the LTT plot and the subsequent diversification-rate analyses, our
290 analyses, indeed, revealed some poorly supported (especially recent) nodes (Fig. 3). However,
291 timetree studies are relatively robust against phylogenetic uncertainties (see e.g., Morvan et al., 2013)
292 as a poorly supported topology does not necessarily affect the timing of rapid speciation events (e.g.,
293 Pagel, 1999). We are therefore confident that the low BPP support of some nodes in our tree had no
294 significant influence on the outcome of our hypothesis testing.

295 Moreover, we also think that our conclusions are not affected by a non-monophyly of the
296 Ohrid group (see random tree #2) as the split between Prespa and Ohrid taxa is, in any event, older
297 than 2 Ma and thus does not affect intra-lacustrine diversification rates within the Lake Ohrid clade.

298 As for the sampling size (i.e., 17 out of 27 nominal species studied), the high diversity of
299 evolutionary lineages found in our phylogenetic analyses indicates that our sampling design likely
300 recovered most major evolutionary lineages within this group. Our sampling includes the majority of
301 genera described except for *Dolapia* (which some authors included in the genus *Ohrigocea*) and
302 *Zaumia*. We also included a variety of specimens collected at various types of habitats and type
303 localities across the lake and its surroundings (see Fig. 2). Moreover, the diversification-rate analysis
304 does account for incomplete sampling and we did infer a single rate shift in two of the random trees
305 tested in the present study. Thus the method used seems to have enough power to detect deviations
306 from a constant diversification rate in our data set.

307

308 **4.3 Ecosystem resilience of Lake Ohrid**

309 If we assume that the rate of diversification in the non-pyrgulinid Hydrobiidae from Lake Ohrid is
310 constant, linking environmental/climatic fluctuations to changes in tempo of diversification becomes
311 impossible (see specific goal iii).

312 However, an important question is whether a non-constant diversification rate could not be
313 demonstrated because Lake Ohrid never experienced massive environmental and/or climatic changes
314 or whether the lake has a high ecosystem resilience that might buffer such perturbations. Preliminary
315 data of the SCOPSCO deep-drilling program based on core catcher data for the last 1.2 Ma and high-
316 resolution data for the last 640 thousand years (ka) so far do not indicate the occurrence of

317 catastrophic environmental events (Francke et al., 2015; Wagner et al., 2014), i.e., events that lead to
318 sudden drastic regime shifts (*sensu* Scheffer and Carpenter, 2003; Scheffer et al., 2001) and thus
319 potentially to mass extinction. By comparison, such events have been observed in other ancient lakes
320 including Lake Titicaca (Kroll et al., 2012; Lavenu, 1992) and Lake Malawi (Cohen et al., 2007; Scholz
321 et al., 2007; Schultheiß et al., 2009, 2011). We do, however, see signatures of severe
322 environmental/climatic perturbations in Lake Ohrid including significant lake-level drops (Lindhorst et
323 al., 2010), volcanic ash deposits (Sulpizio et al., 2010; Wagner et al., 2014), and glacial/interglacial
324 cycles (Lézine et al., 2010; Reed et al., 2010; Wagner et al., 2014).

325 Therefore, we think that the lack of changes in tempo of diversification of Lake Ohrid's non-
326 pyrgulinid Hydrobiidae might be potentially a result of two factors: i) either Lake Ohrid never
327 experienced catastrophic environmental events that resulted in the extinction of all or most of its
328 endemic taxa and thus caused a 'reset' of diversification processes; or ii) Lake Ohrid possibly has a
329 high ecosystem resilience that buffers environmental changes and potentially mitigates extinction
330 events. Note that the two factors might not be mutually exclusive as it has been shown that a loss of
331 resilience may set the scene for a catastrophic event-induced switch to an alternative state (reviewed
332 in Scheffer et al., 2001).

333 The second scenario is supported by the mollusk and diatom fossil records for the past ~100
334 ka (Albrecht et al., 2010) and (Cvetkovska et al., 2015; Jovanovska et al., 2015), respectively. For
335 instance, the Campanian Ignimbrite Y5 tephra influx 39.6 ka ago (see Leicher et al., 2015) altered the
336 water chemistry of Lake Ohrid and increased the content of silica, which in turn, amplified diatom
337 growth rates. However, it did not cause severe changes in diatom community structures or even
338 extinction events (Jovanovska et al., 2015).

339 The suggested high ecosystem resilience of Lake Ohrid might be sustained by several
340 factors including the lake's bathymetry (deep lake with steep flanks, allowing habitats to move
341 vertically with lake-level changes; Lindhorst et al., 2010), ongoing moderate tectonic activities
342 (compensating sedimentation; Hinderer and Einsele, 2001), and its peculiar limnology (the lake is fed
343 by numerous karstic sublacustrine springs, locally buffering environmental changes; Matzinger et al.,
344 2006).

345 Although we think that the patterns inferred from mtDNA sequencing data of extant taxa are
346 highly informative, future analyses utilizing additional (nuclear) markers may help better resolving

347 some of the basal relationships of Ohrid taxa. Moreover, the temporal resolution is still limited, the
348 error rate for time estimates is relatively high, and some important processes such as extinction
349 events are difficult to infer from extant organisms (see also Rabosky, 2010 for a discussion).
350 Therefore, we encourage future palaeontological studies on endemic species using the sediment
351 cores gained during the SCOPSCO deep-drilling campaign. This concerns, for example, diatoms and
352 ostracods. Moreover, besides the main core 'DEEP', obtained from the deepest part of Lake Ohrid,
353 several other cores were retrieved in shallower parts of Lake Ohrid. The latter appears to be relatively
354 rich in mollusk fossils (see Wagner et al., 2014), which could potentially be used to directly study
355 extinction and speciation events (for a proof of principle see Albrecht et al., 2010).

356

357 **5 Conclusions**

358 Our molecular-clock analyses indicate that the non-pyrgulinid Hydrobiidae of ancient Lake Ohrid
359 represent an old endemic group, which is characterized by a constant rate of diversification. We
360 propose that this constant rate has been caused by two factors: i) a possible lack of catastrophic
361 environmental events in Lake Ohrid and/or ii) a high ecosystem resilience, buffering environmental
362 changes. Parameters potentially contributing to Lake Ohrid's ecosystem resilience are its distinct
363 bathymetry, ongoing tectonic activities, and karst hydrology. These findings are not only of interest for
364 one of the overall goals of the SCOPSCO deep-drilling program – inferring the driving forces for biotic
365 evolution in Lake Ohrid. They might also enhance our understanding of how ecosystem resilience, in
366 general, may promote a relative constant diversification in highly isolated ecosystems.

367 However, high-resolution sedimentological data are currently only available for the last 640
368 ka. Therefore, we encourage future studies specifically testing hypotheses about the lack of
369 catastrophic events in Lake Ohrid based on high-resolution data for the entire geological history of the
370 lake, and potentially involving information from the sediment fossil record not only for gastropods but
371 also for other groups with a high share of endemic taxa.

372

373 **Author contribution**

374 T.W. and C.A. conceived the study. K.F. and T.H. collected and identified specimens. K.F. performed
375 lab work. K.F., and B.S. performed the analyses. The manuscript was written by B.S., T.W., and K.F.,
376 with contributions from all co-authors. All authors gave final approval for publication.

377

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389 **References**

- 390 Albrecht, C. and Wilke, T.: Ancient Lake Ohrid: biodiversity and evolution, *Hydrobiologia*, 615, 103–
391 140, doi:10.1007/s10750-008-9558-y, 2008.
- 392 Albrecht, C., Trajanovski, S., Kuhn, K., Streit, B. and Wilke, T.: Rapid evolution of an ancient lake
393 species flock: freshwater limpets (Gastropoda: Ancyliidae) in the Balkan Lake Ohrid, *Org. Divers.*
394 *Evol.*, 6, 294–307, doi:10.1016/j.ode.2005.12.003, 2006.
- 395 Albrecht, C., Hauffe, T., Schreiber, K., Trajanovski, S. and Wilke, T.: Mollusc biodiversity and
396 endemism in the potential ancient Lake Trichonis, Greece, *Malacologia*, 51, 357–375,
397 doi:10.4002/040.051.0209, 2009.
- 398 Albrecht, C., Vogel, H., Hauffe, T. and Wilke, T.: Sediment core fossils in ancient Lake Ohrid: testing
399 for faunal change since the Last Interglacial, *Biogeosciences*, 7, 3435–3446, doi:10.5194/bg-7-3435-
400 2010, 2010.
- 401 Albrecht, C., Föller, K., Clewing, C., Hauffe, T. and Wilke, T.: Invaders versus endemics: alien
402 gastropod species in ancient Lake Ohrid, *Hydrobiologia*, 739, 163–174, doi:10.1007/s10750-013-
403 1724-1, 2014.
- 404 Brooks, J. L.: Speciation in ancient lakes, *Q. Rev. Biol.*, 25, 30–60, 131–176, 1950.
- 405 Cohen, A. S., Stone, J. R., Beuning, K. R. M., Park, L. E., Reinthal, P. N., Dettman, D., Scholz, C. A.,
406 Johnson, T. C., King, J. W., Talbot, M. R., Brown, E. T. and Ivory, S. J.: Ecological consequences of
407 early Late Pleistocene megadroughts in tropical Africa, *Proc. Natl. Acad. Sci. USA*, 104, 16422–16427,
408 2007.
- 409 Cristescu, M. E., Adamowicz, S. J., Vaillant, J. J. and Haffner, G. D.: Ancient lakes revisited: from the
410 ecology to the genetics of speciation, *Mol. Ecol.*, 19, 4837–4851, doi:10.1111/j.1365-
411 294X.2010.04832.x, 2010.
- 412 Cvetkoska, A., Jovanovska, E., Francke, A., Tofilovska, S., Vogel, H., Levkov, Z., Donders, T. H.,
413 Wagner, B. and Wagner-Cremer, F.: Ecosystem regimes and responses in a coupled ancient lake
414 system from MIS 5b to present: the diatom record of lakes Ohrid and Prespa, *Biogeosciences*
415 *Discuss.*, 12, 15051–15086, doi:10.5194/bgd-12-15051-2015, 2015.
- 416 Drummond, A. J. and Rambaut, A.: BEAST: Bayesian evolutionary analysis by sampling trees, *BMC*
417 *Evol. Biol.*, 7, 214, doi:10.1186/1471-2148-7-214, 2007.
- 418 Falniowski, A. and Szarowska, M.: The genus *Daphniola* Radoman, 1973 (Caenogastropoda:
419 Hydrobiidae) in the Peloponnese, Greece, *Folia Malacol.*, 19, 131–137, doi:10.2478/v10125-011-
420 0020-9, 2011.
- 421 Falniowski, A., Szarowska, M., Glöer, P. and Pešić, V.: Molecules vs morphology in the taxonomy of
422 the *Radomaniola/Grossuana* group of Balkan Rissosoidea (Mollusca, Caenogastropoda), *J. Conchol.*,
423 41, 19–36, 2012.
- 424 Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R.: DNA primers for amplification of
425 mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates, *Mol. Mar. Biol.*
426 *Biotechnol.*, 3, 294–299, 1994.
- 427 Francke, A., Wagner, B., Just, J., Leicher, N., Gromig, R., Baumgarten, H., Vogel, H., Lacey, J. H.,
428 Sadori, L., Wonik, T., Leng, M. J., Zanchetta, G., Sulpizio, R. and Giaccio, B.: Sedimentological
429 processes and environmental variability at Lake Ohrid (Macedonia, Albania) between 640 ka and
430 present day, *Biogeosciences Discuss.*, 12, 15111–15156, doi:10.5194/bgd-12-15111-2015, 2015.

- 431 Gould, S. J. and Eldredge, N.: Punctuated equilibria: the tempo and mode of evolution reconsidered,
432 *Paleobiology*, 3, 115–151, 1977.
- 433 Greenwood, P. H., Echelle, A. A. and Kornfield, I.: What is a species flock?, in *Evolution of fish*
434 *species flocks*, edited by A. A. Echelle and I. Kornfield, pp. 13–19, Orono Press, University of Maine,
435 1984.
- 436 Grummer, J. A., Bryson Jr., R. W. and Reeder, T. W.: Species delimitation using Bayes factors:
437 simulations and application to the *Sceloporus scalaris* species group (Squamata: Phrynosomatidae),
438 *Syst. Biol.*, 63, 119–133, doi:10.1093/sysbio/syt069, 2014.
- 439 Hall, T. A.: BioEdit: a user-friendly biological sequence alignment editor and analysis program for
440 Windows 95/98/NT, *Nucleic Acids Symp. Ser.*, 41, 95–98, 1999.
- 441 Hauffe, T., Albrecht, C., Schreiber, K., Birkhofer, K., Trajanovski, S. and Wilke, T.: Spatially explicit
442 analysis of gastropod biodiversity in ancient Lake Ohrid, *Biogeosciences*, 8, 175–188, doi:10.5194/bg-
443 8-175-2011, 2011.
- 444 Hauswald, A. K., Albrecht, C. and Wilke, T.: Testing two contrasting evolutionary patterns in ancient
445 lakes: species flock versus species scatter in valvate gastropods of Lake Ohrid, *Hydrobiologia*, 615,
446 169–179, 2008.
- 447 Hinderer, M. and Einsele, G.: The world's large lake basins as denudation-accumulation systems and
448 implications for their lifetimes, *J. Paleolimnol.*, 26, 355–372, doi:10.1023/A:1012651232541, 2001.
- 449 Ivanov, A. V., Gladkochub, D. P., Déverchère, J. and Ernst, R. E.: Introduction to special issue:
450 geology of the Lake Baikal region, *J. Asian Earth Sci.*, 62, 1–3, doi:10.1016/j.jseaes.2012.12.010,
451 2013.
- 452 Jovanovska, E., Cvetkoska, A., Hauffe, T., Levkov, Z., Wagner, B., Sulpizio, R., Francke, A., Albrecht,
453 C. and Wilke, T.: Differential resilience of ancient sister lakes Ohrid and Prespa to environmental
454 disturbances during the Late Pleistocene, *Biogeosciences Discuss.*, 12, 16049–16079,
455 doi:10.5194/bgd-12-16049-2015, 2015.
- 456 Kilikowska, A., Wysocka, A., Burzyński, A., Kostoski, G., Rychlińska, J. and Sell, J.: Patterns of
457 genetic differentiation and population history of endemic isopods (Asellidae) from ancient Lake Ohrid:
458 combining allozyme and mtDNA data, *Cent. Eur. J. Biol.*, 8, 854–875, doi:10.2478/s11535-013-0204-
459 y, 2013.
- 460 Kroll, O., Hershler, R., Albrecht, C., Terrazas, E. M., Apaza, R., Fuentealba, C., Wolff, C. and Wilke,
461 T.: The endemic gastropod fauna of Lake Titicaca: correlation between molecular evolution and
462 hydrographic history, *Ecol. Evol.*, 2, 1517–1530, doi:10.1002/ece3.280, 2012.
- 463 Lavenu, A.: Formation and geological evolution, in *Lake Titicaca: a synthesis of limnological*
464 *knowledge*, edited by C. Dejoux and A. Ittis, pp. 3–15, Kluwer Academic Publishers, Dordrecht, 1992.
- 465 Leicher, N., Zanchetta, G., Sulpizio, R., Francke, A. and Carlo, P. Del: First tephrostratigraphic results
466 of the DEEP site record from Lake Ohrid, Macedonia, *Biogeosciences Discuss.*, 12, 15411–15460,
467 doi:10.5194/bgd-12-15411-2015, 2015.
- 468 Levkov, Z. and Williams, D. M.: Checklist of diatoms (Bacillariophyta) from Lake Ohrid and Lake
469 Prespa (Macedonia), and their watersheds, *Phytotaxa*, 45, 1–76, 2012.
- 470 Lézine, A. M., von Grafenstein, U., Andersen, N., Belmecheri, S., Bordon, A., Caron, B., Cazet, J. P.,
471 Erlenkeuser, H., Fouache, E., Grenier, C., Huntsman-Mapila, P., Hureau-Mazaudier, D., Manelli, D.,
472 Mazaud, A., Robert, C., Sulpizio, R., Tiercelin, J. J., Zanchetta, G. and Zeqollari, Z.: Lake Ohrid,

- 473 Albania, provides an exceptional multi-proxy record of environmental changes during the last glacial-
474 interglacial cycle, *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 287, 116–127,
475 doi:10.1016/j.palaeo.2010.01.016, 2010.
- 476 Lindhorst, K., Vogel, H., Krastel, S., Wagner, B., Hilgers, A., Zander, A., Schwenk, T., Wessels, M.
477 and Daut, G.: Stratigraphic analysis of lake level fluctuations in Lake Ohrid: an integration of high
478 resolution hydro-acoustic data and sediment cores, *Biogeosciences*, 7, 3531–3548, doi:10.5194/bg-7-
479 3531-2010, 2010.
- 480 Lindhorst, K., Krastel, S., Reicherter, K., Stipp, M., Wagner, B. and Schwenk, T.: Sedimentary and
481 tectonic evolution of Lake Ohrid (Macedonia/Albania), *Basin Res.*, 27, 84–101, doi:10.1111/bre.12063,
482 2015.
- 483 Martens, K.: Speciation in ancient lakes, *Trends Ecol. Evol.*, 12, 177–182, doi:10.1016/S0169-
484 5347(97)01039-2, 1997.
- 485 Martens, K., Goddeeris, B. and Coulter, G.: Speciation in ancient lakes, edited by K. Martens, B.
486 Goddeeris, and G. Coulter, E. Schweitzerbart'sche Verlagsbuchhandlung, Stuttgart., 1994.
- 487 Matzinger, A., Spirkovski, Z., Patceva, S. and Wüest, A.: Sensitivity of ancient Lake Ohrid to local
488 anthropogenic impacts and global warming, *J. Great Lakes Res.*, 32, 158–179, doi:10.3394/0380-
489 1330(2006)32[158:SOALOT]2.0.CO;2, 2006.
- 490 Morvan, C., Malard, F., Paradis, E., Lefébure, T., Konecny-Dupré, L. and Douady, C. J.: Timetree of
491 *Aselloidea* reveals species diversification dynamics in groundwater, *Syst. Biol.*, 62, 512–522,
492 doi:10.1093/sysbio/syt015, 2013.
- 493 Newton, M. A. and Raftery, A. E.: Approximate Bayesian inference with the weighted likelihood
494 bootstrap, *J. R. Stat. Soc. B*, 56, 3–48, 1994.
- 495 Pagel, M.: Inferring the historical patterns of biological evolution, *Nature*, 401, 877–884, 1999.
- 496 Palumbi, S. R., Martin, A., Romano, S., McMillan, W. O., Stice, L. and Grabowski, G.: The simple
497 fool's guide to PCR, University of Hawaii, Honolulu, 1991.
- 498 Paradis, E., Claude, J. and Strimmer, K.: APE: analyses of phylogenetics and evolution in R language,
499 *Bioinformatics*, 20, 289–290, doi:10.1093/bioinformatics/btg412, 2004.
- 500 Pennell, M. W., Harmon, L. J. and Uyeda, J. C.: Is there room for punctuated equilibrium in
501 macroevolution?, *Trends Ecol. Evol.*, 29, 23–32, doi:10.1016/j.tree.2013.07.004, 2014.
- 502 Pešić, V.: A new species of the water mite genus *Hygrobates* Koch, 1837 (Acari: Hydrachnidia:
503 Hygrobatidae) from the ancient Lake Ohrid, *Zootaxa*, 3926, 287–295, doi:10.11646/zootaxa.3926.2.9,
504 2015.
- 505 Posada, D.: jModelTest: phylogenetic model averaging, *Mol. Biol. Evol.*, 25, 1253–1256,
506 doi:10.1093/molbev/msn083, 2008.
- 507 Purvis, A., Orme, C. D. L., Toomey, N. H. and Pearson, P. N.: Temporal patterns in diversification
508 rates, in *Speciation and patterns of diversity*, edited by R. K. Butlin, J. R. Bridle, and D. Schuller, pp.
509 278–300, Cambridge University Press, Cambridge, 2009.
- 510 R Core Team: R: a language and environment for statistical computing. R Foundation for Statistical
511 Computing, Vienna, Austria. URL <http://www.R-project.org>, 2015

- 512 Rabosky, D. L.: Extinction rates should not be estimated from molecular phylogenies, *Evolution*, 64,
513 1816–1824, doi:10.1111/j.1558-5646.2009.00926.x, 2010.
- 514 Radoman, P.: Hydrobioidea, a superfamily of Prosobranchia (Gastropoda). I. Systematics,
515 Monographs Vol. 547, Department of Sciences No. 57, Serbian Academy of Sciences and Arts,
516 Beograd, 1983.
- 517 Radoman, P.: Hydrobioidea, a superfamily of Prosobranchia (Gastropoda). II. Origin, zoogeography,
518 evolution in the Balkans and Asia Minor, Monographs Vol. 1, Institute of Zoology No. 1, Faculty of
519 Science - Department of Biology, Beograd, 1985.
- 520 Rambaut, A. and Drummond, A. J.: Tracer v. 1.5, 2007. Available at <http://tree.bio.ed.ac.uk/software>.
- 521 Reed, J. M., Cvetkoska, A., Levkov, Z., Vogel, H. and Wagner, B.: The last glacial-interglacial cycle in
522 Lake Ohrid (Macedonia/Albania): testing diatom response to climate, *Biogeosciences*, 7, 3083–3094,
523 doi:10.5194/bg-7-3083-2010, 2010.
- 524 Revell, L. J.: phytools: an R package for phylogenetic comparative biology (and other things), *Methods*
525 *Ecol. Evol.*, 3, 217–223, doi:10.1111/j.2041-210X.2011.00169.x, 2012.
- 526 Rossiter, A. and Kawanabe, H.: Ancient lakes: biodiversity, ecology and evolution, Academic Press,
527 San Diego, 2000.
- 528 Salzburger, W., Mack, T., Verheyen, E. and Meyer, A.: Out of Tanganyika: genesis, explosive
529 speciation, key-innovations and phylogeography of the haplochromine cichlid fishes, *BMC Evol. Biol.*,
530 5, 17, doi:10.1186/1471-2148-5-17, 2005.
- 531 Salzburger, W., Van Bocxlaer, B. and Cohen, A. S.: Ecology and evolution of the African Great Lakes
532 and their faunas, *Annu. Rev. Ecol. Evol. Syst.*, 45, 519–545, doi:10.1146/annurev-ecolsys-120213-
533 091804, 2014.
- 534 Scheffer, M. and Carpenter, S. R.: Catastrophic regime shifts in ecosystems: linking theory to
535 observation, *Trends Ecol. Evol.*, 18, 648–656, doi:10.1016/j.tree.2003.09.002, 2003.
- 536 Scheffer, M., Carpenter, S., Foley, J. A., Folke, C. and Walker, B.: Catastrophic shifts in ecosystems,
537 *Nature*, 413, 591–596, doi:10.1038/35098000, 2001.
- 538 Schluter, D.: The ecology of adaptive radiation, edited by R. M. May and P. H. Harvey, Oxford
539 University Press, Oxford, 2000.
- 540 Scholz, C. A., Johnson, T. C., Cohen, A. S., King, J. W., Peck, J. A., Overpeck, J. T., Talbot, M. R.,
541 Brown, E. T., Kalindekaffe, L., Amoako, P. Y. O., Lyons, R. P., Shanahan, T. M., Castañeda, I. S., Heil,
542 C. W., Forman, S. L., McHargue, L. R., Beuning, K. R., Gomez, J. and Pierson, J.: East African
543 megadroughts between 135 and 75 thousand years ago and bearing on early-modern human origins,
544 *Proc. Natl. Acad. Sci. USA*, 104, 16416–21, doi:10.1073/pnas.0703874104, 2007.
- 545 Schön, I. and Martens, K.: Adaptive, pre-adaptive and non-adaptive components of radiations in
546 ancient lakes: a review, *Org. Divers. Evol.*, 4, 137–156, doi:10.1016/j.ode.2004.03.001, 2004.
- 547 Schreiber, K., Hauße, T., Albrecht, C. and Wilke, T.: The role of barriers and gradients in differentiation
548 processes of pyrgulinid microgastropods of Lake Ohrid, *Hydrobiologia*, 682, 61–73,
549 doi:10.1007/s10750-011-0864-4, 2012.
- 550 Schultheiß, R., Van Bocxlaer, B., Wilke, T. and Albrecht, C.: Old fossils–young species: evolutionary
551 history of an endemic gastropod assemblage in Lake Malawi, *Proc. R. Soc. London B*, 276, 2837–
552 2846, doi:10.1098/rspb.2009.0467, 2009.

- 553 Schultheiß, R., Wilke, T., Jørgensen, A. and Albrecht, C.: The birth of an endemic species flock:
554 demographic history of the *Bellamya* group (Gastropoda, Viviparidae) in Lake Malawi, *Biol. J. Linn.*
555 *Soc.*, 102, 130–143, 2011.
- 556 Sherbakov, D. Y.: Molecular phylogenetic studies on the origin of biodiversity in Lake Baikal, *Trends*
557 *Ecol. Evol.*, 14, 92–95, doi:10.1016/S0169-5347(98)01543-2, 1999.
- 558 Slager, D. L., Battey, C. J., Bryson, R. W., Voelker, G. and Klicka, J.: A multilocus phylogeny of a
559 major New World avian radiation: the Vireonidae, *Mol. Phylogenet. Evol.*, 80, 95–104,
560 doi:10.1016/j.ympev.2014.07.021, 2014.
- 561 Stadler, T.: Mammalian phylogeny reveals recent diversification rate shifts, *Proc. Natl. Acad. Sci. USA*,
562 108, 6187–6192, 2011.
- 563 Stadler, T.: TreePar: Estimating birth and death rates based on phylogenies. R package version 3.3.
564 <http://CRAN.R-project.org/package=TreePar>, 2015.
- 565 Stankovic, S.: The Balkan Lake Ohrid and its living world, Dr. W. Junk, The Hague, 1960.
- 566 Stocchino, G. A., Sluys, R., Deri, P. and Manconi, R.: Integrative taxonomy of a new species of
567 planarian from the Lake Ohrid basin, including an analysis of biogeographical patterns in freshwater
568 triclads from the Ohrid region (Platyhelminthes, Tricladida, Dugesiidae), *Zookeys*, 313, 25–43,
569 doi:10.3897/zookeys.313.5363, 2013.
- 570 Suchard, M. A., Weiss, R. E. and Sinsheimer, J. S.: Bayesian selection of continuous-time Markov
571 chain evolutionary models, *Mol. Biol. Evol.*, 18, 1001–1013,
572 doi:10.1093/oxfordjournals.molbev.a003872, 2001.
- 573 Sulpizio, R., Zanchetta, G., D’Orazio, M., Vogel, H. and Wagner, B.: Tephrostratigraphy and
574 tephrochronology of lakes Ohrid and Prespa, Balkans, *Biogeosciences*, 7, 3273–3288,
575 doi:10.5194/bg-7-3273-2010, 2010.
- 576 Szarowska, M.: Molecular phylogeny, systematics and morphological character evolution in the Balkan
577 Risssooidea (Caenogastropoda), *Folia Malacol.*, 14, 99–168, doi:10.12657/folmal.014.014, 2006.
- 578 Szarowska, M., Grzmil, P., Falniowski, A. and Sirbu, I.: *Grossuana codreanui* (Grossu, 1946) and the
579 phylogenetic relationships of the East Balkan genus *Grossuana* (Radoman, 1973) (Gastropoda:
580 Risssooidea), *Hydrobiologia*, 579, 379–391, doi:10.1007/s10750-006-0530-4, 2007.
- 581 Trajanovski, S., Albrecht, C., Schreiber, K., Schultheiß, R., Stadler, T., Benke, M. and Wilke, T.:
582 Testing the spatial and temporal framework of speciation in an ancient lake species flock: the leech
583 genus *Dina* (Hirudinea: Erpobdellidae) in Lake Ohrid, *Biogeosciences*, 7, 3387–3402, doi:10.5194/bg-
584 7-3387-2010, 2010.
- 585 Van Bocxlaer, B., Damme, D. V and Feibel, C. S.: Gradual versus punctuated equilibrium evolution in
586 the Turkana Basin molluscs: evolutionary events or biological invasions?, *Evolution*, 62, 511–520, doi:
587 10.1111/j.1558-5646.2007.00296.x, 2008.
- 588 Wagner, B., Wilke, T., Krastel, S., Zanchetta, G., Sulpizio, R., Reicherter, K., Leng, M. J., Grazhdani,
589 A., Trajanovski, S., Francke, A., Lindhorst, K., Levkov, Z., Cvetkoska, A., Reed, J. M., Zhang, X.,
590 Lacey, J. H., Wonik, T., Baumgarten, H. and Vogel, H.: The SCOPSCO drilling project recovers more
591 than 1.2 million years of history from Lake Ohrid, *Sci. Drill.*, 17, 19–29, doi:10.5194/sd-17-19-2014,
592 2014.
- 593 Wilke, T. and Davis, G. M.: Intraspecific mitochondrial sequence diversity in *Hydrobia ulvae* and
594 *Hydrobia ventrosa* (Hydrobiidae: Risssooidea: Gastropoda): Do their different life histories affect

- 595 biogeographic patterns and gene flow?, *Biol. J. Linn. Soc.*, 70, 89–105, doi:10.1006/bijl.1999.0388,
596 2000.
- 597 Wilke, T., Davis, G. M., Falniowski, A., Giusti, F., Bodon, M. and Szarowska, M.: Molecular
598 systematics of Hydrobiidae (Mollusca: Gastropoda: Rissooidea): testing monophyly and phylogenetic
599 relationships, *Proc. Acad. Nat. Sci. Philadelphia*, 151, 1–21, doi:10.1635/0097-
600 3157(2001)151[0001:MSOHMG]2.0.CO;2, 2001.
- 601 Wilke, T., Davis, G. M., Qiu, D. and Spear, R. C.: Extreme mitochondrial sequence diversity in the
602 intermediate schistosomiasis host *Oncomelania hupensis robertsoni*: another case of ancestral
603 polymorphism?, *Malacologia*, 48, 143–157, 2006.
- 604 Wilke, T., Albrecht, C., Anistratenko, V. V., Sahin, S. K. and Yildirim, Z.: Testing biogeographical
605 hypotheses in space and time: faunal relationships of the putative ancient Lake Egirdir in Asia Minor,
606 *J. Biogeogr.*, 34, 1807–1821, doi:10.1111/j.1365-2699.2007.01727.x, 2007.
- 607 Wilke, T., Schultheiß, R. and Albrecht, C.: As time goes by: a simple fool's guide to molecular clock
608 approaches in invertebrates, *Am. Malacol. Bull.*, 27, 25–45, doi:10.4003/006.027.0203, 2009.
- 609 Wilke, T., Haase, M., Hershler, R., Liu, H.-P., Misof, B. and Ponder, W.: Pushing short DNA fragments
610 to the limit: phylogenetic relationships of “hydrobioid” gastropods (Caenogastropoda: Rissooidea),
611 *Mol. Phylogenet. Evol.*, 66, 715–736, doi:10.1016/j.ympev.2012.10.025, 2013.
- 612 Wilson, A. B., Glaubrecht, M. and Meyer, A.: Ancient lakes as evolutionary reservoirs: evidence from
613 the thalassoid gastropods of Lake Tanganyika, *Proc. R. Soc. London B*, 271, 529–536,
614 doi:10.1098/rspb.2003.2624, 2004.
- 615 Wysocka, A., Kostoski, G., Kilikowska, A., Wróbel, B. and Sell, J.: The *Proasellus* (Crustacea,
616 Isopoda) species group, endemic to the Balkan Lake Ohrid: a case of ecological diversification?,
617 *Fundam. Appl. Limnol. / Arch. für Hydrobiol.*, 172, 301–313, doi:10.1127/1863-9135/2008/0172-0301,
618 2008.
- 619 Wysocka, A., Grabowski, M., Sworobowicz, L., Burzyński, A., Kilikowska, A., Kostoski, G. and Sell, J.:
620 A tale of time and depth: intralacustrine radiation in endemic *Gammarus* species flock from the ancient
621 Lake Ohrid, *Zool. J. Linn. Soc.*, 167, 345–359, doi:10.1111/j.1096-3642.2012.00878.x, 2013.
- 622 Wysocka, A., Grabowski, M., Sworobowicz, L., Mamos, T., Burzyński, A. and Sell, J.: Origin of the
623 Lake Ohrid gammarid species flock: ancient local phylogenetic lineage diversification, *J. Biogeogr.*, 41,
624 1758–1768, doi:10.1111/jbi.12335, 2014.
- 625 Xia, X. and Xie, Z.: DAMBE: Data analysis in molecular biology and evolution, *J. Hered.*, 92, 371–373,
626 doi:10.1093/jhered/92.4.371, 2001.
- 627 Zhang, Z., Schwartz, S., Wagner, L. and Miller, W.: A greedy algorithm for aligning DNA sequences, *J.*
628 *Comput. Biol.*, 7, 203–214, doi:10.1089/10665270050081478, 2000.

Table 1. Taxa studied including locality information, collection and DNA voucher details, and NCBI GenBank accession numbers.

Genus	Species	Locality	Latitude	Longitude	UGSB voucher no.	DNA voucher no.	GenBank accession no. COI	LSU rRNA	Reference
<i>Albaniana</i>	<i>albanica</i>	Albania, Ventrroku, Cave spring	40.67295	20.97403	UGSB 10730	12073	KU170805	KU170882	This study
<i>Belgrandia</i>	<i>maritimesia</i>	Italy, Fonte di S. Cassiano	-	-	UGSB 17130	2332	KU170806	-	This study
<i>Daphniola</i>	<i>exigua</i>	Greece, large spring at Agia Paraskevi, Tembi Valley, N of Larisa	-	-	UGSB 17130	-	JF916470	-	Fainowski and Szarowska (2011)
	<i>graeca</i>	Greece, Dafne Spring	39.891083	22.607222	UGSB 17132	4238	KU170807	-	This study
	<i>lousi</i>	Greece, Athens, spring at Kessariani	37.960729	23.798555	UGSB 17133	4239	KU170808	-	This study
<i>Gocea</i>	<i>ohridana 1</i>	Macedonia, Lake Ohrid, lake bank at Veli Dab	40.97324	20.78668	UGSB 10710	10493	KU170809	-	This study
	<i>ohridana 2</i>	Macedonia, Lake Ohrid, lake bank at Veli Dab	40.97324	20.78668	UGSB 10709	10332	KU170810	KU170883	This study
	<i>ohridana 3</i>	Macedonia, Lake Ohrid, Veli Dab	40.97097	20.78604	UGSB 10681	4299	KU170811	-	This study
<i>Grossuana</i>	<i>codreanul</i>	Bulgaria, Jasenovo	-	-	UGSB 10698	-	EF061920	-	Szarowska et al. (2007)
	<i>delphica</i>	Greece, Delphi, Kastalia Spring	38.483056	22.505278	UGSB 10699	-	EF061922	-	Szarowska et al. (2007)
	<i>serbica</i>	Serbia, Raska River Spring at Sopocani Monastery	43.115833	20.370833	UGSB 10699	-	EF061921	-	Szarowska et al. (2007)
	sp.	Greece, E. of Volos, Oros Pilon, spring E of Anilion	-	-	UGSB 10699	-	KC011768	-	Fainowski et al. (2012)
<i>Vurilana</i>	<i>vurilana</i>	Greece, spring of Louros River	-	-	UGSB 10699	-	EF061923	-	Szarowska et al. (2007)
<i>Islamia</i>	<i>hadzi</i>	Greece, Peloponnese, N Targeos Mts., W of Sparta, spring at Dhiaseleo	-	-	UGSB 10731	12075	JF916473	-	Fainowski and Szarowska (2011)
<i>Lynhdia</i>	<i>gorgjevic 1</i>	Macedonia, Lake Ohrid, in front of Hotel Desaret, village Pestani	40.91208	20.74213	UGSB 10746	12499	KU170812	-	This study
	<i>gorgjevic 2</i>	Macedonia, feeder springs of Sveti Naum	40.91208	20.74213	UGSB 10747	12500	KU170813	-	This study
	<i>gorgjevic 3</i>	Macedonia, feeder springs of Sveti Naum	40.91029	20.74791	UGSB 10685	10286	KU170814	KU170884	This study
	<i>gorgjevic 4</i>	Macedonia, Sveti Naum, small lake with springs	40.91029	20.74791	UGSB 10696	10287	KU170815	KU170885	This study
	<i>stankovici</i>	Macedonia, Lake Ohrid, Trpejca	40.95583	20.76396	UGSB 10697	10291	KU170816	KU170886	This study
<i>Malaprespia</i>	<i>albanica</i>	Albania, Lake Mikri Prespa, spring in the lake	40.67258	20.9892	UGSB 10731	12075	KU170817	KU170887	This study
<i>Ohridohauraffia</i>	<i>depressa 1</i>	Macedonia, Lake Ohrid, in front of Hotel Desaret, village Pestani	41.00927	20.80544	UGSB 10716	10507	KU170818	KU170888	This study
	<i>depressa 2</i>	Macedonia, Lake Ohrid, beach at Gorica Hill	41.08105	20.7974	UGSB 10711	10495	KU170819	KU170889	This study
	<i>minuta 1</i>	Macedonia, Lake Ohrid, spring Studentička	41.10251	20.81491	UGSB 10712	10497	KU170820	KU170890	This study
	<i>minuta 2</i>	Macedonia, spring Bej Bunar	41.11085	20.81905	UGSB 10713	10501	KU170821	KU170891	This study
	<i>minuta 3</i>	Macedonia, spring Bej Bunar	41.11085	20.81905	UGSB 10715	10502	KU170822	KU170892	This study
	<i>rotunda</i>	Macedonia, Lake Ohrid, at camping site "Livadište"	41.12643	20.84184	UGSB 10713	10500	KU170823	KU170893	This study
	<i>sancitnaumi 1</i>	Macedonia, feeder springs of Sveti Naum	40.91414	20.74147	UGSB 10738	12358	KU170824	-	This study
	<i>sancitnaumi 2</i>	Macedonia, feeder springs of Sveti Naum	40.91061	20.74748	UGSB 10737	10510	KU170825	KU170894	This study
<i>Ohridohoratia</i>	<i>carinata 1</i>	Macedonia, Lake Ohrid, Veli Dab	40.97439	20.78746	UGSB 10698	10299	KU170826	KU170895	This study
	<i>carinata 2</i>	Macedonia, Lake Ohrid, Trpejca	40.95868	20.77802	UGSB 10699	10305	KU170827	KU170896	This study
	<i>pygmaea 1</i>	Albania, feeder springs of Tusemisht	40.89874	20.71325	UGSB 10749	12508	KU170828	-	This study
	<i>pygmaea 2</i>	Albania, feeder springs of Tusemisht	40.89886	20.7127	UGSB 10748	12507	KU170829	-	This study
	<i>pygmaea 3</i>	Macedonia, feeder springs of Sveti Naum	40.91019	20.74793	UGSB 10750	12510	KU170830	-	This study
	<i>pygmaea 4</i>	Macedonia, Sveti Naum, spring lake at south end of island with restaurant	40.91393	20.74226	UGSB 10739	12359	KU170831	-	This study
	<i>pygmaea 5</i>	Macedonia, Sveti Naum, spring lake at south end of island with restaurant	40.91393	20.74226	UGSB 10740	12360	KU170832	-	This study
	<i>pygmaea 6</i>	Macedonia, Sveti Naum, springs at pumping station, near chapel	40.9113	20.74744	UGSB 10741	12362	KU170833	-	This study
	<i>pygmaea 7</i>	Albania, feeder springs of Tusemisht	40.89885	20.7136	UGSB 10755	12863	KU170834	-	This study
	<i>pygmaea 8</i>	Albania, Lake Ohrid, south of Lin peninsula	41.06055	20.65186	UGSB 10756	12865	KU170835	-	This study
	<i>pygmaea 9</i>	Macedonia, Lake Ohrid, in bay south of Gorica Hill	41.07304	20.79991	UGSB 10758	12867	KU170836	-	This study
	<i>pygmaea 10</i>	Macedonia, Lake Ohrid, in bay south of Gorica Hill	41.07304	20.79991	UGSB 10757	12866	KU170837	-	This study
	<i>pygmaea 11</i>	Macedonia, Lake Ohrid, northwestern bay, near Podmolje	41.16183	20.7451	UGSB 10742	12439	KU170838	KU170897	This study

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<i>continued</i>											
	<i>pygmaea</i> 12	Macedonia, Lake Ohrid, Veli Dab	40.97439	20.78747	UGSB 10759	12868	KU170839	-	This study		
	<i>pygmaea</i> 13	Macedonia, Lake Ohrid, northwestern bay, near Podmolje	41.16235	20.74395	UGSB 10718	10689	KU170840	KU170898	This study		
	<i>pygmaea</i> 14	Macedonia, Lake Ohrid, northwestern bay, near Podmolje	41.16183	20.7451	UGSB 10743	12440	KU170841	KU170899	This study		
	<i>pygmaea</i> 15	Macedonia, Lake Ohrid, northwestern bay, near Podmolje	41.16183	20.7451	UGSB 10744	12442	KU170842	KU170900	This study		
	<i>pygmaea</i> 16	Macedonia, Lake Ohrid, northwestern bay, near Podmolje	41.16429	20.7396	UGSB 10745	12444	KU170843	KU170901	This study		
<i>Ohrigocea</i>	<i>sarullii</i> 1	Macedonia, feeder springs of Sveti Naum	40.91287	20.74483	UGSB 10752	12514	KU170844	-	This study		
	<i>sarullii</i> 2	Macedonia, Lake Ohrid, south of Pestina	41.09239	20.63361	UGSB 10687	10153	KU170845	KU170902	This study		
	<i>sarullii</i> 3	Macedonia, Lake Ohrid, Veli Dab	40.97439	20.78747	UGSB 10688	10154	KU170846	KU170903	This study		
	<i>sarullii</i> 4	Macedonia, Lake Ohrid, bay south of Gradishte	40.99323	20.79958	UGSB 10689	10156	KU170847	KU170904	This study		
	<i>sarullii</i> 5	Macedonia, Lake Ohrid, lake bank at Veli Dab	40.97324	20.78668	UGSB 10692	10170	KU170848	KU170905	This study		
	<i>stankovici</i> 1	Macedonia, feeder springs of Sveti Naum	40.91029	20.74791	UGSB 10751	12512	KU170849	-	This study		
	<i>stankovici</i> 2	Albania, feeder springs of Tusemisht	40.89874	20.71325	UGSB 10753	12517	KU170850	-	This study		
	<i>stankovici</i> 3	Macedonia, feeder springs of Sveti Naum	40.91298	20.74455	UGSB 10754	12518	KU170851	-	This study		
	<i>stankovici</i> 4	Macedonia, spring at Sveti Naum	40.91135	20.745	UGSB 10690	10164	KU170852	KU170906	This study		
	<i>stankovici</i> 5	Macedonia, Lake Ohrid, Sveti Stefan	41.07306	20.79985	UGSB 10691	10166	KU170853	KU170907	This study		
	<i>stankovici</i> 6	Macedonia, Lake Ohrid, lake bank at Veli Dab	40.97324	20.78668	UGSB 10694	10173	KU170854	KU170908	This study		
	<i>stankovici</i> 7	Macedonia, Lake Ohrid, lake bank at Veli Dab	40.97324	20.78668	UGSB 10693	10172	KU170855	KU170909	This study		
	<i>Polinskiola</i>	<i>polinski</i> 1	Macedonia, feeder springs of Sveti Naum	40.91061	20.74748	UGSB 10725	10730	KU170856	KU170910	This study	
		<i>polinski</i> 2	Macedonia, feeder springs of Sveti Naum	40.91061	20.74748	UGSB 10724	10729	KU170857	KU170911	This study	
		<i>polinski</i> 3	Macedonia, Lake Ohrid, Trpejca	40.95868	20.77802	UGSB 10726	10734	KU170858	KU170912	This study	
		<i>polinski</i> 4	Macedonia, Lake Ohrid, southeast end of the lake	40.9396	20.7783	UGSB 10729	10739	KU170859	KU170913	This study	
<i>polinski</i> 5		Macedonia, Lake Ohrid, Trpejca	40.95752	20.77771	UGSB 10727	10735	KU170860	KU170914	This study		
<i>sturanjvi</i> 1		Macedonia, Lake Ohrid, bay south of Gradishte	40.99323	20.79958	UGSB 10722	10727	KU170861	KU170915	This study		
<i>sturanjvi</i> 2		Macedonia, Lake Ohrid, in bay south of Gorica Hill	41.07304	20.79991	UGSB 10728	10737	KU170862	KU170916	This study		
<i>sturanjvi</i> 3		Macedonia, Lake Ohrid, bay south of Gradishte	40.99323	20.79958	UGSB 10723	10728	KU170863	KU170917	This study		
<i>sturanjvi</i> 4		Macedonia, Lake Ohrid, bay south of Gradishte	40.97324	20.78668	UGSB 10723	10728	KU170863	KU170917	This study		
<i>sturanjvi</i> 5		Greece, Lake Mikri Prespa, at the former Hydrobiological Institute Mikrolimni	40.74215	21.10763	UGSB 10737	12089	KU170864	KU170918	This study		
<i>Prespitolorea</i>	<i>valvataeformis</i> 1	Macedonia, Lake Prespa, southern shore of Golem Grad	40.86655	20.98989	UGSB 10736	12088	KU170865	KU170919	This study		
	<i>valvataeformis</i> 2	Macedonia, Lake Prespa, southern shore of Golem Grad	40.86655	20.98989	UGSB 10735	12087	KU170866	KU170920	This study		
	<i>valvataeformis</i> 3	Macedonia, Lake Prespa, southern shore of Golem Grad	40.86655	20.98989	UGSB 10734	12086	KU170867	KU170921	This study		
	<i>valvataeformis</i> 4	Greece, Lake Prespa, bay on cliff-like coast	40.82032	21.01939	UGSB 10733	12085	KU170868	KU170922	This study		
	<i>valvataeformis</i> 5	Greece, Lake Prespa, bay on cliff-like coast	40.82032	21.01939	UGSB 10732	12084	KU170869	KU170923	This study		
	<i>valvataeformis</i> 6	Greece, Lake Prespa, bay on cliff-like coast	40.82032	21.01939	UGSB 10686	10118	KU170870	KU170924	This study		
<i>Pseudohoratia</i>	<i>brusinae</i>	Macedonia, Lake Ohrid, in front of Ohrid Bay	41.10156	20.78236	UGSB 10700	10313	KU170871	KU170925	This study		
	<i>lacustris</i> 1	Macedonia, Lake Ohrid, 2-5 m depth at northern part of the lake	41.16569	20.71836	UGSB 10702	10316	KU170872	KU170926	This study		
	<i>lacustris</i> 2	Macedonia, Lake Ohrid, 2-5 m depth at northern part of the lake	41.16569	20.71836	UGSB 10703	10319	KU170873	KU170927	This study		
	<i>ohridana</i> 1	Macedonia, Lake Ohrid, south of Gradishte	40.98687	20.79729	UGSB 10707	10330	KU170874	KU170928	This study		
	<i>ohridana</i> 2	Macedonia, Lake Ohrid, in front of Ohrid Bay	41.10156	20.78236	UGSB 10701	10314	KU170875	KU170929	This study		
	<i>ohridana</i> 3	Macedonia, Lake Ohrid, in front of Hotel Desaret, village Pestani	41.01262	20.80513	UGSB 10705	10327	KU170876	KU170930	This study		
<i>ohridana</i> 4	Macedonia, Lake Ohrid, southeast end of the lake	40.94696	20.77385	UGSB 10706	10328	KU170877	KU170931	This study			
<i>ohridana</i> 5	Macedonia, Lake Ohrid, south of Gradishte	40.98687	20.79729	UGSB 10708	10331	KU170878	KU170932	This study			
<i>ohridana</i> 6	Macedonia, Lake Ohrid, in front of Hotel Desaret, village Pestani	41.01262	20.80513	UGSB 10704	10326	KU170879	KU170933	This study			

647 **Table 2.** Reduced output of the diversification-rate analyses (TreePar). *P* values >0.95 indicate that a
 648 single shift in rates explain the tree significantly better than constant diversification rates, that two
 649 shifts explain the tree significantly better than a single shift, and that three shifts explain the tree
 650 significantly better than two shifts (see main text for details).

Random tree #	Max. age (Ma)	Rate shifts	-Log-likelihood	<i>P</i> value	Shift (Ma ago)
1	3.29	0 vs. 1	45.882 vs. 42.043	0.947	
		1 vs. 2	42.043 vs. 38.749	0.914	
		2 vs. 3	38.749 vs. 37.060	0.663	
2	3.06	0 vs. 1	46.292 vs. 45.073	0.514	
		1 vs. 2	45.073 vs. 43.192	0.712	
		2 vs. 3	43.192 vs. 42.478	0.301	
3	2.77	0 vs. 1	36.879 vs. 32.362	0.971	1 shift: 0.1
		1 vs. 2	32.362 vs. 30.577	0.688	▼
		2 vs. 3	30.577 vs. 28.505	0.754	
4	2.40	0 vs. 1	28.083 vs. 26.171	0.712	
		1 vs. 2	26.171 vs. 25.507	0.278	
		2 vs. 3	25.507 vs. 23.337	0.773	
5	2.32	0 vs. 1	18.364 vs. 14.942	0.923	
		1 vs. 2	14.942 vs. 12.978	0.731	
		2 vs. 3	12.978 vs. 10.751	0.784	
6	2.42	0 vs. 1	31.850 vs. 29.342	0.829	
		1 vs. 2	29.342 vs. 27.932	0.580	
		2 vs. 3	27.932 vs. 26.219	0.670	
7	2.64	0 vs. 1	31.770 vs. 29.237	0.833	
		1 vs. 2	29.237 vs. 28.266	0.415	
		2 vs. 3	28.266 vs. 27.571	0.292	
8	2.96	0 vs. 1	34.733 vs. 30.793	0.951	1 shift: 0.1
		1 vs. 2	30.793 vs. 28.986	0.694	▼
		2 vs. 3	28.986 vs. 27.431	0.625	
9	2.85	0 vs. 1	34.152 vs. 31.644	0.829	
		1 vs. 2	31.644 vs. 29.433	0.781	
		2 vs. 3	29.433 vs. 27.109	0.801	
10	2.13	0 vs. 1	31.492 vs. 28.169	0.916	
		1 vs. 2	28.169 vs. 25.885	0.794	
		2 vs. 3	25.885 vs. 23.883	0.739	

Gelöscht: 1 shift: 0.1

Gelöscht: 1 shift: 0.1

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652

655 **Figure captions**

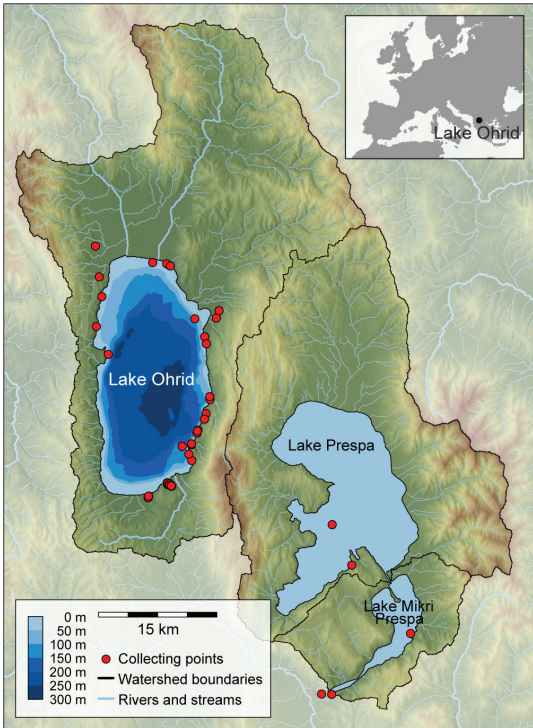


656 **Fig. 1.** Selected representatives of genera belonging to the endemic non-pyrgulinid Hydrobiidae from
657 Lake Ohrid. Scale bar = 1 mm.

658

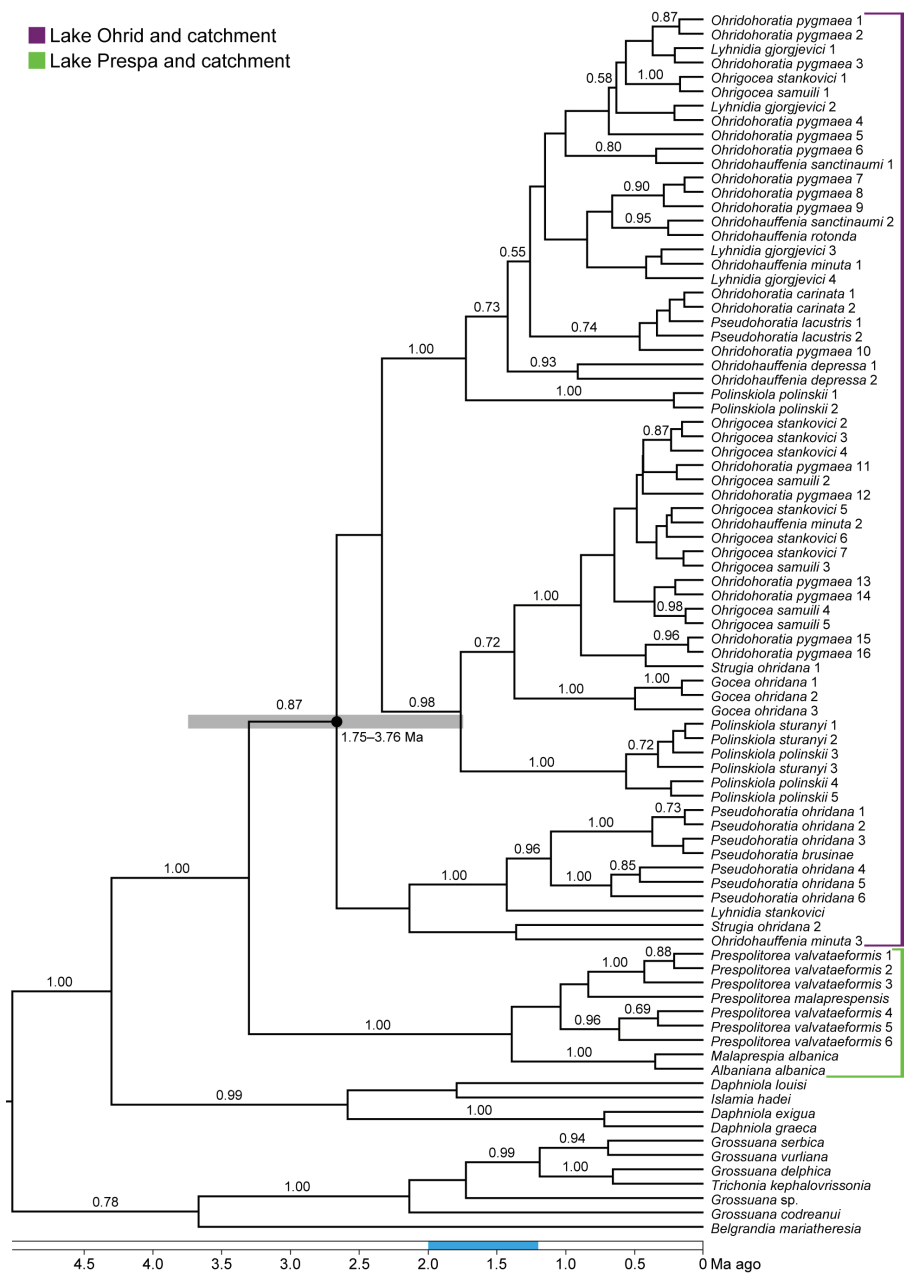
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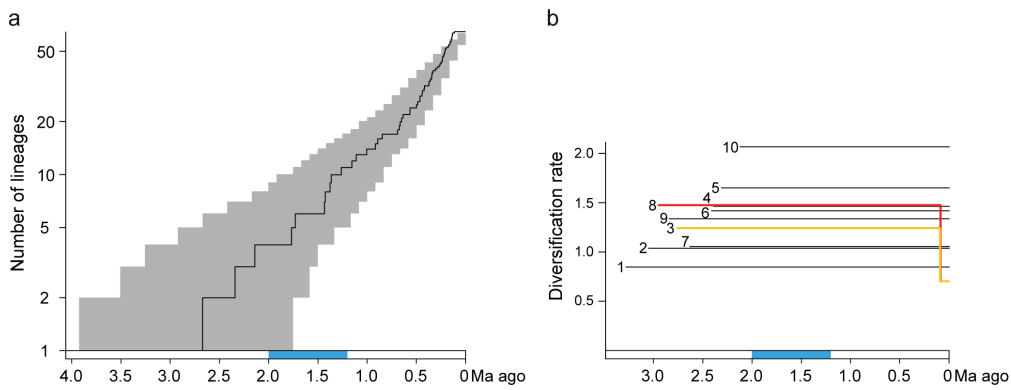


661
 662 **Fig. 2.** Sampling sites for non-pygulinid Hydrobiidae in lakes Ohrid, Prespa and Mikri Prespa, and
 663 their watersheds.

664
 665



666
 667 **Fig. 3.** MCC tree based on a relaxed-clock BEAST analysis for non-pyrgulinid Hydrobiidae from the
 668 Balkans. Posterior probabilities >0.5 are shown at the respective branches. The grey bar indicates the
 669 95% HPD for the age of the MRCA of the Ohrid endemics. The blue bar in the timescale ranging from
 670 1.2–2.0 Ma ago shows the assumed age of Lake Ohrid.



672
 673 **Fig. 4.** (A) LTT plot for the endemic non-pyrgulinid Hydrobiidae in Lake Ohrid. The plot is based on a
 674 relaxed-clock BEAST analysis with the black line showing the BEAST MCC and the shaded area
 675 indicating the 95% confidence interval based on 20,000 post-burnin BEAST trees. The blue bar
 676 ranging from 1.2–2.0 Ma ago shows the assumed age of Lake Ohrid. (B) Diversification rates obtained
 677 from the diversification rate-analyses (TreePar); trees with a single shift in diversification rates at 0.1
 678 Ma (trees #3 and #8) are highlighted in red and yellow.