1 **Early Burst in Body Size Evolution is Uncoupled from Species**

2 **Diversification in Diving Beetles (Dytiscidae)**

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12 **Running head: Early burst of body size evolution in Dytiscidae**

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34 **ABSTRACT**

35 Changes in morphology are often thought to be linked to changes in species diversification,

36 which is expected to leave a signal of Early Burst (EB) in phenotypic traits. However, such

37 signal is rarely recovered in empirical phylogenies, even for groups with well-known

38 adaptive radiation. Using a comprehensive phylogenetic approach in Dytiscidae, which

39 harbors ~4,300 species with as much as 50 fold variation in body size among them, we ask

40 whether pattern of species diversification correlates with morphological evolution.

41 Additionally, we test if the large variation in body size is linked to habitat preference and if

42 the later influences species turnover. We found, in sharp contrast to most animal groups, that

43 Dytiscidae body size evolution follows an Early Burst model with subsequent high

44 phylogenetic conservatism. However, we found no evidence for associated shifts in species

45 diversification, which point to an uncoupled evolution of morphology and species

46 diversification. We recovered the ancestral habitat of Dytiscidae as lentic (standing water),

47 with many transitions to lotic habitat (running water) that are concomitant to a decrease in

48 body size. Finally, we found no evidence for difference in net diversification rates between

49 habitats but evidence for much higher speciation and extinction rates (higher turnover) in

50 lentic species than lotic ones. This result, also recently found for dragonflies, contradicts the

51 current theoretical expectations (Habitat Stability Hypothesis), thus calling for a thorough

52 reassessment of the role of gene flow and local adaptation between lotic and lentic species of

53 Dytiscidae.

54

55

INTRODUCTION

57

58   Understanding why some groups of organisms are more diverse than others and what

59 links species diversification to ecological pattern are fundamental questions in biology. In

60 particular, changes in morphology are often thought to be linked to changes in species

61 diversification. It is especially the case in adaptive radiations where species diversify

62 morphologically when entering new niches. Thus the rate of morphological evolution is

63 expected to be rapid at the beginning of a radiation and slow down as the niche space is filled

64 up (Simpson 1944; Schluter 2000). This pattern was recovered in several studies of

65 individual clades that are known for their adaptive radiation (e.g. Harmon *et al.* 2003;

66 Jønsson *et al.* 2012; Weir & Mursleen 2013). It has then been implied that clades having

67 experienced adaptive radiation should show a pattern of Early Burst (EB) in phenotypic

68 evolution and rate of diversification. (e.g. Harmon *et al.* 2003; Losos *et al.* 2010). However,

69 Harmon *et al.* (2010), in their meta-analysis, found EB of body size evolution in only 2 of 88

70 clades of a wide range of animals. Notably, they failed to identify EB as the best model in

71 larger clades well-known for adaptive radiations such as Galapagos finches, *Anolis* lizards or

72 African cichlids. They concluded that Early Burst in morphology evolution might be rare in

73 the tree of life. Moen & Morlon (2014) hypothesized that patterns of morphological diversity

74 resulting from adaptive radiations may be different at larger time scales, thus more difficult to

75 discover, because many adaptive radiations studied yet are found in relatively young clades

76 while the clades examined by Harmon et al. (2010) are much older. However, EB was

77 identified at larger scale for mammals (Cooper & Purvis 2010) and for birds (Harmon *et al.*

78 2010). Benson *et al.* (2014) combining phylogeny and body-size datasets for fossils, also

79 found strong support for EB of evolution in most dinosaur clades. Recently, this pattern was

80   also found for Gnathostomes using paleontological data (King 2016). These results, taken

81   together, suggest that adaptive radiation also plays a role at larger evolutionary scales.

82

83   In this context, insects represents one of the major ecological and evolutionary

84   diversifications on earth, with more than one million species worldwide, occupying nearly all

85   ecological niches and presenting many different morphologies (Grimaldi & Engel 2005;

86   Footit & Adler 2009). Their high diversity has been explained by several hypotheses such as

87   low extinction rates, acquisition of key innovations (e.g. wings, complete metamorphosis) or

88   appearance of new niches (Farrell 1998; Mayhew 2007). Condamine *et al.* (2016),

89   investigating pattern of diversification in insects using molecular and fossils dataset

90   recovered an Early Burst of diversification rates using fossil data at the whole phylogeny

91   level and at family level in Coleoptera (beetles), the most diverse order while Rainford *et al.*

92   (2016), combining diversification and body size evolution in insect phylogeny, found that

93   patterns of size evolution in insects mainly follow a single stationary peak model of evolution

94   (Ornstein-Uhlenbeck, OU). In Coleoptera, Dytiscidae (predaceous diving beetles) are well

95   suited for the study of patterns of diversification and morphological evolution. With more

96   than 4,300 described species they represent one of the largest and most commonly

97   encountered groups of aquatic insects. They occupy various habitat types, varying from

98   running/lotic (e.g. streams, springs, rivers) to standing/lentic (e.g. swamps, ditches, pools,

99   lakes), permanent to ephemeral water bodies and present large variation in body size (from

100   <1 to almost 50 mm) (Miller & Bergsten 2016).

101

102   Body size is an important trait when studying adaption to different environment

103   pressures and in particular in lentic and lotic environments, which present major differences

104   in their long-term persistence implying different ecological constraints on species inhabiting

4

105   them. It has been shown that species with larger body size are often associated with stagnant

106   water, while smaller species are found in a broader range of water flow regimes (Ribera &

107   Nilsson 1995). The metabolic cost of insect flight varies inversely with body size (Peters

108   1983) while Jenkins *et al.* (2007) showed for a large range of organisms that active dispersers

109   followed a positive dispersal–mass trend. We can thus expect that larger body size in insects

110   would result in better dispersal ability and would be favoured in more ephemeral lentic

111   habitats whereas they would be disadvantaged in more constraining (e.g. with water flows)

112   lotic habitats.

113

114   The difference in habitat stability predicts major differences in dispersal ability and

115   geographic range sizes in lentic and lotic species. The Habitat Stability Hypothesis

116   (Southwood 1962; Ribera 2008; Dijkstra *et al.* 2014) posits that lentic species should have

117   lower speciation and extinction rates because of their higher dispersal ability and thus larger

118   range size mainly due to the lower stability of lentic habitats in space and time. The corollary

119   is that lotic species with smaller geographical range and a higher probability of allopatric

120   speciation would have higher species turnover over time than lentic species. The higher

121   dispersal ability and its link to larger geographical range sizes in lentic species has been

122   demonstrated in many examples of Dytiscidae and Odonata and lower genetic structure

123   among lentic populations has been shown in several groups of aquatic insects, crustaceans

124   and molluscs (see Ribera 2008; Dijkstra 2014 as reviews and references therein). However,

125   no significant turnover difference between lentic and lotic species could be demonstrated yet

126   in Dytiscidae (Ribera *et al.* 2001; Ribera 2008) whereas a recent study on dragonflies (Letsh

127   *et al.* 2016) provides evidence of higher speciation rates in lentic species, which is in

128   contradiction to the Habitat Stability Hypothesis.

129

130   Here, we apply a comprehensive phylogenetic approach in Dytiscidae to answer the

131   following questions:

132

133   -   Is the species-rich and morphologically diverse Dytiscidae a result of adaptive

134   radiation and is it linked to an Early Burst of body size evolution?

135   -   How has body size of Dytiscidae and their habitat preference evolved over time?

136   Were transitions frequent and reversible or can we find strong phylogenetic signal

137   with close species sharing similar morphology and/or habitat?

138   -   Have changes in habitat over time driven the morphological differentiation in

139   Dytiscidae?

140   -   Is the Habitat Stability Hypothesis supported in Dytiscidae?

141

142   METHODS

143

144   *Data Collection*

145

146   We used 164 diving beetles species and 9 outgroup taxa (Gyrinidae, Noteridae,

147   Amphizoidae and Hygrobiidae) from the molecular dataset of the most recent phylogeny of

148   Dytiscidae (Miller & Bergsten 2014). We excluded 4 taxa from the original dataset because

149   of their large proportion of missing data (Lysp376 and Lmsp764 *Laccodytes sp*,

150   Pasp761 *Pachydrus sp*, Hysp731 *Psychopomporus felipi*). The dataset includes 9 loci from

151   mitochondrial and nuclear DNA: 16S rRNA (16S), 12S rRNA (12S), cytochrome c oxidase I

152   (COI), cytochrome c oxidase II (COII), elongator factor 1α (EF1α), arginine kinase (AK),

153   Histone III (H3), RNA polymerase II (RNApol) and wingless (Wnt). We removed portions of

154   the alignment with fewer than 15% of taxa as well as two indel regions of 55bp in AK and

155   76bp in RNApol. Highly variable unalignable regions in 12S and 16S were also excluded

156   resulting in a matrix of 173 taxa and 4972bp.This dataset includes all currently recognized

157   Dytiscidae subfamilies and tribes with the exception of one tribe with two small genera and

158   49% of all genera are present (Table 1, Table S1 Supporting information). Despite the

159   relatively low percentage of genera, we expect that the sampling captures most of the

160   phenotypic diversity in the family as it is scattered throughout the tree.

161

162   *Phylogenetic Reconstruction*

163

164   Phylogenetic tree reconstruction was conducted with MrBayes 3.2.4 (Ronquist *et al.*

165   2012) on the UPPMAX computational cluster, Uppsala, Sweden. The matrix was divided into

166   7 partitions analyzed independently under a GTR model plus a gamma distributed rate

167   variation parameter. The first partition consisted of merged mitochondrial ribosomal regions

168   (12S and 16S), the second to fourth of mitochondrial (COI and COII) regions separated into

169   1st, 2nd and 3rd codon positions and the fifth to seventh of nuclear regions also separated in

170   1st, 2nd and 3rd codon positions. We did two runs of six chains for 25 million generations

171   sampled every 50000 generations. Convergence of the two runs was assessed by visual

172   inspection of the log likelihood and ensuring ESS value above 200 in Tracer 1.6 (Rambaut *et*

173   *al.* 2014) and a burnin of 10 % was discarded. We computed a 50% majority rule consensus

174   tree with posterior probabilities (PP) on each node as support (Fig. S1 Supporting

175   information).

176

177   *Divergence Time Estimation*

178

179   An uncorrelated exponential relaxed molecular clock was used to estimate divergence times.

180   Bayesian analyses were performed using BEAST 2.3.2 (Bouckhaert *et al.* 2014) on the

181   CIPRES Science Gateway (Miller *et al.* 2010). The topology from the 50% majority rule

182   consensus from MrBayes was fixed because allowing BEAST to simultaneously estimate

183   ages and topology consistently led to non-convergence of the MCMC. The few polytomies

184   were randomly broken as BEAST only allows strictly dichotomous trees. We set the

185   outgroup topology according to the most recent beetle phylogeny from the beetle tree of life

186   (McKenna *et al.* 2015). Data were partitioned by locus and codon position (see above), with a

187   separate GTR + γ model of sequence evolution applied to each partition and with a birth-

188   death tree prior. A strict clock like model was not supported as evidenced by the fact that the

189   posterior of the rate variance did not encompass zero. Twelve fossils were selected as

190   calibration points on the tree (Table S2 supporting information). To define calibration points

191   for the five deeper nodes of the phylogeny (A-E), we used the most recent review of

192   paleontological data on the evolution of aquatic beetles and the Mesozoic evolution of

193   Dytiscidae (Ponomarenko & Prokin 2015; Prokin *et al.* 2013) together with a phylogenetic

194   analysis of extant and extinct taxa (Beutel *et al.* 2013). For the shallower nodes (E-L), we

195   used seven fossil descriptions from the literature, and one fossil studied directly (E), all of

196   which could be assigned to extant genera, tribes (I), or subfamilies (E), based on the available

197   documentation (Table S2 supporting information). For the twelve nodes, we used an

198   exponential prior with the lower bounds as the minimum age of the geological period where

199   the fossil was found. Each calibration point was set as stem age for the respective clade (Fig.

200   1, Table S2 Supporting information). The analysis ran twice, with 50 million generations per

201   run and sampled every 25,000 generations. The two runs were combined using

202   LogCombiner, after assessing the convergence of all parameters by visual inspection of the

203   Log likelihood and ensuring ESS value above 200 in Tracer 1.6. The nodes were annotated in

204   TreeAnnotator and this dated tree was used for all analyses described below.

205

206   *Diversification Analyses*

207

208   Using the dated tree obtained from BEAST, we tested the presence of species

209   diversification shift through time using Metropolis-coupled Markov-chain Monte Carlo

210   (MC^3) in BAMMv.2.5 (Rabosky *et al.* 2013), with relevant priors chosen using BAMMtools

211   (Rabosky *et al.* 2014). Recent criticism has been raised against this approach (Moore *et al.*

212   2016) regarding the likelihood estimations and accuracy of the diversification rates. However

213   the debate is still going on and Rabosky *et al.* (2017) recently showed that Moore *et al.*

214   (2016) used wrong parameterization and non-biologically realistic simulations. In regards to

215   this, the results of BAMM for estimating diversification shifts must be taken with caution but

216   those issues are of less concern for phenotypic estimates.

217   Speciation and extinction were inferred using the 'speciation–extinction' module,

218   with correction for differential sampling across genera (Table S1 Supporting information).

219   The sampling fraction of each genus was determined by comparing the number sampled with

220   the total number in the 2015 World Catalogue of Dytiscidae (Nilsson 2001, 2015). The

221   analysis ran for 50 million generations using four chains and sampled every 5,000

222   generations. We assessed the convergence of all parameters by visual inspection of the Log

223   likelihood and ensured ESS value above 200 in Tracer 1.6 and a 10% burnin was

224   consequently removed. BAMMtools was used for summary statistics and Bayes Factors (BF)

225   for each model considered. Posterior probability of change in macroevolutionary regime was

226   evaluated using the most probable credible set of changes. The best shift configuration was

227   estimated and net diversification rate through time was extracted from the results (Fig. S2

228   Supporting information).

229   *Body Size Evolution and Ancestral Estimation*

230

231   *Data.*

232   The minimum and maximum body size measures (mm) per species was taken from

233   the database of the World Catalogue of Dytiscidae 2015, which comprises all recognized

234   species with up-to-date classification, zoogeographic distributions and species numbers

235   (Nilsson 2001, 2015). As the mean body size per species was not available, we estimated it

236   by using half the sum of minimum and maximum body size per species and then averaged

237   over genus (genus mean body size). We then used the logarithm of the genus mean body size

238   in the analyses (Table S1 Supporting information).

239

240   *Phylogenetic signal.*

241   We tested for the presence of phylogenetic signal in body size using Pagel's λ (Pagel

242   1999) and Blomberg's K (Blomberg *et al.* 2003) implemented in the phytools package

243   (Revell 2012) in R 3.2.2. Pagel's λ significance was estimated using likelihood ratio test

244   (LRT) and Blomberg's K with 1000 permutations. Those two metrics assume a Brownian

245   motion model of character state evolution as a reference for estimating the phylogenetic

246   signal. Pagel's λ is equal to 0 when the character states evolve independently of the

247   phylogeny, whereas a value of 1 indicates that the character states evolve according to

248   Brownian motion. Values of Blomberg's K lower than 1 indicate that related species

249   resemble each other less than expected under Brownian motion, whereas values greater than

250   1 mean that related species are more similar for the character state under study than predicted

251   by this model.

252

253   *Models of evolution.*

254   We fitted models of body size evolution to the phylogenetic tree contrasting

255   Brownian motion (neutral evolution), Early Burst, Ornstein-Uhlenbeck and Pagel's λ and δ

256   using the Akaike information criterion in the Geiger package (Harmon *et al.* 2008) using the

257   FitContinuous function in R 3.2.2. The Brownian motion model (BM) (Felsenstein 1973) can

258   be assumed as the neutral model of character evolution with a constant rate through time

259   leading to a correlation between character values and shared ancestry for pairs of species. The

260   other models can be considered as variants of the BM. The Ornstein-Uhlenbeck model (OU)

261   (Butler and King 2004) also assumes a constant rate through time but it adds a tendency

262   towards central values of the characters resulting in a distribution around this value. This

263   model can represent ecological optimum of body size where deviation from the niche

264   optimum decreases fitness and body size has a tendency to remain close to this optimum.

265   The Early Burst model (EB) (Harmon *et al.* 2010), also called the ACDC model

266   (accelerating-decelerating; Blomberg *et al.* 2003), allows for variation of rates through time

267   with either an exponential decrease or increase. A decreasing model can represent a situation

268   where most of the morphological transition, in the case of body size, occurs early in the

269   evolution of the group followed by a rapid decrease in body size rates along the following

270   branches. We also fitted Pagel's λ that measures the relative importance of the phylogeny to

271   explain the character distribution. The last model tested is Pagel's δ, which is similar to an

272   Early Burst model when values of δ are below 1 and indicates recent rapid evolution when

273   above 1.

274

275   In order to test if the sampling can affect the false discovery rate and the power to

276   detect an Early Burst in body size, we conducted simulations. For each simulation, we

277   simulated a complete tree (4303 species) under a pure-birth model with a speciation rate of

278   0.04 to achieve a topology and age close to the observed data. Each tree was scaled so that

279   the root corresponds to the observed root (159.2 Ma). We then simulated a continuous

280   character on this phylogeny under a Brownian motion and under an Early Burst model. We

281   sampled 168 tips from the phylogeny and retrieved the associated value for the character

282   under the two models. On each character, we tested the fit of BM and EB using fitContinous

283   function in "geiger" and report the p-value from the likelihood ratio test between EB and BM.

284   We wanted to test if a reduced sampling can induce a bias toward the detection of an EB

285   (false positive), when the true model is BM, and to assess how much power do we have to

286   detect a signal of EB with reduced sampling when it is present.

287

288   We varied the parameters for the trait simulation in two different types of simulations. First,

289   we simulated a trait 100 times for both BM and EB models using the inferred value from the

290   observed data for sigma^2 (0.027641), which represent the variance of the trait. For the EB

291   model, we simulated the trait using also the inferred parameter "a" (-0.44424), which is the

292   rate modifier in the Early Burst, using the following equation $r[0] = r[0] * \exp(a * t)$. A

293   negative value of "a" means that rates are decelerating through times and the higher the value

294   of "a", the more abrupt is the change close to the root of the tree indicative of a stronger

295   Early Burst. Second, we modeled a range of value for sigma^2, from low to high variance

296   (0.1, 1, 10) and, only for the EB model, a range of values for "a" (-0.1, -0.5, -1) from a

297   smooth decrease in rates to an abrupt change. For each value in those ranges, we simulated

298   100 datasets (tree + trait) under each model. We report the distribution of p-values and the

299   proportion of times an EB model is preferred over BM at 0.05 significance level of the LRT.

300
301   *Ancestral estimation.*

302   Body size evolution was inferred using the continuous 'trait' module in BAMM. This

303   analysis reached convergence less readily and thus ran for 200 million generations using

304   eight chains sampled every 500,000 steps. A burnin of 55% was removed after inspection in

305   Tracer 1.6. The ancestral body size estimation was also implemented in BAMM by adding

306   the two parameters, nodeStateOutfile and nodeStateWriteFreq. BAMMtools was used to

307   determine the posterior probability of change in macroevolutionary regime summarized in the

308   best shift configuration (Fig. 2) and most probable credible set of changes (Fig.S3 Supporting

309   information) and rates of body size change through time were extracted from the results (Fig.

310   3). We obtained a tree with reconstructed body sizes at each sampled iteration of the MC^3.

311   The output was summarized using tree annotator to obtain the median ancestral body size and

312   95%HPD on each node, which was plotted on the BEAST tree (Fig. 2c) using a custom

313   Rscript.

314

315   *Correlation with species diversification.*

316   We tested for a correlation between body size evolution and species richness using

317   linear regression and phylogenetic general least square (PGLS) analysis following Kozak &

318   Wiens (2016). We defined 20 clades representing major lineages of Dytiscidae (Table 1, Fig.

319   1). We extracted body size rate for each clade from the BAMM output. Species richness and

320   clade crown age were used to derive clade-specific diversification rates using Magallon &

321   Sanderson (2001) estimator with low ($\varepsilon = 0$) and high extinction fraction ($\varepsilon = 0.9$). We first

322   tested three models with either the logarithm of species richness or diversification rates as

323   dependent variable and body size rates as predictor using the "lm" function in R and PGLS in

324   the "caper" Rpackages.

325

326   We also estimated Pybus & Harvey's γ statistic (2000) and associated p-value (Table

327   1) considering unsampled species using simulations, where we built a null distribution of γ by

328   simulating 10000 trees totaling all species under a pure birth model and pruned taxa to match

329 the number of taxa sampled in our tree. The γ statistic can be an indicator of diversity-

330 dependent evolution and can be contrasted to morphological rates to test a "niche filling"

331 hypothesis with slowdown in species diversification associated with lower morphological

332 changes. We tested this hypothesis using γ as dependent variable and body size rates using

333 "lm" and PGLS as above.

334

335 *Ancestral Habitat Estimation and Correlates*

336 **Data**

337 We scored the habitat of each species based on their genus habitat (Balke 2005) as discrete

338 character states: lentic (standing water), lotic (running water) or present in both habitats. The

339 genus *Hydrotrupes* present in Hygropetric habitat (i.e. water on a vertical surface, such as

340 waterfalls, seeps) and the monotypic genus *Haideoporus* found in springs aquifers in Texas,

341 considered as stygobiont (i.e. in groundwaters of aquifer), were both scored as lotic. For the

342 43 species for which the genus is found in both habitats, we consulted specialized literature

343 and regional atlases to refine in which habitat the species was found and we obtained only 13

344 species found in both habitats or for which information was not available and thus were kept

345 scored as found in both habitats (see references in Table S1 Supporting information). Thus,

346 of the 164 sampled species, 109 are lentic, 42 lotic and 13 in both habitats. Those 13 species

347 were removed from the HiSSE analysis as it only takes binary characters into account. Our

348 sampling covers 5.2% and 7.8% of all current lentic and lotic species respectively, and is thus

349 not biased towards one state.

350

351   We used Pagel's *λ*, suitable for discrete traits, to test for phylogenetic signal in

352 habitat. We modeled trait evolution with a suite of hiSSE models to first test the hypothesis

353 that lotic diving beetles would have a higher net diversification rate than lentic (as proposed

354 by the Habitat Stability Hypothesis; Ribera 2008) and second, estimate the ancestral states of

355 habitat along the phylogeny.

356

357 Trait evolution is often modeled using a "mk" model with transition rates between states.

358 However, as Maddison *et al.* (2007) pointed out, characters are not independent from the

359 diversification pattern and thus character and diversification should be modeled together.

360 This led to a family of xSSE models of character-associated diversification. These have been

361 strongly criticized recently because of the very high type one error and inadequacy of the null

362 model used (Rabosky & Goldberg 2015; Maddison & FitzJohn 2015). Beaulieu and O'Meara

363 (2015) proposed a new model using a hidden character that represented an unknown

364 associated character and also proposed a new null model.

365

366   We fitted 44 models (Table S3 Supporting information) and selected the best-fit

367 model using the Akaike Information Criterion corrected for sample size (AICc), using a

368 ΔAICc of 2 to select the best-fit model(s) following the approach of Laenen *et al.* (2016).

369 Since our sampling is incomplete, we used a global sampling fraction per states to account

370 for unsampled species. After selection of the best-fit model, we estimated the confidence

371 interval of each parameter, species turnover (τ), extinction fraction (ε) and transition rate (q),

372 by sampling parameter values ensuring that the difference of log-likelihood < 2 as compared

373 to the best-fit model as implemented in the 'SupportRegion' function. τ and ε are transformed

374 to speciation and extinction rates using Eqn 5(a,b) from Beaulieu & O'Meara (2015). We

375 also report the results as speciation (λ), extinction (μ) and net diversification rates ($\lambda - \mu$) to

376 allow comparison with others studies. Ancestral habitat was estimated under the best-fit

377 model and branches with a probability of > 90% are reported on Fig. 2a.

378

379   We also used sister clade analysis following Kaefer & Mousset (2014a) to test if

380   lentic and lotic sister clades differ in species richness and therefore diversification rates. We

381   visually defined 11 sister clades (Table S4 Supporting information) according to the ancestral

382   estimation of habitat. We performed 100,000 resampling iterations as implemented in the

383   Kaefer & Mousset (2014b) Rscript.

384

385   *Test for Phylogenetic Correlation Between Body Size and Habitat*

386

387   We tested for phylogenetic correlation between body size and habitat in two ways,

388   using the Felsenstein threshold model (Felsenstein 2012) and phylogenetic logistic regression

389   (Ives & Garland 2010). The threshold model allows the direct comparison of continuous and

390   discrete characters by fitting a continuous character along the tree, called liability, for each

391   character. The liability represents a continuous character, e.g. amount of genetic drift,

392   underlying the evolution of the real character. When the liability pass a certain threshold then

393   the character state changes. The threshold and liability is estimated using MCMC sampling as

394   implemented in the function ThreshBayes (Revell 2014) in the R package phytools. We also

395   tested whether habitat and body size evolved in a correlated way using Ives & Garland's

396   model (2010) suitable for binary dependent variables. The model was run using the

397   "phyloglm" function in the Rpackage "phylolm" (Ho & Ane 2014) with 2000 bootstrap

398   replicates. Phylogenetic signal is also estimated by the parameter "a" that is related to the

399   transition rates between states 0 and 1. As the transitions rates increases, "a" increases and

400   the phylogenetic signal is lost.

401

402   **RESULTS**

403

Phylogenetic Reconstruction

We found a well-supported back-bone topology which somewhat differ from the topology recovered by Miller & Bergsten (2014) (compared in Fig. S4 Supporting information). The main difference is the grouping of clade A and B (pp. 96) sister to C (pp. 87) while clade C was grouped with B (pp.62) and sister to A (pp.96) in Miller and Bergsten (2014). Inside clade B, we found a better-supported alternative topology with clade B2 and B3 grouped (pp. 100) and sister to B1 (pp. 100). We found a higher support for clade A (pp. 91) and clade C (pp. 96). Another major difference is the placement of Hydrodytinae, sister to the clade ABC whereas it was resolved as sister to Hydroporinae only (clade C) in Miller and Bergsten (2014). This difference is probably due to the use of the morphological characters by Miller and Bergsten (2014) that grouped them together but which are in conflict with molecular data.

The crown age of Hydradephaga superfamily was recovered as 211 Ma (256 - 205 Ma). The divergence of Dytiscidae from the outgroup occurred around 170.8 Ma (155.7-187.7 Ma) and its crowngroup age is around 159.2 Ma (141.5-179.1 Ma). Divergences between the major clades ABC occurred during the Early Cretaceous (145-100 Ma) (Table 1).

Diversification Analyses

The results of the MC^3 in BAMM favour a model with no shift in diversification rate regime with a frequency of 0.66 whereas the model with one shift occurring at the base of clade ABC or at the base of the Matinae were supported with a frequency of 0.2 and 0.12

429   respectively (Supplementary Fig. S2 a, b, c). Those shifts could represent high extinction in

430   Matinae and probably driven by their relatively long branch but do not point to increase in

431   diversification regime in clade ABC. Using Bayes Factors, we found that models with

432   diversification rate shifts were not supported compared to the null model with BF lower than

433   one. Net diversification rate through time show a flat trend with a mean of 0.04 sp/My

434   (Supplementary Fig. S2 d).

435

436   *Body Size Evolution and Ancestral Estimation*

437   There was a strong phylogenetic signal in body size with either Blomberg's K ($K = 3.7230$, p

438    $= 0.001$) or Pagel's λ ($\lambda = 1.01329$, p < 0.0001). Dytiscidae body size evolution shows very

439   strong support for the Early Burst model of character evolution with AICc weight > 0.99

440   compared to all other models (Table 2). This result was supported by simulated complete

441   phylogenies subsampled to equal the number of species present in our phylogeny. Indeed, we

442   found that an Early Burst model was favoured in only 1.7% of the simulations when a

443   Brownian motion was the true model, indicative of low Type I error. However, the power to

444   detect an Early Burst was very high as 100% of the simulations show significance for an

445   Early Burst over a BM when the former was the true model. This result holds when we

446   simulated under different values for the variance of the character and values for the strength

447   of the Early Burst. This indicates that our taxon sampling should be sufficient and robust

448   against false positive model selection (Table S5 and Fig. S5 Supporting information).

449

450   The ancestral body size is estimated to be small (5.53mm) with subsequent

451   derivations of larger but also shifts to smaller sizes (Fig. 2c) and eight major transitions of

452   body size rate change through time were detected in the best shift configuration. The first

453   shift to larger body size was detected on the clade AB at 139 Ma (125 - 155 Ma). Inside clade

454   AB, we recovered a shift to larger and smaller body size respectively at the root of the

455   families Colymbetinae and Agabinae at 65 Ma (37 - 95 Ma) and 83 Ma (53 - 112 Ma) and

456   two shifts inside the clade of Dytiscinae: to larger size for *Dytiscus-Hyderodes* at 74 Ma (35 -

457   111Ma) and to smaller size for another clade at 62 Ma (43 - 83 Ma). There was a shift to

458   smaller size at the root of the clade grouping Laccophilinae, Copelatinae and Cybistrinae at

459   113 Ma (89 - 135 Ma). Two shifts to larger sizes were identified inside the clade C, one

460   inside the tribe Hyphydrini 51Ma (29 - 76 Ma) and one inside the tribe Hygrotini

461   (*Coelambus*) at 43 Ma (24 - 62 Ma).

462

463   We found no support for an association between body size rate change and species

464   richness or species diversification (Table 3) with either linear models or phylogenetically

465   corrected models (PGLS). Models testing the association of slowdown in species

466   diversification (γ) and body size rate change were also non-significant giving no support for

467   the "niche filling" hypothesis (Table 3).

468

469   *Ancestral Habitat Estimation and Correlates*

470

471   A strong phylogenetic signal was recovered for habitat preference using Pagel's λ ($\lambda = 1.013$,

472   p < 0.001).

473   The model 3, which is a BiSSE model with equal transition rate between lotic and

474   lentic, was the best-fitting model with a likelihood value of -783.17 and an AICc weight of

475   0.17. The two following models were model 1, which is the full BiSSE model and model 6

476   which is the BiSSE null model with equal extinction fraction and transition rates, which have

477   respectively likelihood values and AICc weights of -782.4, 0.12 and -784.66, 0.11. There was

478   no significant difference in net diversification rates between lentic and lotic species.

479 However, there were much higher speciation and extinction rates in lentic species than lotic

480 species, which resulted in a higher turnover (Table 4).

481

482   The ancestral habitat estimation resolved the ancestor of Dytiscidae as lentic, with

483 eighteen more recent colonizations to lotic habitat from the end of Cretaceous to the

484 Neogene. There were only two reconstructions of back-colonization from lotic to lentic

485 habitat, *Porhydrus* (Siettitina, Hydroporinae) and *Nebrioporus* (Deronectina, Hydroporinae),

486 the latter found in both lotic and lentic waters (Fig. 2a). The number of transitions estimated

487 here using genera as tips represent major transitions but likely underestimate the real number

488 of events as variation in habitat preference is found within many genera.

489

490   No significant difference in species richness between lentic and lotic sister clades

491 using sister clade comparison was found ($scc_{stat} = 0.36$, p-value = 0.69), which also suggest

492 that net diversification rates do not differ significantly between the two habitats.

493

494 *Test for Phylogenetic Correlation between Body Size and Habitat*

495

496   Assessing the relationship under the threshold model gives a mean correlation

497 coefficient of -0.125 between habitat and body size liability. The posterior distribution of

498 correlation coefficient however encompasses 0 and is thus not statistically significant (Fig. S6

499 Supporting information). The phylogenetic logistic regression returns a significant

500 association between habitat and body size (AIC = 142.23; coeff = -2.07605; bootstrap CI =

501 -4.93798, -1.043; p = 0.02876). A low value of "a" (0.00508) was estimated for the habitat

502 indicative of a very strong phylogenetic signal.

503

DISCUSSION

505   In sharp contrast to most animal groups (Harmon *et al.* 2010), Dytiscidae body size

506   evolution follows an Early Burst model consistent with an explosion of body sizes during the

507   Early Cretaceous with subsequent high phylogenetic conservatism. This high phylogenetic

508   signal is in agreement with the pattern found for the whole insect phylogeny (Rainford *et al.*

509   2016). Even if the high signal of Early Burst in body size evolution in the relatively old

510   Dytiscidae (ca. 160 Ma) is compatible with the niche-filling model of adaptive radiation

511   (Simpson 1944; Schluter 2000; Harmon *et al.* 2010), we found no evidence for shifts in

512   species diversification in the Dytiscidae phylogeny. This result adds to the body of evidence

513   that adaptive radiation and Early Burst of morphological evolution can be uncoupled.

514

515   A more comprehensive phylogeny could help to detect some diversification shifts but we

516   found no relationship between rates of species diversification and rates of morphological

517   evolution among smaller clades, further supporting the fact that species diversification and

518   body size evolution are uncoupled in Dytiscidae. We found no evidence for impact of body

519   size on species richness or decreased diversity over time by niche filling. Consistently,

520   Rainford *et al.* (2016) found no link between diversification and body size evolution in

521   comparative phylogenies of insects, but in their study no evidence for EB was found.

522   Therefore, uncoupled evolution of species diversification and morphology evolution might be

523   more common than previously thought (e.g. Kozak & Wiens 2016).

524

525   Our results show that the origin of the Hydradephaga group at 211 Ma (256 - 205 Ma)

526   is consistent with the age recovered by Hunt *et al.* (2007), 220 Ma (224- 216 Ma), and

527   Toussaint *et al.* (2017), 237 Ma (258-220) but not with McKenna *et al.* (2015), 184 Ma (208-

528   161 Ma). However, McKenna *et al.*'s (2015) ages are clearly too young for several beetle

529   families based on the available fossil record (see Toussaint *et al.* 2017). For clades within

530   Dytiscidae, Colymbetinae crown group age, 65 Ma (37 – 95 Ma) is in line with Morinière *et*

531   *al.* (2016), 56 Ma (69–45 Ma). The Australian radiation of Sternopriscina however is

532   significantly older, 77 Ma (52 – 100 Ma), than the Miocene or Oligocene origin previously

533   reconstructed by mitochondrial clock rates or single fossil (Leys *et al.* 2003; Toussaint *et al.*

534   2015). An early Oligocene origin of Hydroporinae (half of all Dytiscidae at >2250 species) as

535   found by Toussaint *et al.* (2015) implies in our view exceptional and implausible scenarios of

536   speciation rates and dispersal versus vicariance proportions.

537

538   The ancestral body size of Dytiscidae was estimated as small (5.5mm) with

539   subsequent diversification into larger but also smaller sizes. Eight shifts in body size rates

540   through time were detected indicative of several accelerated periods of morphological

541   evolution. We also inferred the ancestral habitat of Dytiscidae as lentic in contrast to the lotic

542   ancestral habitat of dragonflies estimated by Letsch *et al.* (2016). We identified eighteen

543   transitions from lentic to lotic habitats in Dytiscidae with only two back-colonization to lentic

544   waters suggesting that, once gained, attributes associated with lotic habitat might be difficult

545   to reverse which is consistent with niche conservatism. Ribera (2008) already proposed that

546   reversal to lentic habitat is unlikely since lotic species are more specialized, have more

547   structured populations due to lower dispersal reducing geographical ranges and gene flow.

548

549   We further investigated the link between habitat change in Dytiscidae and changes in

550   body sizes through time, which has been neglected in most studies (but see Hjalmarsson *et al.*

551   2015). We found a relationship between habitat and body size evolution and transitions to

552   lotic habitat could be linked to three main shifts in body size rate change identified by the

22

553 BAMM analysis. They occur at the base of the Agabinae, at the split between Cybistrinae,

554 Laccophilinae and Copelatinae and inside Dytiscinae. The five other shifts in body size could

555 not be linked to direct change of habitat. Concerning the first shift, the group Agabinae is

556 separated into a lotic clade with small species (*Hydrotrupes*, 4 – 4.7 mm; *Platynectes*, 4.8 –

557 9.3 mm; *Agametrus*, 6 – 8 mm) and another clade containing both lotic and lentic species

558 with larger sizes on average (*Agabus*, 5.1 – 13.5 mm; *Ilybiosoma*, 6.9 – 13.2 mm; *Ilybius*, 5.3

559 – 14.5 mm). The second shift separates large lentic Cybistrinae (13 – 47 mm) and smaller

560 Laccophilinae (1.5 – 8.6 mm) and Copelatinae (2.9 – 10 mm) found in both habitats. The

561 third shift separates the large lentic *Dytiscus* (22 – 44 mm) and the comparatively smaller

562 lotic *Hyderodes* (19 – 21 mm). Thus the change in body size inside those subfamilies and

563 genera could be explained by a change to lotic habitat and should be further investigated. We

564 can identify two other clades with a change to lotic habitat, one inside Hydroporina and one

565 at the base of the clade encompassing Siettitina, Deronectina and Sternopriscina, all in the

566 Hydroporinae subfamily, however no change in body size was identified by the BAMM

567 analysis in neither of the two clades, probably because most of the 2237 species in

568 Hydroporinae are small (0.91 - 7.8 mm with a median size around 3 mm).

569

570 Whereas we found that habitat played a role in recent transitions (ca. 70 – 10 Ma) of

571 body size in Dytiscidae, it is not clear which factor could have triggered the Early Burst of

572 body size evolution in the Cretaceous, as most Dytiscidae acquired their body size early on.

573 One explanation is that the appearance of new niches at the beginning of their history

574 triggered sympatric differentiations of sizes between different clades. In the Early

575 Cretaceous, several predatorial aquatic beetle families (dytiscoids) went extinct such as

576 Parahygrobiidae, Liadytidae and Coptoclavidae. In particular, the Coptoclavidae were

577 extremely abundant and broadly widespread large predators (~40 mm) that seems to have

578   played an important role in the Early Cretaceous lake ecosystems (Ponomarenko 1995). The

579   extinction of Coptoclavidae might be explained by the rise of teleost fish in the Cretaceous

580   period (Evans 1982) whereas Dytiscidae may have had better protection against fish using

581   powerful defensive secretions (Dettner 2014). Thus, the extinction of those larger predators

582   would have created empty niches (e.g. availability of larger preys), which might have played

583   a role in the body size diversification of Dytiscidae early in their history.

584

585   At the same time, the role of the appearance of angiosperms on aquatic insect

586   evolution has been overlooked in diving beetle studies since the larvae and adults are

587   carnivorous and thus angiosperms are thought to have played a minor role in their evolution

588   (Dijkstra *et al.* 2014). However, it has been proven that vegetation is crucial for many

589   Dytiscidae by providing oviposition sites and specific habitats but also acting as a refuge

590   against predators and wave action (Gioria 2014 for a review). Thus, we can expect that

591   angiosperm emergence might have played a bigger role in Dytiscidae history than previously

592   thought by creating new niches, a line of inquiry that should be further investigated.

593

594   Finally we found no evidence for difference in net diversification rates between

595   habitats at the level of the whole phylogeny or in sister clades comparisons. However, there is

596   evidence (albeit not strongly supported) for much higher speciation and extinction rates

597   (higher turnover) in lentic species than lotic ones. Letsch *et al.* (2016) recently found

598   evidence for a higher net diversification rate in lentic dragonflies than in lotic ones and a

599   positive correlation between speciation and lentic habitat when taking into account the whole

600   phylogeny. Whereas those results slightly differ from ours, both show higher turnover in

601   lentic species and are thus in contradiction with the Habitat Stability Hypothesis (Southwood

602   1962; Ribeira 2008; Dijkstra *et al.* 2014). The higher turnover in lentic species could

603   however be explained by two phenomena. First, species with larger range sizes are more

604   prone to speciation as their range encompass many different habitats or niches (Rosenzweig

605   1995) and/or because larger areas have a higher probability of dissection by geographical

606   barriers (Price *et al.* 2011). Second, the Habitat Stability Hypothesis is built upon the

607   reasoning that low gene flow promotes speciation whereas high gene flow prevents it.

608   However, recent advance in speciation theory and several empirical examples has led to a

609   paradigm shift toward a speciation with gene flow mechanism (Nosil 2008; Feder *et al.*

610   2012). In this context, larger range sizes in lentic species offer more opportunity for local

611   adaptation. Divergence among populations might only accumulate in few key loci while the

612   rest of the genome is still experiencing gene flow through migration. Population

613   differentiation will still be lower in lentic species using only neutral marker as observed in

614   studies on Dytiscidae. However, local adaptation is more likely in lentic habitats over large

615   geographical ranges and will lead to islands of speciation that in turns could lead to higher

616   speciation over long evolutionary timescale. These results call for a thorough reassessment of

617   the role of gene flow and local adaptation between lotic and lentic species of Dytiscidae.

618

627

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Data Accessibility

FIGURE S1. Majority rule consensus tree from MrBayes with posterior probability supports on nodes.

FIGURE S2. a-c) Most probable credible set of changes in diversification rate regime along the Dytiscidae evolution from the BAMM analysis. f represents the frequency of each scenario in the posterior sample and circles are locations of rate shifts. d) Net diversification rates through time derived from the BAMM analysis for the whole Dytiscidae. Shaded blue areas represent 95% confidence intervals.

FIGURE S3. Most probable credible set of changes in body size rate regime along the Dytiscidae evolution from the BAMM analysis. f represents the frequency of each scenario in the posterior sample. Green circles are locations of rate shifts and their size correspond to the magnitude of the shift. The tree is plotted as a phylorate plot with a color gradient from blue to red corresponding to low to high body size rate change.

FIGURE S4. a) Back-bone topology of the majority rule consensus tree compared to b) the one found by Miller and Bergsten 2014. Nodes on the branches are posterior probability supports.

FIGURE S5 Distribution of p-values that test if an EB model is favoured over a BM model when a) a BM was simulated under different variances and b) when an EB was simulated under different variances and strengths of the EB.

FIGURE S6. Posterior distribution of the correlation coefficient between body size and habitat inferred under the threshold model.

TABLE S1. List of all species sampled in the phylogeny with their id from Miller & Bergsten 2014, corresponding habitat (0 = lentic, 1=lotic, 2= both habitats), genus mean body size, number of species sampled per genus, number of species total per genus, sampling fraction and references used for scoring of habitat.

TABLE S2. Fossils used for calibrating the Dytiscidae phylogeny, with node placement, fossil age and parameter of the prior used for calibrating the molecular clock analysis.

TABLE S3. Results from HiSSe on 44 models. Best-fit model using the Akaike Information Criterion corrected for sample size (AICc), using a ΔAICc of 2 to select the best-fit model(s), number of parameters (n), log likelihood (LnL).

TABLE S4. Species richness for 11 lotic and lentic sister clades used in the sister clade analysis of diversification.

923 T ABLE S5. Results of model fitting for simulated characters under a Brownian motion
924 (upper) and an Early Burst (lower) model subsampled to the number of taxa present in this
925 study. Sigma2 represent the simulated values for the variance of the character and "a" the
926 simulated values of the strength of the Early Burst. Values in the table represent the
927 proportion of times the LRT favours an Early Burst model.
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929 AUTHORS CONTRIBUTIONS
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931 A.D., B.L., J.B. designed the study; J.B., A.D., K.B.M collected the data, B.L. and A.D.
932 performed the analyses, A.D. wrote the first draft of the manuscript, BL contributed to the
933 interpretation of the results and writing of the manuscript and all authors contributed to the
934 manuscript revision.
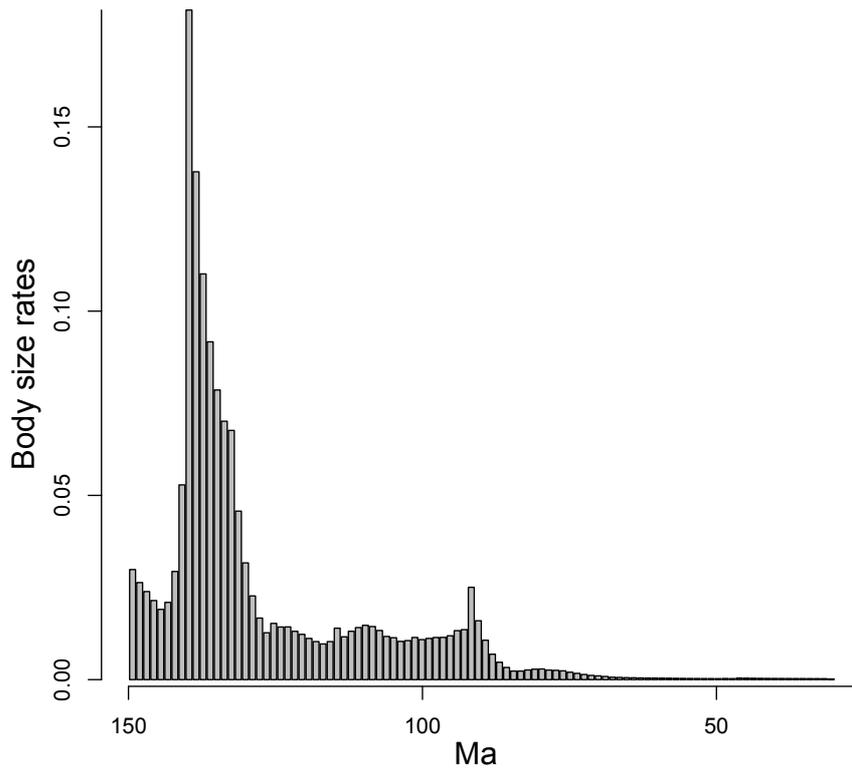935
936 TABLES AND FIGURES

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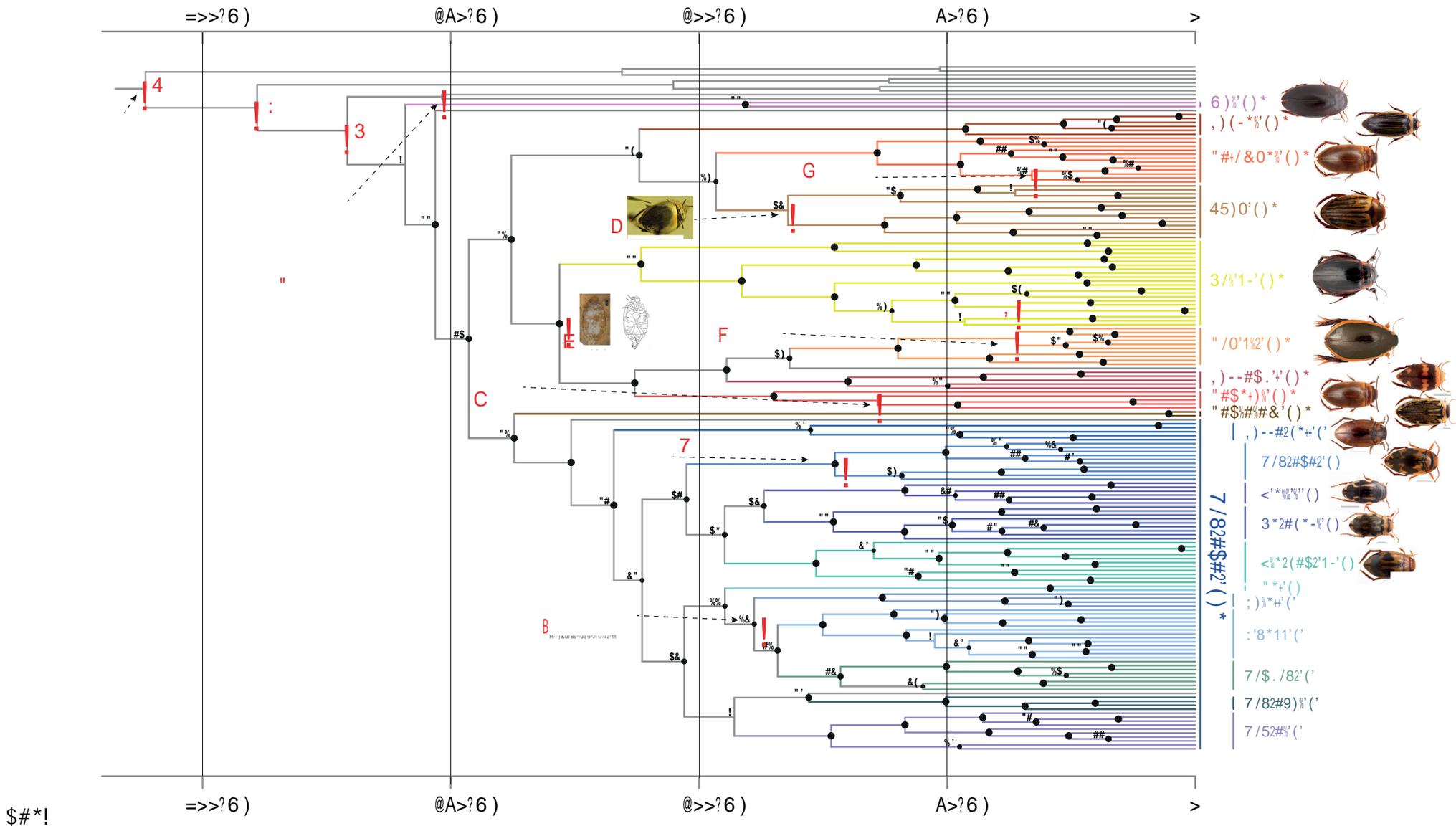
FIGURE 2. a) Ancestral habitat estimation under the HiSSE best-fit model. Bran[...] >90% probabilities are colored in green and orange for lentic and lotic ancestral[...] respectively. Brown branches have <90% probability and grey branches are taxa[...] missing and not included in the reconstruction. Clade A, B and C are indicated.[...] of the mean body size (mm) per genus centered on the overall mean body size a[...] habitat. c) Ancestral body size estimation with branch color following a gradien[...] to orange and thickness proportional to ancestral body size estimation. Stars in l[...] panel are shifts in body size rate regimes detected by BAMM.
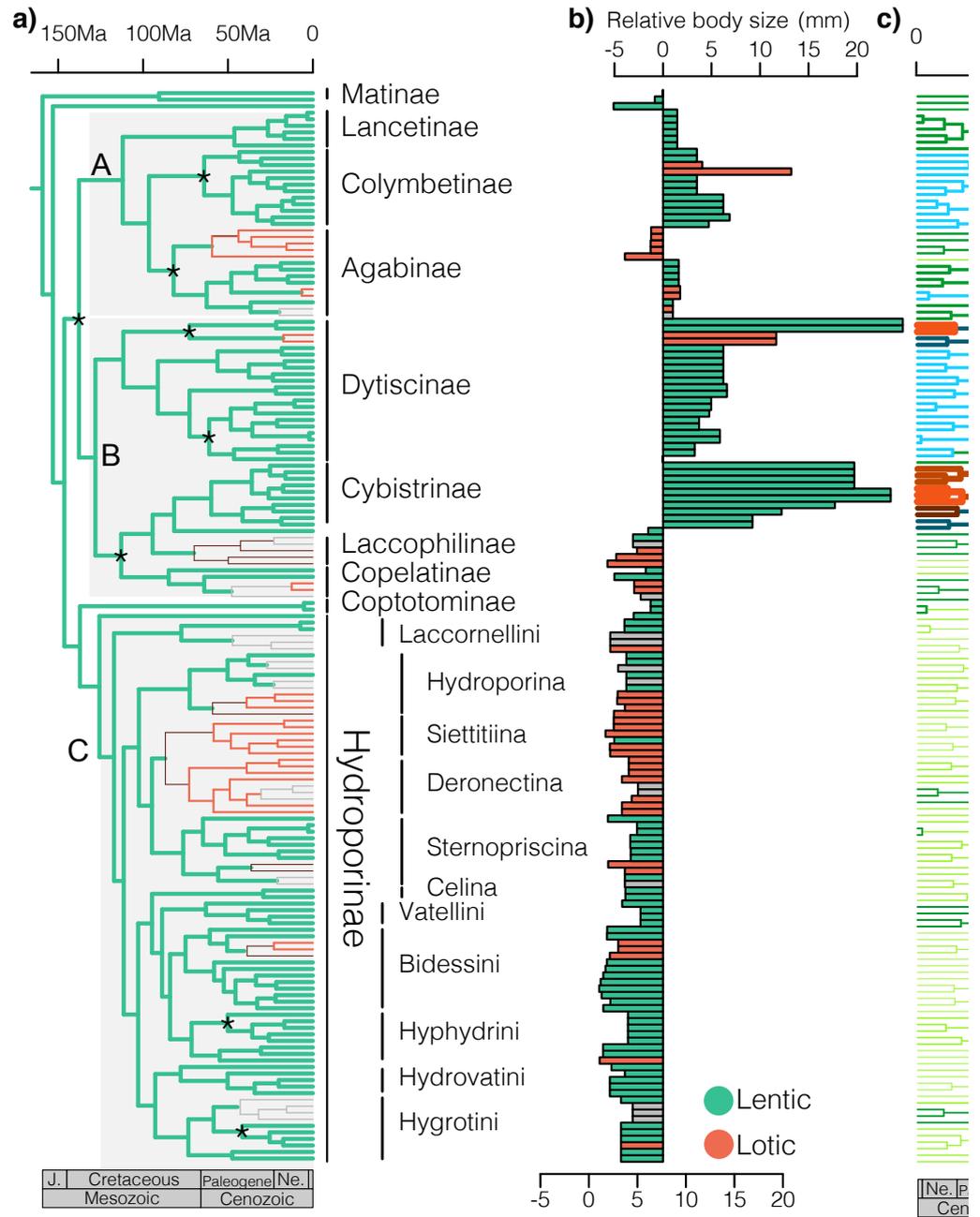
974   F<small>IGURE</small> 3. Body size rates through time (Ma) inferred from BAMM analysis.

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