Early Burst in Body Size Evolution is Uncoupled from Species

Diversification in Diving Beetles (Dytiscidae)

Aurélie Désamoré¹, Benjamin Laenen², Kelly B. Miller³ and Johannes Bergsten¹

1. Swedish Museum of Natural History, Zoology Department, Stockholm, Sweden

2. Stockholm University, Department of Ecology, Environment and Plant Sciences,

Stockholm, Sweden

3. University of New Mexico, Department of Biology and Museum of Southwestern Biology,

Albuquerque, USA

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Corresponding authors: Aurélie Désamoré: Aurelie.desamore@nrm.se and Benjamin Laenen:

Benjamin.laenen@su.se

ABSTRACT

Changes in morphology are often thought to be linked to changes in species diversification, which is expected to leave a signal of Early Burst (EB) in phenotypic traits. However, such signal is rarely recovered in empirical phylogenies, even for groups with well-known adaptive radiation. Using a comprehensive phylogenetic approach in Dytiscidae, which harbors ~4,300 species with as much as 50 fold variation in body size among them, we ask whether pattern of species diversification correlates with morphological evolution. Additionally, we test if the large variation in body size is linked to habitat preference and if the later influences species turnover. We found, in sharp contrast to most animal groups, that Dytiscidae body size evolution follows an Early Burst model with subsequent high phylogenetic conservatism. However, we found no evidence for associated shifts in species diversification, which point to an uncoupled evolution of morphology and species diversification. We recovered the ancestral habitat of Dytiscidae as lentic (standing water), with many transitions to lotic habitat (running water) that are concomitant to a decrease in body size. Finally, we found no evidence for difference in net diversification rates between habitats but evidence for much higher speciation and extinction rates (higher turnover) in lentic species than lotic ones. This result, also recently found for dragonflies, contradicts the current theoretical expectations (Habitat Stability Hypothesis), thus calling for a thorough reassessment of the role of gene flow and local adaptation between lotic and lentic species of Dytiscidae.

Understanding why some groups of organisms are more diverse than others and what links species diversification to ecological pattern are fundamental questions in biology. In particular, changes in morphology are often thought to be linked to changes in species diversification. It is especially the case in adaptive radiations where species diversify morphologically when entering new niches. Thus the rate of morphological evolution is expected to be rapid at the beginning of a radiation and slow down as the niche space is filled up (Simpson 1944; Schluter 2000). This pattern was recovered in several studies of individual clades that are known for their adaptive radiation (e.g. Harmon *et al.* 2003; Jönsson *et al.* 2012; Weir & Mursleen 2013). It has then been implied that clades having experienced adaptive radiation should show a pattern of Early Burst (EB) in phenotypic evolution and rate of diversification. (e.g. Harmon *et al.* 2003; Losos *et al.* 2010). However, Harmon *et al.* (2010), in their meta-analysis, found EB of body size evolution in only 2 of 88 clades of a wide range of animals. Notably, they failed to identify EB as the best model in larger clades well-known for adaptive radiations such as Galapagos finches, *Anolis* lizards or African cichlids. They concluded that Early Burst in morphology evolution might be rare in the tree of life. Moen & Morlon (2014) hypothesized that patterns of morphological diversity resulting from adaptive radiations may be different at larger time scales, thus more difficult to discover, because many adaptive radiations studied yet are found in relatively young clades while the clades examined by Harmon *et al.* (2010) are much older. However, EB was identified at larger scale for mammals (Cooper & Purvis 2010) and for birds (Harmon *et al.* 2010). Benson *et al.* (2014) combining phylogeny and body-size datasets for fossils, also found strong support for EB of evolution in most dinosaur clades. Recently, this pattern was

also found for Gnathostomes using paleontological data (King 2016). These results, taken together, suggest that adaptive radiation also plays a role at larger evolutionary scales.

In this context, insects represents one of the major ecological and evolutionary diversifications on earth, with more than one million species worldwide, occupying nearly all ecological niches and presenting many different morphologies (Grimaldi & Engel 2005; Footit & Adler 2009). Their high diversity has been explained by several hypotheses such as low extinction rates, acquisition of key innovations (e.g. wings, complete metamorphosis) or appearance of new niches (Farrell 1998; Mayhew 2007). Condamine *et al.* (2016), investigating pattern of diversification in insects using molecular and fossils dataset recovered an Early Burst of diversification rates using fossil data at the whole phylogeny level and at family level in Coleoptera (beetles), the most diverse order while Rainford *et al.* (2016), combining diversification and body size evolution in insect phylogeny, found that patterns of size evolution in insects mainly follow a single stationary peak model of evolution (Ornstein-Uhlenbeck, OU). In Coleoptera, Dytiscidae (predaceous diving beetles) are well suited for the study of patterns of diversification and morphological evolution. With more than 4,300 described species they represent one of the largest and most commonly encountered groups of aquatic insects. They occupy various habitat types, varying from running/lotic (e.g. streams, springs, rivers) to standing/lentic (e.g. swamps, ditches, pools, lakes), permanent to ephemeral water bodies and present large variation in body size (from <1 to almost 50 mm) (Miller & Bergsten 2016).

Body size is an important trait when studying adaption to different environment pressures and in particular in lentic and lotic environments, which present major differences in their long-term persistence implying different ecological constraints on species inhabiting

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.

130 Here, we apply a comprehensive phylogenetic approach in Dytiscidae to answer the
131 following questions:

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- 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?

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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 *Psychopompomorus 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 1 α (EF1 α), 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

76bp in RNAPol. Highly variable unalignable regions in 12S and 16S were also excluded resulting in a matrix of 173 taxa and 4972bp. This dataset includes all currently recognized Dytiscidae subfamilies and tribes with the exception of one tribe with two small genera and 49% of all genera are present (Table 1, Table S1 Supporting information). Despite the relatively low percentage of genera, we expect that the sampling captures most of the phenotypic diversity in the family as it is scattered throughout the tree.

Phylogenetic Reconstruction

Phylogenetic tree reconstruction was conducted with MrBayes 3.2.4 (Ronquist *et al.* 2012) on the UPPMAX computational cluster, Uppsala, Sweden. The matrix was divided into 7 partitions analyzed independently under a GTR model plus a gamma distributed rate variation parameter. The first partition consisted of merged mitochondrial ribosomal regions (12S and 16S), the second to fourth of mitochondrial (COI and COII) regions separated into 1st, 2nd and 3rd codon positions and the fifth to seventh of nuclear regions also separated in 1st, 2nd and 3rd codon positions. We did two runs of six chains for 25 million generations sampled every 50000 generations. Convergence of the two runs was assessed by visual inspection of the log likelihood and ensuring ESS value above 200 in Tracer 1.6 (Rambaut *et al.* 2014) and a burnin of 10 % was discarded. We computed a 50% majority rule consensus tree with posterior probabilities (PP) on each node as support (Fig. S1 Supporting information).

Divergence Time Estimation

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

Log likelihood and ensuring ESS value above 200 in Tracer 1.6. The nodes were annotated in TreeAnnotator and this dated tree was used for all analyses described below.

Diversification Analyses

Using the dated tree obtained from BEAST, we tested the presence of species diversification shift through time using Metropolis-coupled Markov-chain Monte Carlo (MC³) in BAMMv.2.5 (Rabosky *et al.* 2013), with relevant priors chosen using BAMMtools (Rabosky *et al.* 2014). Recent criticism has been raised against this approach (Moore *et al.* 2016) regarding the likelihood estimations and accuracy of the diversification rates. However the debate is still going on and Rabosky *et al.* (2017) recently showed that Moore *et al.* (2016) used wrong parameterization and non-biologically realistic simulations. In regards to this, the results of BAMM for estimating diversification shifts must be taken with caution but those issues are of less concern for phenotypic estimates.

Speciation and extinction were inferred using the ‘speciation–extinction’ module, with correction for differential sampling across genera (Table S1 Supporting information). The sampling fraction of each genus was determined by comparing the number sampled with the total number in the 2015 World Catalogue of Dytiscidae (Nilsson 2001, 2015). The analysis ran for 50 million generations using four chains and sampled every 5,000 generations. We assessed the convergence of all parameters by visual inspection of the Log likelihood and ensured ESS value above 200 in Tracer 1.6 and a 10% burnin was consequently removed. BAMMtools was used for summary statistics and Bayes Factors (BF) for each model considered. Posterior probability of change in macroevolutionary regime was evaluated using the most probable credible set of changes. The best shift configuration was estimated and net diversification rate through time was extracted from the results (Fig. S2

Supporting information).

Body Size Evolution and Ancestral Estimation

Data.

The minimum and maximum body size measures (mm) per species was taken from the database of the World Catalogue of Dytiscidae 2015, which comprises all recognized species with up-to-date classification, zoogeographic distributions and species numbers (Nilsson 2001, 2015). As the mean body size per species was not available, we estimated it by using half the sum of minimum and maximum body size per species and then averaged over genus (genus mean body size). We then used the logarithm of the genus mean body size in the analyses (Table S1 Supporting information).

Phylogenetic signal.

We tested for the presence of phylogenetic signal in body size using Pagel's λ (Pagel 1999) and Blomberg's K (Blomberg *et al.* 2003) implemented in the phytools package (Revell 2012) in R 3.2.2. Pagel's λ significance was estimated using likelihood ratio test (LRT) and Blomberg's K with 1000 permutations. Those two metrics assume a Brownian motion model of character state evolution as a reference for estimating the phylogenetic signal. Pagel's λ is equal to 0 when the character states evolve independently of the phylogeny, whereas a value of 1 indicates that the character states evolve according to Brownian motion. Values of Blomberg's K lower than 1 indicate that related species resemble each other less than expected under Brownian motion, whereas values greater than 1 mean that related species are more similar for the character state under study than predicted by this model.

Models of evolution.

We fitted models of body size evolution to the phylogenetic tree contrasting Brownian motion (neutral evolution), Early Burst, Ornstein-Uhlenbeck and Pagel's λ and δ using the Akaike information criterion in the Geiger package (Harmon *et al.* 2008) using the FitContinuous function in R 3.2.2. The Brownian motion model (BM) (Felsenstein 1973) can be assumed as the neutral model of character evolution with a constant rate through time leading to a correlation between character values and shared ancestry for pairs of species. The other models can be considered as variants of the BM. The Ornstein-Uhlenbeck model (OU) (Butler and King 2004) also assumes a constant rate through time but it adds a tendency towards central values of the characters resulting in a distribution around this value. This model can represent ecological optimum of body size where deviation from the niche optimum decreases fitness and body size has a tendency to remain close to this optimum. The Early Burst model (EB) (Harmon *et al.* 2010), also called the ACDC model (accelerating-decelerating; Blomberg *et al.* 2003), allows for variation of rates through time with either an exponential decrease or increase. A decreasing model can represent a situation where most of the morphological transition, in the case of body size, occurs early in the evolution of the group followed by a rapid decrease in body size rates along the following branches. We also fitted Pagel's λ that measures the relative importance of the phylogeny to explain the character distribution. The last model tested is Pagel's δ , which is similar to an Early Burst model when values of δ are below 1 and indicates recent rapid evolution when above 1.

In order to test if the sampling can affect the false discovery rate and the power to detect an Early Burst in body size, we conducted simulations. For each simulation, we simulated a complete tree (4303 species) under a pure-birth model with a speciation rate of

0.04 to achieve a topology and age close to the observed data. Each tree was scaled so that the root corresponds to the observed root (159.2 Ma). We then simulated a continuous character on this phylogeny under a Brownian motion and under an Early Burst model. We sampled 168 tips from the phylogeny and retrieved the associated value for the character under the two models. On each character, we tested the fit of BM and EB using `fitContinuous` function in "geiger" and report the p-value from the likelihood ratio test between EB and BM. We wanted to test if a reduced sampling can induce a bias toward the detection of an EB (false positive), when the true model is BM, and to assess how much power do we have to detect a signal of EB with reduced sampling when it is present.

We varied the parameters for the trait simulation in two different types of simulations. First, we simulated a trait 100 times for both BM and EB models using the inferred value from the observed data for σ^2 (0.027641), which represent the variance of the trait. For the EB model, we simulated the trait using also the inferred parameter "a" (-0.44424), which is the rate modifier in the Early Burst, using the following equation $r[t] = r[0] * \exp(a * t)$. A negative value of "a" means that rates are decelerating through times and the higher the value of "a", the more abrupt is the change close to the root of the tree indicative of a stronger Early Burst. Second, we modeled a range of value for σ^2 , from low to high variance (0.1, 1, 10) and, only for the EB model, a range of values for "a" (-0.1, -0.5, -1) from a smooth decrease in rates to an abrupt change. For each value in those ranges, we simulated 100 datasets (tree + trait) under each model. We report the distribution of p-values and the proportion of times an EB model is preferred over BM at 0.05 significance level of the LRT.

Ancestral estimation.

Body size evolution was inferred using the continuous 'trait' module in BAMM. This analysis reached convergence less readily and thus ran for 200 million generations using

eight chains sampled every 500,000 steps. A burnin of 55% was removed after inspection in Tracer 1.6. The ancestral body size estimation was also implemented in BAMM by adding the two parameters, nodeStateOutfile and nodeStateWriteFreq. BAMMtools was used to determine the posterior probability of change in macroevolutionary regime summarized in the best shift configuration (Fig. 2) and most probable credible set of changes (Fig.S3 Supporting information) and rates of body size change through time were extracted from the results (Fig. 3). We obtained a tree with reconstructed body sizes at each sampled iteration of the MC³. The output was summarized using tree annotator to obtain the median ancestral body size and 95%HPD on each node, which was plotted on the BEAST tree (Fig. 2c) using a custom Rscript.

Correlation with species diversification.

We tested for a correlation between body size evolution and species richness using linear regression and phylogenetic general least square (PGLS) analysis following Kozak & Wiens (2016). We defined 20 clades representing major lineages of Dytiscidae (Table 1, Fig. 1). We extracted body size rate for each clade from the BAMM output. Species richness and clade crown age were used to derive clade-specific diversification rates using Magallon & Sanderson (2001) estimator with low ($\epsilon = 0$) and high extinction fraction ($\epsilon = 0.9$). We first tested three models with either the logarithm of species richness or diversification rates as dependent variable and body size rates as predictor using the “lm” function in R and PGLS in the “caper” Rpackages.

We also estimated Pybus & Harvey’s γ statistic (2000) and associated p-value (Table 1) considering unsampled species using simulations, where we built a null distribution of γ by simulating 10000 trees totaling all species under a pure birth model and pruned taxa to match

the number of taxa sampled in our tree. The γ statistic can be an indicator of diversity-dependent evolution and can be contrasted to morphological rates to test a “niche filling” hypothesis with slowdown in species diversification associated with lower morphological changes. We tested this hypothesis using γ as dependent variable and body size rates using “lm” and PGLS as above.

Ancestral Habitat Estimation and Correlates

Data

We scored the habitat of each species based on their genus habitat (Balke 2005) as discrete character states: lentic (standing water), lotic (running water) or present in both habitats. The genus *Hydrotrupes* present in Hygropetric habitat (i.e. water on a vertical surface, such as waterfalls, seeps) and the monotypic genus *Haideoporus* found in springs aquifers in Texas, considered as stygobiont (i.e. in groundwaters of aquifer), were both scored as lotic. For the 43 species for which the genus is found in both habitats, we consulted specialized literature and regional atlases to refine in which habitat the species was found and we obtained only 13 species found in both habitats or for which information was not available and thus were kept scored as found in both habitats (see references in Table S1 Supporting information). Thus, of the 164 sampled species, 109 are lentic, 42 lotic and 13 in both habitats. Those 13 species were removed from the HiSSE analysis as it only takes binary characters into account. Our sampling covers 5.2% and 7.8% of all current lentic and lotic species respectively, and is thus not biased towards one state.

We used Pagel’s λ , suitable for discrete traits, to test for phylogenetic signal in habitat. We modeled trait evolution with a suite of hiSSE models to first test the hypothesis that lotic diving beetles would have a higher net diversification rate than lentic (as proposed

by the Habitat Stability Hypothesis; Ribera 2008) and second, estimate the ancestral states of habitat along the phylogeny.

Trait evolution is often modeled using a “mk” model with transition rates between states. However, as Maddison *et al.* (2007) pointed out, characters are not independent from the diversification pattern and thus character and diversification should be modeled together. This led to a family of xSSE models of character-associated diversification. These have been strongly criticized recently because of the very high type one error and inadequacy of the null model used (Rabosky & Goldberg 2015; Maddison & FitzJohn 2015). Beaulieu and O’Meara (2015) proposed a new model using a hidden character that represented an unknown associated character and also proposed a new null model.

We fitted 44 models (Table S3 Supporting information) and selected the 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) following the approach of Laenen *et al.* (2016). Since our sampling is incomplete, we used a global sampling fraction per states to account for unsampled species. After selection of the best-fit model, we estimated the confidence interval of each parameter, species turnover (τ), extinction fraction (ε) and transition rate (q), by sampling parameter values ensuring that the difference of log-likelihood < 2 as compared to the best-fit model as implemented in the ‘SupportRegion’ function. τ and ε are transformed to speciation and extinction rates using Eqn 5(a,b) from Beaulieu & O’Meara (2015). We also report the results as speciation (λ), extinction (μ) and net diversification rates ($\lambda - \mu$) to allow comparison with others studies. Ancestral habitat was estimated under the best-fit model and branches with a probability of $> 90\%$ are reported on Fig. 2a.

We also used sister clade analysis following Kaefer & Mousset (2014a) to test if lentic and lotic sister clades differ in species richness and therefore diversification rates. We visually defined 11 sister clades (Table S4 Supporting information) according to the ancestral estimation of habitat. We performed 100,000 resampling iterations as implemented in the Kaefer & Mousset (2014b) Rscript.

Test for Phylogenetic Correlation Between Body Size and Habitat

We tested for phylogenetic correlation between body size and habitat in two ways, using the Felsenstein threshold model (Felsenstein 2012) and phylogenetic logistic regression (Ives & Garland 2010). The threshold model allows the direct comparison of continuous and discrete characters by fitting a continuous character along the tree, called liability, for each character. The liability represents a continuous character, e.g. amount of genetic drift, underlying the evolution of the real character. When the liability pass a certain threshold then the character state changes. The threshold and liability is estimated using MCMC sampling as implemented in the function ThreshBayes (Revell 2014) in the R package phytools. We also tested whether habitat and body size evolved in a correlated way using Ives & Garland's model (2010) suitable for binary dependent variables. The model was run using the "phyloglm" function in the Rpackage "phylolm" (Ho & Ane 2014) with 2000 bootstrap replicates. Phylogenetic signal is also estimated by the parameter "a" that is related to the transition rates between states 0 and 1. As the transitions rates increases, "a" increases and the phylogenetic signal is lost.

RESULTS

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 crown group 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 MC³ 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

respectively (Supplementary Fig. S2 a, b, c). Those shifts could represent high extinction in Matinae and probably driven by their relatively long branch but do not point to increase in diversification regime in clade ABC. Using Bayes Factors, we found that models with diversification rate shifts were not supported compared to the null model with BF lower than one. Net diversification rate through time show a flat trend with a mean of 0.04 sp/My (Supplementary Fig. S2 d).

Body Size Evolution and Ancestral Estimation

There was a strong phylogenetic signal in body size with either Blomberg's K ($K = 3.7230$, $p = 0.001$) or Pagel's λ ($\lambda = 1.01329$, $p < 0.0001$). Dytiscidae body size evolution shows very strong support for the Early Burst model of character evolution with AICc weight > 0.99 compared to all other models (Table 2). This result was supported by simulated complete phylogenies subsampled to equal the number of species present in our phylogeny. Indeed, we found that an Early Burst model was favoured in only 1.7% of the simulations when a Brownian motion was the true model, indicative of low Type I error. However, the power to detect an Early Burst was very high as 100% of the simulations show significance for an Early Burst over a BM when the former was the true model. This result holds when we simulated under different values for the variance of the character and values for the strength of the Early Burst. This indicates that our taxon sampling should be sufficient and robust against false positive model selection (Table S5 and Fig. S5 Supporting information).

The ancestral body size is estimated to be small (5.53mm) with subsequent derivations of larger but also shifts to smaller sizes (Fig. 2c) and eight major transitions of body size rate change through time were detected in the best shift configuration. The first shift to larger body size was detected on the clade AB at 139 Ma (125 - 155 Ma). Inside clade

AB, we recovered a shift to larger and smaller body size respectively at the root of the families Colymbetinae and Agabinae at 65 Ma (37 - 95 Ma) and 83 Ma (53 - 112 Ma) and two shifts inside the clade of Dytiscinae: to larger size for *Dytiscus-Hyderodes* at 74 Ma (35 - 111Ma) and to smaller size for another clade at 62 Ma (43 - 83 Ma). There was a shift to smaller size at the root of the clade grouping Laccophilinae, Copelatinae and Cybistrinae at 113 Ma (89 - 135 Ma). Two shifts to larger sizes were identified inside the clade C, one inside the tribe Hyphydrini 51Ma (29 - 76 Ma) and one inside the tribe Hygrotini (*Coelambus*) at 43 Ma (24 - 62 Ma).

We found no support for an association between body size rate change and species richness or species diversification (Table 3) with either linear models or phylogenetically corrected models (PGLS). Models testing the association of slowdown in species diversification (γ) and body size rate change were also non-significant giving no support for the “niche filling” hypothesis (Table 3).

Ancestral Habitat Estimation and Correlates

A strong phylogenetic signal was recovered for habitat preference using Pagel’s λ ($\lambda = 1.013$, $p < 0.001$).

The model 3, which is a BiSSE model with equal transition rate between lotic and lentic, was the best-fitting model with a likelihood value of -783.17 and an AICc weight of 0.17. The two following models were model 1, which is the full BiSSE model and model 6 which is the BiSSE null model with equal extinction fraction and transition rates, which have respectively likelihood values and AICc weights of -782.4, 0.12 and -784.66, 0.11. There was no significant difference in net diversification rates between lentic and lotic species.

However, there were much higher speciation and extinction rates in lentic species than lotic species, which resulted in a higher turnover (Table 4).

The ancestral habitat estimation resolved the ancestor of Dytiscidae as lentic, with eighteen more recent colonizations to lotic habitat from the end of Cretaceous to the Neogene. There were only two reconstructions of back-colonization from lotic to lentic habitat, *Porhydrus* (Siettitina, Hydroporinae) and *Nebrioporus* (Deronectina, Hydroporinae), the latter found in both lotic and lentic waters (Fig. 2a). The number of transitions estimated here using genera as tips represent major transitions but likely underestimate the real number of events as variation in habitat preference is found within many genera.

No significant difference in species richness between lentic and lotic sister clades using sister clade comparison was found ($scc_{stat} = 0.36$, $p\text{-value} = 0.69$), which also suggest that net diversification rates do not differ significantly between the two habitats.

Test for Phylogenetic Correlation between Body Size and Habitat

Assessing the relationship under the threshold model gives a mean correlation coefficient of -0.125 between habitat and body size liability. The posterior distribution of correlation coefficient however encompasses 0 and is thus not statistically significant (Fig. S6 Supporting information). The phylogenetic logistic regression returns a significant association between habitat and body size ($AIC = 142.23$; $coeff = -2.07605$; bootstrap CI = -4.93798, -1.043; $p = 0.02876$). A low value of “a” (0.00508) was estimated for the habitat indicative of a very strong phylogenetic signal.

DISCUSSION

In sharp contrast to most animal groups (Harmon *et al.* 2010), Dytiscidae body size evolution follows an Early Burst model consistent with an explosion of body sizes during the Early Cretaceous with subsequent high phylogenetic conservatism. This high phylogenetic signal is in agreement with the pattern found for the whole insect phylogeny (Rainford *et al.* 2016). Even if the high signal of Early Burst in body size evolution in the relatively old Dytiscidae (ca. 160 Ma) is compatible with the niche-filling model of adaptive radiation (Simpson 1944; Schluter 2000; Harmon *et al.* 2010), we found no evidence for shifts in species diversification in the Dytiscidae phylogeny. This result adds to the body of evidence that adaptive radiation and Early Burst of morphological evolution can be uncoupled.

A more comprehensive phylogeny could help to detect some diversification shifts but we found no relationship between rates of species diversification and rates of morphological evolution among smaller clades, further supporting the fact that species diversification and body size evolution are uncoupled in Dytiscidae. We found no evidence for impact of body size on species richness or decreased diversity over time by niche filling. Consistently, Rainford *et al.* (2016) found no link between diversification and body size evolution in comparative phylogenies of insects, but in their study no evidence for EB was found. Therefore, uncoupled evolution of species diversification and morphology evolution might be more common than previously thought (e.g. Kozak & Wiens 2016).

Our results show that the origin of the Hydradeephaga group at 211 Ma (256 - 205 Ma) is consistent with the age recovered by Hunt *et al.* (2007), 220 Ma (224- 216 Ma), and Toussaint *et al.* (2017), 237 Ma (258-220) but not with McKenna *et al.* (2015), 184 Ma (208-

161 Ma). However, McKenna *et al.*'s (2015) ages are clearly too young for several beetle families based on the available fossil record (see Toussaint *et al.* 2017). For clades within Dytiscidae, Colymbetinae crown group age, 65 Ma (37 – 95 Ma) is in line with Morinière *et al.* (2016), 56 Ma (69–45 Ma). The Australian radiation of Sternopriscina however is significantly older, 77 Ma (52 – 100 Ma), than the Miocene or Oligocene origin previously reconstructed by mitochondrial clock rates or single fossil (Leys *et al.* 2003; Toussaint *et al.* 2015). An early Oligocene origin of Hydroporinae (half of all Dytiscidae at >2250 species) as found by Toussaint *et al.* (2015) implies in our view exceptional and implausible scenarios of speciation rates and dispersal versus vicariance proportions.

The ancestral body size of Dytiscidae was estimated as small (5.5mm) with subsequent diversification into larger but also smaller sizes. Eight shifts in body size rates through time were detected indicative of several accelerated periods of morphological evolution. We also inferred the ancestral habitat of Dytiscidae as lentic in contrast to the lotic ancestral habitat of dragonflies estimated by Letsch *et al.* (2016). We identified eighteen transitions from lentic to lotic habitats in Dytiscidae with only two back-colonization to lentic waters suggesting that, once gained, attributes associated with lotic habitat might be difficult to reverse which is consistent with niche conservatism. Ribera (2008) already proposed that reversal to lentic habitat is unlikely since lotic species are more specialized, have more structured populations due to lower dispersal reducing geographical ranges and gene flow.

We further investigated the link between habitat change in Dytiscidae and changes in body sizes through time, which has been neglected in most studies (but see Hjalmarsson *et al.* 2015). We found a relationship between habitat and body size evolution and transitions to lotic habitat could be linked to three main shifts in body size rate change identified by the

BAMM analysis. They occur at the base of the Agabinae, at the split between Cybistrinae, Laccophilinae and Copelatinae and inside Dytiscinae. The five other shifts in body size could not be linked to direct change of habitat. Concerning the first shift, the group Agabinae is separated into a lotic clade with small species (*Hydrotrupes*, 4 – 4.7 mm; *Platynectes*, 4.8 – 9.3 mm; *Agametrus*, 6 – 8 mm) and another clade containing both lotic and lentic species with larger sizes on average (*Agabus*, 5.1 – 13.5 mm; *Ilybiosoma*, 6.9 – 13.2 mm; *Ilybius*, 5.3 – 14.5 mm). The second shift separates large lentic Cybistrinae (13 – 47 mm) and smaller Laccophilinae (1.5 – 8.6 mm) and Copelatinae (2.9 – 10 mm) found in both habitats. The third shift separates the large lentic *Dytiscus* (22 – 44 mm) and the comparatively smaller lotic *Hyderodes* (19 – 21 mm). Thus the change in body size inside those subfamilies and genera could be explained by a change to lotic habitat and should be further investigated. We can identify two other clades with a change to lotic habitat, one inside Hydroporina and one at the base of the clade encompassing Siettitina, Deronectina and Sternopriscina, all in the Hydroporinae subfamily, however no change in body size was identified by the BAMM analysis in neither of the two clades, probably because most of the 2237 species in Hydroporinae are small (0.91 - 7.8 mm with a median size around 3 mm).

Whereas we found that habitat played a role in recent transitions (ca. 70 – 10 Ma) of body size in Dytiscidae, it is not clear which factor could have triggered the Early Burst of body size evolution in the Cretaceous, as most Dytiscidae acquired their body size early on. One explanation is that the appearance of new niches at the beginning of their history triggered sympatric differentiations of sizes between different clades. In the Early Cretaceous, several predatorial aquatic beetle families (dytiscoids) went extinct such as Parahydrobiidae, Liadytidae and Coptoclavidae. In particular, the Coptoclavidae were extremely abundant and broadly widespread large predators (~40 mm) that seems to have

played an important role in the Early Cretaceous lake ecosystems (Ponomarenko 1995). The extinction of Coptoclavidae might be explained by the rise of teleost fish in the Cretaceous period (Evans 1982) whereas Dytiscidae may have had better protection against fish using powerful defensive secretions (Dettner 2014). Thus, the extinction of those larger predators would have created empty niches (e.g. availability of larger preys), which might have played a role in the body size diversification of Dytiscidae early in their history.

At the same time, the role of the appearance of angiosperms on aquatic insect evolution has been overlooked in diving beetle studies since the larvae and adults are carnivorous and thus angiosperms are thought to have played a minor role in their evolution (Dijkstra *et al.* 2014). However, it has been proven that vegetation is crucial for many Dytiscidae by providing oviposition sites and specific habitats but also acting as a refuge against predators and wave action (Gioria 2014 for a review). Thus, we can expect that angiosperm emergence might have played a bigger role in Dytiscidae history than previously thought by creating new niches, a line of inquiry that should be further investigated.

Finally we found no evidence for difference in net diversification rates between habitats at the level of the whole phylogeny or in sister clades comparisons. However, there is evidence (albeit not strongly supported) for much higher speciation and extinction rates (higher turnover) in lentic species than lotic ones. Letsch *et al.* (2016) recently found evidence for a higher net diversification rate in lentic dragonflies than in lotic ones and a positive correlation between speciation and lentic habitat when taking into account the whole phylogeny. Whereas those results slightly differ from ours, both show higher turnover in lentic species and are thus in contradiction with the Habitat Stability Hypothesis (Southwood 1962; Ribeiro 2008; Dijkstra *et al.* 2014). The higher turnover in lentic species could

however be explained by two phenomena. First, species with larger range sizes are more prone to speciation as their range encompass many different habitats or niches (Rosenzweig 1995) and/or because larger areas have a higher probability of dissection by geographical barriers (Price *et al.* 2011). Second, the Habitat Stability Hypothesis is built upon the reasoning that low gene flow promotes speciation whereas high gene flow prevents it. However, recent advance in speciation theory and several empirical examples has led to a paradigm shift toward a speciation with gene flow mechanism (Nosil 2008; Feder *et al.* 2012). In this context, larger range sizes in lentic species offer more opportunity for local adaptation. Divergence among populations might only accumulate in few key loci while the rest of the genome is still experiencing gene flow through migration. Population differentiation will still be lower in lentic species using only neutral marker as observed in studies on Dytiscidae. However, local adaptation is more likely in lentic habitats over large geographical ranges and will lead to islands of speciation that in turns could lead to higher speciation over long evolutionary timescale. These results call for a thorough reassessment of the role of gene flow and local adaptation between lotic and lentic species of Dytiscidae.

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Data Accessibility

Additional supporting information may be found in the online version of this article.
The dated tree from the Beast analysis is available on Figshare under accession XXX.

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=lentic, 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 Δ AICc 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.

TABLE S5. Results of model fitting for simulated characters under a Brownian motion (upper) and an Early Burst (lower) model subsampled to the number of taxa present in this study. Sigma² represent the simulated values for the variance of the character and “a” the simulated values of the strength of the Early Burst. Values in the table represent the proportion of times the LRT favours an Early Burst model.

AUTHORS CONTRIBUTIONS

A.D., B.L., J.B. designed the study; J.B., A.D., K.B.M collected the data, B.L. and A.D. performed the analyses, A.D. wrote the first draft of the manuscript, BL contributed to the interpretation of the results and writing of the manuscript and all authors contributed to the manuscript revision.

TABLES AND FIGURES

937 **TABLE 1.** Information on the number of species sampled (Sp.), the total number of species in the clade (Clade size), number of sampled genera
938 per clade (Gen. nb.), percentage of genera sampled per clade (gen (%)), stem and crown clade age with 95% confidence interval (CI 95), net
939 diversification rates with two extinction fractions (ϵ), mean body size rates derived from the BAMM analysis with its standard deviation (SD)
940 and γ statistic for the major clades in the Dytiscidae phylogeny. All p-value associated with γ statistic were non significant. Dash represents data
941 not used or that could not be computed. The 20 major clades used in the analyses are represented in bold.

(Sub)family	(Sub)tribe	Sp.	Clade size	Gen. nb.	Gen. (%)	Crown age Ma (CI 95)	Stem age Ma (CI 95)	Div. rate ($\epsilon = 0$)	Div. rate ($\epsilon = 0.9$)	Body size rate (SD)	γ
Dytiscidae		168	4303	87	49	159.19 (141.5-179.1)	170.8 (155.7-187.7)	0.0482	0.0378	-	-5.56
Matinae		2	9	2	100	90.63 (34.6-160.1)	159.19 (141.5-179.1)	0.0166	0.0059	0.00243 (0.00493)	-
Lancetinae		6	22	1	100	47.24 (19.4-88.4)	113 (75.5-127.8)	0.0508	0.0228	0.00011 (0.00356)	0.07
Colymbetinae		12	137	6	75	65.05 (37-95.4)	97.55 (65.9-127.8)	0.0650	0.0404	0.00208 (0.00482)	-1.94
Agabinae		14	409	5	45	83.03 (53.1-112.3)	97.55 (65.9-127.8)	0.0641	0.0443	0.00757 (0.00667)	-1.36
Dytiscinae		22	244	10	83	112.67 (84-135.4)	129.08 (122-141.4)	0.0426	0.0282	0.00355 (0.00857)	-1.14
Cybistrinae		10	128	5	71	60.87 (37.4-81.5)	82.72 (59.3-110)	0.0683	0.0422	0.00032 (0.00114)	-1.71
Laccophilinae		5	416	5	38	70.94 (44.7-98.3)	95.41 (71.6-120.6)	0.0752	0.0521	0.00076 (0.00071)	-1.1
Copelatinae		5	692	4	50	85.91 (58-114.2)	113.92 (88.8-135.3)	0.0681	0.0489	0.00231 (0.00278)	-0.53
Coptotominae		2	5	1	100	6.3 (1.31-18.7)	138.21 (118.8-158.7)	0.1454	0.0442	0.00222 (0.00554)	-
Hydroporinae		84	2237	47	41	126.73 (105.9-148)	138.21 (118.8-158.7)	0.0554	0.0423	-	-
Hydroporinae	Laccornellini	5	37	2	100	78.49 (34.9-122.3)	118.14 (97.8-139.7)	0.0372	0.0188	0.00379 (0.00796)	0.3
Hydroporinae	Hydroporina	10	282	6	75	73.57 (46.6-99.7)	103.48 (83.1-123)	0.0673	0.0451	0.00018 (0.00007)	-2.09
Hydroporinae	Siettitina	6	52	5	50	59.48 (33.8-83.3)	87.9 (65.6-106.5)	0.0548	0.0295	0.00015 (0.00004)	-1.08
Hydroporinae	Deronectina	9	191	4	50	73.89 (55-95.1)	87.9 (65.6-106.5)	0.0617	0.0398	0.00015 (0.00003)	-1.46
Hydroporinae	Sternopriscina	11	149	7	64	77.39 (51.9-100.3)	95.78 (76.1-116.7)	0.0557	0.0350	0.00029 (0.00249)	-1.27
Hydroporinae	Celina	2	34	1	100	30.21 (7.36-62.6)	95.75 (77.2-115.8)	0.0938	0.0466	0.00028 (0.00053)	-
Hydroporinae	Vatellini	4	57	2	100	64.02 (38.7-90.5)	89.84 (71.1-109.4)	0.0523	0.0287	0.00019 (0.00011)	-0.68
Hydroporinae	Bidessini	13	676	10	22	76.03 (57.3-95.8)	85.13 (67.5-104.4)	0.0766	0.0549	0.00020 (0.00098)	-2.69
Hydroporinae	Emphidromini	8	371	3	21	72.45 (50.4-93.7)	85.13 (67.5-104.4)	0.0721	0.0405	0.00172 (0.00628)	-1.48

943 TABLE 2. Log-likelihood, with associated AICc and akaike weights, for the diffi
 944 of body size evolution.
 945

	K	LogLik	AICc	AICc_weight
Early burst	3	187.23	-368.31	1
δ	3	156.88	-307.60	6.56×10^{-14}
Brownian motion	2	141.43	-278.79	3.64×10^{-20}
Ornstein– Uhlenbeck	3	141.43	-276.72	1.29×10^{-20}
λ	3	156.33	-306.52	3.82×10^{-14}

946
 947 TABLE 3. Results of the linear regression and PGLS models contrasting body si
 948 diversification rates, clades size, clade age and γ statistic for the 20 major clades
 949 Dytiscidae defined on Fig. 1. R^2 and p-values are reported for each model.
 950

	R^2	p-value
Linear regression		
$\ln(\text{species}) \sim \text{body size rate}$	0.004	0.803
Diversification rates ($\epsilon = 0$) $\sim$ body size rate	0.017	0.584
Diversification rates ($\epsilon = 0.9$) $\sim$ body size rate	0.024	0.511
$\gamma \sim \text{body size rate}$	0.030	0.507
PGLS		
$\ln(\text{species}) \sim \text{body size rate}$	0.073	0.293
Diversification rates ($\epsilon = 0$) $\sim$ body size rate	0.039	0.446
Diversification rates ($\epsilon = 0.9$) $\sim$ body size rate	0.001	0.899
$\gamma \sim \text{body size rate}$	0.019	0.597

951

952 TABLE 4. Parameter estimates, with 95% confidence intervals, under the best-fit model of
 953 HiSSE analysis for lentic and lotic Dytiscidae species. Parameters are speciation rates (λ),
 954 extinction rates (μ), net diversification rates ($\lambda - \mu$), turnover (τ), extinction fraction (ϵ) and
 955 transition rates between lentic and lotic state.

	Lentic	Lotic
λ	0.180 (0.147 - 0.240)	0.077 (0.064 - 0.097)
μ	0.146 (0.109 - 0.206)	0.031 (0.022 - 0.056)
Net div. ($\lambda - \mu$)	0.036 (0.030 - 0.042)	0.045 (0.038 - 0.055)
τ ($\lambda + \mu$)	0.326 (0.256 - 0.446)	0.108 (0.085 - 0.153)
ϵ (λ / μ)	0.793 (0.710 - 0.863)	0.386 (0.307 - 0.564)
Transition rates	0.002 (0.001 - 0.002)	0.002 (0.001 - 0.002)

956 **FIGURE 1.** Majority rule consensus tree of Dytiscidae dated using a relaxed molecular clock. Black dots correspond to 100% posterior
957 probability support, 50 to 99% supports are indicated on the node and nodes with no black dots have <50% support. Black stars indicate
958 randomly splitted polytomies. The 20 clades presented in Table 1 are colored and illustrated by one representative species on the right side.
959 (Photos reproduced from Miller & Bergsten, 2016 with permission). Red stars and capital letters represent the position of the twelve fossil
960 calibration points and illustrations. (Fossil images and photos have been reproduced here with permission from copyright holders, for references
961 of each image see Table S2 Supporting information.)

963 **FIGURE 2.** a) Ancestral habitat estimation under the HiSSE best-fit model. Bran-
 964 >90% probabilities are colored in green and orange for lentic and lotic ancestral
 965 respectively. Brown branches have <90% probability and grey branches are taxa
 966 missing and not included in the reconstruction. Clade A, B and C are indicated.
 967 of the mean body size (mm) per genus centered on the overall mean body size a
 968 habitat. c) Ancestral body size estimation with branch color following a gradien-
 969 to orange and thickness proportional to ancestral body size estimation. Stars in l-
 970 panel are shifts in body size rate regimes detected by BAMM.
 971

974 **FIGURE 3.** Body size rates through time (Ma) inferred from BAMM analysis.

975