**Habitat preference and diversification rates in a speciose lineage of diving beetles**

Running title: Habitat type does not affect diversification

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**Abstract**

The long-term geological stability of aquatic habitats has been demonstrated to be a determinant in the evolution of macroinvertebrate fauna, with species in running (lotic) waters having lower dispersal abilities, smaller ranges and higher gene flow between populations than species in standing (lentic) environments. Lotic species have been hypothesized to be more specialised, but the diversification dynamics of both habitat types have not been studied in detail. Using a speciose lineage of water beetles we test here whether diversification rates are related to the habitat preference of the species and its consequences on turnover, which we expect to be higher for lotic taxa. Moreover, we tested whether life in lotic environments is acting as an evolutionary dead-end as it is considered an ecological specialisation. We built a comprehensive molecular phylogeny with 473 terminals representing 421 of the 689 known species of the tribe Hydroporini (Coleoptera, Dytiscidae), using a combination of sequences from four mitochondrial and two nuclear genes plus 69 mitogenomes obtained with NGS. We found a general pattern of gradual acceleration of diversification rate with time, with 2–3 significant diversification shifts. However, habitat is not the main factor driving diversification in Hydroporini based on SecSSE analyses. The most recent common ancestor of Hydroporini was reconstructed as a lotic species, with multiple shifts to lentic environments. Most frequent transitions were estimated from lentic and lotic habitats to the category "both", followed by transitions from lotic to lentic and lentic to lotic respectively, although with very similar rates. Contrary to expectations, we found little evidence for differences in diversification dynamics between habitats, with lotic environments clearly not acting as evolutionary dead-ends in Hydroporini.

## 1. Introduction

The unevenness in species richness across the Tree of Life is one of the most striking features of biological diversity. Two major hypotheses have been proposed to explain differences in richness among clades (Wiens, 2017): time as the main diversification factor (clade age hypothesis, Li and Wiens, 2019), and/or differential diversification rates among clades (rates hypothesis, Hugall and Stuart-Fox, 2012, Baker et al., 2014, McGuire et al., 2014, Wiens, 2015a, Seeholzer et al., 2017). A possible cause for differential speciation or extinction rates between clades is the constraint posed by the habitats that the species live in (the "habitat templet" concept; Southwood, 1977). This idea has been tested multiple times in both terrestrial and aquatic environments, such as the shift from saline to freshwater in amphibians (Hou et al., 2011), the type of nesting of some birds (Irestedt et al., 2009), or the habitat preference in lizards (Collar et al., 2010).

In aquatic environments, the main habitat constraint is the one set between running (lotic) and standing (lentic) waters, with species tending to be restricted to one of them (e.g. Ribera et al., 2001, Ribera et al., 2008, Kalkman et al., 2018, Ye et al., 2019). The instability at geological time-scales of small, isolated lentic water bodies forces a higher dispersal capability of the species living in them, and in consequence broader geographical ranges with higher gene flow between populations for lentic than for lotic species (Ribera and Vogler, 2000, Marten et al., 2006, Hof et al., 2006, Abellán et al., 2009, Sánchez-Fernández et al., 2012). The combination of larger geographic ranges and an increased gene flow between populations should lead to a lower probability of speciation and extinction in lentic species (Papadopoulou et al., 2008, Abellán et al., 2009; see Ribera, 2008 for a review). When habitat preferences are maintained across speciation events, or the physical setting of an area favours the dominance of either lotic or lentic habitats for long periods of time, it is expected that the consequences of habitat constraints will extend to macroevolutionary patterns of whole lineages. Thus, it could be expected that the lower probability of speciation and extinction in lentic species would result in their lower evolutionary turnover. However, the total number of species within a clade at any given time will depend on the balance between speciation and extinction. As the same general

factors promoting speciation also favour extinction (Stanley, 1979, Hubbell, 2001, Gould, 2002, Jablonski, 2007), there are no clear predictions as to which habitat type would promote higher species diversity at any given time.

Limited efforts have been made to understand whether diversification rates are influenced by habitat preference in aquatic environments, perhaps due to the difficulties to estimate diversification rates (Rabosky, 2009) and the need for comprehensive phylogenies to account for precise rates (Barraclough and Nee, 2001). Ribera et al. (2001) compared species-level phylogenies of the lentic diving beetle *Ilybius* and the lotic *Deronectes*, but did not find any significant difference in the lineage through time plots of the linearized trees, intended to reflect the diversification pattern of a lineage (Barraclough and Nee, 2001). *Deronectes* displayed a higher frequency of recent splits than *Ilybius*, consistent with a higher evolutionary turnover and a higher frequency of recent species, but differences were not significant compared to expected patterns under a constant speciation rate null model. Letsch et al. (2016) demonstrated heterogeneous speciation rates across Odonata, with lentic environments linked to higher rates. However, an incomplete taxonomic sampling (c. 17% of recognized anisopteran species) and the heterogeneous complex biological traits across species may have affected their results. More recently, Désamoré et al. (2018) suggested no differential diversification rates between lotic and lentic environments in diving beetles, but again with a very incomplete sampling (ca. 4% of dytiscid species) that unavoidably underestimated the number of habitat shifts within genera (e.g. the 189 described species of *Hydroporus* have very heterogeneous habitats, but in Désamoré et al., 2018 only five of them were included and it was considered an homogeneous lentic genus).

The higher persistence of local populations, together with smaller geographical ranges and reduced gene flow, led Ribera (2008) to hypothesize that lotic species may show the characteristics of an ecological specialisation. If this was the case, it seems likely that this could bias the probability of habitat shifts, as once a lotic specialisation is established the possibility of a transition to a lentic habitat may be impaired.

In this work we tested whether diversification rates are related to the habitat preference of the species, and whether habitat transitions can be associated to changes in diversification rates. To test if life in lotic environments can lead to an ecological specialisation (Ribera, 2008), we estimated the probabilities of habitat transitions between habitats. To investigate these questions, we used the tribe Hydroporini of the diving beetle family Dytiscidae with a comprehensive dataset including ca. 65% of its 689 named species (Nilsson and Hájek, 2019). Species of Hydroporini are more homogeneous in morphology and general life style than other tribes within Dytiscidae, but they are highly diverse and with numerous species in either lotic or lentic environments.

## 2. Methods

### 2.1. Molecular data

Molecular sequences were obtained from 473 Hydroporini specimens, with representatives of all genera except *Amurodytes*, *Etruscodytes*, *Siamoporus* and *Tassilodytes*, all of them monotypic (Nilsson and Hájek, 2019). We included in the analyses only species of Hydroporini as currently understood (Nilsson and Hájek, 2019), following the most recent phylogenies of Dytiscidae (Désamoré et al., 2018). We compiled available published sequences plus 500 newly obtained sequences (Table S1). DNA was extracted with a non-destructive protocol using commercial kits, mostly Qiagen DNeasy Tissue Kit (Hildesheim, Germany). Voucher specimens and DNA samples are kept in the collections of Institute of Evolutionary Biology (IBE, Barcelona, Spain) and Museo Nacional de Ciencias Naturales (MNCN, Madrid). DNA was amplified by PCR for six gene fragments in five reactions for a set of mitochondrial and nuclear genes: i) 5′ end of cytochrome *c* oxidase subunit I (COI-5, the barcode, Hebert et al., 2004), ii) 3′ end of cytochrome *c* oxidase subunit I (COI-3), iii) 5′ end of the large ribosomal RNA unit (16S) plus the complete leucine tRNA (tRNA-L1) plus the 5′ end of NADH dehydrogenase subunit I (NAD1), iv) the 5′ end of the small ribosomal unit (18S) and v) an internal fragment of the Histone 3 gene (H3). PCR primers and conditions can be found in Table S2.

Additionally, we sequenced 69 mitochondrial genomes of selected species from an equimolar bulk of DNA following a Mitochondrial Metagenomic protocol adapted from Crampton-Platt et al. (2016). Our pipeline includes preparation of DNA libraries with TruSeq Nano DNA kit, sequencing in an Illumina MiSeq (insert size 300 bp) and *de novo* assemblies with different software to validate each contig. To assure correct identification of contigs, BLAST searches (Madden et al., 2009) were made against a custom database with Sanger sequences of the COI and 16S genes (see Villastrigo et al., 2020 for the detailed pipeline). All sequences were trimmed using Geneious v10.2.5 (Kearse et al., 2012) and uploaded to the ENA database (accession numbers LR998984-LR999418 and MW465237-MW465304, see Table S1 for a complete list of voucher specimens, mitochondrial genomes and accession numbers).

## 2.2. Phylogenetic reconstruction

Sequences from both mitochondrial genomes and Sanger sequencing were aligned using MAFFT 7.409 (Katoh et al., 2009) with the G-INS-I algorithm. To avoid alignments with partitions with very unequal amounts of missing data, the partition scheme was based on the type and source of the sequence: (i) nuclear genes (18S plus H3), (ii) leucine tRNA, present in both Sanger and mitogenomes sequences, (iii) tRNAs present only in mitogenome sequences, (iv) coding sequences present in both Sanger and mitogenome sequences (COI plus NAD1), (v) coding sequences present only in mitogenome sequences, (vi) rRNA present in both Sanger and mitogenome sequences (16S) and (vii) rRNA present only in mitogenome sequences (12S). We opted for a simple model selection approach, considering two complex evolutionary models (HKY + G + I and GTR + G + I) in combination with two different clock models (strict and uncorrelated lognormal) following recent published recommendations (Nascimento et al., 2017, Abadi et al., 2019). We run BEAST v1.10.4 (Suchard et al., 2018) for 100 My generations using a Yule speciation process as prior. Phylogenies were calibrated with the estimated crown age of Hydroporini from the most recent phylogeny of Dytiscidae (103.6 Ma), obtained using a combination of fossils (Désamoré et al., 2018). To reduce computation time,

the monophyly of the subtribes was constrained based on the results of a previous analysis without any topological constraint with IQ-TREE v2.0 (Minh et al., 2020) with the same partition scheme, allowing the software to estimate the best evolutionary model per partition (Kalyaanamoorthy et al., 2017) and assessing branch support using SH-aLRT and ultrafast bootstrap (Hoang et al., 2018) (Fig. S1). Convergence was assessed using Tracer v1.7 (Rambaut et al., 2018).

## 2.3. Evolution of habitat preference

Habitat preferences of the species were compiled using published data and our own observations (Table S1). Ancestral habitat was reconstructed using Beast v1.10.4 under an asymmetrical model with three states: i) Lotic, ii) Lentic, and iii) species that are habitually found in both habitats (i.e. category 'Both'). Phylogenetic signal of habitat preference was calculated using a likelihood ratio test that compared the maximized likelihood of a model with strong signal ($\lambda = 1$) and a model without signal ($\lambda = 0$). Transitions rates between habitat categories were obtained in Beast.

## 2.4. Patterns of diversification and their relationship with habitat

Diversification rates were calculated for all genera with more than two sampled terminals in BAMM v2.5.0 (Rabosky, 2014). In addition to the consensus tree obtained in Beast, we randomly selected a set of 100 trees from the post-burnin posterior distribution to account for phylogenetic uncertainty, considering in both cases the sampled fraction of species following BAMM specifications. We also estimated diversification rates of the same clades using the method of moments estimator (MM-estimator, Magallón and Sanderson, 2001) using the *geiger* R-package (Harmon et al., 2008) for both crown and stem ages under different extinction scenarios (no extinction, $\varepsilon = 0$; intermediate, $\varepsilon = 0.5$; and high extinction, $\varepsilon = 0.9$). To test whether habitat preference types had different diversification rates we used a state-dependent speciation and extinction approach that account for alternative models and concealed states with the R package SecSSE (Herrera-Alsina et al., 2019). We tested three alternative models: i) a constant rate model (CR) where all species share the same speciation and extinction

rates, ii) the examined-trait dependent diversification model (ETD), where the speciation and extinction rates may vary only between the examined states and iii) a concealed-trait-dependent diversification model (CTD) where speciation an extinction rates may vary only between concealed states. The three models were compared using Akaike Information Criterion weights (Wagenmakers and Farrell, 2004).

We also estimated the contemporary diversification pattern in each habitat type. Mean diversification rates of extant species (tip rates) plus their variance were obtained from the set of 100 post-burnin trees, and the *slouch* R-package (Hansen et al., 2008) was used to obtain pairwise comparison of each habitat type trends. We used the estimated tip rates and their variance, and habitat preference as reconstructed in Beast was included as a fixed factor. The *slouch* package can integrate a measure of variance within the response, minimizing errors attributed to an uneven number of measures across taxa.

## 3. Results

### 3.1. Molecular phylogeny and taxonomic status

The phylogenetic reconstruction included 473 terminals representing 423 species and 21 subspecies, plus 29 unnamed terminals (Table S3), covering ca. 62% of the recognized taxa of Hydroporini (688 species plus 27 subspecies, Nilsson and Hájek, 2019). Analyses under uncorrelated lognormal clock did not reach an adequate effective sample size, whilst analyses with a strict clock did with essentially the same topology (results not shown). The HKY + G + I model obtained a larger effective sample size than the GTR + G + I and therefore was selected for subsequent analyses.

The consensus phylogeny (Fig. S2) fully resolved the relationships between major clades (posterior probability [pp] > 1 for subtribe relationships) and recovered most genera as monophyletic, except for *Tiporus* (which included the monotypic genus *Sekaliporus*), a clade including the American genera *Neoporus* plus *Heterosternuta* plus *Haideoporus*, and a clade with *Hydrocolus* plus *Hydroporus*. The only unexpected result was the placement

of *Lioporeus* within Siettitiina (Fig. S1, S2), while it is currently considered within Hydroporina (Nilsson and Hájek, 2019). A collapsed phylogeny showing major clades is provided in Fig. 1.

### 3.2. Evolution of habitat preference

Beast reconstructed the most recent common ancestor of Hydroporini as a lotic species, with multiple shifts to lentic environments (Fig. 2). In addition, some genera reconstructed as lotic such as *Graptodytes*, *Megaporus* or *Strenopriscus* contained nested subclades that represented reversals to other habitat state. Transitions from Lentic to Lotic occurred multiple times mostly on terminal branches (except in a clade within *Hydroporus*), with a similar pattern for species of category Both.

The impact of habitat preference on diversification rates was tested on SecSSE. The examined-trait dependent model estimated a higher diversification rate for species living in both habitats, followed by lentic species, which have a slightly higher diversification rate than lotic species (Table 1). It was also estimated a contrasting turnover between lotic and lentic species, being more than four times higher for species inhabiting lotic habitats as a result of fivefold extinction rate than lentic habitats. Nonetheless, when alternative state-dependent models were compared, SecSSE estimated that the concealed-trait dependent model (CTD) was the best scoring model based on AIC weights (Table 2). The contemporary diversification trends estimated in *slouch* for the terminals considered, as in the ETD model by SecSSE (Table 1), that lentic species tended to increase their diversification rates in comparison with lotic species (contrast 0.0004 ± 0.0002) or species in category 'both' (contrast 0.0007 ± 0.0006); raw

contemporary rates for extant taxa are available at Table S5. Differences between states Lotic and Both were not significant due to large standard errors.

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## 4. Discussion

### 4.1. Molecular phylogeny

We found some unexpected phylogenetic relationships, in disagreement with the current classification of Hydroporini. Previous authors noticed the morphological resemblance of *Neoporus* and *Heterosternuta* (e.g. Larson et al., 2000, Miller and Bergsten, 2016), considering them as closely related genera with overlapping distributions (Larson et al., 2000) and similar habitat preferences (Table S2). The main morphological character separating both genera is the apex of the aedeagus (bifid in *Heterosternuta*), and our samples were identified based on this character. Our results point to a more complex scenario, with *Heterosternuta* as a mononophyletic lineage derived within a larger clade including both *Neoporus* and *Haideoporus*, suggesting the need for a taxonomic revision of the group. Miller et al. (2013) reported a similar result, with a clade

including *Haideoporus*, *Heterosternuta* and *Neoporus*. Another novel result was the ascription of *Lioporeus* to subtribe Siettitiina, formerly considered to be part of the *Hydroporus pulcher-undulatus* group by Fall (1923) (see also Miller and Bergsten, 2016). The morphological resemblance of the species of *Lioporeus* to other Siettitiina species, especially in the male genitalia, supports this new interpretation.

The paraphyly of *Hydroporus* was also not surprising, as *Hydrocolus* was erected for the former *Hydroporus oblitus* group (Larson et al., 2000). Recent studies considered *Hydrocolus* as closely related to *Hydroporus* (Ribera et al., 2008, Miller and Bergsten, 2014), but further studies with a more complete sampling are needed prior to rearranging the taxonomy of this diverse clade.

*Tiporus* is a homogeneous group of species inhabiting lotic environments in northern Australia (Miller and Bergsten, 2016). The low support for its monophyly (pp = 0.85) may be due to the inclusion of *Sekaliporus* in some of the topologies of the Bayesian analysis. Toussaint et al. (2015) found the same result, with a well supported clade including both *Sekaliporus* and *Tiporus*.

Another interesting result was the relationship between subtribes. We recovered Siettitiina as sister to the rest of Hydroporini, followed by Deronectina and a clade formed by Hydroporina plus Sternopriscina, all nodes with very strong support. Désamoré et al. (2018) found Hydroporina as sister to the rest, with Siettitiina sister to Deronectina, but with a more incomplete sampling and lower support.

## 4.2. Evolution of habitat preference

Our analyses showed strong evidence of a marked phylogenetic signal for habitat preference. According to the habitat reconstruction in Beast, the most common recent ancestor of Hydroporini lived in lotic habitats, with most of the basal nodes being also lotic. The phylogenetic position of Hydroporini within the wider Hydroporinae is still uncertain (e.g. Ribera et al., 2008, Désamoré et al., 2018), and in consequence it is not possible to consider the habitat preference of putative sister groups, which may influence the reconstruction

of the ancestral habitat of the tribe. The expected probability of habitat transitions suggested

by Ribera (2008) was that the more specialised habitats should have a lower frequency of

transitions to other habitats, therefore, transitions from lotic habitats should be lower than those

from lentic habitats. Contrary to this expectation, we found similar transition rates between lotic

and lentic habitats, demonstrating that lotic habitats are not an ecological specialisation or an

evolutionary dead-end (Kelley and Farrell, 1998). It should be noted that habitat constraints are

not symmetrical: populations of lentic species have to disperse when the habitat disappears, but

populations of lotic species do not need to move that frequently. Thus, while among lentic

species the frequency of species with small ranges is expected to be low, it is not rare to find

widespread species in lotic lineages (Ribera and Vogler, 2000, García-Vázquez et al., 2016).

These widespread running species may act as "diversity pumps", extending the range of existing

clades when conditions are favourable (e.g. immediately after glacial periods), and originating

new species by subsequent isolation when these favourable conditions change (Ribera et al.,

2011, García-Vázquez et al., 2017). The process would be similar to the "taxon cycle" or "taxon

pulse" (see e.g. Liebherr and Hajek, 1990 for a review), or to that described by Janz et al.

(2006) with butterflies, in which the high diversity may be kept through cycles of range

expansion by host switching and subsequent diversification by fragmentation. Specialisation is

only a pruning process and cannot generate diversity on its own; to generate diversity repeated

cycles of generalists with a subsequent specialisation would be necessary (Janz et al., 2006).

Several recent studies have also found that habitat specialisation does not imply an evolutionary

dead-end, such as the adaptation to the subterranean environment in beetles (Cieslak et al.,

2014) or crayfishes (Stern et al., 2017), in pollinator-specific and host-specific plants (Day et

al., 2016), or in beetles in hypersaline habitats (Villastrigo et al., 2020).

### 4.3. Patterns of diversification and their relationship with habitat

Despite the recent controversy on the accuracy of diversification rates estimated by BAMM

(Moore et al., 2016, Rabosky et al., 2017, Meyer et al., 2018, Meyer and Wiens, 2018, Rabosky,

2018, Rabosky, 2019), our results are an example of its power when using comprehensive

phylogenies. Rabosky (2019) showed how rates were an inverse function of clade size and how variance should be taken into account when estimating diversification rates. The low number of species and associated higher variances may explain the differences between the estimates of BAMM and the *method of moments* in some species-poor genera (*Barretthydrus*, *Carabhydrus*, *Chostonectes*, *Iberoporus*, *Megaporus*, *Mystonectes*, *Porhydrus* and *Stictotarsus*).

The general trend of diversification through time in Hydroporini was a gradual acceleration, contrary to the general trend of a slowdown generally reported in the literature (McPeek, 2008, Morlon et al., 2010, Moen and Morlon, 2014). A recent study (Henao Diaz et al., 2019) suggested generalized heterogeneous patterns within clades, with higher rates at their origin followed by a slow-down with time. This pattern was not observed in Hydroporini, that have undergone diversification events that have maintained or increased the rates of diversification in recent times. Cusimano and Renner (2010) considered that the general pattern of slow-down with time may be caused by non-random sampling, overestimating diversification events in deep nodes. However, some accelerated diversification rates can also be found linked to niche expansions (Nürk et al., 2015). The increase in diversification rates toward the present could be also related to the effects of Pleistocene glaciations, as suggested for North American tiger beetles (Barraclough and Vogler, 2000). This idea may be supported by the high frequency of speciation events of Pleistocene origin found in Hydroporini (see also Ribera and Vogler, 2004 for other Dytiscidae lineages). BAMM analyses revealed a heterogeneous diversification rate across Hydroporini, with at least two main core shifts for clades within *Sternopriscus* and *Boreonectes*, and in some cases in the clade formed by *Hydrocolus* plus *Hydroporus*. Main shifts occurred in clades formed by species mostly linked to lentic environments, although *Hydroporus* plus *Hydrocolus* has a more heterogeneous habitat preference. However, the number of species of *Hydroporus* plus *Hydrocolus* that are not strictly lotic is much lower than the species in the lentic or both categories (40 strict lotic species against 94 species in Lentic or Both categories).

Overall, we did not find a clear signal of differential diversification rates based on habitat preference under the examined trait model (ETD). However, we found a contrasting evolutionary turnover between lotic and lentic habitats due to markedly lower extinction rates in lentic species. These findings are consistent with the hypothesis that species turnover is higher in lotic lineages due to the different constraints imposed by lotic and lentic habitats on aquatic organisms (Ribera, 2008). The instability at geological time-scales of lentic habitats forces a higher dispersal capability of the species living in them, and in consequence broader geographical ranges with higher gene flow between populations for lentic than for lotic species (Hof et al., 2006, Monaghan et al., 2005, Ribera and Vogler, 2000, Ribera et al., 2001, Sánchez-Fernández et al., 2012), which would lead to a lower probability of extinction in lentic species, resulting in a lower evolutionary turnover (Ribera, 2008). Regardless of a heterogeneous diversification rate across Hydroporini phylogeny, the preferred trait-dependent diversification model was the concealed trait model (CTD), suggesting that habitat preference is not the most important factor driving the diversification pattern within Hydroporini. Letsch et al. (2016) compared speciation and extinction rates in dragonflies with a similar diversification pattern: lentic clades displayed higher speciation rates than lotic counterparts, which they linked with the broader geographical ranges of lentic species compared with lotic taxa. The only similar study in aquatic beetles have been made by Désamoré et al. (2018), but they did not recover any differential net diversification for diving beetles. The absence of contrasting diversification patterns by Désamoré et al. (2018) was hypothesized to be linked to an increased probability of fragmentation in lentic species due to their broader geographical ranges, something already suggested by Darwin (1859). Additionally, the enhanced capability of dispersal in lentic species may fuel speciation through frequent long-distance dispersal events. However, Papadopoulou et al. (2008) showed that even very sporadic dispersal events among populations are sufficient to prevent the differentiation of populations in good disperser species. Ribera et al. (2001) suggested the possibility that lentic and lotic clades may indeed display similar patterns of diversification, with similar levels of evolutionary turnover, but the evolutionary processes may take place at very different spatial scales. Thus, it could be that two

species have similar metapopulation dynamics over their whole range, with similar overall probabilities of fragmentation or extinction, so that both species may be indistinguishable when studied in a scale-free context. But they may still have very contrasting absolute sizes of the geographical range, which implies that if confined to a similar geographic space both may have very different evolutionary dynamics, still in agreement with the habitat stability set of hypotheses.

Our data also supported a considerable higher turnover for lotic species, even if the general diversification pattern was essentially the same as lentic species and lentic clades where the ones that displayed diversification shifts. Other studies have also tried to match differential diversification or speciation rates with the habitat of the species with limited success, but none of them focused on the general turnover pattern of aquatic taxa in an evolutionary scope. Some examples are fishes living in freshwater or in marine habitats (Bloom et al., 2013), coral reef-associated sharks (Sorenson et al., 2014), tenrecs in the humid forest in Madagascar (Everson et al., 2016), or the division between terrestrial and aquatic environments (Wiens, 2015a). At larger scales, habitat has also been found to explain only a moderate amount of the total variation in diversification rates among animal phyla (Wiens, 2015b).

## 5. Data Accessibility

The data that supports the findings of this study are available in the supplementary material (Table S1) of this article.

## CRediT authorship contribution statement

**Adrián Villastrigo:** Conceptualization, Methodology, Software, Writing - original draft, Writing - review & editing, Visualization. **Pedro Abellán:** Conceptualization, Methodology, Software, Writing - original draft, Writing - review & editing. **Ignacio Ribera:** Conceptualization, Methodology, Resources, Writing - original draft, Supervision, Project administration, Funding acquisition.

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# Tables

Table 1. Estimated speciation and extinction rates for habitat states using SecSSE sensu ETD model.

| Model | Speciation | Extinction | Diversification | Turnover |
|-------|-----------|-----------|----------------|----------|
| Lotic | 0.0519 | 0.0101 | 0.0418 | 0.1935 |
| Both | 0.0806 | $8.40 \times 10^{-14}$ | 0.0806 | $1.04 \times 10^{-12}$ |
| Lentic | 0.0489 | 0.0021 | 0.0468 | 0.0433 |

Table 2. Maximum Likelihood estimations of SecSSE for different models of trait-dependent diversification

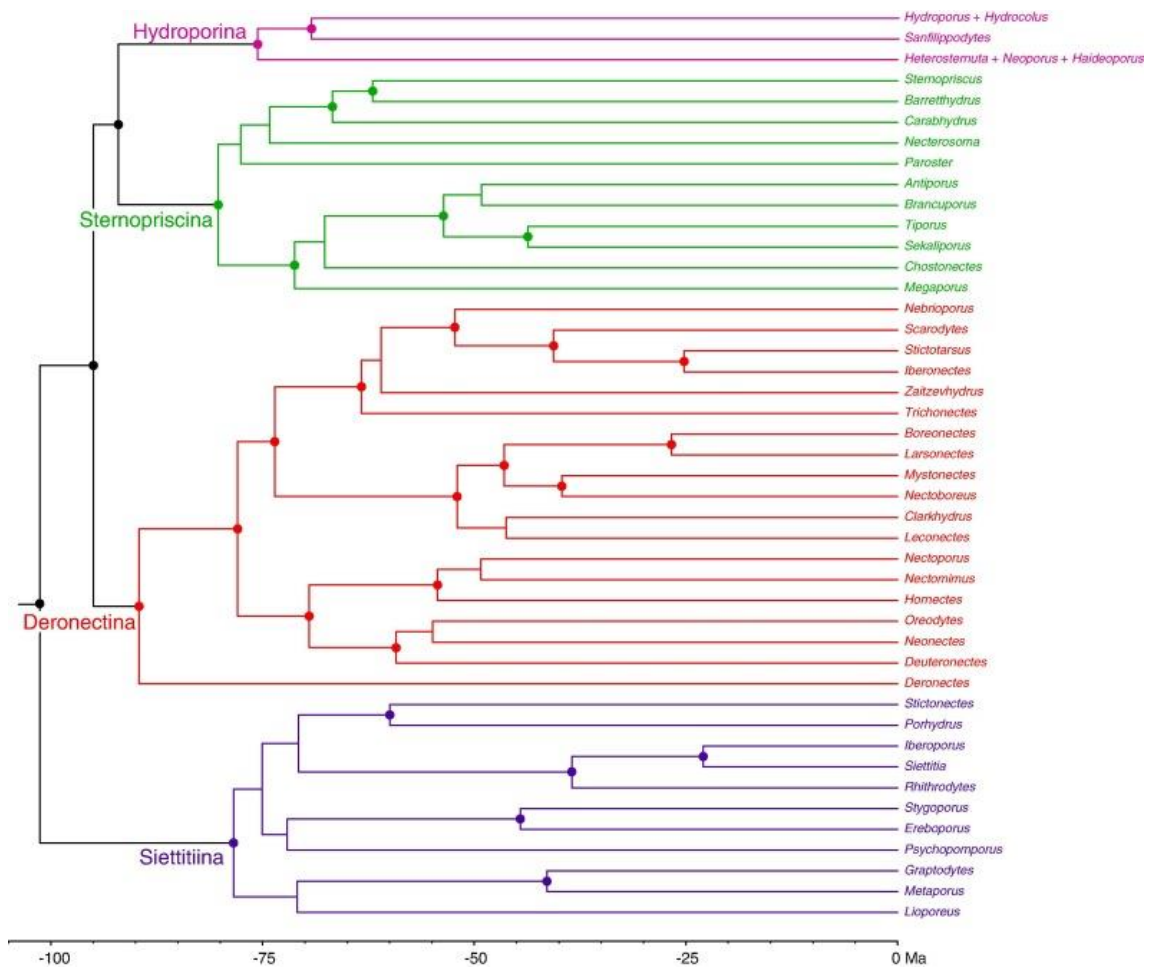| Model | $k$ | ML | AICw |
|-------|-----|------|------|
| CR | 8 | -2258.928 | $6.035 \times 10^{-10}$ |
| ETD | 12 | -2245.494 | $7.549 \times 10^{-6}$ |
| CTD | 12 | -2233.699 | 0.99999 |

**Figure 1.** Collapsed molecular phylogeny of the genera of Hydroporini, obtained with BEAST. Circles in nodes represent posterior probabilities = 1. The monophyly of the subtribes was constrained according to the results of a previous IQTREE search, which obtained maximum support for all of them (Fig. S1).
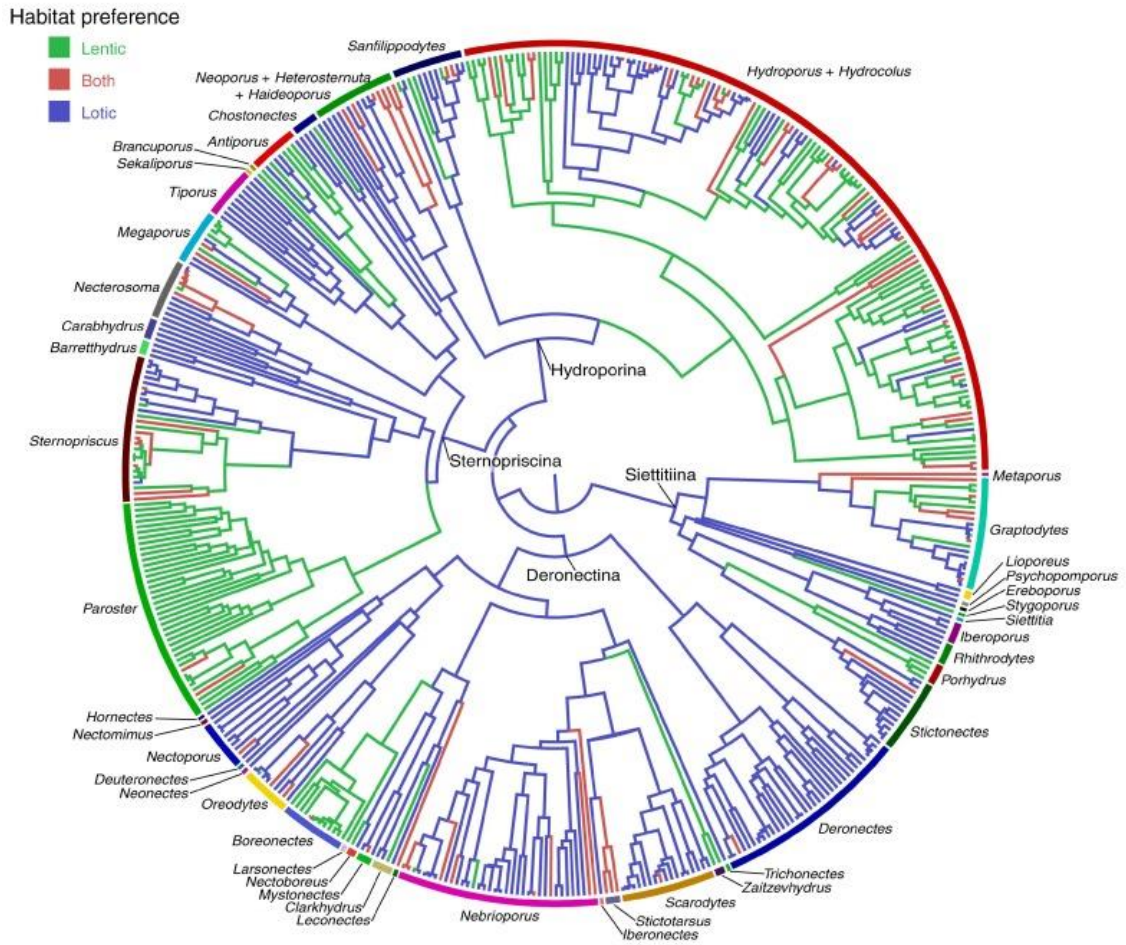
**Figure 2**. Reconstruction of the habitat preference in Hydroporini using BEAST with an asymmetric transition probability model.
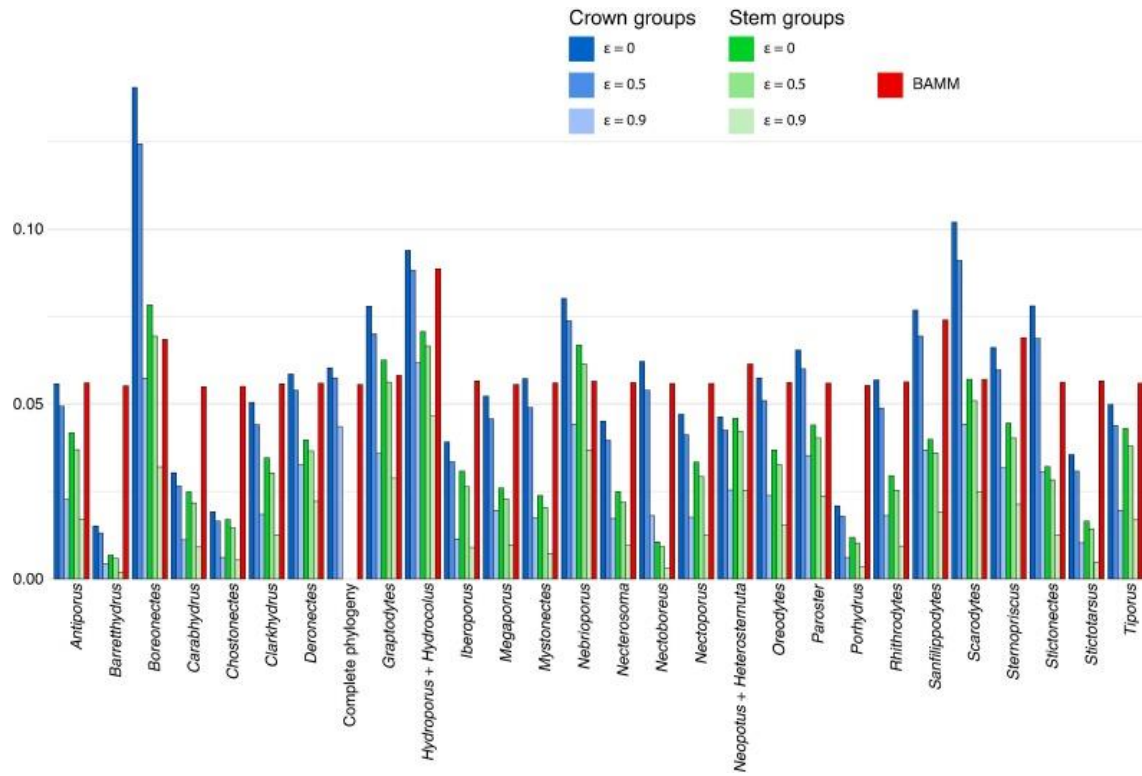
Figure 3. Mean diversification rates for the major clades for the stem and crown groups and different levels of extinction.
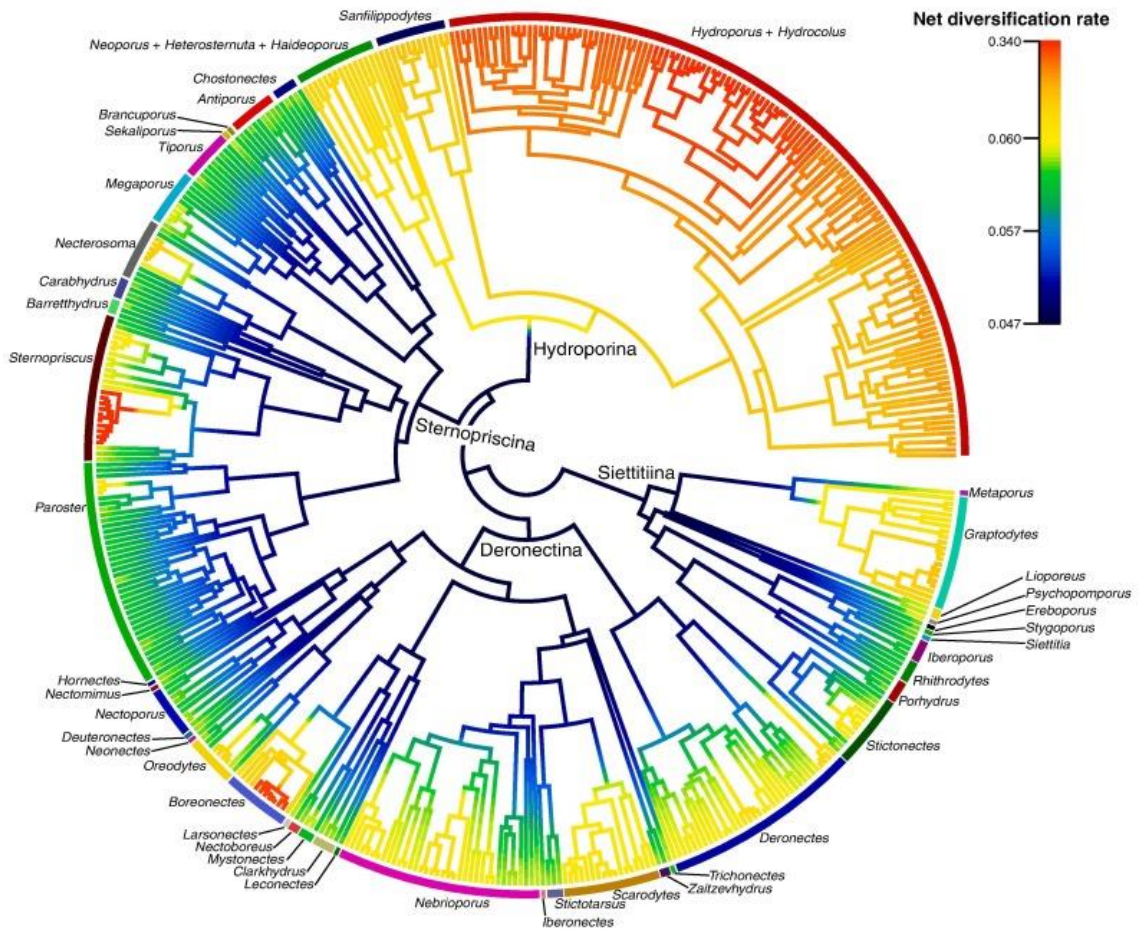
Figure 4. BAMM estimation of the diversification rates in Hydroporini.

**Additional information**

Table S1. List of material used in the study, including habitat preference, voucher numbers, locality data, and accession numbers of mitogenomes and sequences. In bold, newly obtained sequences.

Table S2. List of PCR primers (A) and typical PCR conditions (B).

Table S3. Number of currently recognized species per genus of Hydroporini (following Nilsson and Hájek 2019), with the number included in the phylogeny, plus the number of unidentified terminals.

Table S4. Mean diversification rates and standard deviations obtained with the MM-estimator under three different extinction ($\varepsilon$) scenarios and with BAMM

Table S5. Contemporary rates estimated for extant taxa (speciation, extinction, diversification and its standard deviation - sd).

Figure S1. Phylogeny of Hydroporini obtained with IQTREE v.2.0. Numbers in nodes represent SH-aLRT support (%) and ultrafast bootstrap support (%) respectively.

Figure S2. Majority rule topology of Hydroporini obtained in BEAST. Numbers in nodes, posterior probability.