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# Fifth mass extinction event triggered the diversification of the largest family of freshwater gastropods (Caenogastropoda: Truncatelloidea: Hydrobiidae)

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**Abstract**

The fifth mass extinction event (MEE) at the Cretaceous–Palaeogene (K–Pg) boundary 66 million years ago (Ma) led to massive species loss but also triggered the diversification of higher taxa. Five models have been proposed depending on whether this diversification occurred before, during or after the K–Pg boundary and the rate of species accumulation. While the effects of the K–Pg MEE on vertebrate evolution are relatively well understood, the impact on invertebrates, particularly in freshwater ecosystems, remains controversial. One example is the hyperdiverse Hydrobiidae—the most species-rich family of freshwater gastropods. Whereas some studies place its origin in the Jurassic or even Carboniferous, most fossil records postdate the K–Pg event. We therefore used robustly time-calibrated multi-locus phylogenies of >400 species representing >100 hydrobiid genera to unravel its evolutionary history and patterns of diversification. We found that the family started diversifying shortly after the K–Pg boundary (~60 Ma; 95% highest posterior density 52–69 Ma). Lineage richness gradually increased to the present and phylogenetic diversity until ~25 Ma. These findings suggest that diversification was not initially driven by ecological opportunity. Combining the two criteria of timing and rate of diversification, a soft-explosive diversification model of aquatic vertebrates best fits the patterns observed. We also show that most higher hydrobiid taxa (i.e. subfamilies) diversified from the Middle Oligocene to Middle Miocene (i.e. 12–28 Ma). Two of the 15 major clades delimited are described here as new subfamilies (i.e. Bullaregiinae n. subfam. and Pontobelgrandiellinae n. subfam.), whose members are restricted to subterranean waters. Our results are an important contribution to understanding how the fifth MEE has shaped evolution and patterns of biodiversity in continental aquatic systems. Given the high extinction risks faced by many hydrobiids today, they also emphasise the need to study the biodiversity of vulnerable ecosystems.



## Introduction

The fifth mass extinction event (MEE) 66 million years ago (Ma) marked the end of the Cretaceous (K) period and the beginning of the Palaeogene (Pg). It has long been associated with massive faunal turnovers in terrestrial and aquatic ecosystems that resulted in the extinction of several higher taxa and their subsequent replacement by new ones (Wahlberg et al., 2009; O'Leary et al., 2013; Alfaro et al., 2018). However, it is controversial whether the evolution of these new taxa actually started before the K–Pg MEE (Archibald and Deutschman, 2001; Landman et al., 2015). Five models have been proposed according to whether initial diversification occurred before (short-fuse model), during (long-fuse and trans-KPg model) or after (soft-explosive and explosive models) the K–Pg boundary (Springer et al., 2019). Previously, placental mammals were thought to follow the explosive model, with a high speciation rate and major clades originating after the fifth MEE (O'Leary et al., 2013). More recent studies utilising enhanced genomic sampling and

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methodological improvements showed an increase in speciation rate ca. 20 million years (Myr) earlier and supraspecific diversification across the K–Pg boundary, implying a long-fuse or soft-explosive model of mammal evolution (Álvarez-Carretero et al., 2022; Quintero et al., 2022). Similarly, the supraspecific diversification in butterflies followed the long-fuse model (Heikkilä et al., 2011). While avian speciation rates, in general, were relatively constant over the past 115 Myr, with bird orders originating before the K–Pg boundary (i.e. a long-fuse model), songbirds adhere to the soft-explosive model with an origin shortly before the K–Pg boundary and elevated rates of speciation and supraspecific diversification (Jetz et al., 2012; Maliet and Morlon, 2022). Higher taxa of aquatic vertebrates survived the K–Pg MEE and diversified rapidly thereafter (Bertozzi et al., 2016; Feng et al., 2017; Alfaro et al., 2018; Guinot and Condamine, 2023), which is also consistent with the soft-explosive model.

Freshwater gastropods are another group of aquatic animals with a high diversity turnover during the K–Pg transition. Unlike aquatic vertebrates, fossil data indicate that 92.5% of all recorded European species became extinct during the fifth MEE (Neubauer et al., 2021). However, most gastropod genera and families originating before the K–Pg boundary survived, although with reduced richness. Their diversification rate returned to Late Cretaceous levels in two phases: an initial dynamic period of ~5 Myr characterised by high speciation and extinction rates, followed by a recovery period of another ~7 Myr in which the extinction rate reverted to background level but speciation rates were twice the long-term average (Neubauer et al., 2021). As a result, niches left vacant by the K–Pg MEE were filled (Neubauer et al., 2022b). While this would imply an explosive model of diversification, the fact that very few families of continental aquatic gastropods have emerged in the late Cretaceous (Neubauer et al., 2016) suggests a short-fuse model of supraspecific diversification. However, some modern families with inconspicuous shells that occurred in ecosystems with a limited fossilisation potential (e.g. spring, subterranean, and karstic waters) are poorly represented in the fossil record (Strong et al., 2008). These characteristics widely apply to the largest family of freshwater gastropods, the Hydrobiidae W. Stimpson, 1865. Although it includes approximately one-fifth of all described continental aquatic gastropods, there is little knowledge about its major clades. Therefore, our picture of species diversification and turnover of supraspecific taxa derived from the fossil record of continental aquatic gastropods might be biased (Neubauer et al., 2021, 2022b).

Gastropods of the family Hydrobiidae are a well-suited group to test models of diversification in aquatic organisms. The family's estimated diversity ranges from ~910 (Miller et al., 2018) to >1200 (Strong et al., 2008) extant species in ~180 genera (MolluscaBase eds., 2023). Although predominantly found in springs and subterranean waters, they also occur in other aquatic habitats, from coastal marine and brackish waters to rivers, lakes and wetlands (Wilke and Delicado, 2019). The taxon is widely distributed across North America, continental Europe, northern Africa and western and central Asia. Disjunct occurrences are known from some Atlantic islands and South Africa (Wilke and Delicado, 2019). Despite this wide distribution, many taxa show restricted geographical ranges and exhibit a high degree of endemism (Miller et al., 2018), particularly in ancient lakes such as Lake Ohrid on the Balkan Peninsula (Hauffe et al., 2011) and in the Caspian Sea (Wesselingh et al., 2019).

Unfortunately, this combination of often restricted range, small body size (0.5–8 mm, rarely 15 mm long) and inconspicuous shells hampers inferences about hydrobiid diversity and systematic relationships. As a consequence, past systematic studies based on morphological characters found little consensus on the classification of supraspecific taxa (subfamilies) (Kabat and Hershler, 1993; Wilke et al., 2001). More recent studies using either morphology or DNA data (Wilke et al., 2013; Boeters and Falkner, 2017; Anistratenko et al., 2021) led to the current recognition of 11 nominal subfamilies within the Hydrobiidae (Appendix S1). However, the overall systematic relationships within the Hydrobiidae remain poorly understood. The problem is exacerbated by conflicts among previous phylogenetic hypotheses (Szarowska, 2006; Wilke et al., 2013). Moreover, while most researchers agree that the Hydrobiidae is an evolutionarily old group, its phylogenetic age remains controversial. The earliest fossils assigned to the 'Hydrobiidae' date to the early Carboniferous (i.e. ~350 Ma; Knight et al., 1960; Solem and Yochelson, 1979). However, the family assignment of these fossils has been questioned (Ponder, 1988). Thompson (1979) suggested that the origin of the North American hydrobiids predates the opening of the North-Central Atlantic Ocean in the middle-late Jurassic (150–170 Ma; Klitgord and Schouten, 1986). In contrast, most hydrobiid fossils postdate the K–Pg event (FreshGEN database; Neubauer et al., 2014), suggesting a more recent origin of the family. Crown ages of several dated molecular phylogenies (e.g. for the subfamilies Pseudamnicolinae and Pyrgulinae; Wilke et al., 2007; Delicado et al., 2015) also suggest a post-K–Pg diversification.

Yet most previous studies were conducted over restricted spatial and temporal ranges and/or with a subset of taxa. Thus, a robust time-calibrated molecular phylogeny of the family, ideally involving a complete taxon sampling, multi-locus phylogenetic

datasets, robust calibration points and consistency of tree topologies across phylogenetic approaches (Wilke et al., 2009), is still lacking. This paucity of reliable age estimates, along with conflicts within and between phylogenetic and paleontological data, hampers our understanding of the evolutionary history of this family. In particular, the timing and drivers of diversification remain largely unknown, including a potential differential impact of MEEs on species accumulation and origination of supraspecific taxa.

In this study, we therefore used robustly time-calibrated multi-locus phylogenies of >400 species representing >100 hydrobiid genera to unravel the evolutionary history and patterns of diversification of this hyperdiverse taxon. Our specific goals are to:

1. reconstruct the phylogenetic relationships within the Hydrobiidae using a set of phylogenetic approaches;
2. infer the onset of diversification within the family using a molecular-clock approach;
3. unravel the dynamics of lineage richness through time in light of the above-described diversity models; and
4. disentangle the subfamily-level systematic relationships within the Hydrobiidae using the phylogenetic criteria of monophyly, evolutionary age and phylogenetic distinctiveness.

Based on preliminary paleontological evidence (Neubauer et al., 2021), the overarching working hypothesis of this study is that the diversification of the Hydrobiidae was triggered by the MEE at the K–Pg boundary, and that major clades subsequently evolved rapidly, filling vacant ecological niche space. Lineage richness would thus rapidly increase in the Palaeogene, as predicted by the explosive diversification model.

## Materials and methods

### *Taxon sampling*

We analysed DNA sequences from 407 (391 valid and 16 undescribed) species belonging to 102 genera, generating new sequencing data for ~120 species and increasing the sequence coverage for another ~130 species (Table S1). Specimens of ~160 species were collected from their type locality ("topotypes"), or nearby regions, which allowed direct assignment to described species. Individuals from non-type localities were identified using a literature survey of original descriptions and published records of the species from the same locality (e.g. Wilke et al., 2001; Hershler et al., 2003; Vandendorpe et al., 2019). Most undescribed species have been previously delimited using molecular methods (Wilke et al., 2007; Delicado et al., 2018; Miller et al., 2022). Based on the hydrobioid phylogeny inferred by Wilke et al. (2013), we selected as outgroups specimens of *Lithoglyphus naticoides* (C. Pfeifer, 1828) (family Lithoglyphidae) sensu Wilke et al. (2013) and *Oncomelania hupensis* Gerdler, 1881 (family Pomatiopsidae) for the phylogenetic analyses that require outgroups (see below). The DNA extracted from specimens was deposited in the following public collections, where the original samples were housed (Table S1): the University of Giessen Systematics and Biodiversity collection (UGSB; Diehl et al., 2018) in Giessen, Germany; the Smithsonian Institution's National Museum of Natural History collection (USNM), Washington DC, USA; and the Molluscs collection of the National Museum of Natural Sciences (MNCN), Madrid, Spain.

### *Sequence data generation and alignment*

Total genomic DNA was extracted from entire snails using a CTAB protocol described in Wilke et al. (2006) and the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany). The mitochondrial gene fragments cytochrome *c* oxidase subunit I (COI) and the large ribosomal subunit (16S), as well as the nuclear large ribosomal subunit (28S), were amplified using the primer pairs of Boulaassafer et al. (2020). The universal metazoan primers of Holland et al. (1991) served to amplify a fragment of the nuclear small ribosomal subunit (18S). Amplification conditions for COI, 16S and 18S were the same as those described in Delicado et al. (2012) with an annealing temperature of 48–50°C. The 28S PCR conditions were those of Boulaassafer et al. (2020). The amplified PCR products were Sanger-sequenced on an ABI 3730 XL sequencer (Life Technologies, Carlsbad, CA, USA) using a Big Dye Terminator kit v. 3.1 (Life Technologies). Forward and reverse sequences were assembled and edited in SEQUENCHER v. 4.1.4 (Gene Codes Corp., Ann Arbor, MI, USA).

We generated 669 sequences and analysed them along with 620 previously published sequences from the same or other members of the family Hydrobiidae (GenBank accession numbers in Table S1). Final alignments included 404 sequences of the COI gene, 394 of 16S, 333 of 28S and 158 of 18S. Protein-coding COI sequences were aligned in MEGA v. 11.0.10 (Tamura et al., 2021). The 16S and 18S sequence alignments were performed automatically in MAFFT v. 7.402 (Katoh et al., 2019), according to the secondary-structure alignments designed for Hydrobiidae and other related families (Wilke et al., 2013), and default settings for gap penalties (gap opening penalty = 1.53). The secondary structure of the 28S fragment was inferred using a 28S structural alignment for several truncatelloidean families. The resulting hydrobiid sequences were incorporated in MAFFT v. 7.402 as the reference to align our 28S sequence dataset with default settings for gap penalties. The software Gblocks v. 0.91b (Castresana, 2000) detected that about 10% of the nucleotide positions in the sequence alignments of ribosomal DNA were ambiguously aligned and therefore removed. All the sequence alignments with their respective secondary structure in dot-bracket format are available on Figshare (https://doi.org/10.6084/m9.figshare.22317709). Potential substitution saturation in each individual gene-partition dataset was assessed by conducting a saturation test (Xia et al., 2003; Xia and Lemey, 2009) in DAMBE v. 7.0.28 (Xia, 2018), based on the proportion of invariant sites obtained in jModelTest v. 2.1.4 (Darriba et al., 2012) (see below).

### *Phylogenetic analyses*

PartitionFinder v. 2.1.1 (Lanfear et al., 2017) was used to select the best-fit model of nucleotide substitution by codon position within our COI dataset according to the corrected Akaike's information criterion (Akaike, 1974; Sugiura, 1978; Hurvich and Tsai, 1989) and the greedy algorithm (Lanfear et al., 2012) for model fitting. Following the identification of a single-partition scheme across codon positions, further analyses were conducted without codon partitioning. The best nucleotide substitution model for each gene partition was then identified in jModelTest according to the corrected Akaike's information criterion. The selected models were as follows: TrN (Tamura and Nei, 1993) + I (invariable sites) + G (rate variation

among sites) for the first, second and third codon position of the COI fragment, TPM1uf (TPM1 model with unequal base frequencies; Kimura, 1981) + I + G for COI without internal partitions, TIM3 (Posada, 2008) + I + G for 16S, TrNef (Tamura–Nei model with equal base frequencies; Tamura and Nei, 1993) + I + G for 18S and TrNef + I + G for 28S.

In order to identify subfamily-level clades in the group, phylogenetic relationships within the Hydrobiidae were estimated based on the individual datasets and the concatenation of the four gene fragments under maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) methods. We used the package PHANGORN v. 2.9.0 (Schliep, 2011) for the R v. 4.2.1 statistical programming language (R Core Team, 2022) to perform an MP tree search, with the parsimony ratchet (Nixon, 1999) and a stopping-rule of 250 rearrangements without improvement in parsimony score. Branch support was calculated through transfer bootstrap expectation based on 1000 MP pseudoreplicates. Transfer bootstrap expectation is a better measure for branch support for species-rich phylogenies than Felsenstein's (1985) non-parametric bootstrapping approach (Lemoine et al., 2018). Maximum likelihood analyses of single and concatenated datasets were conducted in RAxML-NG v. 1.0.2 (Kozlov et al., 2019) with 200 random starting trees under the abovementioned substitution models for each gene partition selected by jModelTest. Transfer bootstrap expectation was calculated using the autoMRE (cutoff: 0.03) bootstrapping convergence criterion with a maximum of 5000 bootstrap replicates.

Providing structural information for truncatelloidean ribosomal RNA improves branch support in Bayesian phylogenetic trees (Wilke et al., 2013). Accordingly, four schemes of BI analyses were defined and run in the software MrBayes v. 3.2.7a (Ronquist et al., 2012): (i) each gene dataset separately with its corresponding substitution nucleotide model jointly inferred with the phylogeny during the analysis by the mixed substitution model function of MrBayes; (ii) 16S, 18S and 28S datasets partitioned by stem and loop positions, utilising the doublet and four-by-four structural models, respectively for the two partitions; (iii) a concatenated dataset of the four gene fragments, for each of which substitution models were jointly inferred with the phylogeny as in scheme (i); and (iv) a concatenated dataset of the four gene fragments divided into seven partitions (COI, stem 16S, loop 16S, stem 18S, loop 18S, stem 28S and loop 28S), using mixed substitution models for COI and the corresponding structural models for the stem and loop partitions. The phylogenies using schemes (i) and (ii) were inferred through two independent runs of four chains simultaneously for 25 million Markov chain Monte Carlo (MCMC) generations (75 million generations for the COI dataset), sampling every 2000th tree. Markov chain Monte Carlo chains with 80 million generations each were run for the 16S dataset analysis of scheme (ii), sampling every 8000th tree. For schemes (iii) and (iv), the same settings were applied: 50 million MCMC generations and sampling every 10 000th tree but setting the heating temperature of the chains to 0.05 to improve exploration of parameter space and convergence for scheme (iv). Convergence in combined runs was considered when the effective sample size (ESS) of each parameter was greater than 200 (20% burn-in in the analysis of scheme (iii), 10% burn-in for all remaining analyses), which we checked in Tracer v. 1.7.1 (Rambaut et al., 2018). The first 10% (or 20%) of the sampled trees were discarded as burn-in before constructing the 50% majority-rule consensus tree. Trees and branch support values (i.e. Bayesian posterior probabilities, BPPs) were visualised in FigTree v. 1.4.3 (Rambaut, 2016).

## *Time-calibrated phylogenetic analyses*

We inferred a time-calibrated tree of the family Hydrobiidae using the four-gene dataset, comprising a total of 406 taxa, in BEAST v. 2.6.6 (Bouckaert et al., 2019). As recommended in a BEAST manual (Drummond and Bouckaert, 2015), we excluded the non-Hydrobiidae outgroups and the species *Montenegrospeum bogici*, which, according to our MP, ML and BI phylogenetic results, does not belong to Hydrobiidae. The input files (.xml) were created using BEAUti v. 2.6.6, specifying an uncorrelated relaxed log-normal molecular clock (Drummond et al., 2006). The analysis was conducted using a birth–death model (Gernhard, 2008) to infer the phylogenetic tree (birth and death rates drawn from default uniform priors), and the best-fitting nucleotide substitution models were jointly inferred with the tree through the 'Beast Model test' option (package bModelTest v. 1.2.1; Bouckaert and Drummond, 2017). We used the most likely tree estimated for the concatenated dataset in the RAxML analysis as a starting tree to improve the convergence of the BEAST analysis.

For our node dating, the age of eight nodes was constrained based on a geological event (i.e. Lake Ohrid basin formation) and the ages of six fossils. Placing fossils within the hydrobiid phylogeny is challenging owing to morphological similarities in shell shape among distantly related groups (e.g. Delicado et al., 2019). However, most hydrobiid genera, particularly those in springs and subterranean waters, have specific habitat preferences (Miller et al., 2018), and the low estimated dispersal rates in biogeographic reconstructions (e.g. Delicado et al., 2015; Van Dam and Matzke, 2016) suggest that their narrow geographic distribution does not change substantially over evolutionary timescales. This allows for more robust fossil identification at higher taxonomic levels despite morphological convergence. Here, we included fossils that could be unambiguously assigned to extant clades according to their shell morphology, the type of sediment from which the fossil was recovered and their geographic location. Given the lack of continuous fossil records for Hydrobiidae, the oldest fossil occurrence for each time-calibrated clade (corresponding to genera or subfamilies) was selected by querying the databases MolluscaBase and FreshGEN (Neubauer et al., 2014). We applied a lognormal prior to all fossil calibrations, setting the offset to the younger boundary of the stratigraphic age interval that is reported for the respective fossil and the mean (in real space) to a value that places the expected (mean) age of the prior halfway through the interval. The shape values of the priors were always set to one. Nodes used for the calibration were: (i) and (ii) the origin of two Lake Ohrid species flocks of the subfamilies Horatiinae and Pyrgulinae (normal prior 1.36 ± 0.50 Ma, offset 0 Ma) based on the estimated age of the lake basin formation (Wagner et al., 2019); (iii) the most recent common ancestor (MRCA) of the *Islamia* clade (log-normal prior 1.00 ± 1.00 Ma, offset 7.00 Ma) based on the earliest known record of the genus (*I. bambolii*; age: Miocene, Late Tortonian (ca. 7–9 Ma); Esu and Girotti, 2015); (iv) the MRCA of the *Pseudamnicola* clade (log-normal prior 1.00 ± 1.00 Ma, offset 7.20 Ma) based on the earliest known record of the genus (*P. lobostoma*; age: Miocene, Tortonian (7–11 Ma); Schütt and Besenecker, 1973); (v) the MRCA of the *Pyrgulopsis* clade (log-normal prior 1.50 ± 1.00 Ma, offset 5.30 Ma) based on the earliest known record of the genus (*P. truckeensis*; age: Late Miocene (ca. 5–8 Ma); Hershler and Sada, 2002); (vi) the MRCA of the Belgrandiinae/Caspiinae clade (log-normal prior 2.00 ± 1.00 Ma, offset 28.10 Ma) based on the earliest known record of the clade (*Martinietta tenuiplicata*; age: Oligocene, Rupelian (27–34 Ma); Glibert and de Heinzelin, 1954); (vii) the MRCA of the Hydrobiinae clade (log-normal prior 2.00 ± 1.00 Ma, offset 41.20 Ma) based on the earliest known record of the subfamily (*Polycirsus varicosus*; age: Eocene, Lutetian (41–48 Ma); D'Orbigny, 1837; Rey, 1977); and (viii) the MRCA of the Pyrgulinae clade (log-normal prior 2.00 ± 1.00 Ma, offset 15.97 Ma) based on the earliest known record of the subfamily (*Pseudodianella haueri*; age: Miocene, Burdigalian (16–21 Ma); Neubauer et al., 2013).

We performed four separate MCMC runs using the adaptive parallel tempering algorithm (Müller and Bouckaert, 2020) with a heating temperature of 0.05, a chain length of 300 million and a thinning

interval of 20 000 for each run. Next, we loaded the log files into Tracer to verify that the ESS of each of the parameters was close to or >200. With a 10% burn-in, ESS values above 300 were recovered for most parameters. Consequently, the tree files of all runs could be combined using LogCombiner v. 2.6.6. The maximum clade credibility tree was identified using TreeAnnotator v. 2.6.6, and the mean divergence time estimates with 95% highest posterior density (HPD) intervals were visualised in FigTree.

We assessed the impact of the priors for node calibration on divergence times through sampling from the priors only and compared the resulting node ages with the posterior distribution of node ages obtained from the inference including the sequence data. These distributions might have a similar mean but differ in shape, with a more peaked posterior indicating age information derived from the sequence data. We quantified the difference between both distributions by the Kullback–Leibler (KL) divergence measure, commonly used to assess whether two probability distributions represent the same information. We then compared the observed divergence with the null assumption that prior and posterior distributions are identical, which was obtained by randomly shuffling the node ages between the two distributions. The R package LaplacesDemon v. 16.1.6 (Statisticat, 2021) was used to quantify the KL divergence between the observed prior and posterior distributions of node ages and the 10 000 randomisations approximating the case of no differences. To assess the sensitivity of tree topology and divergence times to the choice of priors, we ran a new BEAST2 analysis without the fossil calibrations that did not differ from their prior distribution, using the same settings as before.

### *Estimation of diversity indices over time*

Considering the controversy over identifying speciation and extinction rates (Louca and Pennell, 2020), we calculated lineage richness over time to assess the diversification dynamics of the Hydrobiidae and to understand how that is influenced by the MEE at the K–Pg boundary. Moreover, we tested whether lineage richness and phylogenetic diversity (herein referred to as phylodiversity) over time are decoupled as a proxy for the phylogenetic distinctiveness of major hydrobiid clades. This was done by identifying periods where their trajectories are significantly different. By increasing the phylogenetic branch lengths, both trends become decoupled, leading to a slower accumulation of lineages that are phylogenetically more distinct and therefore belong to different major clades. For lineage richness, we quantified the number of extant lineages at a moment in time. For phylodiversity, we calculated the phylogenetic diversity per species with the equation $(P_t - t)/N_t$ (Richter et al., 2021), where $P_t$ is the total branch length of all extant lineages $N$ until time $t$ (i.e. phylogenetic diversity sensu Faith, 1992).

However, the extinct lineages and extant species that are not included in our time-calibrated molecular phylogeny may bias the comparison of lineage richness and phylodiversity over time. Data augmentation can mitigate this by allocating additional branches to the observed phylogeny to represent missing lineages (i.e. extinct and unsampled extant species) based on the inferred diversification rate and clade-specific sampling fractions (Table S2). MolluscaBase provided us with the total extant richness of every subfamily, from which we calculated clade-specific sampling fractions. Adding branches to a phylogeny to complete it with extinct and unsampled species is done in a Bayesian framework, in which a set of speciation and extinction rates is proposed for each observed branch in a given time interval. The diversification process, generating additional lineages and terminating them by extinction events, is then simulated towards the present using the proposed speciation and extinction rates. The MCMC proposal will be rejected if the process terminates earlier than the present because all lineages have gone extinct. If, in contrast, it reaches the present with the number of tips equaling the sum of extant and missing species, the proposal is accepted, resulting in a tree augmented by lineages representing extinct and unsampled extant species (e.g. Maliet and Morlon, 2022). We used the cladogenetic diversification rate shift model (ClaDS; Maliet et al., 2019; Maliet and Morlon, 2022) implemented in the PANDA v. 0.0.7 package (https://github.com/hmorlon/PANDA.jl) for the Julia language v. 1.8.5 (Bezanson et al., 2017), with default priors and convergence criteria to obtain 100 MCMC samples that are augmented by missing extant and extinct lineages for our BEAST maximum clade credibility tree and 50 random post-burning trees. We then calculated for all 51 × 100 augmented trees lineage richness and phylodiversity along 0.1 Myr increments between the root age and the present using the R package paleotree v. 3.4.5 (Bapst, 2012).

To test the decoupling relationship between the two temporal trajectories, we first scaled them to the range [0, 1] because of their unequal units (richness vs. phylogenetic diversity per species). Then, we obtained the probability of overlap in their 95% HPD intervals at each moment in time and the proportion of trees that showed an identical directional change in their trajectory. For the latter, we segmented time with a rolling window of 10 Myr and compared the regression slope between lineage richness or phylodiversity and time within the window.

## Results

### *Tree topologies and robustness*

The length of the concatenated dataset was 2462 bp (COI, 658 bp; 16S, 447 bp; 28S, 870 bp; 18S, 487 bp). For all gene partitions, the saturation index ($I_{ss}$) was significantly lower than the critical value ($I_{ss.c}$) (COI, $I_{ss}$ = 0.48, $I_{ss.c}$ = 0.72; 16S, $I_{ss}$ = 0.33, $I_{ss.c}$ = 0.70; 28S, $I_{ss}$ = 0.12, $I_{ss.c}$ = 0.74; 18S, $I_{ss}$ = 0.40, $I_{ss.c}$ = 0.70) for a symmetrical topology ($P < 0.05$), which suggests little or no saturation. With an extremely asymmetrical tree topology, the saturation index did not differ significantly from the critical value for all datasets except for the 28S sequences, suggesting that saturation is unlikely.

Among the single-locus analyses, nodes of the COI and 16S trees received higher support than those inferred from the nuclear gene trees (Figs. S1–S4). While some subfamily-level clades could be retrieved in the 28S trees, only a few nodes were well-supported in the 18S trees. BI analyses, using mixed substitution models for COI and structural models for ribosomal genes, generally produced more robust phylogenies than the MP and ML methods. Branch support and topology similarity among methods increased considerably when concatenating all gene fragments. Adding structural information for the ribosomal RNA partitions in the concatenated BI (scheme (iv)) provided better support values than using mixed substitution models (scheme (iii)); therefore, the results of scheme (iv) are shown in Fig. 2 and Fig. S5. The resulting multilocus phylogenies (Fig. S5) display detailed information about node supports among major retrieved clades. Although basal nodes within the family were still poorly supported by all methods, each of the

concatenated analyses showed high support for most of the critical nodes of this study (i.e. those grouping species into subfamilies and those relating subfamily-level clades).

*Divergence time inference*

According to the divergence time estimates, the Hydrobiidae began to diversify after the K–Pg MEE (mean, 60 Ma; 95% HPD, 52–69 Ma). In 89% of all posterior trees from BEAST2 (i.e. a posterior probability of 0.89), the root node of all Hydrobiidae was younger than 66 Myr (Fig. 1a). The clade attributed to the Hydrobiinae showed the earliest divergence among the established subfamilies with a crown age of approximately 42 Myr (95% HPD, 41–43 Myr). The most recent crown ages were estimated for the clades comprising the recognised subfamilies Mercuriinae and Bullaregiinae n. subfam. (95% HPD, 5–18 Ma).

For six of our eight time-calibration points/bounds, the observed KL divergence was outside what is expected under identical prior and posterior distributions of node ages (Fig. S6). The six calibrations included the oldest one, belonging to the Hydrobiinae clade, as well as fossils from species-rich groups such as the Islamiinae and *Pyrgulopsis*. Our sensitivity test, in which we excluded the two fossil calibrations that did not differ from their prior distribution, showed a high congruence in tree topology and node ages with the inference using all eight calibration points and bounds (Fig. S7). Therefore, we assume that divergence times were informed mainly by sequence divergence and not by priors alone.

*Trends in diversity*

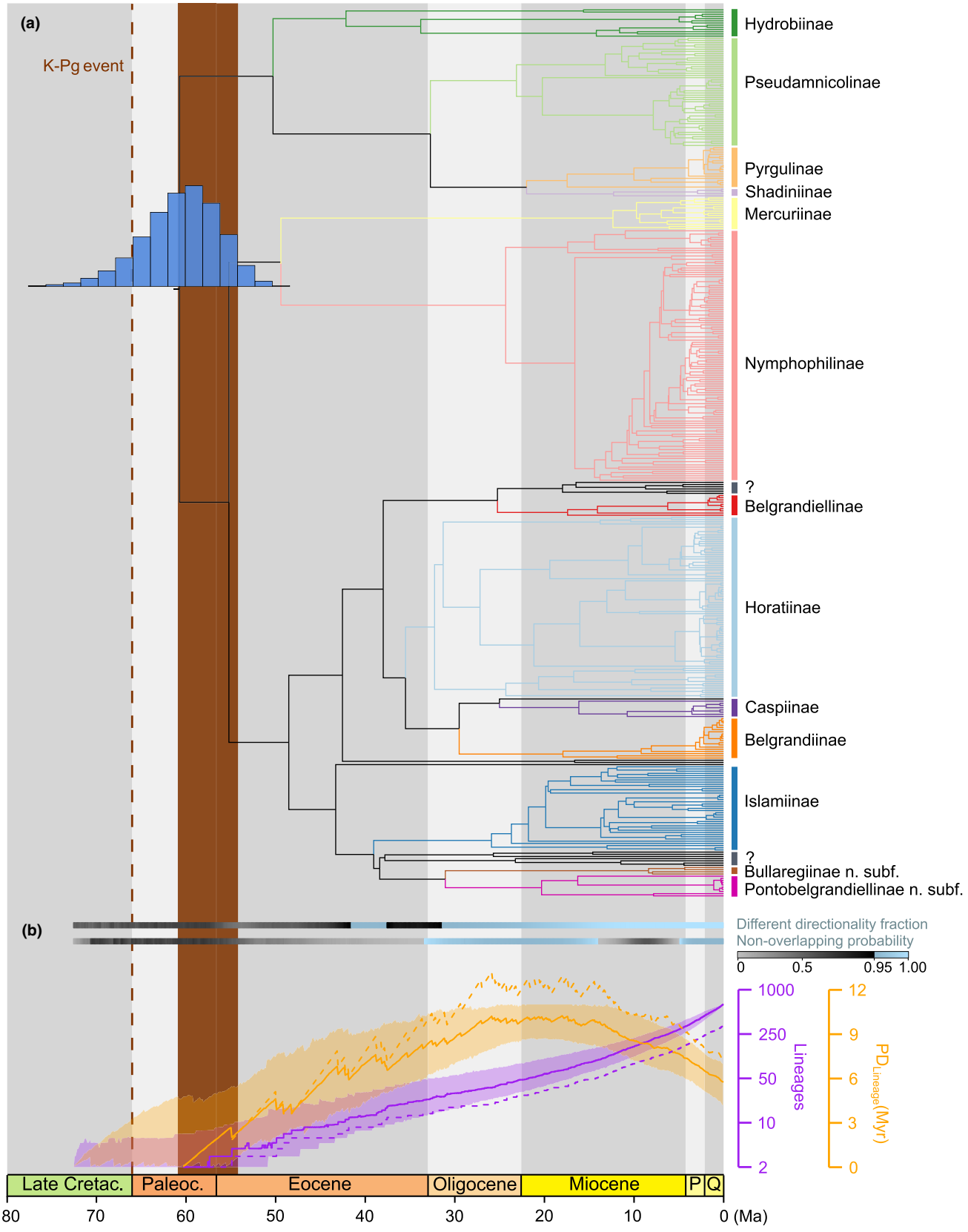
The lineages and phylodiversity through time plots showed a steady increase until ~33 Ma (interrupted by a brief decline in phylodiversity ~39–45 Ma), with overlapping 95% HPD intervals indicating a coupling between both diversity measures (Fig. 1b). Phylodiversity was later decoupled from lineage richness. Both diversity measures continued to increase, but with the rise in phylodiversity being more rapid and the probability of non-overlapping uncertainty intervals exceeded 0.95. Phylodiversity formed a plateau from the Middle Oligocene to the Middle Miocene with an average phylogenetic distance among lineages of ~10 Ma, after which it declined to 60% of that value by the present day. In contrast, lineage richness continued to increase to the present (although augmenting the phylogenies with extinct branches could also result in a decline).

*Subfamily-level clades and their systematic relationships*

Fifteen subfamily-level clades were identified based on the criteria reciprocal monophyly (bootstrap support (BS) > 75% by ML, BPP > 0.95 by BI; Fig. S5), clade age, phylogenetic diversity (Figs. 1 and 2) and morphological/ecological similarity of individuals from the same clade (see Appendix S1). The MP analysis yielded 17 major clades (BS > 75%), most coinciding with clades retrieved by the other two methods. Eleven of the 15 subfamily-level clades emerged from the Middle Oligocene to Middle Miocene (i.e. 12–28 Ma), a period of increased phylogenetic diversity in the family (Fig. 1b). Two subfamily-level clades are older and two are younger. Among the 11 clades of intermediate age, we identified eight as nominal subfamilies based on their nominotypical taxa, one as a potential new subfamily (i.e. Pontobelgrandiellinae n. subfam.; see Appendix S1), and the remaining two as taxonomically unidentified clades. The two older clades were attributed to the subfamilies Hydrobiinae (average crown age 42 Myr; 95% HPD, 41–43 Myr) and Horatiinae (32 Myr; 95% HPD, 27–37 Myr) based on similar morphological/ecological features among their respective taxa, including the nominotypical species. The two younger clades (5–18 Myr) were considered subfamilies, given the large interval of more than 20 Myr between the crown and stem ages and the morphological/ecological similarity of individuals from the same clade. These clades were assigned to the Mercuriinae and a new subfamily, the Bullaregiinae n. subfam. (Appendix S1).

In the MP, ML and BI analyses, the Hydrobiinae, Pseudamnicolinae, Pyrgulinae, Shadiniinae, Mercuriinae and Nymphophilinae formed a well-supported monophyletic group (BS > 75%, BPP > 0.91). In this group, the Pyrgulinae and Shadiniinae are sister taxa. They cluster with the Pseudamnicolinae in the MP (BS > 95%), ML (BS > 75%) and BI (BPP > 0.95) analyses. All three phylogenetic analyses placed the

Fig. 1. Divergence time estimates and diversity dynamics over time within Hydrobiidae. (a) Time-calibrated phylogenetic tree of Hydrobiidae, displaying the frequency of crown age distribution across all posterior trees from the BEAST2 analysis at the tree root. The family began to diversify during the recovery period of European freshwater gastropods (brown bar) after the K–Pg MEE (Neubauer et al., 2021). Coloured vertical bars on the right represent subfamily-level assignments. (b) Number of lineages and phylogenetic diversity per lineage through time, with dashed lines corresponding to the trajectories inferred for the time-calibrated tree. Solid lines represent the trajectories after augmenting the time tree with extinct lineages and missing species through a probabilistic model. The shaded area displays the 95% highest posterior density (HPD) interval for 50 randomly chosen and augmented BEAST2 posterior trees. Horizontal bars indicate the frequency for a different direction in the two trajectories and the probability of no overlap between their HPD intervals, with any blue-shaded intervals indicating decoupling times between lineage and phylodiversity.



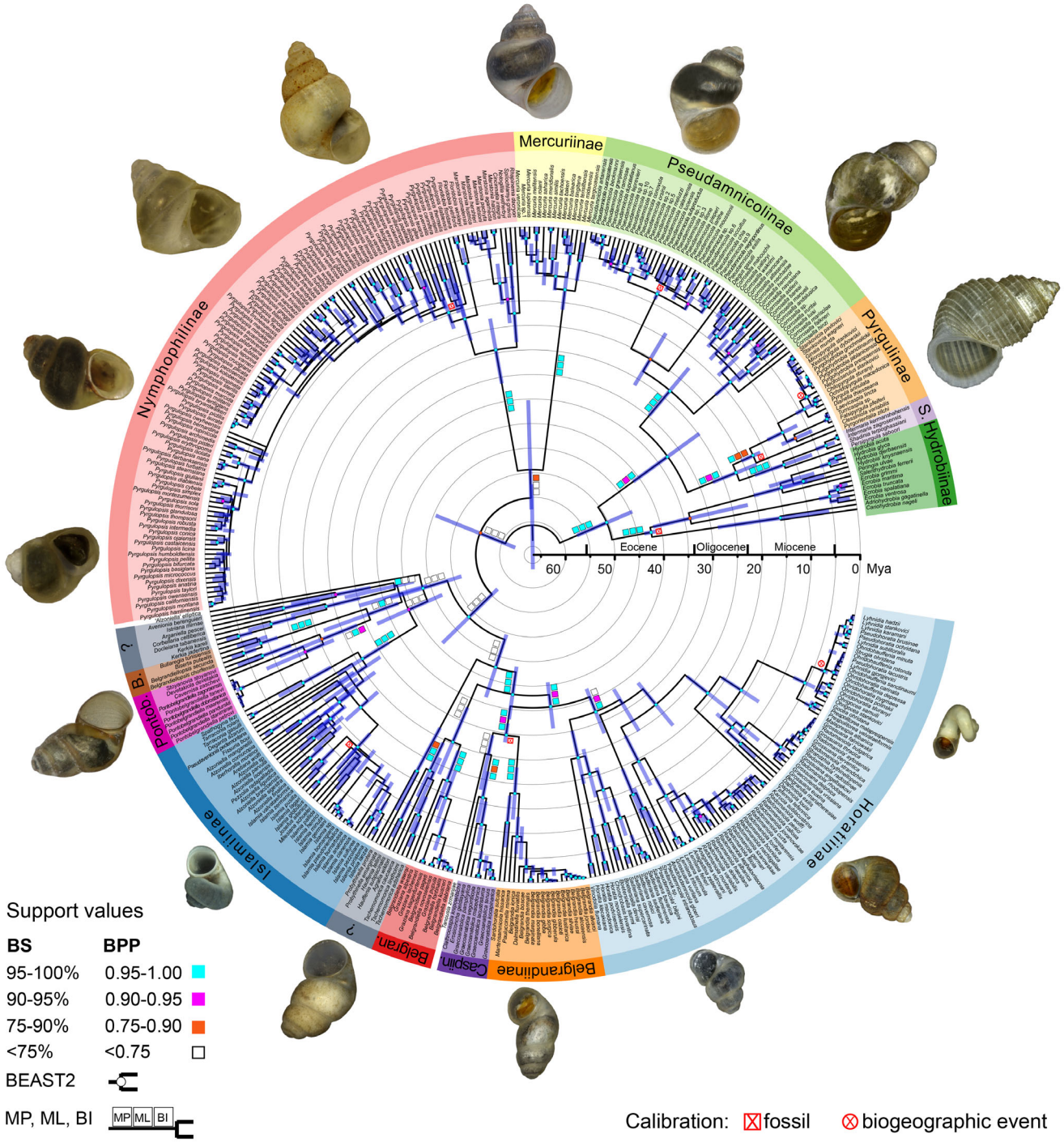



Fig. 2. Maximum clade credibility tree of the family Hydrobiidae inferred using a Bayesian relaxed clock approach (BEAST2), based on four gene fragments and calibrated with six fossils and two biogeographic events. The blue bars show 95% highest posterior density (HPD) intervals of the age estimates. The coloured dots at the nodes represent Bayesian posterior probabilities (BPPs) from BEAST2. The coloured squares at supraspecific (i.e. subfamily level) branches indicate bootstrap support (BS) values from the maximum parsimony (MP) and maximum likelihood (ML) analyses and BPPs from the Bayesian inference (BI) analysis (all based on the concatenated dataset). The detailed results of each phylogenetic analysis are presented in Fig. S5. Subfamily labels S. and B. refer to the Shadiniinae and Bullaregiinae.

Hydrobiinae as sister to the Pseudamnicolinae–Pyrgulinae–Shadiniinae group with high bootstrap and Bayesian support. The phylogenetic position of the Mercuriinae clade (as depicted in Fig. 2) was well supported only by the MP analysis (BPP > 0.95). In all three analyses, a clade comprising the subfamilies

Horatiinae, Belgrandiinae, Caspiinae and Belgrandiellinae was recovered, which was sister to that of *Agrafia*, *Hauffenia* and *Tschernomorica* (BS > 95% and BPP > 0.95). The clade comprising the Belgrandiinae and Caspiinae was sister to the Horatiinae (BS > 75% and BPP > 0.95) and all together clustered in a clade with the Belgrandiellinae (BS > 90% and BPP > 0.95). The recovered phylogenetic positions of the Islamiinae and the other three remaining subfamily-level clades, as well as the genera *Probythinella*, *Arganiella*, *Avenionia* and *Istriana*, and the species *Alzoniella elliptica*, were generally poorly supported in all analyses. The sister relationship between the new subfamilies Bullaregiinae and Pontobelgrandiellinae was well supported only in the ML analysis (BS > 95%).

## Discussion

In this study, we generated multi-locus phylogenies of the hyperdiverse snail family Hydrobiidae based on 406 taxa belonging to 101 genera to test diversification models related to the K–Pg MEE. The divergence time inference revealed that the Hydrobiidae began to diversify in the Palaeocene ~60 Ma (95% HPD, 52–69 Ma), shortly after the K–Pg event (Fig. 1a). Lineage and phylogenetic diversity jointly increased steadily until 33 Ma and then decoupled into different trajectories (Fig. 1b). However, most of the 15 major clades identified did not emerge until the Oligocene (Fig. 2).

### *Onset of Hydrobiidae diversification and the K–Pg MEE*

Our analysis dates the earliest split within the Hydrobiidae to the Palaeocene ~60 Ma (95% HPD, 52–69 Ma), probably after the K–Pg boundary (~66 Ma), for 89% of all posterior trees (Fig. 1a). It roughly corresponds to the beginning of the recovery period of European freshwater gastropods after the K–Pg MEE (~61 Ma; Neubauer et al., 2021). The temporal framework thus points to a younger origin of the family than the Jurassic, as previously suggested based on Carboniferous fossil records or biogeographic events (Knight et al., 1960; Solem and Yochelson, 1979; Thompson, 1979). Instead, our divergence time estimates based on internal calibrations are consistent with divergence times estimated from phylogenies calibrated with external molecular clock rates (Liu and Hershler, 2005; Wilke et al., 2007; Delicado et al., 2015; Miller et al., 2022). There are only a few other freshwater gastropod families with initial diversification times comparable with the Hydrobiidae, such as the Melanopsidae H. Adams & A. Adams, 1854, for which the fossil record dates back to shortly before the K–Pg boundary (Neubauer et al., 2016). An onset of diversification just after the K–Pg boundary has also been suggested for some groups of freshwater fish (Imoto et al., 2013; Kappas et al., 2016).

### *Gradual evolution after the K–Pg MEE*

The time-calibrated phylogeny for the Hydrobiidae (Fig. 1a) shows a gradual increase in lineage richness from the onset of diversification ~60 Ma to the present. A slightly different picture emerges for the phylogenetic diversity, which increases from ~60 to ~25 Ma, plateauing until ~15 Ma, and then decreasing to the present (Fig. 1b). Comparing the two trajectories, the decoupling between lineage and phylodiversity did not start until ~33 Ma. Moreover, an early rapid diversification in the family's history in response to the high ecological opportunity associated with the massive loss of gastropod species during the K–Pg MEE (Neubauer et al., 2021) can be rejected. This pattern of a gradual evolution of the Hydrobiidae after the K–Pg boundary also contrasts with the overall rapid increase in species richness of gastropods 55–61 Ma inferred from the fossil records (Neubauer et al., 2021). These differences could be due to a differential resilience of freshwater ecosystems to the perturbations of the K–Pg event. Unlike most other freshwater gastropods, hydrobiids are predominantly associated with springs and subterranean waters. These environments yielded more survivors than other inland waters, such as shallow lakes, wetlands or coastal ecosystems, as they acted as thermal refugia during the prolonged global winter following the K–Pg event (Robertson et al., 2013). Therefore, lower ecological opportunity in springs and subterranean waters may have prevented an early rapid diversification of hydrobiids in the Palaeocene owing to the higher prevalence of the resident fauna.

Our molecular data evidence the emergence of fast-diversifying clades in the Miocene (e.g. Pseudamnicolinae, Nymphophilinae and Caspiinae), resulting in an accelerated lineage accumulation of hydrobiids that is consistent with the diversification rates inferred for Palaearctic and Nearctic gastropod species based on fossil data (Neubauer et al., 2015, 2021, 2022a). This has been attributed to intense topographic isolations, paleoclimatic change and the evolution of several long-lived lake systems (in the Palaearctic) during the last 20 Myr, leading to highly diverse faunas (Neubauer et al., 2022a, 2022b). These factors potentially stimulated speciation across different hydrobiid lifestyles and subfamilies, such as the Pseudamnicolinae of mountain and lowland springs (Delicado et al., 2018), the desert-spring species belonging to Nymphophilinae (Liu and Hershler, 2005) and the mainly lacustrine Caspiinae (Wesselingh et al., 2019) and Horatiinae (Föller et al., 2015).

A decoupling of diversity trends owing to declines in phylogenetic diversity (Fig. 1b) since the middle Miocene coincides with a period of increasing landscape transformation and re-shaping of continental hydrological systems. It appears that Miocene tectonics and the subsequent formation of the current river basins (Colgan and Henry, 2009; Tockner et al., 2009) played an important role in the configuration of the present-day hydrobiid fauna. Future research is needed to compare diversification dynamics and processes (e.g. adaptive vs. non-adaptive radiations; see Delicado and Hauffe, 2022) among freshwater gastropod families, especially among the spring and subterranean ones, and to test for heterogeneity in diversification drivers across clades and lifestyles (see Delicado et al., 2018).

### *Hydrobiidae resemble the soft-explosive model of diversification*

Five diversification models have been proposed in relation to the impact of the K–Pg MEE (see Introduction). They are based on timing and rate of diversification (Springer et al., 2019). For the Hydrobiidae, we inferred an onset of diversification shortly after the K–Pg MEE (see the Section 'Onset of Hydrobiidae diversification and the K–Pg MEE') and a gradual evolution following this event (see the Section 'Gradual evolution after the K–Pg MEE'). Based on the two criteria of Springer et al. (2019), four models can be discarded: the short-fuse model (crown age and supraspecific diversification before the K–Pg boundary), the long-fuse and trans-KPg models (crown age before the K–Pg boundary with supraspecific diversification occurring across the MEE) and the explosive model (rapid diversification starting after the K–Pg boundary). Instead, the pattern derived for the Hydrobiidae most closely matches the soft-explosive model with gradual diversification, differing only in that diversification starts at the K–Pg boundary rather than shortly before it. Therefore, the overarching working hypothesis of this study, i.e. the K–Pg MEE triggered the rapid evolution of the Hydrobiidae in conjunction with the rapid occupation of vacant ecological niche space, must be rejected. Apparently, the Hydrobiidae did not benefit from the massive loss of other gastropod species during the K–Pg MEE (Neubauer et al., 2021), resulting in a gradual diversification.

These patterns thus contrast with those of most other families of freshwater gastropods, which originated before the K–Pg boundary (Liu et al., 2014; Stelbrink et al., 2020; Neubauer and Harzhauser, 2022) and may therefore follow the short-fuse model (Bininda-Emonds et al., 2007). Instead, the patterns observed in the Hydrobiidae bear some resemblance to the soft-explosive diversification of aquatic vertebrates (Bertozzi et al., 2016; Feng et al., 2017; Alfaro et al., 2018), in which several families arose soon after the K–Pg boundary.

### *Implications for subfamily-level systematics within the Hydrobiidae*

Our phylogenetic criteria (i.e. monophyly, evolutionary age and phylogenetic distinctiveness) indicate the presence of at least 15 subfamily-level taxa within Hydrobiidae (Fig. 2). Of these 15 taxa, 11 were previously described as nominal subfamilies (i.e. Hydrobiinae, Pseudamnicolinae, Pyrgulinae, Shadiniinae, Mercuriinae, Nymphophilinae, Belgrandiellinae, Horatiinae, Belgrandiinae, Caspiinae and Islamiinae). Two (i.e. a clade containing the genera *Hauffenia*, *Agrafia* and *Tschernomorica* and a clade containing the genera *Kerkia*, *Docleiana* and *Corbellaria*) are considered as potential subfamilies, and two (i.e. Bullaregiinae n. subfam. and Pontobelgrandiellinae n. subfam.) are here described as new subfamilies (Appendix S1).

Of the 11 subfamilies previously described, nine have already been confirmed using molecular data (Hershler et al., 2003; Wilke et al., 2013; Anistratenko et al., 2017). The status of the remaining two subfamilies (i.e. Caspiinae and Mercuriinae) is supported here by DNA sequence data. The two clades with unknown subfamily assignment are primarily composed of small hydrobiid taxa living in subterranean waters of southern Europe and western Asia (Bodon et al., 2001; Szarowska and Falniowski, 2011a; Delicado et al., 2019, 2021; Vinarski and Palatov, 2019). They would deserve separate status as distinct subfamilies according to a crown age similar to other subfamily-level clades (i.e. $\sim$20 Myr) and their phylogenetic distinctiveness. However, describing them as subfamilies would require additional systematic and ecological studies of these groundwater-restricted gastropods (see Appendix S1).

We here describe the other newly recorded clades as two new subfamilies because our study includes most species previously discussed as belonging to them. One clade corresponds to the new subfamily Bullaregiinae n. subfam., which includes subterranean species that occur in North Africa and have unusual anatomical characteristics among hydrobiids (see Appendix S1). This has led to previous discussions about their subfamily classification (Khalloufi et al., 2017, 2020). Pontobelgrandiellinae n. subfam., the second newly described subfamily, also consists of subterranean species distributed in the northern regions of the Balkan Peninsula (Osikowski et al., 2017).

A limited understanding of hydrobiid biodiversity and the increasing anthropogenic pressures on springs and subterranean waters (Szarowska and Falniowski, 2011b; Hershler et al., 2014) emphasise a need for conserving this hyperdiverse taxon. This is an

essential point, given that our systematic results show that entire modern subfamilies of subterranean aquatic gastropods have hitherto been overlooked. Moreover, these taxa exhibit an evolutionary history that differs from some better-studied groups as vertebrates. Information on the evolutionary history of a taxon and phylogenetic diversity is increasingly used to assess the conservation needs of species groups or geographic regions (Forest et al., 2007; Kling et al., 2019). For example, Miller et al. (2018) emphasised the importance of considering evolutionary history along with factors that influence dispersal (e.g. geographic connectivity or biogeographic affiliation) when identifying hotspots of high conservation value for hydrobiids. Given the high extinction risks faced by freshwater gastropods today (Neubauer et al., 2021) and the threatened status of many hydrobiid species (note that of the ~600 species of "Hydrobiidae" reported in the IUCN Red List (IUCN, 2022), only 74 (= 12%) are considered Least Concern), there is an urgent need to study the diversity and evolutionary history of hydrobiids, particularly in vulnerable ecosystems.

### *Methodological limitations of this study*

Unravelling evolutionary history and patterns of diversification from molecular phylogenies is vulnerable to two principal problems. The first problem concerns the reliable calibration of a time-dated tree based on internal calibration points. The fossil record of the Hydrobiidae is limited because specimens are tiny in size and usually found in springs and subterranean ecosystems where shells are often not sufficiently preserved (Strong et al., 2008). However, we could provide eight calibration points/bounds across our phylogeny (see Materials and methods for details). Subsequent dating revealed that the age of the root node of Hydrobiidae is younger than 66 Ma in 89% of all posterior trees. These time estimates are supported by previous molecular-clock studies of Hydrobiidae using external clock rates (see the Section 'Onset of Hydrobiidae diversification and the K–Pg MEE') and a comparison with the time-tree priors (Fig. S6). We therefore think that the limited fossil record for the Hydrobiidae has not affected the main conclusions of our study.

The second problem relates to the limited taxon sampling in the context of diversification-rate estimations. Incomplete taxon sampling and missing information on extinction events in phylogenies of extant species might lead to an underestimation of lineage richness and an overestimation of phylodiversity through time owing to longer internal and shorter terminal branches (Richter et al., 2021). In our study, we covered almost 50% of the know species and nearly 60% of the known genera of the Hydrobiidae. Moreover, we used data augmentation informed by clade-specific sampling fractions to infer the position and length of missing phylogenetic branches. In this way, we were able to provide a robust and unbiased perspective on the decoupling dynamics of lineage accumulation and phylodiversity.

## Conclusions

Our study clearly evidenced that, unlike most modern families of freshwater gastropods, the Hydrobiidae started to diversify shortly after the K–Pg MEE. While this overlaps with the recovery period of freshwater gastropods, lineages and phylodiversity did not rapidly accumulate during the early stages of the Hydrobiidae evolution. This suggests that diversification was not initially driven by ecological opportunity. Accordingly, our working hypothesis—the K–Pg MEE triggered the explosive diversification of the Hydrobiidae as a result of the rapid occupation of vacant ecological niche space—must be rejected. The estimated time of origin and temporal diversity patterns indicate that the Hydrobiidae follow the soft-explosive diversification model with gradual supraspecific branching starting after the K–Pg MEE. In contrast, most freshwater gastropod families originated before the K–Pg boundary and probably evolved according to a short-fuse model.

Most of the 15 subfamily-level clades (identified using the phylogenetic criteria of monophyly, evolutionary age and phylogenetic distinctiveness) did not emerge before the Oligocene, the period of increasing phylogenetic diversity in the family. Eleven of these 15 taxa correspond to nominal subfamilies, two are considered potential subfamilies and two (i.e. Bullaregiinae n. subfam. and Pontobelgrandiellinae n. subfam.) are described here as new subfamilies. These hitherto overlooked clades emphasise the need to further study the diversity and evolution of hydrobiids, especially in vulnerable ecosystems like springs and subterranean waters that are increasingly exposed to anthropogenic pressure.

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## Conflict of interest

None declared.

## Data availability statement

New sequences were deposited in GenBank (Table S1) and sequence alignments are available at: https://doi.org/10.6084/m9.figshare.22317709.

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## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Fig. S1.** Phylogenetic relationships among higher clades of hydrobiid gastropods reconstructed with maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) using the COI gene fragment.

**Fig. S2.** Phylogenetic relationships among higher clades of hydrobiid gastropods reconstructed with maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) using the 16S gene fragment.

**Fig. S3.** Phylogenetic relationships among higher clades of hydrobiid gastropods reconstructed with maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) using the 28S gene fragment.

**Fig. S4.** Phylogenetic relationships among higher clades of hydrobiid gastropods reconstructed with maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) using the 18S gene fragment.

**Fig. S5.** Phylogenetic relationships among major clades of hydrobiid gastropods reconstructed with maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) using the concatenated dataset of four gene fragments.

**Fig. S6.** Comparison of the prior and posterior distribution inferred in the BEAST2 time-tree analyses for the age of each of the eight calibration nodes.

**Fig. S7.** Robustness of Bayesian time calibration against prior choice.

**Table S1.** Species name and GenBank accession numbers for the taxa studied.

**Table S2.** Sampling fraction applied to the 15 subfamily-level clades to quantify phylodiversity over time.

**Appendix S1.** Systematic descriptions of hydrobiid subfamilies.