

Original Article

Molecular insights into the taxonomy and biogeography of subterranean Sphaeromatidae in Europe (Crustacea: Isopoda)

Teo Delić^{1, }, Rudi Verovnik¹, Jana Bedek^{2, }, Maja Zagmajster¹, Fabio Stoch^{3, },
Simona Prevorčnik¹

¹Biotechnical Faculty, Department of Biology, SubBioLab, University of Ljubljana, 1000 Ljubljana, Slovenia

²Croatian Biospeleological Society, 10000 Zagreb, Croatia

³Evolutionary Biology and Ecology, Université libre de Bruxelles, B-1050 Brussels, Belgium

*Corresponding author. Biotechnical Faculty, Department of Biology, SubBioLab, University of Ljubljana, 1000 Ljubljana, Slovenia. E-mail: teo.delic@bf.uni-lj.si

ABSTRACT

European subterranean crustaceans represent a significant component of freshwater biodiversity; however, many taxa remain poorly studied. Among them are the groundwater-dwelling isopods of the family Sphaeromatidae, which are distributed across France, Switzerland, Italy, Slovenia, and the Dinaric Karst. We examined specimens spanning the full geographic range of all known species and subspecies, integrating morphological analyses with multilocus DNA data to identify molecular operational taxonomic units (MOTUs) and to test long-standing taxonomic hypotheses. Our results reveal a well-supported clade of European subterranean Sphaeromatidae comprising 29–32 MOTUs, with contrasting patterns of diversity: some morphologically uniform taxa show deep genetic divergence, while others display extensive morphological variation but limited genetic differentiation. The phylogeny supports two geographically distinct genera—*Caecosphaeroma* in the west and *Monolistra* in the east—but does not support the traditional subgeneric classification, suggesting paraphyletic or polyphyletic origins. Time-calibrated analyses indicate that the group originated in the Cretaceous, pre-dating the Alpine and Dinaric orogeny, which probably played a major role in subsequent diversification. This study provides the first comprehensive phylogenetic framework for European subterranean Sphaeromatidae, highlights pronounced mismatches between morphological and genetic diversity, and establishes a foundation for future research on their evolution and biogeographic history.

Keywords: Pre-Alps; Dinaric Karst; homoplasy; *Monolistra*; phylogeny; trans-Adriatic

INTRODUCTION

Sphaeromatidae Latreille, 1925 are a species-rich family of isopod crustaceans, comprising more than 100 genera with more than 700 species (Wetzer *et al.* 2018, Boyko *et al.* 2026). Sphaeromatids are mainly detritus feeders; most species are marine bottom-dwellers, inhabiting a wide variety of habitats, from sandy beaches and gravel seabed to macroalgae and sponges (Poore and Bruce 2012). In addition to seawater, a small fraction of species independently colonized continental freshwaters (Wilson 2008). To date, only four genera (*Gnorimosphaeroma* Menzies, 1954; *Exosphaeroides* Holdich and Harrison, 1983; *Bilistra* Sket and Bruce, 2004; and *Makarasphaera* Bruce, 2005) have been reported

from freshwater habitats, in the amphi-Pacific region (Holdich and Harrison 1983, Nunomura 1998, Sket and Bruce 2003, Bruce 2005), one (*Thermosphaeroma* Cole and Bane, 1978) from eastern North America (Cole and Bane 1978), and three genera (*Monolistra* Gerstaecker, 1856; *Caecosphaeroma* Dollfus, 1896 and *Merozoon* Sket, 2012) from Europe (Wetzer *et al.* 2018, Boyko *et al.* 2026).

The European genera allegedly form a monophylum and were traditionally assigned to the tribe Monolistrini (Wetzer *et al.* 2018). With 37 species, European representatives stand for the most species-rich group of freshwater sphaeromatids worldwide (Boyko *et al.* 2026). Interestingly, all members of the group live

Received 22 October 2025; revised 26 March 2026; accepted 10 April 2026

© The Author(s) 2026. Published by Oxford University Press on behalf of The Linnean Society of London.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

exclusively in subterranean freshwater (Sket 1986, 2012). Two alternative hypotheses were proposed to explain the colonization of inland subterranean habitats. The first suggests that the marine ancestors crossed two ecological boundaries, i.e. the marine–freshwater barrier and the surface–subterranean barrier (Sket 1986, 2012); the taxon is thus considered as ‘limnicoid stygobiont’ (Coineau and Boutin 1992). The second (Wägele 1990) considers this group of direct marine origin (ie, a ‘thalassoid stygobiont’). The opposing views make subterranean Sphaeromatidae an intriguing model system for ecological and evolutionary studies.

European freshwater sphaeromatids are currently split into a Western and an Eastern group of species. The less diverse Western group, represented by the sole genus *Caecosphaeroma* Dollfus, 1896, with only two known species and a subspecies, is distributed in France (Sket 1986). The Eastern group comprises two genera, the peculiar and enigmatic *Merozoon* Sket, 2012, described from a fragment of a single specimen found in an anchialine cave on the Adriatic coast (Sket 2012), and the highly diversified genus *Monolistra* Gerstaecker, 1856. The latter is distributed along the central and eastern Southern Limestone Pre-Alps (Switzerland, Italy, Slovenia) (Ruffo and Stoch 2005, Stoch and Galassi 2010) and in the Dinaric Karst (Italy, Slovenia, Croatia, Bosnia and Herzegovina, Montenegro, and Albania) (Sket 1964, Prevorčnik et al. 2010). Recently, a remote and isolated population was discovered on the Tyrrhenian coast of peninsular Italy (reported herein). Currently, there are 34 formally described *Monolistra*

species and subspecies. The genus *Monolistra* is currently comprising five subgenera: *Monolistra* Gerstaecker, 1856, *Typhlosphaeroma* Racovitza, 1910, *Microlistra* Racovitza, 1929, *Pseudomonolistra* Hubault, 1937, and *Monolistrella* Sket, 1964 (Fig. 1) (Sket 1964).

The current taxonomic structure and phylogenetic relationships of the subterranean sphaeromatids have been inferred mainly from their morphology and geographic distribution. Likewise, the global molecular phylogenetic analysis of relationship among sphaeromatid genera (Wetzer et al. 2018) did not include representatives of this group and, therefore, their phylogenetic placement and division were unknown. Consequently, the subgeneric subdivision of the genus *Monolistra* relied on morphological traits such as sexual dimorphism, prehensile pereopods, uropod reduction, and cuticle ornamentation (Sket 1964). The only exception was a phylogenetic analysis of the subgenus *Microlistra* (Prevorčnik et al. 2010), which suggested the monophyly of this subgenus. However, studies of different subterranean taxa showed that a discrepancy between morphological and genetic variation is a rule rather than an exception (Zakšek et al. 2009, Bilandžija et al. 2013, Recknagel et al. 2024, Lapadula et al. 2025), with two emerging commonalities. The first is the high morphological similarity of genetically distinct species, the so-called cryptic species. Studies of subterranean amphipods, aselloid isopods (Eme et al. 2017) and Italian Pre-Alpine *Monolistra* (Lapadula et al. 2025) populations suggested that morphologically distinct species might include genetically distinct



Figure 1. Map of European freshwater sphaeromatids sampling localities included in this study, shown together with the major mountainous areas (grey) and the outline of the Dinaric Karst (light red). Examples of morphological diversity within the genus *Monolistra* are presented on the right, including species with and without cuticular ornamentation and with varying uropod presence and elongation. From left to right: *M. spinosa* (Racovitza, 1929); *M. pretneri* Sket, 1965; *M. coeca* Gerstaecker, 1856; *M. hercegovinensis* Absolon, 1916, and *M. bolei* (Sket, 1960).

lineages, considered as putative cryptic species. The second commonality is a high degree of convergent evolution leading to homoplasy, masking the true phylogenetic relationship among species (Fišer *et al.* 2008, Lukić *et al.* 2019). Hence, to exploit the potential of subterranean sphaeromatids in ecological, evolutionary, and biogeographic studies, a comprehensive taxonomic analysis using DNA-based tools was deemed necessary.

To fill this knowledge gap, we employed extensive molecular revision of the European freshwater Sphaeromatidae, including all available material from the entire geographic range and all described taxa. First, we examined the molecular diversity of freshwater Sphaeromatidae and tested for cryptic diversity using unilocus species' delimitation methods. Second, we employed multilocus molecular phylogenetics and tested whether current morphologically defined generic and subgeneric classifications reflects phylogenetic relationships. By comparing diagnostic morphological traits and molecular diversification, we examined the overlap between morphologically described species and molecular operational taxonomic units (MOTUs). Finally, using a time-calibrated phylogeny, we correlated the main cladogenetic events with the palaeogeographic history of the eastern Mediterranean region providing a basis for further evolutionary and biogeographical studies.

MATERIALS AND METHODS

Geographical scope and taxa analysed

We collected samples of two European sphaeromatid genera from their whole geographic range, from France, over the southern Alps to the eastern Dinarides, including the recently discovered disjunct population from peninsular Italy. We included both known species of *Caecosphaeroma*, 31 out of 34 described species or subspecies of *Monolistra*, and several *Monolistra* populations with undetermined species affiliations (Supporting information, S1).

Altogether, we analysed samples from 82 caves, 38 springs, and four wells (Supporting information, S1). In addition to specimens from 26 type localities, we included samples distributed throughout the entire species or subspecies ranges. If material from the type localities was unavailable (five taxa), we included the individuals collected at the nearest known locality. Overall, we obtained samples from 35% of all known *Monolistra* sites (Zagmajster *et al.* 2014), a total of 169 individuals (Supporting information, S1). The outgroups comprised 12 individuals of the Mediterranean Sphaeromatidae marine and brackish water genera *Lekanesphaera*, *Sphaeroma*, *Paravireia*, *Dynamene*, and *Campepeopea*. All individuals were fixed in 96% ethanol at the time of collection and identified to the species/subspecies level based on their external morphology. The morphological samples are stored in the Zoological Collection at the Subterranean Biology Laboratory (SubBioLab), Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia. Information on localities, sampling dates, collectors and GenBank accession numbers are given in Supporting information, S1.

DNA extraction, PCR amplification, and DNA sequencing

We extracted genomic DNA from 179 individuals using the GeneElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich) and amplified two mitochondrial (12S rRNA and

16S rRNA) and three nuclear (28S rRNA, histone *H3*, and aminoacyl-tRNA synthetase—*EPRS*) gene fragments. Amplified polymerase chain reaction (PCR) products were purified with Exonuclease I and Thermosensitive Alkaline Phosphatase (Thermo Fisher Scientific Inc., US) and were sequenced in both directions by Macrogen Europe (Amsterdam, Netherlands) using the same primers used for amplification. We visually checked and edited the resulting sequences in GENEIOUS 8.0.4. (Biomatters Ltd, New Zealand) and aligned in MAFFT v.7 (Katoh and Standley 2013) using the E-INS-i algorithm. A list of primers and amplification protocols used is available in Supporting information, S2.

DNA-based species' delimitation

To acquire a robust support for the DNA-based 'species candidates' or molecular operational taxonomic units (hereafter MOTUs), we applied two unilocus species' delimitation procedures (Carstens *et al.* 2013, Fontaneto *et al.* 2015) using two mitochondrial gene fragments, 12S rRNA and 16S rRNA, resulting in four separate analyses.

We employed the distance-based assemble species by automatic partitioning (ASAP: Puillandre *et al.* 2021) method and the tree-based Poisson tree processes (PTP) model (Zhang *et al.* 2013). The ASAP species' delimitation method was implemented using the ASAP web-server available at <https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html#>, under the Kimura two parameter substitution model (K80 v.2.0) and the default settings.

While ASAP sorts the sequences into 'candidate species' based solely on the distribution of genetic pairwise distances, without an a priori species hypothesis, PTP uses a phylogenetic input tree to infer putative species boundaries (Tang *et al.* 2014). We ran two alternative PTP models, using (i) the maximum likelihood solution (PTP), and (ii) the Bayesian implementation (bPTP). 12S rRNA and 16S rRNA phylogenetic trees, inferred in PhyML (Guindon *et al.* 2010), were used as an input for the analyses. Species' delimitations in bPTP were conducted after running 500000 generations, sampling every 500 generations, and discarding the first 20% of the samples as a burn-in.

Phylogenetic analysis

Phylogenetic relationships were reconstructed using Bayesian inference in MrBayes v.3.2.6 (Ronquist *et al.* 2012) and maximum likelihood using IQtree v.1.6.7 (Nguyen *et al.* 2015). Individual gene fragment alignments were concatenated to produce a final, 3509-bp long alignment for 179 individuals, which was used in the phylogenetic analyses (available in Supporting information, S3).

The best-fitted partitioning scheme and the appropriate evolutionary model for each of the gene partitions were selected via PartitionFinder 2.1.1 (Lanfear *et al.* 2016). Due to the protein-coding nature of *H3* and *EPRS* genes, codon-specific evolutionary models were selected for these two genes. Information on the partitioning schemes and selected evolutionary models are available in Supporting information, S3. Bayesian phylogenetic relationships were inferred after running two parallel Markov chain Monte Carlo (MCMC) runs of four cold chains for 15 million generations in MrBayes, sampling every 2000 generations; the first 25% of the sampled trees were discarded as burn-in.

Maximum likelihood analyses were run using the partition-specific models with node support assessed by performing 1000 ultrafast bootstrap replicates (Hoang *et al.* 2018) and testing alternative topologies by an SH-aLRT test (Guindon *et al.* 2010) with 1000 replicates.

Estimation of divergence times

To assess the temporal diversification of subterranean Sphaeromatidae, we reconstructed a time-calibrated multilocus phylogeny using the package BEAST 2.6.3 (Bouckaert *et al.* 2019). The same dataset as above, comprising 179 individuals and five molecular markers, was analysed using partitioning schemes with distinct parameter settings, following the best-fit models of evolution proposed by bModelTest (Bouckaert and Drummond 2017). To avoid over-parametrization, we reduced the original number of partitions (nine), to gene-specific partitions (five). Substitution and clock models were unlinked for all partitions, while the tree partitions were linked to generate a single dated phylogeny. The marginal likelihood of birth–death and Yule speciation tree priors were compared in BEAST’s extension Path Sampler. Based on the marginal likelihood, we used a Yule speciation tree prior for the final analyses (results not shown). Based on a low coefficient of variation, lower than 0.1 under a log-normal clock, the analysis was run using a strict molecular clock (Drummond *et al.* 2006). The tree was calibrated based on the similarity of the distributional pattern to the one present in the trans-Adriatic representatives of the subterranean amphipod genus *Niphargus*, distributed in the Dinaric Karst and the Apennine Peninsula (Delić *et al.* 2020). The trees were calibrated by using (i) Mid-Miocene land-bridge (14 Mya); (ii) Messinian salinity crisis land-bridge; and (iii) Pleistocene land-bridge. As the first and the last options gave results that expressed large discrepancies with the estimated origins of the Dinaric subterranean fauna (Delić *et al.* 2020, Borko *et al.* 2021), 254 Mya and 8 Mya, respectively, we calibrated the tree using the age of the well-documented Messinian salinity crisis (5.96–5.33 Mya). At the time, the two peninsulas were connected by a land-bridge across the Adriatic Sea, which has been recognized to have a crucial impact on distribution of subterranean taxa (Delić *et al.* 2020). The calibration point was set to a normal distribution with a 95% confidence interval in the period of Messinian Salinity Crisis, between 5.33 Mya and 5.96 Mya. Four independent runs of 100 million generations, sampling every 10000 steps, were performed, and combined using LogCombiner 2.6.3. The stationarity of singles run was checked in TRACER and the first 20% trees were discarded as burn-in. All the remaining samples from the posterior distribution were summarized and subsampled using TreeAnnotator 2.6.3 and the maximum clade credibility (MCC) tree was visualized in FigTree 1.4.4 (Rambaut 2018).

Morphological dataset

We constructed a data matrix of ‘taxa x characters’. The taxa were represented by previously recognized MOTUs, including putative species. We included the following morphological characters that were used as diagnostic characters in morphological species’ delimitation: (i) the body length ratio of males versus females (character states: males larger than females; smaller than females; equal to females); (ii) the shape of male pereopod II (character

states: prehensile, with a developed claw; non-prehensile); (iii) the development of uropods [character states: normally developed (biarticulated), residual (small remnant raised on a small bump), reduced or absent (either as a small unraised remnant or absent)]; and (iv) presence of dorsal pereopod spines or protuberances (character states: pereopod smooth; pereopod with spines, bumps, tubercles).

We measured at least one adult individual per each sex per MOTU. Every individual was measured under a dissection microscope Olympus SZX9 with a mounted digital camera, ColorViewIII. In some cases, when the adult individuals of either sex were damaged or missing, we used measurements and data from the descriptions in the literature. Measurements are given in Supporting information, S1.

RESULTS

Molecular diversity of *Monolistra*

Both species’ delimitation methods suggested that *Monolistra* comprised 29 to 32 MOTUs (Supporting information, S1, S4). The upper limit of MOTU diversity, considering all MOTUs identified in at least one analysis, is 36. However, not all currently valid species/subspecies were supported as separate MOTUs, as out of 31 extant taxa included in our study, only 22 MOTUs were identified. There were 14 additional MOTUs identified, representing putative distinct species. Most of the latter were placed within the subgenera *Monolistra* and *Typhlosphaeroma*. In the case of *Monolistra coeca* Gerstaecker, 1856, morphologically recognized as a single species, four distinct allopatric MOTUs are recognized. On the other hand, none of the delimitation approaches revealed MOTUs that would be matching the species described within the morphologically highly diversified and monophyletic subgenus *Microlistra*.

Phylogeny and subgenera of *Monolistra*

Both methods, maximum likelihood and Bayesian inference, resulted in similar tree topologies, indicating the robustness of the results (Supporting information, S5). The monophyly and sister-relationship of the two genera, western *Caecosphaeroma* and eastern *Monolistra*, were supported by both approaches, justifying the present taxonomic subdivision of subterranean Sphaeromatidae at the generic level (Fig. 2; Supporting information, S5). Bayesian analysis recovered strong support for individual lineages within *Monolistra* (PP ≥ 95), but intrageneric branches remained short and mostly unsupported. The ranges of well-supported clades broadly overlapped (Fig. 3).

The subgeneric structure of *Monolistra* was only partially recovered. Two north-west and central Dinaric subgenera, *Microlistra* and *Monolistrella*, received high support, but *Monolistra*, *Typhlosphaeroma* and *Pseudomonolistra*, turned out to be paraphyletic or polyphyletic. The polyphyly of the nominal subgenus, *Monolistra*, is the most outstanding as it includes at least seven independent geographically defined lineages, scattered across the entire distribution range of the genus, i.e. from the Alps in the north-west to Albania in the south-east (Fig. 3; Supporting information, S5). Likewise, the subgenus *Typhlosphaeroma* is subdivided into several lineages distributed across the whole range of the genus. Paraphyly of the subgenus *Pseudomonolistra* emerged

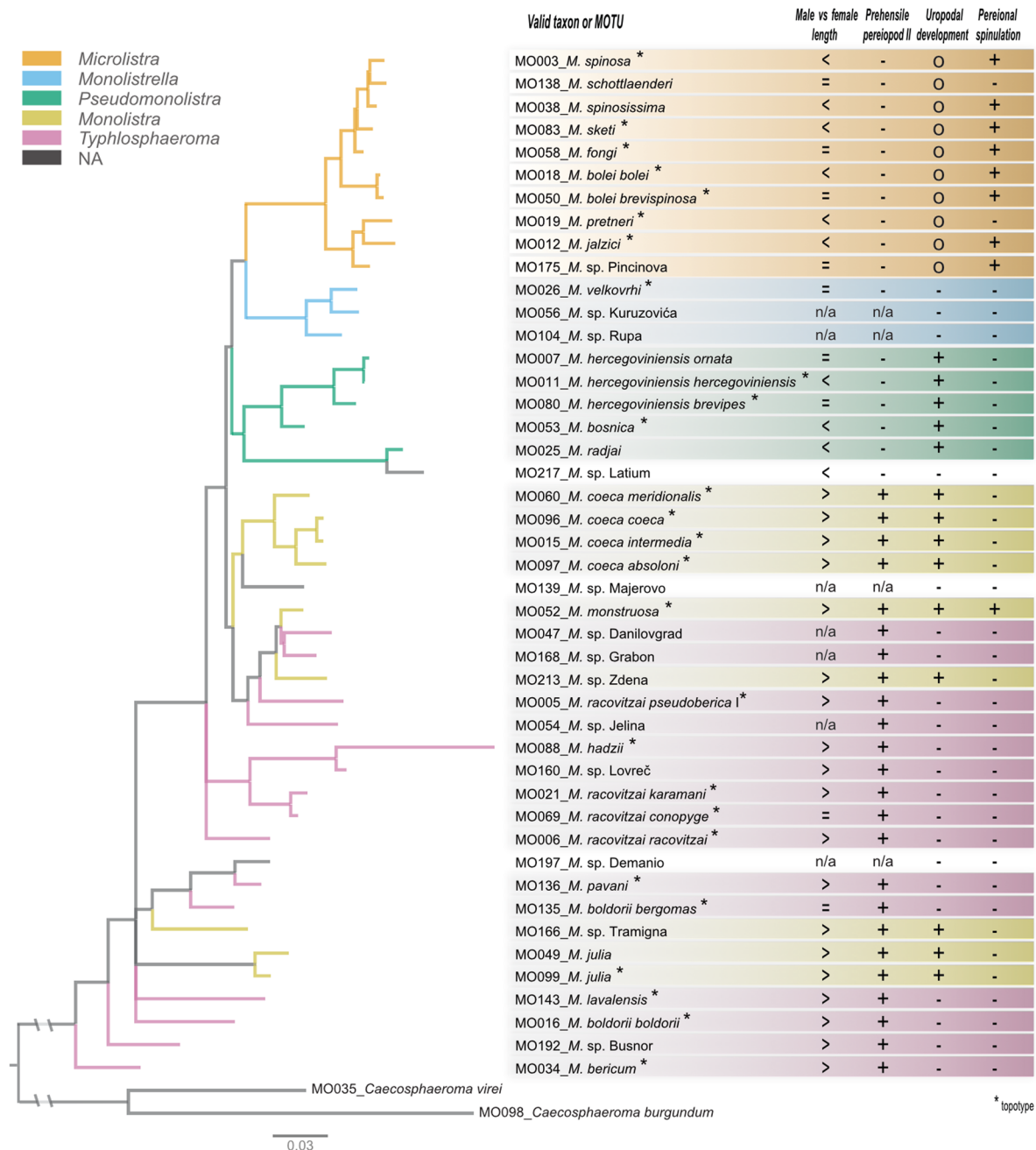


Figure 2. Simplified phylogeny of subterranean Sphaeromatidae, based on the Bayesian inference tree inferred from mitochondrial (12S and 16S) and nuclear (28S, H3, and EPRS) molecular markers (original tree is available in [Supporting information, S5](#)). The names of the formally described taxa are in italics, whereas the newly recognized MOTUs (without taxonomic status) are given in regular font. The states of four morphological characters, used for morphospecies designation, are indicated next to the specimen label. Coloured branches correspond to the morphologically recognised subgenera.

after showing its sister-relationship with *M. radjai*, distributed along the eastern Adriatic coast, and the recently discovered species from the Tyrrhenian coast of the Italian Peninsula ([Fig. 3](#)). The yet undescribed species has some 'pseudomonolistran' traits (i.e. larger males than females, non-prehensile male pereopod II) but lacks well-developed uropods, which are characteristic of the subgenus ([Fig 2](#); [Supporting information, S5](#)).

Most diagnostic characters used to define the subgenera were homoplastic ([Fig. 2](#)). Only two characters exhibited potential synapomorphies, each represented by a single character state: a

reduced uropod in *Microlistra* and non-prehensile male pereopods in the lineage comprising *Microlistra* + *Monolistrella* + *Pseudomonolistra* + the undescribed Tyrrhenian species. Notably, the currently employed subgeneric diagnostic traits do not adequately distinguish the individual clades on a morphological basis.

The age of splits

The age of the common ancestor of Sphaeromatidae was dated to the Early Cretaceous, with a mean estimate of 116 Mya (95% confidence interval 67–169 Mya), falling into the period of the

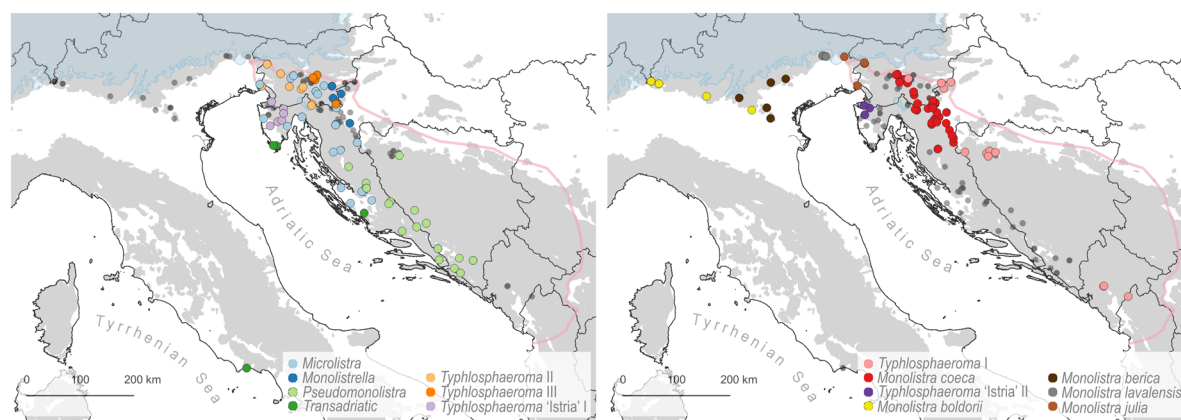


Figure 3. Geographic distribution of the major phylogenetic lineages within the genus *Monolistra*, as inferred from the phylogenetic analysis. The area with the highest species diversity, the Dinaric Karst, is delimited by a pink line. Major mountainous areas are displayed in grey, whereas the extent of glaciers during the Last Glacial Maximum (about 21 000 years ago) is indicated in light blue. Due to the large number of lineages, the figure is split in two parts, with the colour-coding of the major lineages matching lineages-specific colour-coding in Figure 4.

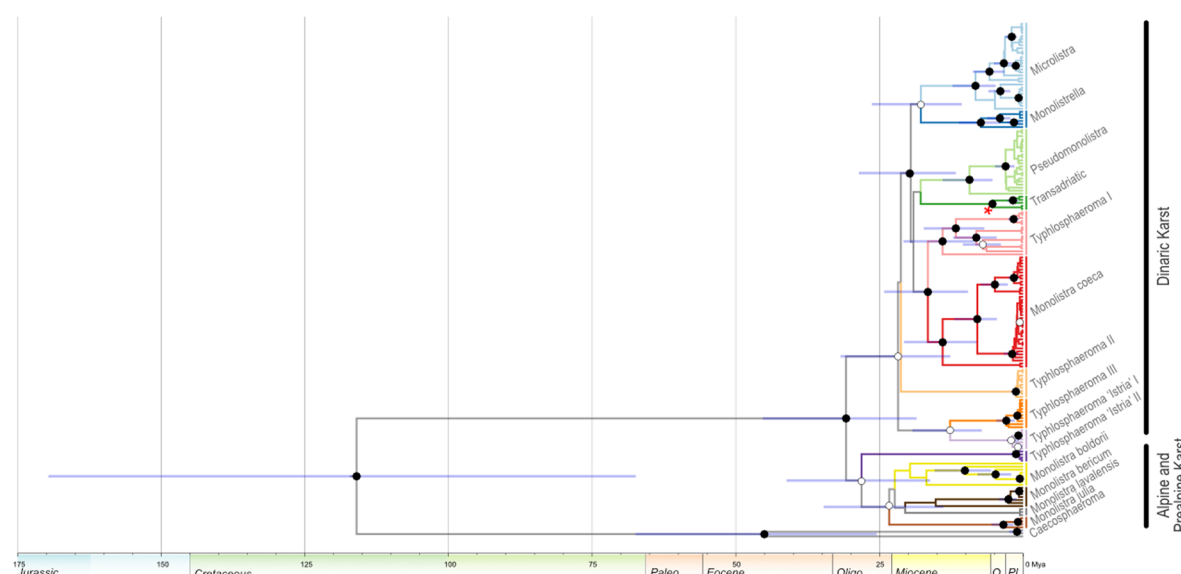


Figure 4. Time-calibrated phylogeny of European the subterranean Sphaeromatidae based on mitochondrial (12S and 16S) and nuclear (28S, *H3*, and *EPRS*) gene fragments, with names of the main phylogenetic lineages and broad geographical distribution. The full tree is available in the [Supporting information, S6](#). Node supports larger than 0.95 are indicated with white circles and a support of 1 with black circles. Blue node bars represent the 95% confidence interval for node ages. The red asterisk denotes the node used for the calibration of the tree: division of 'transAdriatic' lineages present on both sides of the Adriatic Sea.

emerging European landmasses ([Supporting information, S6](#)). The common ancestor of the genus *Monolistra*, presently distributed in the Dinaric Karst, southern Alps, and Italian Peninsula, was estimated at a mean age of 30.8 Mya (95% confidence interval 18.5–45.3 Mya) ([Fig. 4](#)), corresponding with the onset of the main uplift of the Dinaric Karst and the final stages of the Alpine orogenesis. Most of the later splits fall within the dynamic palaeogeographic development of the region during the Miocene, while the most recent splits, including those between closely related but morphologically very distinct species, and most of the intraspecific ones, appear to be of Pleistocene age.

DISCUSSION

Molecular insights into the diversity of the freshwater subterranean Sphaeromatidae yielded three principal findings. First, there is a clear incongruence between morphology-based and molecular-based taxonomy at both the species and subgeneric level ([Fig. 2](#); [Supporting information, S5](#)). Second, most of the studied morphological variation appears to be homoplastic, possibly resulting from convergent evolution. Third, patterns of origin and diversification are associated with major geological events in Europe. Each of these three findings is discussed in detail below.

Molecular species' delimitation analyses revealed that morphology-based species boundaries do not correspond to the

MOTUs identified within *Monolistra* (see also: [Lapadula et al. 2025](#)). Although such discrepancies are not completely unexpected, it is notable that among the 31 morphospecies and subspecies included in this study, only 22 MOTUs were recovered. This reduction in number of MOTUs with respect to the described species and subspecies contrasts with patterns observed in other subterranean taxa, particularly in the amphipod genus *Niphargus* ([Fišer et al., 2009](#)), where molecular approaches often uncover greater diversity.

A marked decrease in species' number was observed in the *Microlistra* clade, which includes the most morphologically diverse taxa, primarily distinguished by variation in pereopodal spines. These spines probably function as anti-predator defence—especially against the olm, *Proteus anguinus* Laurenti, 1768 ([Premate et al. 2021b](#))—and their development may covary with local predation pressure. Interestingly, some subterranean amphipod species, first described as independent species (e.g. *Niphargus dolichopus* [Fišer et al., 2006](#)) are also armed with spines ([Zakšek et al. 2019](#)), suggesting that anti-predation traits have poor diagnostic value at the species-level ([Jugovic et al. 2010](#)).

A more anticipated outcome was the identification of 14 MOTUs corresponding to eight formally described species or subspecies within the subgenera *Monolistra* and *Typhlosphaeroma* (Supporting information, S4, S5). Morphologically similar species, including cryptic species, are common in subterranean taxa, probably due to shared selective pressures ([Delić et al. 2017](#), [Eme et al. 2017](#), [Recknagel et al. 2024](#)). The mismatches among MOTUs, morphologically defined species, and subspecies in *Monolistra*, therefore, supports the general patterns observed in subterranean environments. However, the MOTUs defined in our delimitation analysis should be interpreted with caution, as they rely exclusively on mitochondrial markers. Mitochondrial markers may generate a false-positive species' hypothesis ([Hupalo et al. 2023](#), [Knüsel et al. 2023](#)) and an overestimation of the number of 'putative' species. A more robust taxonomic assessment should, therefore, incorporate nuclear markers and, ideally, morphological and behavioural variability within an integrative-iterative taxonomic framework ([Padial et al. 2010](#), [Yeates et al. 2011](#)).

One of the most significant outcomes of our phylogenetic analysis is that only two subgenera (*Microlistra* and *Monolistrella*) were recovered as monophyletic, and both were nested within the subgenus *Monolistra*. In contrast, the remaining subgenera (*Monolistra*, *Typhlosphaeroma*, and *Pseudomonolistra*) proved to be polyphyletic or paraphyletic. Diagnostic characters traditionally used to delimit these subgenera were shown to be largely homoplastic, while putative synapomorphies corresponded only to single character states: reduced uropod for *Microlistra* and *Typhlosphaeroma*, and non-prehensile male pereopods in the clade comprising *Microlistra*, *Monolistrella*, *Pseudomonolistra*, and the undescribed Tyrrhenian species of *Monolistra* s.s. Importantly, the morphological traits used in classical morphotaxonomy did not reliably discriminate the clades identified by molecular phylogeny. On this basis, we formally synonymize the subgenera *Monolistra*, *Typhlosphaeroma*, *Pseudomonolistra*, *Microlistra*, and *Monolistrella* as junior synonyms of *Monolistra* Gerstaecker, 1856.

Convergent evolution is expected among subterranean lineages, where strong local selection, combined with low dispersal ability ([Trontelj et al. 2009](#), [Bregović et al. 2019](#), [Cooper et al.](#)

[2023](#)), may obscure true phylogenetic relationships. Consequently, many diagnostic characters are homoplastic, leading to artificial generic and subgeneric classifications ([Fišer et al. 2008](#), [Lukić et al. 2020](#)). Such traits may include: (i) functional characters subjected to natural selection, such as protective spines ([Jugovic et al. 2010](#), [Premate et al. 2021b](#)); (ii) features influenced by fecundity or sexual selection, including sexual size dimorphism and prehensile male pereopods, which may drive convergent behavioural ([Berisha et al. 2023](#)), morphological ([Premate et al. 2024](#)), and life history evolution ([Fišer et al. 2013](#), [Premate et al. 2021a](#)); and (iii) traits shaped by relaxed selection, such as the degree of uropod reduction. These interpretations must be treated with caution, as the function and heritability of these characters remain untested ([Schluter 2009](#)).

Although support values were not uniformly high across our time-calibrated phylogeny, the inferred divergence times allow tentative correlations between the evolutionary history of *Monolistra* and *Caecosphaeroma* and major palaeogeographic events in Europe. The initial split between the eastern and western genera was dated (95% CI) to 67–169 Mya. Considering the absence of representatives of this genus group in North America, colonization probably occurred after the opening of the North Atlantic Ocean, broadly consistent with our temporal estimates ([Voo 1993](#)). [Wetzer et al. \(2018\)](#) suggested, based solely on morphological characters, that the 'Monolistra group', including *Caecosphaeroma*, forms a monophyletic clade due to similarities in pleopod, antennule, and antenna morphology. Whether both lineages derive from a common marine ancestor remains uncertain, due to unresolved relationships with distantly related marine genera. Nevertheless, the estimated timing of divergence corresponds to the early stages of the European continent formation, beginning in the western part of the present landmass ([Popov et al. 2004](#)), which may account for the north-western distribution of *Caecosphaeroma* in France ([Sket 1986](#)).

Diversification within the eastern lineage (*Monolistra*) began approximately 31 Mya (95% confidence interval 18.5–45.3 Mya), corresponding with the extensive uplift of carbonate massifs along the southern Alpine slopes and the Dinaric Karst ([Popov et al. 2004](#)). These patterns parallel the evolutionary history of the subterranean amphipod genus *Niphargus*, which colonized continental waters approximately ~60–80 Mya in fragmented Western European territories ([McInerney et al. 2014](#)), followed by subsequent diversification in southern and south-eastern Europe ([Borko et al. 2021](#)). Another notable similarity between *Niphargus* and *Monolistra* is the close relatedness between species inhabiting the Eastern Adriatic coast and those from the Tyrrhenian region, suggesting relatively recent connectivity across the Adriatic Basin. In *Niphargus*, multiple dispersal events across the Adriatic have been inferred during periods of sea regressions, including the Messinian Salinity Crisis (5.33–5.96 Mya) and Pleistocene glaciations ([Delić et al. 2020](#)).

An alternative hypothesis ([Sket 1986, 1994](#)) posits that present-day distributions reflect ancient palaeo-drainage systems, implying that ancestral *Monolistra* colonized surface freshwater habitats, diversified epigeally, and subsequently invaded subterranean environments multiple times. Although no extant European sphaeromatids occur in surface waters, several genera

of the family were present in springs, brooks, and ponds during the Cenozoic (Van Straelen 1928). These genera do not closely resemble the *Monolistra* group, yet this scenario cannot be excluded. Testing it rigorously will require broader genomic sampling, particularly the inclusion of multiple nuclear markers (Recknagel *et al.* 2024).

Monolistra emerges as a promising model system, comparable to *Niphargus*, and is well suited for studying patterns of subterranean diversification and possible adaptive radiation. Although there is still a long way to associate morphological disparity with functional traits, selection regimes, and fitness consequences, and to develop genome-wide markers for more resolved phylogenetic inference, such efforts may illuminate general mechanisms underlying diversification. Parallel adaptive radiations, occurring within the same geographic region and time frame across distinct taxa, may help explain why the studied area constitutes a global hotspot of subterranean biodiversity [see discussions by Simpson (1953) and Morinaga *et al.* (2023)].

ACKNOWLEDGEMENTS

The authors are deeply thankful to all collectors, persons accompanying them in the fieldwork, and personnel, which enabled them to fill the goals of the paper, including Arne Hodalič, Tonči Rađa, Andrej Hudoklin, Peter Trontelj, Cene Fišer, Branko Jalžić, Jure Jugovic, Valerija Zakšek, Matej Simonič, Jos Notenboom, Roman Ozimec, Predrag Rade, Helena Bilandžija, Luca Dorigo, Erminio Piva, Gianfranco Caoduro, Gianfranco Tomasin, Bruno Delprat, etc.. In addition, we wish to thank Cene Fišer for his review of an early version of the manuscript.

This study was supported by the Slovenian Research Agency (core program P1-0184), with the DarCo project by Biodiversa+, the European Biodiversity Partnership under the 2021–22 BiodivProtect joint call for research proposals, co-funded by the European Commission (GA N°101052342) and with the funding organizations Ministry of Universities and Research (Italy), Agencia Estatal de Investigación—Fundación Biodiversidad (Spain), Fundo Regional para a Ciência e Tecnologia (Portugal), Suomen Akatemia—Ministry of the Environment (Finland), Belgian Science Policy Office (Belgium), Agence Nationale de la Recherche (France), Deutsche Forschungsgemeinschaft e. V.—BMBF-VDI/VDE INNOVATION + TECHNIK GMBH (Germany), Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung (Switzerland), Fonds zur Förderung der Wissenschaftlichen Forschung (Austria), Ministry of Higher Education, Science and Innovation (Slovenia), and the Executive Agency for Higher Education, Research, Development and Innovation Funding (Romania) and with Biodiversa+, the European Biodiversity Partnership, in the context of the Sub-BioMon—Developing and testing approaches to monitor subterranean biodiversity in karst project under the 2022–23 BiodivMon joint call, co-funded by the European Commission (GA N°101052342) and the following funding organizations: Ministry of Higher Education, Science and Innovation (Slovenia), The Belgian Science Policy (Belgium), Ministry of Universities and Research (Italy), National Research, Development and Innovation Office (Hungary), Executive Agency for Higher Education, Research, Development and Innovation Funding

(Romania) and self-financing partner National Museum of Natural History Luxembourg (Luxembourg). Part of the equipment used in this study was purchased for the project ‘Development of research infrastructure for the international competitiveness of the Slovenian RRI space –RI-SI-LifeWatch’. The operation is co-financed by the Republic of Slovenia, Ministry of Education, Science and Sport and the European Union from the European Regional Development Fund.

The authors would like to jointly express their gratitude and dedicate this work to the late Boris Sket, who initiated research on this topic. We also thank all cavers, colleagues, and researchers who contributed to the collection of materials.

SUPPLEMENTARY DATA

Supplementary data is available at *Zoological Journal of the Linnean Society* online.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

FUNDING

The study was funded by Slovenian Agency for Research and Innovation (ARIS) through the core program Integrative Zoology and Speleobiology P1-0184 and Critical Ecosystem Partnership Fund project 116863, with a support of the infrastructural center ‘Microscopy of biological samples’, (MRIC UL, I0-0022-0481-08) of Biotechnical Faculty, University of Ljubljana. Finally, the study was supported by the Biodiversa+, the European Biodiversity Partnership, in the context of the ‘Sub-BioMon—Developing and testing approaches to monitor subterranean biodiversity in karst’ project under the 2022-2023 BiodivMon joint call. It was co-funded by the European Commission (GA N°101052342) and the following funding organisations: Ministry of Higher Education, Science and Innovation (Slovenia), The Belgian Science Policy (Belgium), Ministry of Universities and Research (Italy), National Research, Development and Innovation Office (Hungary), Executive Agency for Higher Education, Research, Development and Innovation Funding (Romania) and self-financing partner National Museum of Natural History Luxembourg (Luxembourg). Further on, it was funded also by Biodiversa+, the European Biodiversity Partnership, in the context of the ‘Darco—The vertical dimension of conservation—a cost-effective plan to incorporate subterranean ecosystems in post-2020 biodiversity and climate change agendas’ under the 2021–22 BiodivProtect joint call for research proposals, co-funded by the European Commission (GA N°101052342) and with the funding organisations Ministry of Universities and Research (Italy), Agencia Estatal de Investigación—Fundación Biodiversidad (Spain) Ministerio de Ciencia, Innovación y Universidades—Agencia Estatal de Investigación (MICIU/AEI/10.13039/501100011033) and NextGenerationEU/PRTR (PCI2022-135076-2), Fundo Regional para a Ciência e Tecnologia (Portugal), Suomen Akatemia—Ministry of the Environment (Finland), Belgian Science Policy Office (Belgium),

Agence Nationale de la Recherche (France), Deutsche Forschungsgemeinschaft e. V. (Germany), Schweizerischer Nationalfonds (Grant No. 31BD30_209583, Switzerland), Fonds zur Förderung der Wissenschaftlichen Forschung (Austria), Ministry of Higher Education, Science and Innovation (Slovenia) and the Executive Agency for Higher Education, Research, Development and Innovation Funding (Romania).

DATA AVAILABILITY

All data upon which this study is based are available either within the [supplementary materials](#) accompanying this article or through public data repositories. Genetic sequence data have been deposited in GenBank, and additional datasets, including morphological and analytical data, are available via the Dryad Digital Repository. Accession numbers and DOIs for all datasets are provided in the [supplementary materials](#).

REFERENCES

- Berisha H, Horváth G, Fišer Z, *et al.* Sex-dependent increase of movement activity in the freshwater isopod *Asellus aquaticus* following adaptation to a predator-free cave habitat. *Current Zoology* 2023;**69**:418–25. <https://doi.org/10.1093/cz/zoac063>
- Bilandžija H, Morton B, Podnar M *et al.* Evolutionary history of relict *Congerina* (Bivalvia: Dreissenidae): unearthing the subterranean biodiversity of the Dinaric Karst. *Frontiers in Zoology* 2013;**10**:5. <https://doi.org/10.1186/1742-9994-10-5>
- Bouckaert RR, Drummond AJ. bModelTest: Bayesian phylogenetic site model averaging and model comparison. *BMCEvolutionary Biology* 2017;**17**:1–11. <https://doi.org/10.1186/s12862-017-0890-6>
- Bouckaert R, Vaughan TG, Barido-Sottani J *et al.* BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 2019;**15**:e1006650.
- Boyko CB, Campos-Filho IS, Hadfield KA *et al.* (eds). World Marine, Freshwater and Terrestrial Isopod Crustaceans Database. Sphaeromatidae Latreille, 1825, 2026. Accessed through: World Register of Marine Species at: <https://www.marinespecies.org/aphia.php?p=taxdetails&id=118277> on 2026-02-25
- Borko Š, Trontelj P, Seehausen O *et al.* A subterranean adaptive radiation of amphipods in Europe. *Nature Communication* 2021;**12**:3688. <https://doi.org/10.1038/s41467-021-24023-w>
- Bregović P, Fišer C, Zagamajster M. Contribution of rare and common species to subterranean species richness patterns. *Ecology Evolution* 2019;**9**:11606–18. <https://doi.org/10.1002/ece3.5604>
- Bruce NL. New sphaeromatids (Crustacea: Isopoda: Sphaeromatidea) from coastal and freshwater habitats in New Zealand. *Zootaxa* 2005;**1002**:20. <https://doi.org/10.11646/zootaxa.1002.1.1>
- Carstens BC, Pelletier TA, Reid NM *et al.* How to fail at species' delimitation. *Molecular Ecology* 2013;**22**:4369–83. <https://doi.org/10.1111/mec.12413>
- Coineau N, Boutin C. Biological processes in space and time: colonization, evolution and speciation in interstitial stygobionts. In: Camacho AI (ed.), *The Natural History of Biospeleology*. Madrid: Museo Nacional de Ciencias Naturales, 1992, 423–51.
- Cole GA, Bane CA. *Thermosphaeroma subequalum*, n.gen., n.sp. (Crustacea: Isopoda) from Big Bend National park, Texas. *Hydrobiologia* 1978;**59**:223–8.
- Cooper S, Fišer C, Zakšek V *et al.* Chapter 7 - Phylogenies reveal speciation dynamics: case studies from groundwater. *Groundwater Ecology and Evolution* 2023;165–83.
- Delić T, Trontelj P, Rendoš M *et al.* The importance of naming cryptic species and the conservation of endemic subterranean amphipods. *Scientific Reports* 2017;**7**:3391. <https://doi.org/10.1038/s41598-017-02938-z>
- Delić T, Stoch F, Borko Š *et al.* How did subterranean amphipods cross the Adriatic Sea? Phylogenetic evidence for dispersal–vicariance interplay mediated by marine regression–transgression cycles. *Journal of Biogeography* 2020;**47**:1875–87. <https://doi.org/10.1111/jbi.13875>
- Drummond AJ, Ho SYW, Phillips MJ *et al.* Relaxed phylogenetics and dating with confidence. *PLoS Biology* 2006;**4**:e88. <https://doi.org/10.1371/journal.pbio.0040088>
- Eme D, Zagamajster M, Delić T *et al.* Do cryptic species matter in macroecology? Sequencing European groundwater crustaceans yields smaller ranges but does not challenge biodiversity determinants. *Ecography* 2017;**41**:424–36. <https://doi.org/10.1111/oik.02629>
- Fišer C, Sket B, Trontelj P. A phylogenetic perspective on 160 years of troubled taxonomy of *Niphargus* (Crustacea: Amphipoda). *Zoologica Scripta* 2008;**37**:665–80. <https://doi.org/10.1111/j.1463-6409.2008.00347.x>
- Fišer C, Zagamajster M, Zakšek V. Coevolution of life history traits and morphology in female subterranean amphipods. *Oikos* 2013;**122**:770–8. <https://doi.org/10.1111/j.1600-0706.2012.20644.x>
- Fontaneto D, Flot J-F, Tang CQ. Guidelines for DNA taxonomy, with a focus on the meiofauna. *Marine Biodiversity* 2015;**45**:433–51. <https://doi.org/10.1007/s12526-015-0319-7>
- Guindon S, Dufayard J-F, Lefort V *et al.* New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 2010;**59**:307–21. <https://doi.org/10.1093/sysbio/syq010>
- Hoang DT, Vinh LS, Flouri T *et al.* MPBoot: fast phylogenetic maximum parsimony tree inference and bootstrap approximation. *BMC Evolutionary Biology* 2018;**18**:11. <https://doi.org/10.1186/s12862-018-1131-3>
- Holdich DM, Harrison K. Sphaeromatid Isopods (Crustacea) from Brackish Waters in Queensland, Australia. *Zoologica Scripta* 1983;**12**:127–40.
- Hupalo K, Copilaş-Ciocianu D, Leese F, Weiss M. Morphology, nuclear SNPs and mate selection reveal that COI barcoding overestimates species diversity in a Mediterranean freshwater amphipod by an order of magnitude. *Cladistics* 2023;**39**:129–43. <https://doi.org/10.1111/cla.12520>
- Jugović J, Prevorcnik S, Aljancic G, Sket B. The atyid shrimp (Crustacea: Decapoda: Atyidae) rostrum: phylogeny versus adaptation, taxonomy versus trophic ecology. *Journal of Natural History* 2010;**44**:2509–33. <https://doi.org/10.1080/00222933.2010.502258>
- Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 2013;**30**:772–80. <https://doi.org/10.1093/molbev/mst010>
- Knüsel M, Borko S, Alther R *et al.* Phylogenetic structure and molecular species delimitation hint a complex evolutionary history in an Alpine endemic *Niphargus* clade (Crustacea, Amphipoda). *Zoologischer Anzeiger* 2023;**306**:27–36.
- Lanfear R, Frandsen PB, Wright AM *et al.* PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 2016;**34**:772–773. <https://doi.org/10.1093/molbev/msw260>
- Lapadula S, Manenti R, Barzaghi B *et al.* Population genomic divergence reveals uncertainties in species identification of cave-dwelling isopods. *Journal of Zoological Systematics and Evolutionary Research* 2025;1–14. <https://doi.org/10.1155/jzs/6395480>
- Lukić M, Delić T, Pavlek M *et al.* Distribution pattern and radiation of the European subterranean genus *Verhoeffiella* (Collembola, Entomobryidae). *Zoologica Scripta* 2019;**49**:86–100. <https://doi.org/10.1111/zsc.12392>
- McInerney CE, Maurice L, Robertson AL, *et al.* The ancient Britons: groundwater fauna survived extreme climate change over tens of millions of years across NW Europe. *Molecular Ecology* 2014;**23**:1153–66. <https://doi.org/10.1111/mec.12664>

- Morinaga G, Wiens JJ, Moen DS. The radiation continuum and the evolution of frog diversity. *Nature Communications* 2023;**14**:1–12. <https://doi.org/10.1038/s41467-023-42745-x>
- Nguyen LT, Schmidt HA, Von Haeseler A *et al.* IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 2015;**32**:268–74. <https://doi.org/10.1093/molbev/msu300>
- Nunomura N. On the genus *Gnorimosphaeroma* (Crustacea, Isopoda, Sphaeromatidae) in Japan with description of six new species. *Bulletin of the Toyama Science Museum* 1998;**21**:23–54.
- Padial JM, Miralles A, De la Riva I *et al.* The integrative future of taxonomy. *Frontiers in Zoology* 2010;**7**:16. <https://doi.org/10.1186/1742-9994-7-16>
- Poore GCB, Bruce NL. Global diversity of marine isopods (except asellota and crustacean symbionts). *PLoS One* 2012;**7**:e43529. <https://doi.org/10.1371/journal.pone.0043529>
- Popov SV, Rogl F, Rozanov AY, *et al.* Lithological-Paleogeographic maps of Paratethys. 10 maps Late eocene to Pliocene. *Courier Forschungsinstitut Senckenberg* 2004;**250**:1–46.
- Premate E, Borko S, Kralj-Fišer S, *et al.* No room for males in caves: Female-biased sex ratio in subterranean amphipods of the genus *Niphargus*. *Journal of Evolutionary Biology* 2021a;**34**:1653–61. <https://doi.org/10.1111/jeb.13917>
- Premate E, Fišer C. Functional trait dataset of European groundwater Amphipoda: Niphargidae and Typhlogammaridae. *Scientific Data* 2024;**11**:188. <https://doi.org/10.1038/s41597-024-03020-w>
- Premate E, Zagmajster M, Fišer C. Inferring predator–prey interaction in the subterranean environment: a case study from Dinaric caves. *Scientific Reports* 2021b;**11**:21682. <https://doi.org/10.1038/s41598-021-01249-8>
- Prevorčnik S, Verovnik R, Zagmajster M *et al.* Biogeography and phylogenetic relations within the Dinaric subgenus *Monolistra* (*Microlistra*) (Crustacea: Isopoda: Sphaeromatidae), with a description of two new species. *Zoological Journal of the Linnean Society* 2010;**159**:1–21. <https://doi.org/10.1111/j.1096-3642.2009.00593.x>
- Rambaut A. *FigTree v1.4.4: Tree Figure Drawing Tool*. Edinburgh: Institute of Evolutionary Biology, University of Edinburgh, 2018. <http://tree.bio.ed.ac.uk/software/figtree/> (14 March 2024, date last accessed).
- Recknagel H, Zakšek V, Delić T *et al.* Multiple transitions between realms shape relict lineages of *Proteus* cave salamanders. *Molecular Ecology* 2024;**33**:0. <https://doi.org/10.1111/mec.16868>
- Ronquist F, Teslenko M, Van Der Mark P *et al.* MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 2012;**61**:539–42. <https://doi.org/10.1093/sysbio/sys029>
- Ruffo S, Stoch F. 16 Memoire del Museo Civico di Storia Naturale di Verona, 2. serie, Sezione Scienze della Vita Checklist e distribuzione della fauna Italiana. 10.000 Specie Terrestri E Delle Acque Interne. Museo Civico di Storia Naturale Verona, Verona, 2005. <https://doi.org/10.1017/CBO9781107415324.004>
- Schluter D. Evidence for ecological speciation and its alternative. *Science* 2009;**323**:737–41. <https://doi.org/10.1126/science.1160006>
- Simpson GG. *The major features of evolution*. Columbia University Press, 1953. <https://doi.org/10.7312/simp93764>
- Sket B. Östliche Gruppe der Monolistrini (Crust. Isopoda) I. Systematischer Teil. *International Journal of Speleology* 1964;**1**:163–89. <https://doi.org/10.5038/1827-806X.1.1.14>
- Sket B. Isopoda: Sphaeromatidae. In: Botosaneanu L (ed.), *Stygofauna Mundi: A Faunistic, Distributional and Ecological Synthesis of the World Fauna Inhabiting Subterranean Waters (Including the Marine Interstitial)*. Amsterdam: Institute of Taxonomic Zoology, 1986, 423–27.
- Sket B. Distribution patterns of some subterranean Crustacea in the territory of the former Yugoslavia. *Hydrobiologia* 1994;**287**:65–75.
- Sket B, Bruce NL. Sphaeromatids (Isopoda, Sphaeromatidae) from New Zealand fresh and hypogean waters, with description of *Bilistra* n. gen. and three new species. *Crustaceana* 2004;**76**:1347–70.
- Sket B. *Merozoon vestigatum* g. n., sp. n., a new freshwater subterranean isopod (Isopoda: Sphaeromatidae) from a cave in Croatia. *Acta Biologica Slovenica* 2012;**55**:71–6.
- Stoch F, Galassi DMP. Stygobiotic crustacean species richness: a question of numbers, a matter of scale. *Hydrobiologia* 2010;**653**:217–34. <https://doi.org/10.1007/s10750-010-0356-y>
- Tang CQ, Humphreys AM, Fontaneto D *et al.* Effects of phylogenetic reconstruction method on the robustness of species delimitation using single locus data. *Methods in Ecology and Evolution* 2014;**5**:1086–94.
- Trontelj P, Douady CJ, Fišer C, *et al.* A molecular test for cryptic diversity in ground water: how large are the ranges of macro-stygobionts?. *Freshwater Biology* 2009;**54**:727–44. <https://doi.org/10.1111/j.1365-2427.2007.01877.x>
- Voo R van der. The opening of the Atlantic Ocean. In: *Paleomagnetism of the Atlantic, Tethys and Iapetus Oceans*. Cambridge University Press, 1993:123–42.
- Van Straelen V. Contribution à l'étude des isopodes Méso-et Cénozoïques. *Mémoires de l'Académie Royale de Belgique. Classe des Sciences* 1928;**9**:1–66.
- Wägele J-W. Aspects of the evolution and biogeography of stygobiontic Isopoda (Crustacea: Peracarida). *Bijdragen tot de Dierkunde* 1990;**60**:145–50.
- Wetzer R, Bruce NL, Pérez-Losada M. Relationships of the Sphaeromatidae genera (Peracarida: Isopoda) inferred from 18S rDNA and 16S rDNA genes. *Arthropod Systematics & Phylogeny* 2018;**76**:1–30. <https://doi.org/10.3897/asp.76.e31934>
- Wilson GDF. Global diversity of Isopod crustaceans (Crustacea: Isopoda) in freshwater. *Hydrobiologia* 2008;**595**:231–40. <https://doi.org/10.1007/s10750-007-9019-z>
- Yeates DK, Seago A, Nelson L *et al.* Integrative taxonomy, or iterative taxonomy? *Systematic Entomology* 2011;**36**:209–17. <https://doi.org/10.1111/j.1365-3113.2010.00558.x>
- Zagmajster M, Eme D, Fišer C *et al.* Geographic variation in range size and beta diversity of groundwater crustaceans: insights from habitats with low thermal seasonality, 2014, 1–11. <https://doi.org/10.1111/geb.12200>
- Zakšek V, Delić T, Fišer C, Jalžić B, Trontelj P. Emergence of sympatry in a radiation of subterranean amphipods. *Journal of Biogeography* 2019;**46**:657–69. <https://doi.org/10.1111/jbi.13514>
- Zakšek V, Sket B, Gottstein S *et al.* The limits of cryptic diversity in groundwater: Phylogeography of the cave shrimp *Troglocaris anophthalmus* (Crustacea: Decapoda: Atyidae). *Molecular Ecology* 2009;**18**:931–46. <https://doi.org/10.1111/j.1365-294X.2008.04061.x>
- Zhang J, Kapli P, Pavlidis P *et al.* A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* 2013;**29**:2869–76. <https://doi.org/10.1093/bioinformatics/btt499>