SYSTEMATICS, BIOGEOGRAPHY AND EVOLUTIONARY PATTERNS OF THE FAMILY HYDROBIIDAE (MOLLUSCA: CAENOGASTROPODA)

SISTEMÁTICA, BIOGEOGRAFÍA Y PATRONES EVOLUTIVOS DE LA FAMILIA HYDROBIIDAE (MOLLUSCA: CAENOGASTROPODA)

DOCTORAL THESIS

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Systematics, biogeography and evolutionary patterns of the Hydrobiidae family (Mollusca: Caenogastropoda)

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忍の才能はそんなとこにありやしねえ。まだ分からねーのか 忍者とは忍び堪える者のことなんだよ。一つてめーに教えといてやる 忍びの才能で一番大切なのは持ってる術の数なんかじゃねェ 大切なのは、あきらめねェど根性だ

“(…) You're wrong, that's not what makes a shinobi (…) A real ninja is one who endures no matter what gets thrown at him (...) Let me explain something to you, there is only one thing that matters if you are a shinobi and it isn't the number of techniques you know. All you do need, is the guts to never give up!”

“(…) Estás equivocado, eso no es lo que hace a un shinobi. Un verdadero ninja es aquel que aguanta sin importar lo que le arrojen (…) Déjame explicarte algo, solo hay una cosa que importa si eres un shinobi, y no es el número de técnicas que conozcas. ¡Todo lo que necesitas son las agallas para nunca rendirte!"

Jiraiya, Sato no Kyōki

A mi familia, mi tío, mi tía, mi papá, mis primas.

En especial a mi abuela Verónica Ruíz Padrón

Y a todos los que creyeron en mi

y nunca perdieron su fe.

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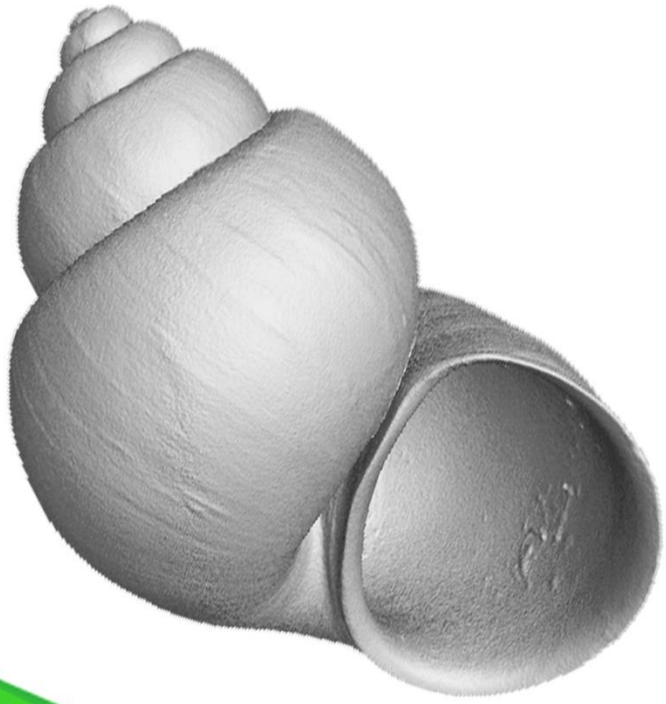

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ABSTRACT

Within Mollusca, freshwater gastropods are one of the most diverse groups. Previous studies have reported that this group is comprised of approximately 4,000 valid nominal species, but they have also concluded that this estimate may represent only 25% of its actual diversity. Freshwater snails are found on every continent, except Antarctica and in nearly every aquatic habitat (lotic and lentic), including lakes, swamps, rivers, streams, springs, underground aquifers and interstitial waters, among others. The truncatelloidean family Hydrobiidae Stimpson, 1865 is the most specious assemblage of freshwater gastropods, with ca. 1,000 recognised species. However, until the application of molecular tools, most brackish or freshwater snails that were small (between 0.5 and 8 mm) and had featureless shells were typically classified as hydrobioids (= Hydrobiidae *sensu lato* [*s. l.*]). This likely resulted in an overestimation of the family's actual species richness. In fact, the modern *sensu stricto* (*s. str.*) definition of the family, based on morphological and molecular evidence, recognised some former hydrobiid subfamilies as independent families. Taken altogether, these points highlight our incomplete understanding of the species richness and the global geographic distribution of Hydrobiidae *s. str.* New investigations of global-scale patterns of hydrobiid species richness and endemicity are needed to provide a baseline from which to infer the factors influencing the evolutionary processes and geographic distribution of Hydrobiidae.

Historical biogeography is of special importance for understanding the evolutionary processes that have led to the geographic distribution and species richness of extant clades. The scarcity of these types of studies is due, in part, to the fact that the great majority of the hydrobiid genera inhabiting Mediterranean continental waters consist of narrow-range endemics in species-poor assemblages. Only a few Mediterranean genera are diverse and geographically distributed enough to be good candidates for studies aiming to reconstruct biogeographic histories and infer speciation processes. Suitable taxa for such studies include *Corrosella* Boeters, 1970, a genus comprised of 17 known species distributed mainly in mountain springs in the Iberian Peninsula and the lowland genera *Pseudamnicola* Paulucci, 1878, *Islamia* Radoman, 1973 and *Mercuria* Boeters, 1971, with 70, 45 and 26 currently recognised extant species, respectively. The latter three genera occur in a wide range of freshwater ecosystems throughout the Mediterranean basin in both Europe and North Africa. *Pseudamnicola* has also been reported for the Ponto–Caspian region and *Mercuria* for Atlantic coastal regions. The four studied genera are notable as they occupy different habitats along an elevational gradient (e.g., from the highland species of *Corrosella* to the

saline species of *Mercuria*). Among these genera, *Mercuria* is the assemblage with the largest number of species delineated on the basis of conchological features. Given the questionable use of shell features to identify hydrobiid species, the taxonomy of *Mercuria* populations needs to be re-evaluated within an integrative framework.

Although there are numerous taxonomic works that described the morphology of hydrobiids, an exhaustive review of the total number of species and an explorative analysis inferring which evolutionary factors have determined their present-day richness have not yet been performed. Furthermore, although there are various phylogenetic works describing the relationships of genera inhabiting mountainous regions, coastal streams and lakes or brackish environments, there are currently no studies comparing the evolutionary patterns (e.g., divergence times, rates of genetic substitution or diversification and biogeographic patterns) of these genera. Such studies would provide a better understanding of how the biota found in these habitats evolved. To this end, the major goal of this doctoral thesis was to test which ecological and geographic factors may have influenced (i) the disparity in species richness among regions and taxonomic groups and (ii) the evolutionary patterns among hydrobiid genera.

To achieve this goal, we first conducted a comprehensive literature and biodiversity database review in order to determine the number of total, endemic and threatened species per freshwater ecoregion and compile information on them. We classified ecoregions as hotspots if each biodiversity index was in the top 25% of its range and assessed the effect of environmental and evolutionary factors on species richness using generalized linear models. We identified 906 extant species and 157 genera of Hydrobiidae *s. str.* that show a mainly Nearctic–Palearctic distribution and 11 biodiversity hotspots, most located across the Mediterranean basin. According to our dataset, 83% of the species are endemic to a single ecoregion. Out of the 43% of non-data deficient species, nearly three times more were classified as threatened than non-threatened and extinction risk peaked at 1,500 m.a.s.l. Species richness was unequally distributed over biogeographic realms, increasing with greater connectivity among ecoregions and was negatively related to annual temperature range. Latitude and precipitation seasonality partially explained the variation in richness by a non-linear relationship. The identified hotspots correspond to those of other freshwater taxa.

Multilocus phylogenies were used to compare the evolutionary patterns and historical biogeography of the selected hydrobiid genera. Combined data from mitochondrial (mtCOI and 16S rRNA) and nuclear ribosomal (28S rRNA) gene fragments were analysed for 183 individuals of *Mercuria*, 116 of *Pseudamnicola*, 34 of *Islamia* and 102 of *Corrosella*. To obtain sequences for *Mercuria*, 129

locations were sampled. For *Islamia*, many of the sequences were generated *de novo* using samples deposited in the University of Giessen Systematics and Biodiversity (UGSB) collection. Sequences of *Corrosella* and *Pseudamnicola* were retrieved mainly from GenBank (NCBI).

Our integrative study revealed changes in the taxonomy and geographic distribution of the species of *Mercuria* studied. Based on the applied species delimitation methods and morphological characterisations, 14 putative species were identified in the dataset: nine correspond to species recognised through traditional taxonomic classifications and five are new to science. Among the applied molecular delimitation methods, automatic barcode gap discovery (ABGD) was the most efficient at recognising *Mercuria* species (match ratio value of 0.85), confirming 12 of the 14 species delimited in the study. By contrast, the tree-based methods [i.e., generalised mixed Yule coalescent (GMYC) and Poisson tree processes (PTP)] presented a relatively low match ratio value, coinciding with an overestimation of the number of species. Several authors have recognised that these methods should be interpreted with caution for groups with low vagility and poor dispersal capacities such as gastropods, contrasting the validity of the delimited species with morphological data under an integrative taxonomic approach. Morphological similarity among *Mercuria* species was scored on the basis of a geometric morphometric analysis of shell shape using a total of 1,148 shells from 46 populations and 21 landmarks and semilandmarks. In the PCA, the first two components accounted for 75.3% of the variation in shell shape; however, only 50% of the species were successfully delimited on the basis of the shell morphometry. Generally, the shell, operculum and genitalia of *Mercuria* present wide intra- and interspecific variability; therefore, the use of these characters should be used cautiously when assessing the taxonomic status of populations of this genus. Instead, we propose the use of integrative taxonomy, adding genetic information to morphological data. Under this approach, various misidentifications of previous records of *Mercuria* were revealed in our study, representing a significant advancement in our knowledge of this genus.

Bayesian inference and maximum likelihood analyses recovered similar topologies (in terms of the degree of geographic restriction of clades and divergence times) for the genera *Mercuria* and *Pseudamnicola* and for *Islamia* and *Corrosella*. By applying an external calibration rate, our dated species trees showed a similar age for *Corrosella* and *Islamia* (ca. 11 Mya) and also for *Mercuria* and *Pseudamnicola* (ca. 7 Mya), with a notably younger age estimated for the latter pair. Taking these estimated ages into account, we placed the origin of the diversification of the four genera in the Miocene (from 23 to 5.3 Mya). According to the diversification principle, older groups should present more species as they have had a longer time to diversify; however, when we contrasted

the ages of these genera with the number of species in each, we found discrepancies demonstrating that they do not follow this principle. We found a notable difference between the number of extant species presented by *Corrosella* (17) and *Islamia* (45) and a greater difference between that by *Mercuria* (26) and *Pseudamnicola* (70). The disparity in the observed species richness could be related to differences in environmental preferences such as in habitat type, elevational range or water parameters and also in dispersal strategies.

To test these hypotheses, the ancestral area of species was inferred in the program BioGeoBEARS using the calibrated species trees [i.e., maximum clade credibility (MCC) trees] and the geographic distribution of each species coded by freshwater ecoregion. This analysis revealed high speciation within peninsular regions, which supports previous assumptions on the role of the Mediterranean peninsulas as evolutionary centres for hydrobiids. The inferred biogeographic history of *Corrosella* suggests a pattern of isolation by distance produced by vicariance processes. Cladogenesis within *Islamia* involved a combination of founder events from the Apennine Peninsula to the east and the west and narrow (within-ecoregion) sympatry, whereas in *Pseudamnicola* and *Mercuria*, it involved a series of dispersal and colonisation events followed by subsequent isolation.

The BioGeoBEARS analyses showed that rates of anagenetic range expansion (*d*) were higher in species that occur at high (*Corrosella*) and low (*Mercuria*) elevations, whereas those that occur at medium elevations (*Pseudamnicola* and *Islamia*) tended to have low dispersal rates. Also, species that occur at high elevation (i.e., *Corrosella*) presented the lowest founder event (jump dispersal) speciation parameter (*j*) at cladogenesis, while species that occur at medium elevations (i.e., *Pseudamnicola* and *Islamia*) showed a propensity for jump dispersals. In order to assess the potential relationship between altitudinal range and dispersal, we calculated, for each genus, the dispersal ratio ($d_{ratio} = (N_d + N_j) / N_{lin}$) from the number of dispersal or range expansion events (N_d), jump-dispersal or founder events (N_j) and total lineages (N_{lin}) and plotted this against elevation occurrences. We observed that the dispersal ratio decreased with elevation, from 0.42 for the lowland genus *Mercuria* to 0.14 for the headwater genus *Corrosella.* Our data, unlike for other organisms, did not evidence a direct relationship between dispersal and body size.

Overall, this study confirms that the family Hydrobiidae is a highly diverse group with hotspots of richness, endemism and threat, mainly in freshwater ecoregions of the Mediterranean basin. This distribution of the species richness was determined by geographic and climatic factors such as latitude, watershed connectivity, precipitation, seasonality and annual temperature range, but not by

elevation and geological heterogeneity (probably because the spatial aggregation of these two factors concealed their true variability). Therefore, we conclude that global hotspots of Hydrobiidae richness represent areas of climatic stability with moderate precipitation and temperature seasonality that are well connected to other hydrological basins. Our study also indicates that the current patterns of species richness have been affected by the dispersal strategy of groups, influenced by the elevation range in which they occur, rather than by age or habitat type. Better access for dispersal vectors and greater habitat connectivity at the lower elevations may have facilitated long-distance dispersal, resulting in founder event (cladogenetic) speciation. On the contrary, highly specialised and isolated groups, such as the *Corrosella* species inhabiting mountain-top springs, are less likely to disperse and speciate (and are probably more prone to extinction) compared with those inhabiting lower elevations. Taken altogether, our results illustrate that both evolutionary and environmental factors determine global species patterns and that future changes of the latter factors may affect the species richness of hydrobiids.

RESUMEN

Dentro del fílum Mollusca, los gasterópodos de agua dulce son uno de los grupos más diversos. Estudios anteriores han informado que este grupo está compuesto por aproximadamente 4,000 especies nominales válidas, pero también han concluido que esta estimación puede representar solo el 25% de su diversidad real. Los caracoles de agua dulce se encuentran en todos los continentes, excepto en la Antártida, y en casi todos los hábitats acuáticos (lóticos y lénticos), incluidos lagos, pantanos, ríos, arroyos, manantiales, acuíferos subterráneos y aguas intersticiales, entre otros. La familia Hydrobiidae Stimpson, 1865 (Truncatelloidea) constituye el grupo más diverso de gasterópodos de agua dulce, con ca. 1.000 especies reconocidas. Sin embargo, hasta la aplicación de herramientas moleculares, la mayoría de los caracoles de agua dulce o salobre que eran pequeños (entre 0,5 y 8 mm) y tenían conchas sin caracteres específicos se clasificaban típicamente como hidrobioides (= Hydrobiidae sensu lato [*s. l.*]). Esto probablemente resultó en una sobreestimación de la riqueza real de especies de la familia. De hecho, la definición moderna sensu stricto (*s. str.*) de la familia, basada en evidencias morfológicas y moleculares, reconoció a algunas antiguas subfamilias de hidróbidos como familias independientes. Tomados en conjunto, estos puntos resaltan nuestra comprensión incompleta de la riqueza de especies y la distribución geográfica global de Hydrobiidae *s. str*. Se necesitan nuevas investigaciones de patrones a escala global de riqueza y endemicidad de especies de hidróbidos para proporcionar una línea de base a partir de la cual inferir los factores que influyen en los procesos evolutivos y la distribución geográfica de Hydrobiidae.

La biogeografía histórica es de especial importancia para comprender los procesos evolutivos que han llevado a la distribución geográfica y la riqueza de especies de los clados existentes. La escasez de este tipo de estudios se debe, en parte, al hecho de que la gran mayoría de los géneros de hidróbidos que habitan las aguas continentales mediterráneas consisten en especies endémicas de distribución restringida en ecosistemas pobres en especies. Solo unos pocos géneros mediterráneos son lo suficientemente diversos y geográficamente distribuidos como para ser buenos candidatos para estudios destinados a reconstruir la biogeografía histórica e inferir procesos de especiación. Los taxones adecuados para tales estudios incluyen *Corrosella* Boeters, 1970, un género compuesto por 17 especies conocidas distribuidas principalmente en manantiales de montaña en la Península Ibérica, y los géneros que habitan a menor elevación *Pseudamnicola* Paulucci, 1878, *Islamia* Radoman, 1973 y *Mercuria* Boeters, 1971, con 70, 45 y 26 especies existentes actualmente reconocidas, respectivamente. Los últimos tres

géneros se encuentran en una amplia gama de ecosistemas de agua dulce en toda la cuenca mediterránea tanto en Europa como en el norte de África. También se ha encontrado de *Pseudamnicola* en la región Ponto-Caspia y *Mercuria* para las regiones costeras del Atlántico. Los cuatro géneros estudiados son notables porque ocupan diferentes hábitats a lo largo de un gradiente de elevación (por ejemplo, desde las especies de las zonas altas de *Corrosella* hasta las especies salobres de *Mercuria*). Entre estos géneros, *Mercuria* es el conjunto con el mayor número de especies delimitadas sobre la base de características conquiliológicas. Dado el uso cuestionable de los caracteres de la concha para identificar especies de hidróbidos, la taxonomía de las poblaciones de *Mercuria* deben reevaluarse dentro de un enfoque integrador.

Si bien existen numerosos trabajos taxonómicos que describen la morfología de los hidróbidos, aún no se ha realizado una revisión exhaustiva del número total de especies y un análisis exploratorio que infiera qué factores evolutivos han determinado su riqueza actual. Además, aunque existen varios trabajos filogenéticos que describen las relaciones de géneros que habitan en regiones montañosas, arroyos costeros y lagos o ambientes salobres, actualmente no hay estudios que comparen los patrones evolutivos (por ejemplo, tiempos de divergencia, tasas de sustitución o diversificación genética y patrones biogeográficos) de estos géneros. Dichos estudios proporcionarían una mejor comprensión de cómo evolucionó la biota que se encuentra en estos hábitats. Con este fin, el objetivo principal de esta tesis doctoral fue estimar qué factores ecológicos y geográficos pueden haber influido (i) en la disparidad en la riqueza de especies entre regiones y grupos taxonómicos y (ii) en los patrones evolutivos entre los géneros de hidróbidos.

Para lograr este objetivo, primero se llevó a cabo una revisión exhaustiva de la bibliografía y las bases de datos de biodiversidad para determinar el número total de especies endémicas y amenazadas por ecorregión de agua dulce y recopilar información sobre ellas. Clasificamos las ecorregiones como hotspots si cada índice de biodiversidad dentro del percentil superior 25% de su rango, y evaluamos el efecto de los factores ambientales y evolutivos sobre la riqueza de especies utilizando modelos lineales generalizados. Identificamos 906 especies existentes y 157 géneros de Hydrobiidae *s. str*. que muestran una distribución principalmente Neártica-Paleártica, y 11 puntos calientes (hotspots) de biodiversidad, la mayoría ubicados en la cuenca mediterránea. Según nuestro conjunto de datos, el 83% de las especies son endémicas de una sola ecorregión. Del 43% de las especies no deficientes de datos, casi tres veces más se clasificaron como amenazadas que no amenazadas, y el riesgo de extinción alcanzó su máximo

a 1.500 m s.n.m. La riqueza de especies se distribuyó de manera desigual en los reinos biogeográficos, aumentando con una mayor conectividad entre las ecorregiones, y se relacionó negativamente con el rango de temperatura anual. La latitud y la estacionalidad de las precipitaciones explicaron parcialmente la variación en la riqueza con una relación no lineal. Los hotspots identificados corresponden a similares estudiados en otros taxones de agua dulce.

Se utilizaron filogenias multilocus para comparar los patrones evolutivos y la biogeografía histórica de los géneros de hidróbidos seleccionados. Se analizaron datos combinados de fragmentos de genes mitocondriales (mtCOI y 16S rRNA) y ribosomales nucleares (28S rRNA) para 183 individuos de *Mercuria*, 116 de *Pseudamnicola*, 34 de *Islamia* y 102 de *Corrosella*. Para obtener secuencias para *Mercuria*, se muestrearon 129 ubicaciones. Para *Islamia,* muchas de las secuencias se generaron *de novo* utilizando muestras depositadas en la colección de Sistemática y Biodiversidad de la Universidad de Giessen (UGSB). Las secuencias de *Corrosella* y *Pseudamnicola* se recuperaron principalmente de GenBank (NCBI).

Nuestro estudio integrador reveló cambios en la taxonomía y distribución geográfica de las especies de *Mercuria* estudiadas. Con base en los métodos aplicados de delimitación de especies y caracterizaciones morfológicas, se identificaron 14 especies putativas en el conjunto de datos: nueve corresponden a especies reconocidas a través de clasificaciones taxonómicas tradicionales y cinco son nuevas para la ciencia. Entre los métodos de delimitación molecular aplicados, el «Automatic Barcode Gap Discovery» (ABGD) fue el más eficiente para reconocer especies de *Mercuria* (con un match ratio de 0,85), lo que confirma 12 de las 14 especies delimitadas en el estudio. Por el contrario, los métodos basados en árboles [es decir, procesos coalescentes mixtos de Yule generalizados (GMYC) y de árboles de Poisson (PTP)] presentaron un valor de relación de coincidencia relativamente bajo, coincidiendo con una sobreestimación del número de especies. Varios autores han reconocido que estos métodos deben interpretarse con cautela para grupos con baja vagilidad y escasa capacidad de dispersión como los gasterópodos, contrastando la validez de las especies delimitadas con los datos morfológicos bajo un enfoque taxonómico integrador. La similitud morfológica entre las especies de *Mercuria* se puntuó sobre la base de un análisis de morfometría geométrica de la forma de la concha utilizando un total de 1.148 conchas de 46 poblaciones y 21 landmarks y semilandmarks. En el PCA, los dos primeros componentes representaron el 75,3% de la variación en la forma de la concha; sin embargo, solo el 50% de las especies se delimitaron con éxito sobre la base de la morfometría de la concha. Generalmente, la concha, el opérculo y los

genitales de *Mercuria* presentan una amplia variabilidad intra e interespecífica; por lo tanto, el uso de estos caracteres debe usarse con cautela al evaluar el estado taxonómico de las poblaciones de este género. En cambio, proponemos el uso de taxonomía integradora, agregando información genética a los datos morfológicos. Bajo este enfoque, en nuestro estudio se revelaron varias identificaciones erróneas de registros anteriores de *Mercuria*, lo que representa un avance significativo en nuestro conocimiento de este género.

La inferencia bayesiana y los análisis de máxima verosimilitud recuperaron topologías similares (en términos del grado de restricción geográfica de clados y tiempos de divergencia) para los géneros *Mercuria* y *Pseudamnicola*, y para *Islamia* y *Corrosella*. Aplicando una tasa de calibración externa, nuestros árboles de especies fechadas mostraron una edad similar para *Corrosella* e *Islamia* (ca. 11 millones de años), y también para *Mercuria* y *Pseudamnicola* (ca. 7 millones de años), con una edad notablemente más joven estimada para el último par. Teniendo en cuenta estas edades estimadas, ubicamos el origen de la diversificación de los cuatro géneros en el Mioceno (de 23 a 5,3 millones de años). Según el principio de diversificación, los grupos más antiguos deberían presentar más especies ya que han tenido más tiempo para diversificarse; sin embargo, cuando contrastamos las edades de estos géneros con el número de especies en cada uno, encontramos discrepancias que demuestran que no siguen este principio. Encontramos una diferencia notable entre el número de especies existentes presentadas por *Corrosella* (17) e *Islamia* (45), y una diferencia mayor entre el de *Mercuria* (26) y *Pseudamnicola* (70). La disparidad en la riqueza de especies observada podría estar relacionada con diferencias en las preferencias ambientales, como el tipo de hábitat, el rango de altitud o los parámetros del agua, y también en las estrategias de dispersión.

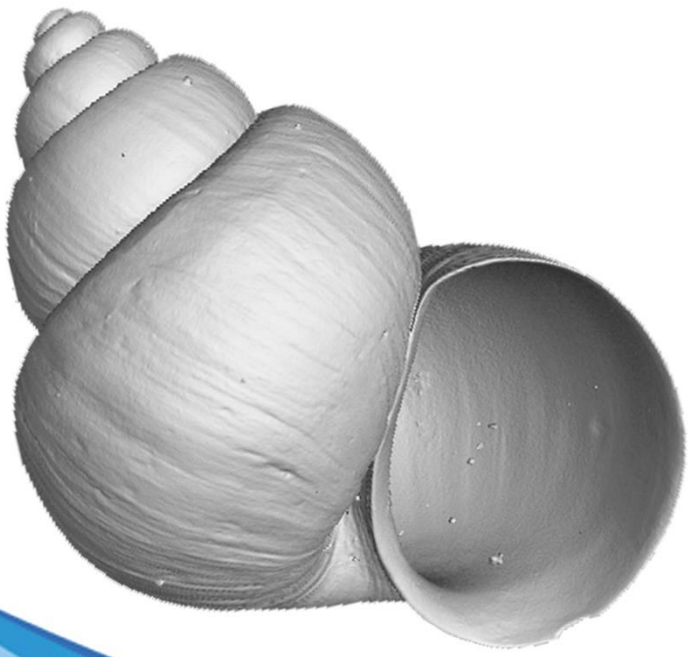
Para evaluar estas hipótesis, el área ancestral de las especies se infirió en el programa BioGeoBEARS utilizando los árboles de especies calibrados [es decir, árboles de máxima credibilidad de clado (MCC)] y la distribución geográfica de cada especie codificada por ecorregión de agua dulce. Este análisis reveló una alta especiación dentro de las regiones peninsulares, lo que respalda los supuestos previos sobre el papel de las penínsulas mediterráneas como centros evolutivos de los hidróbidos. La historia biogeográfica inferida de *Corrosella* sugiere un patrón de aislamiento por distancia producido por procesos de vicarianza. La cladogénesis dentro de *Islamia* involucró una combinación de eventos fundadores de la Península de los Apeninos hacia el este y el oeste, y simpatía estrecha (dentro de la ecorregión), mientras que en *Pseudamnicola* y *Mercuria*, involucró una serie de eventos de dispersión y colonización seguidos de un aislamiento posterior.

Los análisis de BioGeoBEARS mostraron que las tasas de expansión del rango anagenético (*d*) fueron más altas en las especies que ocurren en zonas altas (*Corrosella*) y bajas (*Mercuria*), mientras que aquellas que ocurren en elevaciones medias (*Pseudamnicola* e *Islamia*) tendían a tener tasas de dispersión bajas. Además, las especies que ocurren en elevaciones elevadas (es decir, *Corrosella*) presentaron el parámetro de especiación (*j*) del evento fundador más bajo (dispersión de salto) en la cladogénesis, mientras que las especies que ocurren en elevaciones medias (es decir, *Pseudamnicola* e *Islamia*) mostraron una propensión a la dispersión de saltos. Para evaluar la relación potencial entre rango altitudinal y dispersión, calculamos, para cada género, la relación de dispersión ($d_{ratio} = (N_d + N_j) / N_{lin}$) a partir del número de eventos de dispersión o expansión de rango (N_d), salto-dispersión o eventos fundadores (N_j) y linajes totales (N_{lin}), y se representaron gráficamente estos contra los datos de presencia y elevación. Observamos que el índice de dispersión disminuyó con la elevación, de 0,42 para el género *Mercuria* de zonas bajas a 0,14 para el género *Corrosella* de zonas altas. Nuestros datos, a diferencia de otros organismos, no evidenciaron una relación directa entre la dispersión y el tamaño corporal.

En general, este estudio confirma que la familia Hydrobiidae es un grupo muy diverso con hotspots de riqueza, endemismo y amenaza, principalmente en las ecorregiones de agua dulce de la cuenca mediterránea. Esta distribución de la riqueza de especies estuvo determinada por factores geográficos y climáticos como latitud, conectividad de cuencas, precipitación, estacionalidad y rango de temperatura anual, pero no por elevación y heterogeneidad geológica (probablemente porque la agregación espacial de estos dos factores ocultaba su verdadera variabilidad). Por lo tanto, concluimos que los hotspots globales de riqueza de Hydrobiidae representan áreas de estabilidad climática con precipitación moderada y estacionalidad de temperatura que están bien conectadas con otras cuencas hidrológicas. Nuestro estudio también indica que los patrones actuales de riqueza de especies se han visto afectados por la estrategia de dispersión de los grupos, influenciada por el rango de elevación en el que ocurren, más que por la edad o el tipo de hábitat. Un mejor acceso para los vectores de dispersión y una mayor conectividad del hábitat en las elevaciones más bajas pueden haber facilitado la dispersión a larga distancia, lo que resultó en la especiación (cladogenética) por evento fundador. Por el contrario, los grupos altamente especializados y aislados, como las especies de *Corrosella* que habitan en los manantiales de alta montañas, tienen menos probabilidades de dispersarse y especiarse (y probablemente son más propensos a la extinción) en comparación con los que habitan en elevaciones más bajas. Tomados en conjunto, nuestros

resultados ilustran que tanto los factores evolutivos como los ambientales determinan los patrones globales de especies y que los cambios futuros de estos últimos factores pueden afectar la riqueza de especies de hidróbidos.

INTRODUCTION



1.1 The family Hydrobiidae Stimpson, 1865: morphological versus molecular systematics

Within Mollusca, freshwater gastropods are one of the most diverse groups of organisms. This group is comprised of approximately 4,000 valid nominal species (Strong et al. 2008); however, this estimate may represent only 25% of its actual diversity. These snails are found on every continent, except Antarctica and in nearly every aquatic habitat (lotic and lentic), including lakes, swamps, rivers, streams, underground aquifers and springs, among others (Strong et al. 2008, Lydeard and Cummings 2019). The truncatelloidean family Hydrobiidae Stimpson, 1865 is the most specious assemblage of freshwater gastropods, with species occupying several biotopes, from the clear waters of mountain springs to the shallow waters of estuarine environments. Anatomical and systematic studies of this group are challenging due to the small size of most species (typically from 1 to 5 mm, occasionally up to 15 mm) and their reduced and simplified morphology, which yields few characters for diagnosis at the gross morphological level (Kabat and Hershler 1993, Hershler and Ponder 1998).

Troschel (1857) was the first author to refer to Hydrobiidae as 'Hydrobiae' to classify a group of molluscs belonging to the order Ctenobranchiata Schweigger, 1820 with a "range of uncertainty". Nevertheless, the authorship of the family has been attributed to Stimpson (1865) by Bouchet and Rocroi (2005): these authors did not find enough morphological characters in Troschel's works to establish the family rank for some genera. Consequently, this group was not named with terminology corresponding to the family category, similar to what occurred with other groups such as Bithyniae, Litogliphy, Pachichily and Thiarae. Bouchet and Rocroi (2005) considered the name 'Hydrobiae' as a temporal reference that did not denote a family taxonomic category; therefore, they concluded that Stimpson (1865) was the first author to treat Hydrobiidae as a family.

Regarding higher taxonomic levels, the classification of the Hydrobiidae proved to be perplexing for a long time as the limits and interspecific relationships of many extant taxa were poorly known. The high level of evolutionary convergence in shape found among the small, smooth shells of hydrobioids (Figure 1) added to the difficulty in classifying the family (Davis 1979). As a consequence, this family has been classified in three different superfamilies within Caenogastropoda Cox, 1960 (recently ranked as a subclass by Bouchet et al. 2017) on the basis of morphological characters: Rissooidea (Thiele 1926, Wenz 1939, Taylor and Sohl 1962, Taylor 1967, Bernasconi 1992), Hydrobioidea (Radoman 1973, 1983) and Truncatelloidea (Golikov and Starobogatov 1975, Ponder and Warén 1988).

Figure 1. Evolutionary convergence in shell shape among truncatelloidean genera, as reflected within each row of images showing the shells of species belonging to Hydrobiidae *sensu stricto (s. str*.) or Hydrobiidae *sensu lato* (*s. l.)*. **A** – **C**, *Alzoniella*; **D**, *Pristinicola*; **E**, *Bythinella*; **F**, *Erhaia*; **G**, *Corbellaria*; **H**, *Tarraconia*; **I** – **J**, *Valvata*; **K** – **L**, *Mercuria*; **M**, *Potamolithus*; **N**, *Bithynia*; **O**, *Amnicola*; **P**, *Peringia*; **Q**, *Ecrobia*; **R**, *Heleobia*; **S**, *Littoridinops*. Scale bars = 1 mm. Composite figure comprised of some images taken from other resources: **A-C**, **G**, **H**, modified from Fernando García Guerrero; **F**, (Gittenberger et al. 2020); **I**, (Vinarski et al. 2013); **J**, (Odabaşi et al. 2015); **D**, **M**, (Hershler 2020); **N**, (Glöer and Maassen 2009); **O**–**P**, (InvertEBase 2020); **R**, (Szarowska et al. 2014); **S**, (Collado et al. 2019).

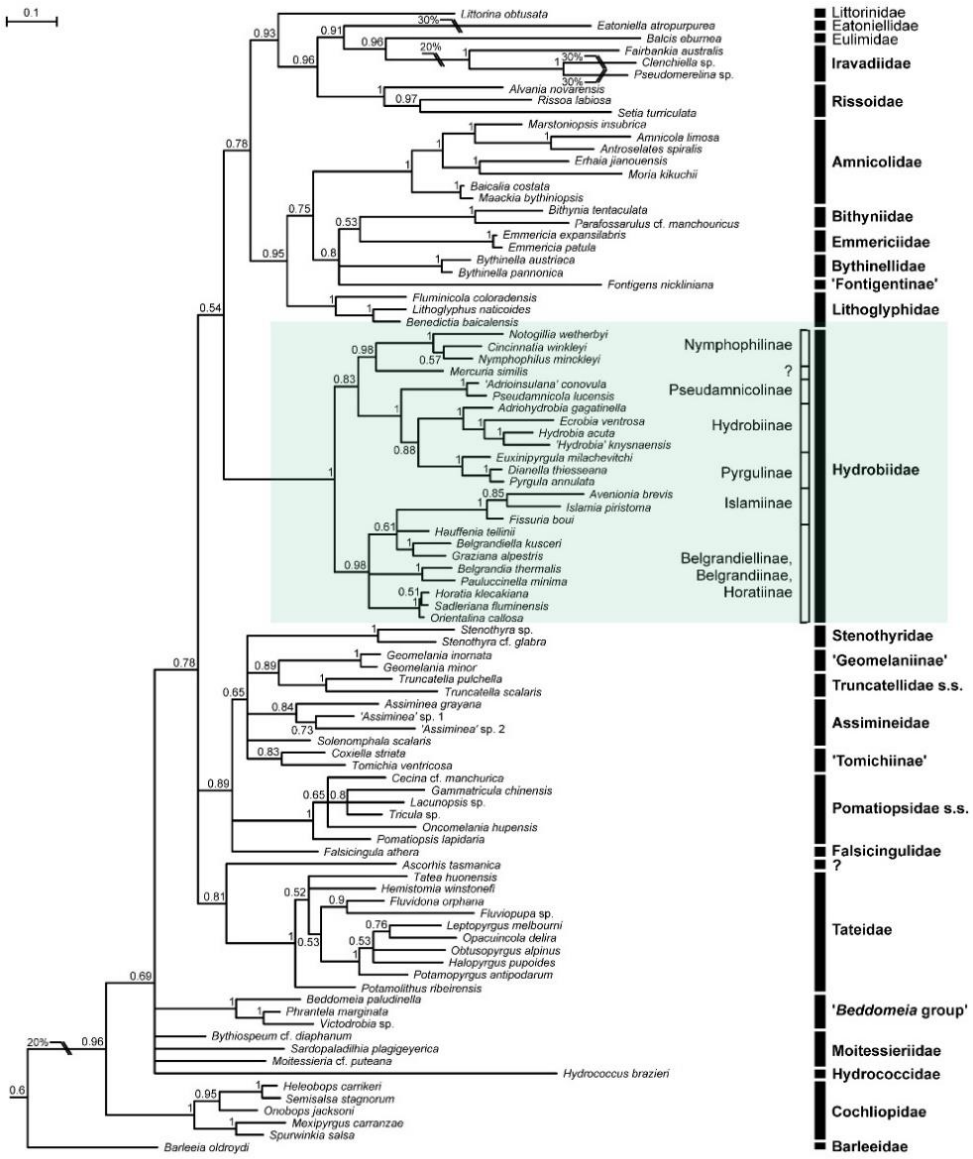


Figure 2. Bayesian tree of Hydrobiidae *s. l.* taxa based on a combined (COI, 16S and 18S) genetic dataset. In green, the monophyletic group comprising Hydrobiidae *s. str.* Modified from Wilke et al. (2013).

Only a few works have tested the hypotheses of the morphology-based higher systematic classifications using genetic data. For instance, Colgan et al. (2007b) and Criscione and Ponder (2013) have both analysed the phylogenetic relationships among several Caenogastroda taxa and have established the phylogenetic limits and positions of some superfamilies based on multilocus

genetic datasets. Only Criscione and Ponder (2013) included a hydrobiid species in their phylogenetic inference, which clustered Hydrobiidae within the superfamily Truncatelloidea. Bouchet et al. (2017) retained this higher taxonomic classification in their prestigious nomenclator of gastropod families and it is the currently accepted superfamily for Hydrobiidae.

Although the review of morphological characters of Hydrobiidae *s. l.* conducted by Hershler and Ponder (1998) established a good morphological basis for descriptions of hydrobiid taxa, morphological species assignments are still difficult to perform. In fact, until the application of molecular tools, brackish and freshwater snails were all typically classified as hydrobioids (= Hydrobiidae *sensu lato* [*s. l.*]; (Davis 1979) due to their small size and featureless shells. Also, cladistic studies based only on morphological data, which contained many homoplastic characters, resulted in inconsistent phylogenies (Bodon et al. 2001, Szarowska 2006, Haase 2008). In a first attempt to combine genetic studies with anatomical data, Wilke et al. (2001) showed the monophyletic origin of the family Hydrobiidae from the classification given by Kabat and Hershler (1993). This study was also supported by anatomical data through its comparison of the general organisation of the female genital system in each of the families. In a more extended work in terms of both taxa and genetic markers, Wilke et al. (2013) confirmed the monophyletic origin of the family and redefined some of its subfamilies (Figure 2).

The modern *sensu stricto* (*s. str.*) definition of the family Hydrobiidae, based on morphological and molecular evidence (Wilke et al. 2001, Criscione and Ponder 2013, Wilke et al. 2013), allowed for the recognition of some former hydrobiid subfamilies, such as Cochliopinae, Lithoglyphinae and Moitessieriinae, as independent families outside Hydrobiidae (see Bouchet and Rocroi 2005, Bouchet et al. 2017). Therefore, the taxon Hydrobiidae, as previously defined based on morphology alone, was not monophyletic. The family Hydrobiidae *s. str.* (herein referred to as hydrobiids) is currently more narrowly delimited and confined to the western Palearctic, eastern Nearctic, northern Neotropic and South Africa (Wilke et al. 2013).

1.2 Species richness distribution in Hydrobiidae *s. str.* and potential underlying factors

Knowledge of species richness and threat patterns is still limited to only a few taxonomic groups (Tisseuil et al. 2013), particularly for freshwater taxa compared with terrestrial ones (Beck et al. 2012). Furthermore, information for freshwater groups is still biased in terms of geography and taxonomy (Dudgeon et al. 2006,

Strayer and Dudgeon 2010). As a result, global species richness patterns of highly diverse freshwater groups, such as gastropods, are poorly understood, despite the importance of these organisms in ecosystem function (Johnson et al. 2013) and references therein) and their rapidly declining levels of diversity (Lydeard et al. 2004). Moreover, discerning global patterns of freshwater gastropod diversity presents enormous difficulties. The number of species is certainly underestimated as a considerable number of freshwater gastropod species are (1) endemic to only a few square metres and can easily remain undetected during field surveys and (2) described based on shell characteristics alone, which can hide greater diversity as most of the species present featureless shells (Strong et al. 2008). Another hindrance is that regions other than North America, Europe and Australia remain poorly surveyed (Lydeard et al. 2004).

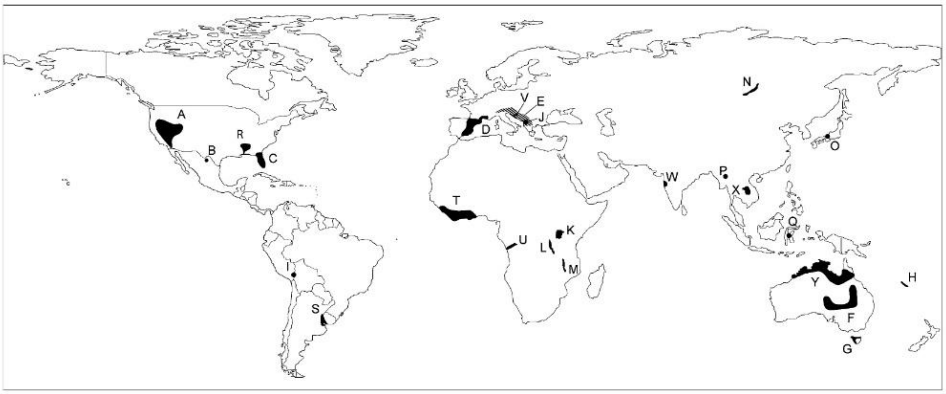


Figure 3. Global diversity hotspots of freshwater gastropods according to Strong et al. (2008).

Kabat and Hershler (1993) and Strong et al. (2008) estimated around 1,000 species of Hydrobiidae *s. l.* distributed worldwide (Figure 3). This estimate suggests that Hydrobiidae account for around a quarter of the total freshwater gastropod diversity; however, due to the aforementioned limitations in species detection and description, these authors also recognised that this value might depict only about 25% of actual species diversity. On the other hand, their application of the Hydrobiidae *s. l.* concept likely resulted in an overestimation of the family's actual species richness. Over the last two decades, molecular data have been used to support taxonomic assignments and to infer evolutionary relationships among hydrobiid taxa. The above-mentioned phylogenetic works redefined our understanding of the family Hydrobiidae *s. str.* and restricted its geographic extension to mainly the Palaearctic and Nearctic regions (Wilke et al. 2001, Wilke et al. 2013).

Within this distribution range, hydrobiids are found in nearly all aquatic continental ecosystems, with the great majority of the species inhabiting spring and cave interstitial systems (Strong et al. 2008). These ecosystems contain a highly endemic hydrobiid fauna of poorly dispersing animals that require perennial waters and specific habitats. As a consequence, their distribution is often restricted to a single or a few sites, making species highly vulnerable to the effects of habitat loss and fragmentation, which can lead to their extinction in extreme cases (e.g., Szarowska 2006, Hershler et al. 2014b). Furthermore, natural stochastic processes and anthropogenic actions have profoundly and diversely impacted hydrobiid species (Sisk et al. 1994, Collen et al. 2014, Cowie et al. 2017), making the group a priority for conservation studies in recent decades (e.g., Bouchet and Gargominy 1998, Hershler et al. 2014a). As a result, 1,107 species of Hydrobiidae *s. l.* are reported in the Red List of Threatened Species (IUCN 2018); of these, 31 are listed as extinct and 526 (~50%) are considered to have a high risk of extinction. Given the new definition of Hydrobiidae *s. str.* based on molecular evidence and the rapid increase in the number of species discovered [around 120 species of Hydrobiidae have been described in the last two decades (e.g., Ramos et al. 2000, Arconada and Ramos 2007, Delicado et al. 2012, Hershler et al. 2013, Radea et al. 2013, Glöer et al. 2015b)], estimates of species richness should be re-examined and adjusted accordingly. This requires new investigations of global-scale patterns of hydrobiid species richness and endemicity, which will provide a baseline for macroecological studies and conservation actions.

Unlike for other taxonomic groups, global biogeographic studies of the family Hydrobiidae are scarce (e.g., Lassen 1975, Davis 1982, Hershler and Liu 2017). However, regional-scale studies of hydrobiid diversity have reported the remarkable richness of spring systems located in the Apennine, Iberian and Balkan peninsulas (Radoman 1983, Bodon et al. 2001, Arconada and Ramos 2003), the Great Basin in North America (Hershler 1998), the ancient lakes Ohrid and Prespa in the Balkan Peninsula (Radoman 1983) and in brackish environments such as the Caspian Sea (Vinarski and Kantor 2017). These studies also provide evidence of the restricted distribution ranges of these gastropods and question the taxonomic delimitation of widely distributed species. In fact, of the few widespread hydrobiids that are known (e.g., Hershler et al. 2011), genetic evidence suggests that some of these taxa actually comprise a complex of cryptic, geographically limited species (Wilke and Pfenninger 2002, Liu et al. 2003, Delicado and Ramos 2012, Hershler et al. 2014a), with a few being old, unique phylogenetic clades (Khalloufi et al. 2017). Further phylogeographic studies are,

therefore, needed to reappraise the taxonomic status of several hydrobiid species and to identify targets for conservation strategies.

Given the dependency of these species to a non-marine aquatic environment, large-scale geography, dispersal processes and climatic conditions could have significantly influenced the observed patterns of species richness of extant hydrobiids. Variation in species richness of freshwater organisms is often associated with latitude (Hof et al. 2008, Perez-Losada et al. 2009, Abell et al. 2011, Pérez-Quintero 2015, Georgopoulou et al. 2016, Iversen et al. 2016), which, in turn, is related to climatic gradients. For instance, the stable temperature and precipitation conditions in the tropics are thought to increase net diversification for a wide range of taxa, resulting in increased species richness (Gaston 2007, Oberdorff et al. 2011a, Pyron and Wiens 2013). Elevation is another potentially important geographic factor influencing species richness. A high number of endemic freshwater species inhabit high elevations, presumably as a result of greater habitat specialisation and isolation affecting montane populations (Hughes and Eastwood 2006, Steinbauer et al. 2016) and more stable climate conditions compared with lower elevations (Jetz et al. 2004, Ohlemüller et al. 2008). Distribution of species richness may also depend on regional factors, such as the ancestral geographic area of the group, dispersal limitations and habitat heterogeneity (Hortal et al. 2009, Iversen et al. 2016). Considering that most hydrobiids are habitat specialists and poor dispersers, we expect to observe a positive relationship between connectivity among ecosystems and species richness, as shown in other organisms (Ribera et al. 2003, Braaker et al. 2017). Given this context, remote areas within the global distribution of hydrobiids or isolated territories, such as peninsulas or islands, might contain fewer species (the classic 'peninsula effect', see Simpson (1964). As with habitat connectivity, habitat heterogeneity also increases species numbers due to more niche space. Indeed, a positive relationship between heterogeneity and diversity has been widely documented for a variety of groups (Kohn and Walsh 1994, Tews et al. 2004, Hortal et al. 2009, Weisberg et al. 2014), including freshwater molluscs (Hauffe et al. 2014).

1.3 Patterns and timing of speciation in hydrobiid genera

In addition to being one of the most abundant families of freshwater molluscs, Hydrobiidae is considered one of the most ancestral. According to the fossil record, it dates back to the Carboniferous period, approximately 280 Mya, with the description of *Bernicia praecursor* Cox 1927, from northern England (Cox 1927). However, Ponder (1988) questioned the identification of these specimens as a hydrobiid given the "meager evidence of Carboniferous fossils" (p. 156),

attributing them instead to the Ampullarioidea. This implied, on the basis of other fossil specimens of hydrobiids (Davis 1979), a later origin of the family during the late Triassic – early Jurassic.

Although there is no consensus about the origin of the family Hydrobiidae, some authors have proposed the origin of all truncatelloidean taxa to the Carboniferous period (around 360 Mya) in the Pangea terrains (Cox 1927, Kabat and Hershler 1993). However, due to the simplicity of the shell shape and convergent evolution, it is quite possible that many of these fossil records were erroneously assigned. In this case, a later origin during the Jurassic in the Laurasian terrains, as hypothesised by Ponder (1988), would be more supported. Regardless, both hypotheses agree on one point: freshwater hydrobioids originated in the western part of the Tethys Sea (Figure 4), during a gradually transition from a marine to continental environment (Ponder 1988).

Much of the diversity of Hydrobiidae *s. str.* is represented by spring snails, which have very poor fossil records (Strong et al. 2008). Moreover, as explained above, there is uncertainty in the fossil taxonomy and date of origin of hydrobiids. To date, the preferred calibration method to calculate divergence times in Hydrobiidae has been the molecular clock approach applied to DNA sequence data. The estimation of divergence time through the use of DNA sequences on phylogenetic trees has become a very interesting approach used by evolutionary biologists in recent decades. Several of the methods are based on the molecular clock hypothesis proposed by Zuckerkandl and Pauling (1965), which postulates that the differences (based on DNA or protein sequences) between two species is a function of the time since the divergence of their most recent common ancestor (Forest 2009).

This advancement paved the way for the inclusion of a temporal framework in many evolutionary studies by linking the evolution of a particular set of morphological characters or ecological traits to geological events or periods, thus placing phylogenies in an evolutionary timescale (Forest 2009). The assumption of regular evolutionary rates, however, proved to be invalid: this clock does not make regular ticks. This is explained by the heterogeneity of substitution rates between lineages due to many factors inherent to the species such as metabolic and mutation rates and population size (Forest 2009).

Several methods for estimating divergence time are found in the literature and can be grouped into three categories:

1- Methods that use a molecular clock assuming one global rate of substitution.

2- Methods that apply a correction for rate heterogeneity.
3- Methods that try to incorporate rate heterogeneity.

The most commonly used method, Bayesian evolutionary analysis by sampling trees (BEAST; (Drummond and Rambaut 2007), falls into the third category (Rutschmann 2006).

Undoubtedly, this framework has generated controversy, especially in its handling of heterogeneity rate and calibration. Though the accuracy of the heterogeneity parameter has improved over the years due to the development of new methodologies that deal with variation in substitution rates, calibration is still regarded as a source of uncertainty.

We consider calibration as the incorporation of independent non-molecular time information into a phylogeny, which transforms relative into absolute divergence time. Most calibration bounds are based on geological events such as tectonic plates drifting or the origins of geographic phenomena such as mountains, islands and valleys, all of which favour allopatric speciation processes. The fossil record, which is regarded as the best source of non-molecular information, is also often used as calibration points. However, erroneous fossil age estimates and the assignment of fossils to erroneous taxonomic categories can lead to incorrect dating assessments (Rutschmann et al. 2007).

Using different molecular methods, the following substitution rates have been estimated from dated phylogenies of hydrobiid taxa calibrated using biogeographic events: 0.81% per million years (percentage of substitutions per lineage My^{-1}) for the North American genus *Pyrgulopsis* (Hershler and Liu 2008) and 0.91% and 0.72% for the subfamily Hydrobiinae (Wilke (2003) and Wilke et al. (2009)). Many authors have used these substitution rates to date the divergence times of several groups of hydrobiids (Wilke 2003, Falniowski et al. 2016, Osikowski et al. 2016); nevertheless, this information remains unknown for the vast majority of groups.

1.3.1. Using dated phylogenies to infer the biogeography of hydrobiid genera

For those groups that lack a robust fossil record, such as hydrobiids, molecular phylogenies can be particularly useful tools for reconstructing the biogeography of species. Historical biogeography is of special importance in the understanding of the evolutionary processes that led to the geographic distribution and species richness of present-day clades (Wiens and Donoghue 2004). Unlike for the North American hydrobiid species (e.g., Hershler et al. 1999, Hershler and Sada 2002, Liu et al. 2013), little is known about the biogeography of the Palaearctic groups.

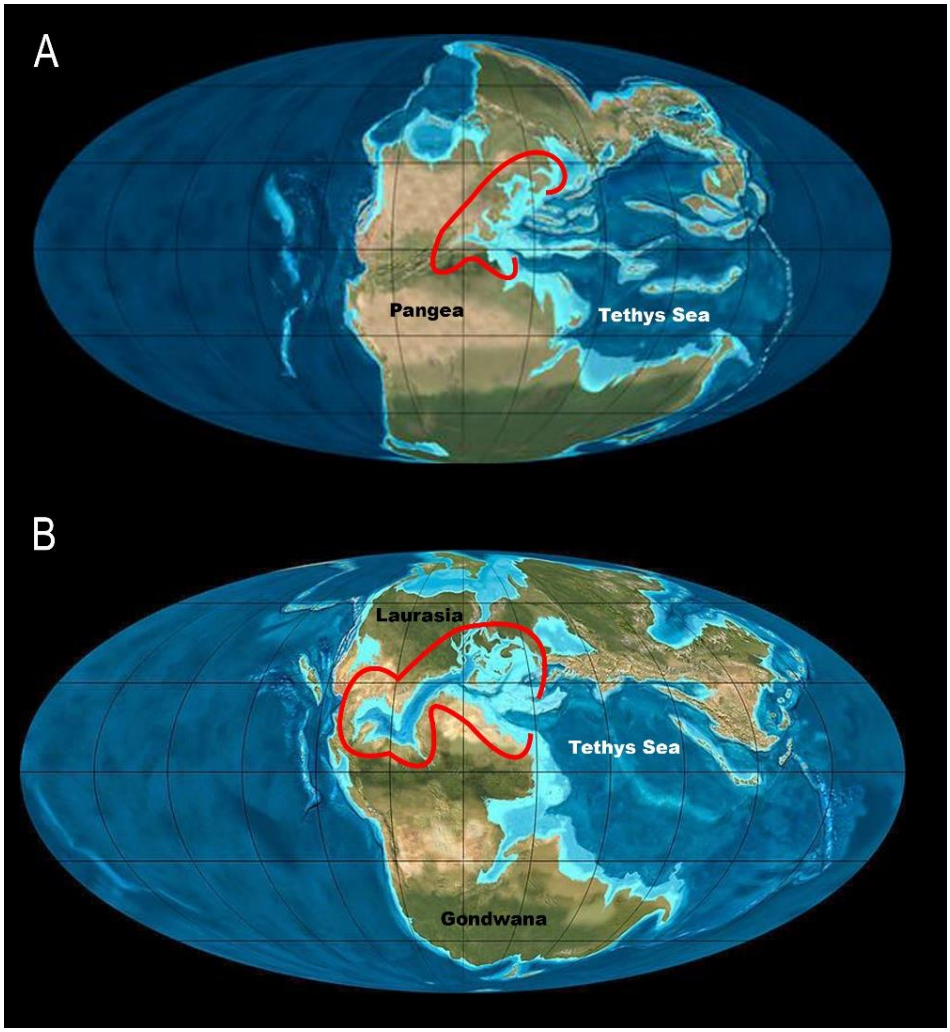


Figure 4. Maps showing the paleogeographic area hypothesised as the origin of the truncatelloidean gastropods. **A**, Carboniferous period ca. 360 Mya; **B**, Late Triassic – early Jurassic period, ca. 120 Mya. Paleogeographic reconstructions were obtained from Blakey (2004).

Most studies of hydrobiid species in the Mediterranean are regional-scale taxonomic descriptions; only a few have explored their phylogenetic relationships and biogeographic history (e.g., Falniowski and Szarowska 2011, Delicado et al. 2014, Delicado et al. 2019, Vandendorpe et al. 2019). The scarcity of these types of studies is due, in part, to the fact that the great majority of the hydrobiid genera inhabiting Mediterranean continental waters consist of narrow-range endemics in species-poor assemblages. Only a few Mediterranean genera are diverse and geographically distributed enough to be good candidates for studies aiming to

reconstruct biogeographic histories and infer speciation processes. Suitable taxa for such studies include the genera *Corrosella* Boeters, 1970; *Pseudamnicola* Paulucci, 1878; *Islamia* Radoman, 1973 and *Mercuria* Boeters, 1971. These genera are also notable as they occupy different habitats along an elevational gradient.

Among the genera studied in this thesis, *Corrosella* is the most species-poor. Of the 17 known species (MolluscaBase 2020), most occur mainly in mountain springs in the Iberian Peninsula, mostly in the south (Delicado et al. 2012, Delicado et al. 2013b). Only one species, *C. astieri* (Dupuy, 1851), is distributed in a few springs throughout southern France (Girardi 2009). All of the Iberian species occur in only a few sites, except the widespread species *C. navasiana* (Fagot, 1907). The phylogenetic relationships among the species of the genus are well known (Delicado and Ramos 2012, Delicado et al. 2013b) as species are well delimited at the molecular level. Nevertheless, basal relationships within *Corrosella* remain unresolved. Previous phylogenetic inferences recovered three well-supported clades: clades I and II are distributed around the central Iberian Peninsula and southern France and clade III, in the southern Iberian Peninsula. Delicado et al. (2013b) dated the divergence of these clades to the Miocene, ca. 10 Mya, with recurrent speciation events occurring within the genus 5 and 2 Mya.

The genus *Pseudamnicola* comprises about 147 nominal species, of which, 70 are extant and 77 are fossil (MolluscaBase 2020). These species are distributed throughout the Mediterranean basin in both Europe and North Africa (Glöer et al. 2015c) and some have also been reported for the Ponto–Caspian region (Glöer and Pešić 2012). They mainly inhabit streams and springs with high water conductivity (Delicado et al. 2014) and can be found from almost sea level (Radea et al. 2015, Falniowski 2016) to ca. 1800 m.a.s.l. (Glöer and Pešić 2012). This wide altitudinal range suggests that the genus assignment of many of the species described as *Pseudamnicola*, based only on conchological studies, may have been wrong, especially of those species described for marginal areas of the supposed distribution of the genus (i.e., Iran; Delicado et al. 2016). Recent phylogenetic studies of *Pseudamnicola* in the Iberian Peninsula, Balearic Islands and Northwest Africa have inferred a close relationship between this genus and *Corrosella* (see Delicado et al. 2014, Delicado et al. 2015, Boulaassafer et al. 2020).

Basal phylogenetic relationships within the genus *Pseudamnicola* were not well supported by previous studies (Delicado et al. 2015). However, with the inclusion of more material from poorly sampled regions in studies, many speciation events linked to long-distance dispersion have been discovered (Delicado et al. 2014, Szarowska et al. 2016, Boulaassafer et al. 2020). Phylogenetic studies of

Pseudamnicola in the Iberian Peninsula and the Balearic Islands (Majorca and Minorca) recovered two major clades whose divergence dated to about 4 Mya (Delicado et al. 2014). Evidence of a dispersal event between populations from the Iberian Peninsula and Minorca was found for one of the clades. More recently, Boulaassafer et al. (2020) found species of *Pseudamnicola* in Northwest Africa that diverged from the Majorca clade around 3.2–1.4 Mya. Taken together, the results of both studies suggest that species from the Balearic Islands had a double origin through two dispersion events, one from Europe and the other from Northwest Africa.

The westernmost part of the geographic range of *Pseudamnicola* largely overlaps with that of *Mercuria*, another hydrobiid genus. In fact, species of both genera have frequently been found co-occurring within the same site. For instance, in Ullals de Baltasar, Santamera and Ribas de Santiuste, all in Spain, *M. similis* (Draparnaud, 1805) and *P. subproducta* (Paladilhe, 1869) [= *P. spiratus* (Paladilhe, 1869)] have been found in sympatry, as have *M. balearica* (Paladilhe, 1869) and *P. meloussensis* Altaba, 2007 in Colarsega, Minorca, Spain. Despite the large phylogenetic distance separating these genera, which belong to two different subfamilies (Wilke et al. 2013), we hypothesise that the biogeographic patterns and processes that have influenced species of both genera are similar given the low (active) dispersal abilities of these snails. However, before these assumptions can be tested, a deep systematic revision of the species of *Mercuria* is needed as the great majority of the nominal species have been described based on only shell and penis features, which are doubtful diagnostic characters for this genus (Boulaassafer et al. 2018). To this end, the delimitation, timing of speciation and biogeography of species of *Mercuria* have been studied here to a greater extent than in any previous study.

The fourth genus characterised in this study is *Islamia*, which includes 49 nominal species, 45 of which are extant (MolluscaBase 2020). The species of this genus can be found in aquatic continental ecosystems throughout the Mediterranean region. Until recently, the genus was thought to occur only in the European continental area, from the Iberian Peninsula to Anatolia (Radoman 1983, Arconada and Ramos 2006, Radea 2011, Bodon and Cianfanelli 2012, Beran et al. 2016, Yildirim et al. 2017); however, it has also been found in North Africa (Glöer et al. 2020) and some Mediterranean islands (Bodon et al. 1995, Radea et al. 2017). It inhabits springs found at low to medium elevation, with species presenting a high level of local endemism (Arconada and Ramos 2006, Bodon and Cianfanelli 2012). More detailed morphological descriptions are generally available for the species of *Islamia* (Radoman 1983, Arconada and Ramos 2006,

Bodon and Cianfanelli 2012) than for those of *Mercuria*; however, *Islamia* has been poorly studied at the molecular level.

The genus *Islamia* is closely related to the genera *Fisuria* Boeters, 1981; *Avenionia* Nicolas, 1882 and, its likely sister group, *Milesiana* Arconada & Ramos, 2006 (Wilke et al. 2013, Delicado et al. 2019). However, recent analyses of the species attributed to *Islamia* revealed taxonomic inconsistencies indicating that the characters that usually define closely related genera within Islaminae Radoman, 1973 (i.e., position and relative size of the two seminal receptacles, presence/absence of a bursa copulatrix and number of seminal receptacles) are not sufficient to assign species to either one genus or another (Delicado et al. 2019). Similar observations made for other genera have resulted in *Alzoniella* Giusti & Bodon, 1984 also being considered a closely related genus within the subfamily (Beran et al. 2016). Given the regional scope of previous studies, a global-scale phylogenetic study of the genus that includes all species from various Mediterranean regions is needed. The unresolved systematics, together with the high convergence in shell shape, has made it challenging to study the taxonomy of this genus. Consequently, very diverse taxa have been recognised as species of *Islamia*, despite the high uncertainty of their placement [e.g., *Deganta azarum* (Boeters & Rolán, 1988), *I. ateni* (Boeters, 1969), *I. coronadoi* (Bourguignat, 1870) and *I. edlingeri* (A. Reischütz & P. L. Reischütz, 2004)]. Due to the lack of general molecular studies of *Islamia*, the speciation times of lineages within the genus and the biogeographic patterns or evolutionary processes that have influenced its species, are unknown. Knowledge of these aspects are essential to understand the current species distribution of the genus.

1.4 The neglected genus *Mercuria* Boeters, 1971

The western Palearctic freshwater snail genus *Mercuria* comprises 30 recognised species (26 extant and 4 extinct; (MolluscaBase 2020) that are primarily distributed in lowland localities of Mediterranean and Atlantic coastal regions (Glöer et al. 2015b). Within the Mediterranean, this genus has been found in the Iberian and Italian peninsulas, southern France, some western Mediterranean islands and northwestern Africa. On the Atlantic coast, confirmed records include the southern British Islands, northern continental Europe and Macaronesia (Kerney 1992, Kerney 1999).

Boeters and Falkner (2017) erected the subfamily Mercuriinae Boeters & Falkner, 2017 for this genus on the basis of the unique set of morphological characters featured by the species of *Mercuria* and the molecular systematics findings of Wilke et al. (2001) and Wilke et al. (2013). The most diagnosable morphological character for the genus is the presence of a penial appendix at the distal end of the

penis (Boeters 1971). Despite this, a low level of variability in this structure has been observed between closely related species, often leading to incorrect classifications (Boulaassafer et al. 2018). Moreover, great inter- and intraspecific variations are generally observed in the shell characters and the size of species (e.g., *M. tensiftensis* in Boulaassafer et al., 2018); yet, in a few cases, some studied species are hardly distinguishable from others and could be categorised as cryptic species (e.g., *M. tachoensis* and *M. bayonnensis*). All of these aspects, along with missing type material and unspecific descriptions of type localities in past taxonomic studies (Giusti et al. 1995), make it challenging to assign populations to a specific species of *Mercuria*.

1.4.1 Taxonomy of *Mercuria* species

Phylum Mollusca*

Class Gastropoda

Subclass Caenogastropoda Cox, 1960

Subcohort Hypsogastropoda Ponder & Lindberg, 1997

Superfamily Truncatelloidea Gray, 1840

Family Hydrobiidae Stimpson, 1865

Subfamily Mercuriinae Boeters & Falkner, 2017

Mercuria anatina (Poiret, 1801)

Mercuria atlasica Mabrouki, Glöer & Taybi, 2021

Mercuria baccinelliana Esu & Girotti, 2015 †

Mercuria bakeri Glöer, Boeters & Walther, 2015

Mercuria balearica (Paladilhe, 1869)

Mercuria baudoniana (Gassies, 1859)

Mercuria bayonnensis (Locard, 1894)

Mercuria boetersi Schlickum & Strauch, 1979 †

Mercuria bourguignati Glöer, Bouzid & Boeters, 2010

Mercuria corsensis Boeters & Falkner, 2017

Mercuria emiliana (Paladilhe, 1869) accepted as *Pseudamnicola emilianus*

Mercuria gauthieri Glöer, Bouzid & Boeters, 2010

Mercuria globulina (Letourneux & Bourguignat, 1887)

Mercuria helicella (Sandberger, 1858) †

Mercuria maceana (Paladilhe, 1869)

Mercuria melitensis (Paladilhe, 1869)

Mercuria meridionalis (Risso, 1826)

Mercuria midarensis Boulaassafer, Ghamizi & Delicado, 2018

Mercuria punica (Letourneux & Bourguignat, 1887)

Mercuria pycnocheilia (Bourguignat, 1862)

Mercuria rolani Glöer, Boeters & Walther, 2015

Mercuria saharica (Letourneux & Bourguignat, 1887)

Mercuria sarahae (Paladilhe, 1869)

Mercuria similis (Draparnaud, 1805)

Mercuria tachoensis (Frauenfeld, 1865)

Mercuria targouasensis Glöer, Boeters & Walther, 2015

Mercuria tensiftensis Boulaassafer, Ghamizi & Delicado, 2018

Mercuria tingitana Glöer, Boeters & Walther, 2015

Mercuria vanparysi Marquet et al., 2008 †

Mercuria vindilica (Paladilhe, 1870)

Mercuria zopissa (Paulucci, 1882)

* The systematic classification of Bouchet et al. (2017) was followed.

† Extinct species.

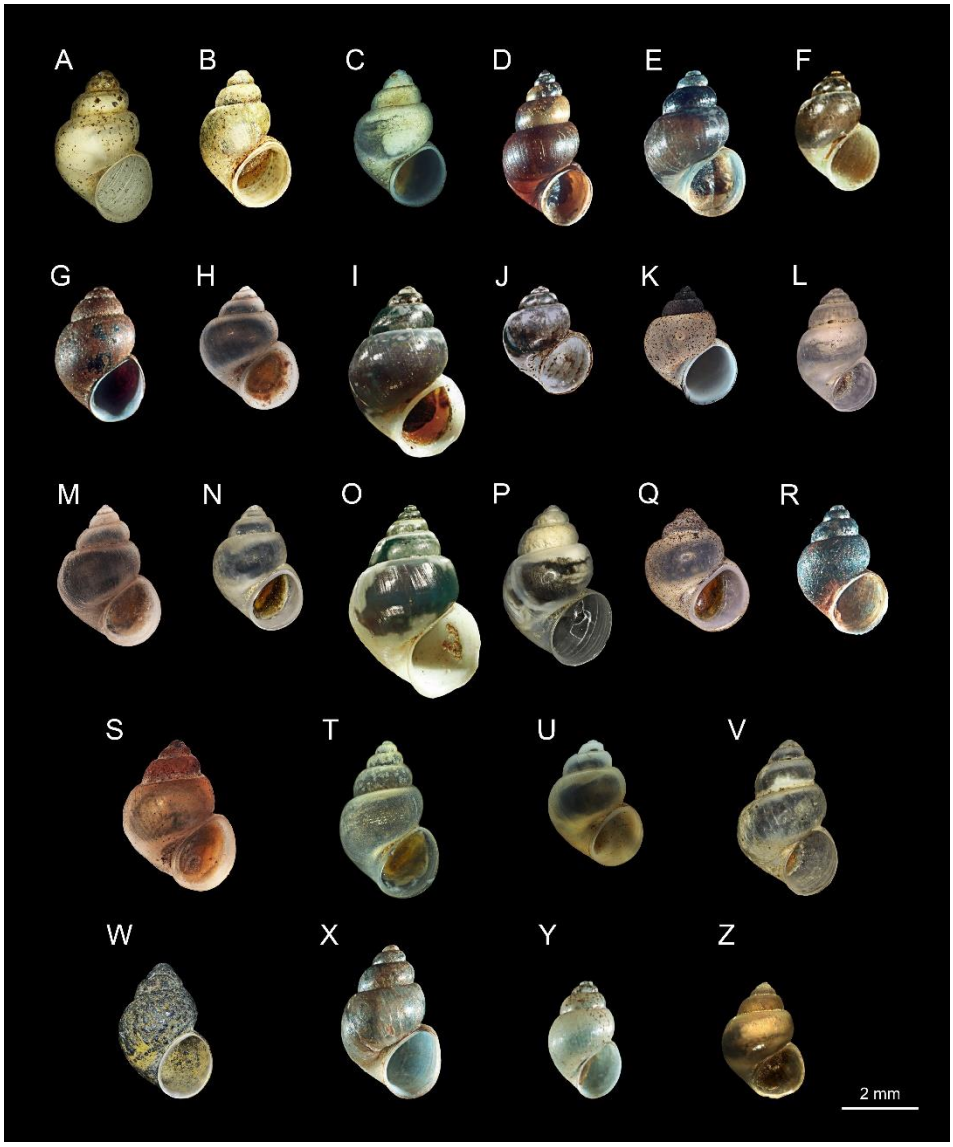


Figure 5. Shells of some extant species of *Mercuria*. **A**, *M. anatina,* Oude Maas at Hoogvliet, Netherlands; **B**, *M. bakeri,* holotype, Tetouan, Morocco; **C**, *M. balearica*, Stream at Sant Joan de Carbonell, Minorca, Spain; **D**, *M. baudoniana*, lectotype, Le Teich, France; **E**, *M. bayonnensis,* lectotype, Lac de le Negresse, Bayonne, France; **F**, *M. bourguignati*, holotype, Ksar el Boukhari, Algeria; **G**, *M. corsensis,* holotype, Couvent de la Trinité, Corsica, France; **H**, *M. emiliana*, Fonte Dame, Salses-le-Château, France; **I**, *M. gauthieri,* holotype, outskirts of Nemours, Algeria; **J**, *M. globulina,* Boufarik, Algeria; **K**, *M. maceana,* C′an Tunis, Barcelona, Spain; **L**, *M. melitensis,* Il-Bahrija, Malta; **M**, *M. meridionalis*, La Foux-de-Draguignan, Var, France.

The current taxonomy of *Mercuria* is based on a complex of conchologically variable species without clear morphological, anatomical and genetic delimitations for many species. Early authors, on the basis of external features, assigned some species currently recognised as *Mercuria* to either the genus *Cyclostoma* Draparnaud, 1805 or *Amnicola* Gould & Haldeman, 1840 (e.g., Moquin-Tandon 1855, Frauenfeld 1863, Paladilhe 1869, Bourguignat 1876, Clessin 1882). One example is represented by the type species *Amnicola confusa* (Frauenfeld, 1863), which was erected after Frauenfeld (1863) dubious conclusion about differences between the operculum of this species and that of *Cyclostoma simile*: "*When listing the species of the genus* Bithynia, *I have shown that the* similis *Drp. is a snail with a concentric operculum, with which a very similar snail with a subspiral operculum has been confused, which Küster has shown very well and to which I ascribe the above name [Ich habe bei der Aufzählung der Arten der Gattung* Bithynia *nachgewiesen, dass die similis Drp. eine Schnecke mit concentrischem Deckel ist, mit welcher eine sehr ähnliche Schnecke mit subspiralem Deckel verwechselt worden, die namentlich Küster sehr gut abgebildet hat und der ich obigen Namen beilege*]" (Frauenfeld 1863) p. 1029). As mentioned, the loss of type material and the imprecise descriptions of the type localities make it difficult to resolve ongoing taxonomic questions, especially those involving the recognition and delimitation of widespread species. For these reasons, many authors have debated about the effectiveness of shell characters to evaluate the diversity of *Mercuria* (e.g., Adam 1940, Boeters and Falkner 2000, Vinarski and Eschner 2016), leading to the conclusion that a taxonomy of the group based on only shell and external characters is really complex and ineffective.

Figure 5. Continuation. **N**, *M. midarensis*, holotype, Midar, Morocco; **O**, *M. pycnocheilia*, Temascin, Algeria; **P**, *M. rolani,* Arco de Calheta, Madeira, Portugal; **Q**, *M. saharica*, Gabes, Tunisia; **R**, *M. sarahae*, lectotype, Loire near Nantes, France; **S**, *M. similis*, Les Cabanes, Bouches-du-Rhône, France; **T**, *M. tachoensis*, São Mamede, Leiria, Portugal; **U**, *M. targouasensis,* Assaka Spring, Sus-Masa-Draa, Morocco; **V**, *M. tensiftensis,* holotype, Sidi Bouzid, near Chichaoua, Morocco; **W**, *M. tingitana,* Tanger, Morocco; **X**, *M. vindilica*, Belle-Île-en-Mer, Morbihan, France; **Y**, *M. zopissa*, Monte dei Sette Fratelli, Sardinia, Italy; **Z**, *M. atlasica*, Aouinat El Hajjaj, Morocco. [**B**, **Y** were taken from Glöer et al. (2015b); **D**, **E**, **G**, **R**, **X** were taken from Boeters and Falkner (2017); **O**, **I**, **J**, **F** were taken from Glöer et al. (2010); **N**, **V**, **W** were taken from Boulaassafer et al. (2018); Z, modified from Mabrouki et al. (2021)

Since Boeters (1971) established the genus, the use of anatomical features to describe *Mercuria* species has increased (e.g., Boeters 1988, Boeters and Falkner 2017, Boulaassafer et al. 2018). Despite this, taxonomic issues related to several species living in Mediterranean coastal areas, for instance, the type species *M. confusa*, remain unresolved. Boeters (1971) designated *Amnicola confusa* as the type species of *Mercuria* and provided, as type material, a lectotype 5.1 mm in height (labelled as MW/1; fig. 10) and three paralectotypes (labelled as MW/3).

This material had been deposited at the Museum of Natural History in Vienna, Austria and was supposedly studied by Frauenfeld. The locality "Gallia mér." was indicated on the original label of the lectotype and one of the paralectotypes (Boeters 1971, Boeters and Falkner 2000), leading Boeters (1971) to interpret it as the type locality of *M. confusa*. This author also extended the morphological description of *M. confusa* after dissecting specimens collected from Foux de Draguignan Spring in southern France (Boeters 1971). In the same publication, Boeters (1971) designated a lectotype for the species *Cyclostoma simile* Draparnaud, 1805. The specimen, the only shell from Draparnaud's collection that was deposited at the Museum of Natural History in Vienna, was assigned to this species by Frauenfeld (1863). This nomenclatural act was rejected by many taxonomists (Giusti 1976, Bodon 1995, Giusti et al. 1995) as the measurements of the shell illustrated by Boeters as the lectotype of *C. simile* (i.e., a shell 7 mm in height; figure 11 in Boeters, 1971) did not match with the one depicted in the original description (i.e., a shell of approximately 4.5 mm; pl. 1, fig. 15 in Draparnaud, 1805). Moreover, in a recent study, (Eschner et al. 2020) suggested that the lectotype designated by Boeter (1971) more likely corresponds to a species of *Bithynia* Leach, 1818 than to one of *Mercuria*. Recognising the discrepancies, Boeters and Falkner (2000) declared the lectotype of *C. simile* as invalid and assigned, as neotype of this species, the lectotype of *A. confusa* depicted by Boeters (1971: fig. 10), thus synonymising these two species under the name *Mercuria similis*. This nomenclature act was based on similarities in shell dimensions between the lectotype of *A. confusa* (Boeters, 1971: fig. 10) and the shell illustrated by Draparnaud in the original description of *C. simile* (Draparnaud, 1805: pl. 1, fig. 15).

This taxonomic paradigm led to various unresolved questions, such as the identity of the lectotype of *C. simile* designated by Boeters (1971) and the location of the shell type of *C. simile* depicted in the original description by Draparnaud (1805). Regarding the first uncertainty, Vinarski and Eschner (2016), based on the taxonomic work of Boeters and Falkner (2000), declared the taxonomic status of the lectotype of *C. simile* (i.e., fig. 11 in Boeters, 1971) as unknown; they also

provided a photograph (figure 4D) and a catalogue number (NHMW-MO 14715) for this specimen. To date, the lectotype of *A. confusa* designated by Boeters (1971; fig. 10), which is still held at the Museum of Natural History in Vienna, seems to have been accepted as a neotype of *Mercuria similis* under catalogue number NHMWMO 92596 (Eschner et al. 2020; figure 1T), although Boeters and Falkner (2017; figure 9A) provided a different catalogue number for the same specimen (i.e., NMW-FRD/1). Due to the imprecise type localities of *A. confusa* and *C. simile*, species delimitation and taxonomic assessments of these species based on an integrative taxonomic approach is almost impossible to perform. However, an intensive field survey of populations across southern France, followed by morphological and molecular studies of the collected specimens, can provide an estimation of the species richness of the genus in this area.

The coastal regions of France are a hotspot for *Mercuria* species. Relying mainly on museum materials, Boeters and Falkner (2017) reviewed the taxonomic status of the species of *Mercuria* occurring in this region and acknowledged that some of the species require additional studies. One example is the species currently recognised as *M. meridionalis*. This species, which was found in areas surrounding Nice and in the Maritime Alps, was originally described as a species of *Bithynia*. Boeters (1971) later synonymised it with *M. confusa* and Glöer et al. (2015b) subsequently considered as a valid species of *Mercuria*, which is its current taxonomic status. However, Boeters and Falkner (2017) highlight that *M. meridionalis* cannot be distinguished from *M. similis* by shell features alone.

Another species with an imprecise type locality and dubious type material is *M. anatina*. According to the original description, the type locality of the species is the surroundings of Paris [Circa Lutetiam?] and the mouth of the Somme River [Ostio fluminis Samarae] in northern France (Poiret 1801). However, Poiret was not convinced about the first location, which was a personal communication from Lamarck. Servain (1870) later cited the species in Spain and the Balearic Islands and in areas surrounding Salses-le-Château in southern France. Westerlund (1885) cited the species as having a much wider distribution: in southern France, Spain, the Balearic Islands, Corsica, Sicily, Italy, Malta and the Bahamian island of Andros). Clearly, his interpretation of this species included a wide range of snails that are, nowadays, consider as several other species. Later, Adam (1940) cited the species near Antwerp, Belgium and Kadolsky (2011), in the Arún River, 1 km northeast of Arudel, Sussex in the British Islands. Kadolsky (2011) considered the original type locality of the "surroundings of Paris" (Poiret 1801) as erroneous since there have been no subsequent reports of the species in or near that locality and suggested the specimen described by Poiret is a tertiary fossil. More recently, the species has been cited in the Netherlands, specifically in the province of Zuid

Holland, including in the Oude Mass River near Hoogvliet and in Spijkenisse, among other localities; and in Biesbosch National Park (Boeters and Falkner 2017). These authors also cited it in Anvers, Belgium.

As for many of the species of *Mercuria* described in the nineteenth century, the type material of *M. anatina* has either been lost or was in doubt. Kadolsky (2011) cited a specimen originally from Draparnaud's collection that was at the Museum of Natural History in Vienna, at least in 1820. However, Locard (1895) stated that this specimen had disappeared. Falkner et al. (2002) mentioned a specimen reported by Frauenfeld (1863), also held at the Museum of Natural History in Vienna; however, this specimen was never photographed or illustrated as it was a teratological specimen. In their characterisation of the species, Boeters and Falkner (2017) deemed the syntype (NWW-FRA) as questionable due to other material collected by Locard at the type locality (MNHN-LOC) and a shell collected from the Netherlands (BOE 0133).

Owing to all of these taxonomic misinterpretations, we hypothesise that *Mercuria* species are very similar conchologically and may even be cryptic. If this is the case, molecular tools are vital to clarify the taxonomic status of the different species. But, in this sense, what is a cryptic species? According to the literature, cryptic species are those taxa so closely related that they are frequently classified into a single nominal species due to a high level of morphological similarity (Sáez and Lozano 2005). Often cryptic species are uncovered through DNA sequence analyses and later confirmed by ethological, ecological and/or morphological data (Bickford et al. 2007, Pfenninger and Schwenk 2007). The species mentioned above may hide unknown diversity or, on the contrary, be less diverse than currently thought, leading to an overestimation of the species richness of the genus. For this reason, molecular techniques are needed to confirm synonyms or to detect potential hidden diversity, especially in widespread species such as *M. similis* (distributed over the Iberian Peninsula, Majorca, southern France and Tunisia) or *M. balearica*, originally described from Minorca (Balearic Archipelago) by Paladilhe (1869) and also registered in the province of Granada (Iberian Peninsula) by Boeters (1988).

In the case of *Mercuria* in the Iberian Peninsula, some authors have argued that the richness of the described species within the genus is lower than previously thought. Holyoak et al. (2017), for example, concluded that *M. edmundi*, a species described from three coastal localities in the western Iberian Peninsula (Boeters 1988), is a junior synonym of the geographically proximate *M. tachoensis*. Likewise, Girardi (2003) concluded that *M. similis* and *M. emiliana* are the same species. *Mercuria emiliana* had long been considered a widespread species of

Mercuria in the Iberian Peninsula and on the Balearic Island of Majorca (Boeters 1988) until its transfer to the genus *Pseudamnicola* by Boeters and Falkner (2017). These authors considered the type material of *A. emiliana* as lost and invalidly designated a specimen given from Folin to Pascal, labeled as "déterm. par Paladilhe", as the neotype (MNHN-IM-2000-32541) of a new combined species, *Pseudamnicola emilianus*. However, according to Breure and Audibert (2017), this neotype should be considered invalid as there is a syntype in Paladilhe's collection at the University of Montpellier (UM.PLD.003). Despite the taxonomic tangle, Boeters and Falkner (2017) acknowledged the taxonomic uncertainty of such a genus assignment. Adding to the chaos, the species *M. emiliana*, *M. similis* and *Pseudamnicola subproducta* (distributed acros the Iberian Peninsula and southern France) have been recovered as different taxa in several phylogenetic studies (Wilke et al. 2000a, Perez et al. 2005, Boulaassafer et al. 2018); however, the populations sequenced for these studies require taxonomic re-evaluation as they were collected from areas distant to the type localities.

Mercuria is also distributed in Northwest Africa, from Tunisia to Morocco through Algeria and in Macaronesia, on the island of Madeira (Glöer et al. 2015b). Though Wollaston (1878) mentioned the presence of *Pseudamnicola similis* in the Azores, this record has not been confirmed in modern times. To date, a total of 12 species have been described from Northwest Africa. Many of these descriptions, particularly those from the nineteenth century, are based mainly on conchological characteristics. Though the anatomy and the phylogenetic relationships of the species inhabiting Morocco are relatively well known (Boulaassafer et al. 2018), those of the species from Algeria and Tunisia have been scarcely studied. Moreover, taxonomic reviews of *Mercuria* in Tunisia or Algeria have been based only on old material found in museum collections (Glöer et al. 2010, Glöer et al. 2015b).

This complicated and entangled overview of *Mercuria* reveals that many of its species require taxonomic revision. Despite this, many recent newly described species (e.g., Glöer et al. 2010, Glöer et al. 2015a) have been erected in the same manner as during Draparnaud's time: on scarce shell measurements and, in some cases, no anatomical details at all or, in other cases, on geographic distance (assuming allopatric speciation for the genus). This adds, in most cases, more confusion than information to an already variegated mosaic.

Delimiting species using genetic and morphological data to build a "species hypothesis" in which thresholds to assign individuals to a species are determined is a practical approach to address the question of what is a cryptic species. All species delimitation methods rely on a theoretical frame of what a species is, that

is, a species concept (Schlick-Steiner et al. 2010). A plethora of species concepts (more than 20) have been developed in the last century. The most common concepts applied in taxonomy include the biological species concept (Mayr 2000), the Hennigian species concept (Hennig 1950), the evolutionary species concept (Simpson 1961), the ecological species concept (Van Valen 1976) and the phylogenetic species concept (Eldredge and Cracraft 1980, Cracraft 1983). The last one has been repeatedly used to define hydrobiid species (e.g., Delicado et al. 2012, Falniowski et al. 2016, Boulaassafer et al. 2018, Delicado et al. 2019). According to this concept, a species is understood as a set of populations with a common evolutionary origin that can be diagnosed by having a unique combination of morphological states.

The phylogenetic species concept has been accommodated within a novel approach developed this century: "integrative taxonomy" (Dayrat 2005). This term refers to a multisource (or multidisciplinary) approach that combines molecular, morphological, ecological and geographical data to more accurately delimit species (Padial et al. 2010, Schlick-Steiner et al. 2010, Yeates et al. 2011).

1.4.2 Fossil record

As aptly stated by Sagan (1980): "*You have to know the past to understand the present*". In this context, it is worth mentioning the extinct species of *Mercuria*. The known fossil record of *Mercuria* includes only four fossil species (Figure 6); however, some of the genus assignments are questionable due to convergence in shell shape (see section 1.1). The ages of these fossils range from the Oligocene (33.9 Mya) to the Miocene (11.62 Mya) (Sandberger 1858, Schlickum and Strauch 1979, Marquet and Lenaerts 2008, Esu and Girotti 2015a).

Differences between extant and extinct species of *Mercuria* are immediately noticable (compare Figures 5 and 6): none of the modern species have as pronounced a lip of a circular aperture as in *M. boetersi* (Figure 6A-B) or as small an aperture as in *M. helicella* (Figure 6E-H). These observations suggest that more detailed taxonomic studies should be carried out to clarify the taxonomic position of these fossils, as well as of several others discovered on the Iberian Peninsula. For instance, some imprecise fossil records assigned as *Pseudamnicola* sp./*Bithynia* sp. appear to be very similar to *Mercuria* in shell shape, suggesting the possibility of misclassification. Moreover, Adrover (1975) noticed the presence of *Bithynia* and *Pseudamnicola* on Upper Miocene bedrocks near the locality of Teruel, in the province of Aragon in northern Spain and Anadón et al. (2008) reported the presence of *Pseudamnicola* and *Hydrobia* W. Hartmann, 1821 on Pliocene lacustrine deposits from the La Rioja region, also in northern Spain.

More precise is the work of Sesé et al. (2004), who identified *M. confusa* in the flood plains of the Tagus River near the city of Toledo in central Spain; and De Marfà (2007), who suggested the presence of *M. emiliana* on lacustrine sediments from the Lower Pliocene in the province of Granada in southern Spain.

Considering these findings, we can only conclude that *Mercuria* has been present in the Iberian Peninsula for, at least, the last 5 Mya. Due to the scarcity of preserved material and the vagueness of original descriptions, which, in some cases, are clearly erroneous, the fossil record is not used as a calibration bound in the molecular clock model utilised in this thesis. Instead, a dating approach involving substitution rates is used, as it has been successfully applied to date phylogenies of other hydrobiid genera (see section 1.3).

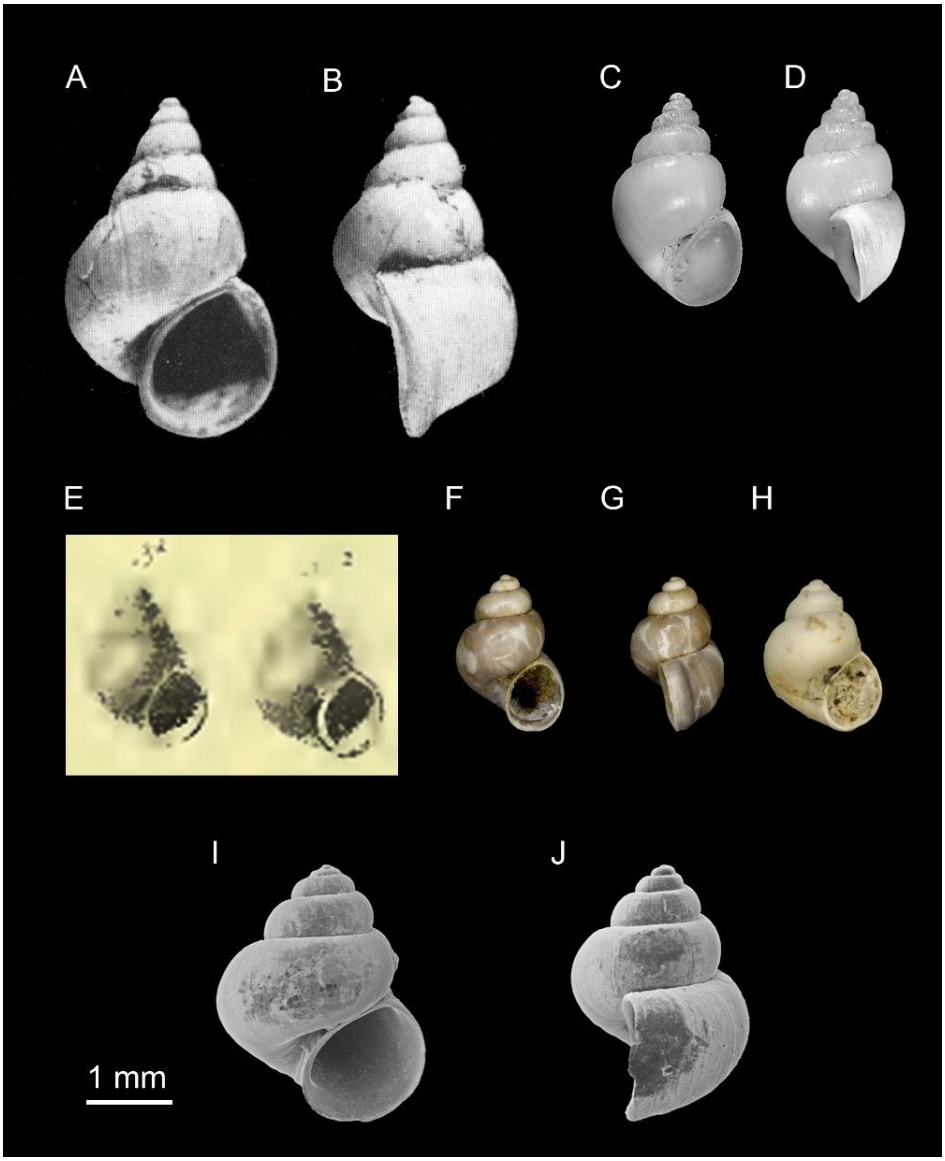
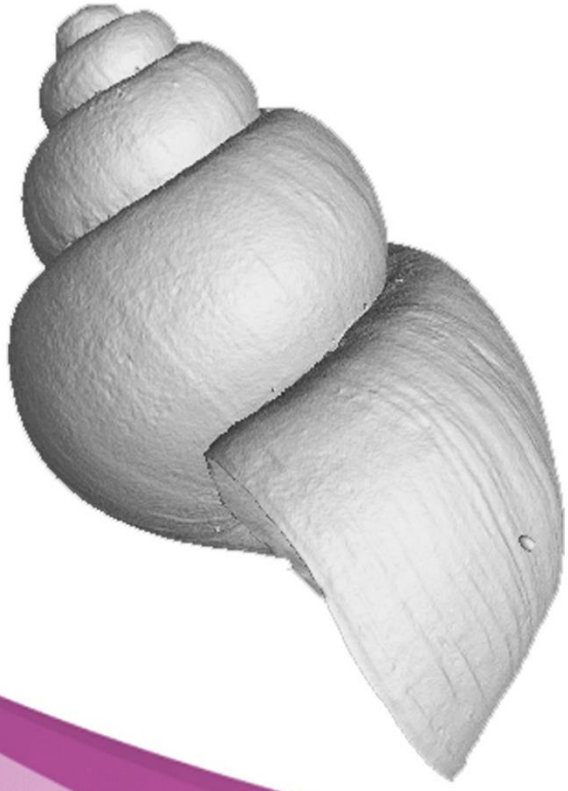


Figure 6. Shells of extinct species of *Mercuria*. **A** – **B**, *M. boetersi*, holotype, opencast mine of the Rhenish lignite mines, age: Pliocene (Schlickum and Strauch 1979); **C** – **D**, *M. baccinelliana*, holotype, Baccinello, Tuscany, Italy, age: Late Tornonian, Miocene (Esu and Girotti 2015a); **E**, *M. helicella*, drawing in the original description, Hackenheim, Rhenish, age: Tertiary (Sandberger 1858); **F** – **G**, *M. helicella*, MNHN.F.A42222, Chemin d'Orgemont, Île-de-France, France, age: Rupelian, Oligocene (Lozouet and Maestrati 2012); **H**, *M. helicella*, NMR3432, Vlaams Brabant, Hoogbutsel, Belgium, age: Late Priabonian – early Rupelian, Oligocene (https://www.nmr-pics.nl/); **I** – **J**, *M. vanparysi*, holotype NNM RGM 550-04, Maison Vleminckx, Vlaams Brabant, Belgium, age: Rupelian (Marquet and Lenaerts 2008).

HYPOTHESES AND OBJECTIVES



Despite the great richness of gastropod species in inland waters (around 4,000 species, Strong et al. 2008), there are few evolutionary studies that investigate the origin and distribution of this diversity. The Hydrobiidae family represents an excellent taxonomic model to carry out such studies due to its high species richness, its wide distribution and its adaptation to different continental habitats. Although there are numerous taxonomic works that describe new species of hydrobiids (Ramos et al. 2000, Arconada and Ramos 2001, Arconada and Ramos 2006, Arconada and Ramos 2007, Boeters and Glöer 2007, Erőss and Petró 2008, Georgiev 2013, Glöer and Pesic 2014, Glöer and Pešić 2015) an exhaustive review of the total number of species of hydrobiids and an explorative analysis on which evolutionary, geographic and climatic factors have determined the richness distribution of the family have not yet been carried out. Another important point to consider is that, although phylogenetic works exist for genera inhabiting mountain-top springs (e.g., (Hershler et al. 2003, Szarowska 2006, Delicado et al. 2013, Delicado et al. 2019), coastal streams (e.g., (Delicado et al. 2014)), lakes (e.g., (Wilke et al. 2007, Föller et al. 2015) or brackish environments (e.g., (Wilke et al. 2000, Vandendorpe et al. 2019), a comparative work integrating the evolutionary patterns (i.e., speciation times, rates of genetic substitution or biogeographic patterns) of hydrobiid groups occurring in different ecosystems and at different levels of elevation within the river continuum is still missing. Such a study will help to understand how the biota of these different habitats evolves. Therefore, the major goal of this doctoral thesis is to test for the ecological and geographical factors that may influence (i) the disparity of species richness among regions and taxonomic groups and (ii) the evolutionary patterns among hydrobiid genera. Previous knowledge on this gastropod family suggests the following hypothetic scenarios:

Hypothesis 1: Given their strong dependency to a nonmarine aquatic environment, abiotic factors such as elevation and latitude significantly influence the geographic distribution of the richness patterns of extant Hydrobiidae *s. str*. species.

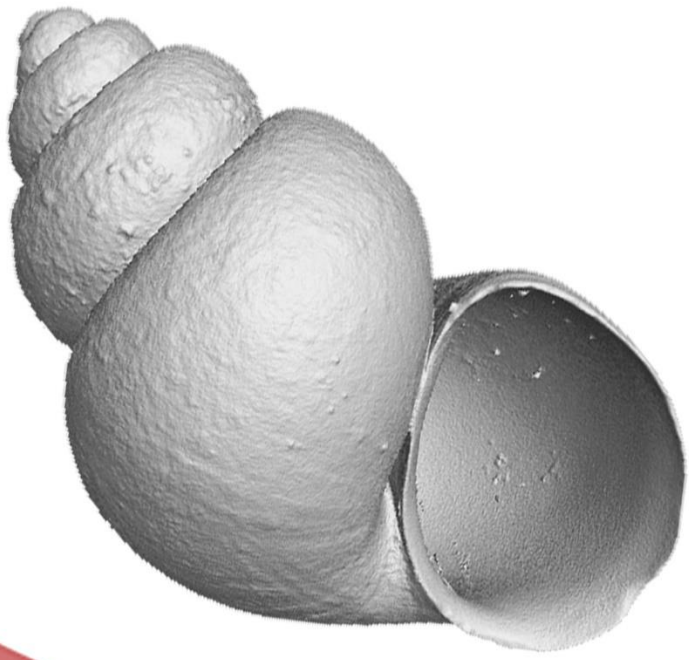
Hypothesis 2: As some populations of the genera *Mercuria* and *Pseudamnicola*, which are classified into different subfamilies, co-occur in the same Mediterranean habitats, a parallelism is expected in their phylogenetic patterns and biogeographic histories. This would reflect the importance of the habitat type and the elevation range where the species live in the evolution of the Hydrobiidae taxa.

Hypothesis 3: The elevation factor may influence the dispersal strategies and therefore the phylogenetic and biogeographic patterns, of the genera that inhabit headwater springs and those that live in lowland environments. This may cause differences in the number of lineages and speciation times, which translates into different rates of diversification and species richness.

The working hypotheses will be addressed through four specific objectives:

1. To analyse the influence of the geographical component on the diversification of this family. For this purpose, a bibliographic review of the species richness of the hydrobiid genera and their geographic distribution (elevation and latitude) will be carried out.
2. To compare the evolutionary patterns and species richness of the genera that cohabit in some Mediterranean coastal streams and other saline ecosystems (specifically between *Mercuria* and *Pseudamnicola*).
3. To assess the role of elevation in the dispersal modes and biogeographic patterns in four hydrobiid genera (i.e., *Mercuria*, *Pseudamnicola*, *Islamia* and *Corrosella*) that live in different elevational zones and present different species richness.
4. To contribute to the systematic knowledge and evolutionary history of the Hydrobiidae family through the systematic revision of poorly known genera such as *Mercuria*.

MATERIAL AND METHODS



2.1 Hotspots of Hydrobiidae *s. str.*

To identify putative hotspots of Hydrobiidae *s. str.* species diversity, a variety of biodiversity measures were analysed in this thesis. To obtain these measures, first, a global database of the geographical distribution of extant Hydrobiidae *s. str.* species was generated by conducting a comprehensive literature search for systematics and species distribution information in the original descriptions (see Appendix 1). Taxonomic incongruences and duplicated information caused by synonyms were avoided by cross-referencing several renowned biodiversity websites including the International Union for Conservation of Nature (IUCN 2018), the Pan-European Species Directories Infrastructure, PESI (de Jong et al. 2015), Fauna Europaea (Bank 2016), the Integrated Taxonomic Information System, ITIS (System 2010), the World Register of Marine Species, WORMS (Horton et al. 2017), MolluscaBase (MolluscaBase 2020) and AnimalBase (AnimalBase 2016). Owing to the lack of detailed range maps for many species and precision of some of the type localities, the number of hydrobiid species per freshwater ecoregion (Abell et al. 2008) was used to quantify species richness. These ecoregions represent mainly drainage basins and were delineated based on freshwater vertebrate assemblages. Moreover, they were previously used as biogeographical units to calculate species richness and to create conservation plans for numerous freshwater invertebrate groups (Perez-Losada et al. 2009).

A list of species was compiled and the following information was registered: type locality coordinates, presence in ecoregions, endemism and conservation status. Endemic species were identified as those occupying a single ecoregion. The conservation status of each species was assigned as one of the following IUCN Red List categories (IUCN, 2018): Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT), Least Concern (LC) and Data Deficient (DD). Species not yet assessed by the IUCN were assigned as data deficient.

Biodiversity hotspots have been identified based on different operational criteria, for instance, considering the top 2.5% of grid cells or 5% of the total land area (Orme et al. 2005, Huang et al. 2016) of highest species richness. These thresholds may not be suitable for a low number of geographic units comprised of large areas, such as the freshwater ecoregions in this study. Therefore, hotspots were defined as the richest 25% of ecoregions in terms of species richness, endemics and threatened species (i.e., those in the categories CR, EN and VU) following Abell et al. (2011), who used the same geographic units as in this study. Additional scenarios (top 5% and 10%) were applied to assess the efficiency of the selection. Hydrobiid species richness, endemics, threatened species and hotspots were mapped in ArcGIS 10.4 (ESRI 2011).

The robustness of the hotspot identification was evaluated against species misidentification and erroneous occurrence reports by randomly re-assigning 50% of the species of an ecoregion to other species and repeating the hotspot selection. A completely random species assignment is an unrealistic scenario because typically the regional species pool is considered by taxonomists and ecologists. Therefore, species were sampled from the same biogeographic realm with a probability inversely proportional to the geographic distance to the focal ecoregion. The simulation was run 1,000 times in the R 3.3.2 statistical environment (Core-Team 2017) and the distances among ecoregions were calculated using the geosphere 1.5-5 package (Hijmans 2016).

2.1.1 Geographic and climatic data

The effect of 13 geographic and climatic predictors (see Appendix 2) on hydrobiid species richness was assessed. These variables constitute proxies for different geographical, ecological and evolutionary processes affecting the distribution of species richness.

Several of these variables may explain variation in hydrobiid species richness at the global scale. Utilising the freshwater ecoregion maps published by Abell et al. (2008), the latitude and longitude of each region were obtained using the coordinates of their centroids by applying the functions Feature to Point and Calculate Geometry in ArcGIS. Only the absolute values of latitude were used as a predictor of hydrobiid richness, whereas both measures were needed to evaluate the robustness of our statistical analyses (see below). Ecoregion area was calculated using the geosphere package. Latitude and area are not direct drivers of species richness but often are co-correlated with numerous ecological factors, such as primary productivity and habitat diversity, respectively. Based on the digital elevation model GMTED2010 with a spatial resolution of 30 s, the mean elevation of each ecoregion and its elevational range were calculated using 3D Analyst tools in ArcGIS. Bioclimatic variables were extracted following the same method but using the digital raster model from the WorldClim database (Hijmans et al. 2006) with a spatial resolution of 30 s for the variables BIO1 = Annual Mean Temperature, BIO7 = Annual Temperature Range, BIO12 = Annual Precipitation and BIO15 = Precipitation Seasonality. The annual mean temperature of the Last Glacial Maximum according to the CCSM4 climatic projection was sourced from the same database with a spatial resolution of 2.5 min.

Proxies for the influence of evolutionary and dispersal processes included the following smaller-scale factors: connectivity among ecoregions, peninsula effect, biogeographic affiliation (Udvardy 1975, Holt et al. 2013) and habitat

heterogeneity. As a measure of connectivity, the number of neighbours for each ecoregion was obtained using the rgdal 1.2-4 package for R (Bivand et al. 2014). The peninsula effect was coded by zero (for ecoregions not on any peninsula) and one (for ecoregions on a peninsula). As a proxy for habitat heterogeneity, the number of geological entities per ecoregion was estimated from a lithological map of the world gridded to 0.5° spatial resolution (Hartmann and Moosdorf 2012) using the Join and Statistic functions in ArcGIS. Because the number of geological units increases with area, this variable was standardised by ecoregion size. The matrix used for the analyses with all the data is available in the supplementary material section (see Appendix 3).

2.1.2 Statistical analysis

An ordinal regression model was used to evaluate the influence of elevation on extinction risk. The IUCN Red List category served as the ordered response and the linear, quadratic and cubic terms of elevation as the predictor. Because the position of the data deficient category within the ordered sequence of extinction risk is not defined, those species were excluded. Given the narrow range of most hydrobiid species, the elevation of each type locality was used as an indicator of elevational range (Appendix 3). The regression model was fitted using a vector generalised linear model with the proportional odds family implemented in the VGAM 1.0-4 package for R (Yee 2017). The model was selected with the lowest Akaike Information Criterion (AIC) (Akaike 1974). Using the likelihood ratio test, the proportional odds assumption was tested. This test evaluates whether individual risk categories differ in their response to elevation, such as whether the proportion of one category increases with elevation as another one decreases.

Regression analysis of species richness was performed as described by Zuur et al. (2010). In order to standardise and compare regression coefficients of individual predictors, all variables were centred to zero and scaled to one standard deviation. Potential outliers were detected by boxplots of each variable in R. Strong collinearity among predictors may affect the estimation of the regression parameters. Latitude, mean annual temperature and temperature of the Last Glacial Maximum were correlated with a Pearson's correlation coefficient > |0.7| (Dormann et al. 2013). Therefore, the latter two predictors were excluded from the models. The effect of the selected variables on species richness was tested using a generalised linear model (GLM). Preliminary analyses using a Poisson error distribution, which is typically recommended for count data (Cameron and Trivedi 2013), indicated a problem with overdispersion, resulting from the variance in hydrobiid richness exceeding its mean and from biased regression models. Therefore, the GLM of the MASS 7.3-45 package for R (Venables and

Ripley 2002) was utilised with a negative binomial distribution of model errors. Following Zuur et al. (2010), potential violations of GLM assumptions were assessed by inspecting the QQ-plots.

Using stepwise selection of predictors, the model with the lowest AIC was identified. In addition to excluding correlated predictors prior to model fitting, the car 2.1-3 package for R (Fox et al. 2010) was used to calculate the generalised variance inflation factor, which indicates remaining multicollinearity by values greater than 10 (Quinn and Keough 2002). However, in this case, all values were well below this threshold.

A lack of independence between observations can produce potential biases in estimated regression coefficients (e.g., Dormann et al. 2007, Zuur et al. 2009). Therefore, model residuals were tested for spatial autocorrelation using Moran's I statistic (Moran 1950), ranging from –1 to +1. The correlog function of the pgirmess 1.6.5 package for R (Giraudoux 2013) indicated low but significant spatial autocorrelation for the first distance class ($I_{780km} = 0.2$ $p < 0.001$), after applying the progressive Bonferroni correction (Hewitt et al. 1997). Only a few methods to account for spatial autocorrelation can handle count data (reviewed in Dormann et al. 2007); however, none of these classical methods could remove the spatial autocorrelation observed here, likely due to it being lower than that reported by Dormann et al. (2007). Therefore, the residuals autocovariate (RAC) GLM was used. In simulation studies, this model revealed unbiased regression coefficients (Crase et al. 2012). It is a regression with all previously identified predictors plus the RAC. In this case, the RAC is, for each ecoregion, the sum of the non-spatial GLM residuals weighted by the inverse squared distance to the focal ecoregion. The residuals of this regression analysis showed no spatial autocorrelation. A second AIC-based model selection identified the predictors of hydrobiid species richness that were previously only selected because of unaccounted spatial autocorrelation.

2.2 Systematic of the genus *Mercuria*

2.2.1 Material examined and collecting methods

Systematic studies, as in other fields, rely on the validity of the results, which among other things, depends on the sampling methods used. In order to cover most of the geographical range of *Mercuria* (see Introduction), specimens were collected from 129 localities (Figure 7), ranging from the westernmost (e.g., Madeira Island and Portugal) to the easternmost (e.g., Tunisia and Italy) and the northernmost (e.g., United Kingdom and Netherlands) to the southernmost (Morocco) regions of its distribution. Fieldwork was conducted from March 2017

to October 2018. However, resources and efforts were focused mainly on populations within the Ibero-Balearic region and points cited in the bibliography as type locations. The main reasons for such a sampling design were the high number of *Mercuria* populations reported in the literature for this territory, taxonomic incongruences, habitat loss and the limited time of a Ph.D. thesis.

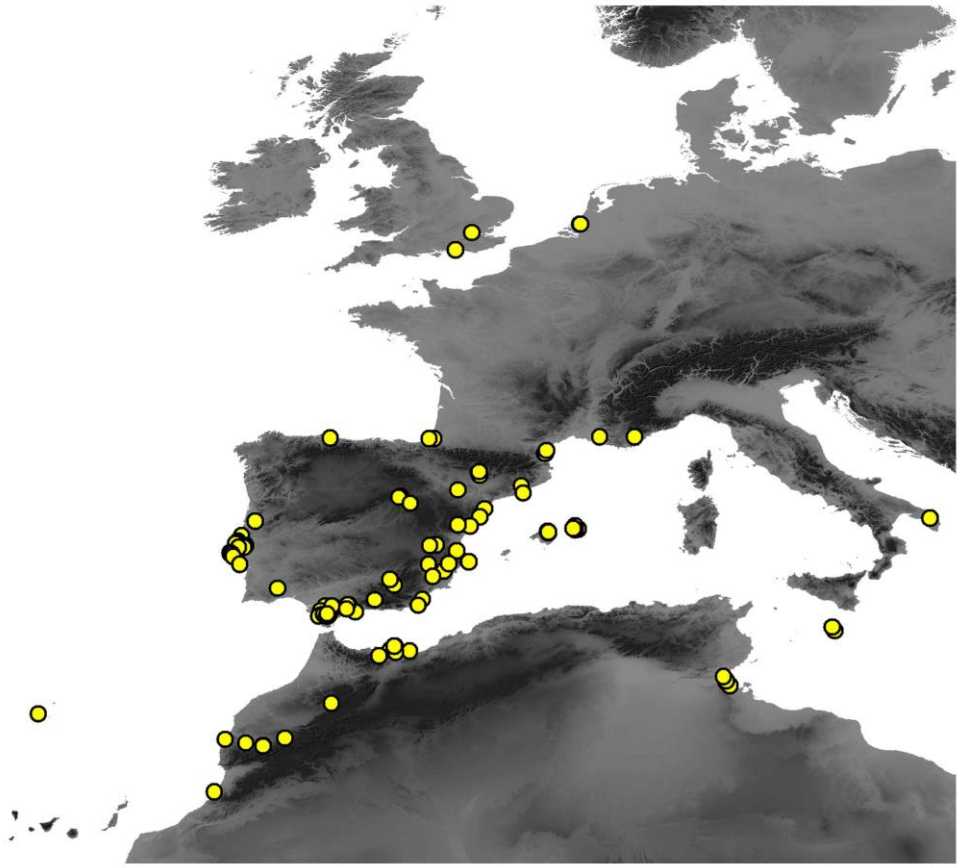


Figure 7. Distribution map of the studied populations of *Mercuria*.

One relevant aspect to consider in relation to the distribution of the *Mercuria* species is their wide range of habitat preferences. The types of water bodies inhabited by this genus are mainly springs, ditches, ullals (eyes in Catalan language, refers to an underwater source, especially in wetland areas), streams, ponds and even river sections, all with permanent water and a slow flow, which help clean and oxygenate the water (Figure 8). However, in many cases, *Mercuria* snails have also been found in environments associated with a high degree of salinity caused by Keuper facies. These facies comprise Upper Triassic sediments that contain gypsiferous mudstones facies, changing to alluvial fans and playa

deposits (Sopeña et al. 1988). Following this criterion, new putative localities in Spain focused on places with outcropped Keuper facies or those exposed on the continental territory. Usually, these types of places correspond to ancient salt mines. Therefore, another criterion for potential areas to explore was their proximity to salt mines or to those with a toponym referencing salt including rivers, springs, or other water bodies and even streets in villages.

To determine the salt tolerance of the different species, water conductivity was used as a proxy as it measures the water's ability to pass electrical flow and is directly related to the concentration of ions in the water, whereas salinity in its most basic definition only indicates the total mass of salts in the water. Conductivity and pH were measured *in situ* using a HANNA HI 9033 multi-range conductivity meter and a HANNA HI9025 pH meter, respectively. Conductivity measurements were taken after first stabilising the meter for 5 min in the water.

Collection techniques varied according to the substrate where the snails were found. *Mercuria* is thought to inhabit mainly coastal streams, although in many cases, snails were found outside the water (which may be correlated with amphibian activity), either on the base of *Phragmites* spp., *Typha* spp., *Juncus* spp., or other shoreline vegetation or covered by a mud layer, embedded in small cavities at the margins of the water bodies. In these cases, the specimens were collected by hand. A set of three sieves (with mesh sizes of 4 mm, 2 mm and 0.125 mm) was used to collect animals living underwater in springs or streams. Material was collected from the surface bottom and rinsed with abundant water. The specimens were collected from the sieve with the smallest mesh.

All data from each locality were registered *in situ* using the Epicollect5 application (Aanensen et al. 2009), which records the coordinates of each point. However, the Magellan eXplorist 200 GPS was preferred as it presents coordinates as decimal degrees. Once the samples were assigned a unique lot number, the sample and associated coordinate data were added to a database. Information on the localities, collection dates and collectors is provided in Appendix 4.

2.2.2 Material preparation

In the laboratory, live specimens were stored at 4ºC to slow down the snails' metabolism and to avoid death due to oxygen deficiency in the water. Once ready to be processed, samples were warmed up to room temperature (approx. 25ºC) and checked for activity. Then, menthol crystals were added to the water to maximally relax the animals. After achieving relaxation but prior to fixation, a brief thermal shock was applied by placing the animals into a strainer and dipping

them into a water bath previously heated to 60-70ºC for five seconds (Araujo et al. 1995, Ramos et al. 2000b).

Table 1. List of all the collectors who provided the samples included in this work and their associated abbreviations. Appendix 4 details which samples and localities are associated with each collector.

Collector	Abbr.	Collector	Abbr.
Jordi Corbella Alonso	JC	*Rui M. da Costa Mendes*	RM
Francisco de Erit Vázquez Toro	FVT	Dietrich Kadolsky	DK
Javier Ripoll Rodríguez	JR	Geraldine A. Holyoak	GH
Juan Sebastián Torres Alba	STA	Diego Moreno Lampreave	DM
Amanda Aguado Bachiller	AAB	José Manuel Amarillo Vargas	JMA
Miguel Carrillo Pacheco	MCP	Torsten Hauffe	TH
Félix Ríos Jiménez	FRJ	Diana Delicado Iglesias	DD
Fernando García-Guerrero	FGG	Khadija Boulaassafer	KB
Gerson Cárdenas García	GCG	Mohamed Ghamizi	MG
Antonio José García Meseguer	AGM	Younes Mabrouki	YM
Luis Murillo Guillén	LM	Afef Brahmi	AM
Cristobal Rubio Millán	CR	Mustapha Bejaoui	MB
Marian Ramos Sanchez	MR	Noureddine Khalloufi	NK
Sergio Quiñonero Salgado	SQ	Alberto Sánchez Vila	AS
Martin Willing	MW	Ramón Álvarez Halcón	RAH
Josep Quintana Cardona	JQ	Agustín López Pla	ALP

Institution	Abbr.	Institution	Abbr.
Museo Nacional de Ciencias Naturales (CSIC)	MNCN / FW	Museu Zoologico Barcelona	MZB
Université de Montpellier	UM	University of Giessen Systematics and Biodiversity Collection	UGSB

Specimens were then fixed according to their final purpose. For molecular studies, they were either frozen at -80ºC or preserved in absolute ethanol and kept at - 20ºC. For anatomical studies, they were fixed in 70% ethanol and stored at room

temperature. For histological studies, the animals were fixed using Bouin's solution (saturated picric acid, formaldehyde and acetic acid solution), then dehydrated through a graded ethanol series (40%-70%) and finally stored in 70% ethanol at 4ºC.

For dissection of the soft bodies, the shells of the specimens selected for anatomical studies were removed using a decalcifying 5% aqueous solution of Ethylenediaminetetraacetic acid (EDTA) (Ramos et al. 2000b).

All of the collected material, after its study and proper labelling, will be deposited into the Malacology and the Tissues-DNA collections at the National Museum of Natural Science (MNCN-CSIC), Madrid, Spain.

2.2.3 Molecular and phylogenetic studies

DNA isolation, amplification and sequencing

A total of 79 *Mercuria* populations was genetically analysed in this study. From each population, 2-3 specimens were DNA amplified and sequenced. Genomic DNA was extracted from a small piece of the foot tissue using the DNeasy Blood and Tissue kit (QIAGEN, Hilden, Germany). Fragments of two mitochondrial genes, cytochrome *c* oxidase subunit I (COI) and 16S rRNA (16S) and one nuclear gene, 28S rRNA (28S), were amplified with the primer pairs indicated in Table 2. These fragments have been successfully used to detect genetic differences between not only hydrobiid species (Delicado et al. 2013, Delicado et al. 2015, Delicado et al. 2019) but also *Mercuria* species (Boulaassafer et al. 2018). Moreover, they provided robust supports in phylogenetic analyses (e.g., Delicado et al. 2019).

Each PCR tube contained 1-5 µl of DNA, 2.5 µl of 10x Buffer, 0.25 µl of $MgCl_2$ (25mM solution), 0.6 µl of dNTPs mix, 0.6 of each primer (10 mM), 0.4 µl of Taq DNA polymerase (5 U/µl – Takara) and 17 µl of purified distilled water.

The following PCR conditions were used: an initial denaturation step at 94ºC for 4 min, followed by 35 cycles at 94ºC for 45 s, XºC (48ºC for COI; 50ºC for 16S and 51ºC for 28S) for 45 s and 72ºC for 45 s. A final extension was performed at 72ºC for 10 min. To determine the quantity of PCR product in each reaction, 1 µl was run on a 1% agarose gel, then PCR products were visualised under UV light with SYBR Safe (Invitrogen, USA). From those samples presenting a bright band, 10 µl of the PCR product were cleaned using 5 µl of EXOSAP (Thermo Fisher Scientific, USA), diluted 1/10 in distilled water. The reaction was then equally aliquoted into two tubes and 5 µl of either the forward or reverse primer (5 mM) were added to each tube and sent to be sequenced by Macrogen Spain (Madrid).

Phylogenetic analysis and molecular clock approach

The obtained sequences of 209 *Mercuria* specimens were edited using SEQUENCHER v.5.4.6 (Gene Codes Corp., Ann Arbor, MI, USA). *Pyrgulopsis californiensis* (GenBank accession numbers: COI, AF367651; 16S, MG697201; and 28S, MG697212) and *Pseudamnicola lucensis* (accession numbers: COI, AY627924; 16S, AY627981; and 28S, MG697212) were used as outgroups.

The protein-coding gene COI was aligned manually using MEGA v.7.0.14 (Kumar et al. 2016). The 16S and 28S sequences were aligned using MAFFT v.7.312 (Katoh and Standley 2013), with default settings [gap opening penalty (GOP) = 1.53]. Sequence divergences (uncorrected *p*-distances) were calculated in MEGA.

Table 2. Primers used to amplify mitochondrial (COI and 16S) and nuclear (28S) DNA fragments. Nucleotide sequences and references are also provided.

Primers	Direction	Sequence	Reference
16Sar-L	Forward	5'- CGACTGTTTAWCAAAAACAT -3'	Modified from Palumbi (1991) by Delicado et al. (2019)
16Sbr-H	Reverse	5'- CCGGTCTGAACTCAGATCA -3'	
LCO1490	Forward	5'- GGTCAACAAATCATAAAGATATTG -3'	Folmer et al. (1994)
COR722b	Reverse	5'- TAAACTTCAGGGTGACCAAAAAATYA -3'	Wilke and Davis (2000)
28S F63	Forward	5'- ACCCGCTGAAYTTAAGCATA -3'	Park and Ó Foighil (2000). Modified by Benke et al. (2009)
28S LSU3	Reverse	5'- TCCTGAGGGAAACTTCGG -3'	

Figure 8. Different habitats where *Mercuria* species can be found: springs (**A** – Nascente Senhor Jordão, Alpedriz, Portugal. **B** – Fonte das Mouras, Alpedriz, Portugal. **C** – Fuente Tebia, Camoca, Asturias, Spain. **D** – Buddle in La Palme, Provence-Alpes-Côte d'Azur, France. **E** – Fonte dos Amores, Coimbra, Portugal; streams (**F** – Marsh in Peñíscola, Spain. **G** – Las Negras ravine, Almeria, Spain. **H** – Arroyo Salado, stream in Guadalajara, Spain); ponds (**I** – Galayo Pond, Albacete, Spain).

Phylogenetic relationships of *Mercuria* species were assessed based on maximum likelihood (ML) and Bayesian Inference (BI) approaches. Prior to these analyses, each gene partition was analysed using jModelTest 2.1.6 (Darriba et al. 2012) in order to obtain the best-fit model of nucleotide evolution under the corrected Akaike's information criterion (AICc) (Akaike 1974). The selected substitution models were HKY (Hasegawa et al. 1985) + Γ (rate variation among sites) for COI; K80 (Kimura 1980) + Γ for 16S and TrN (Tamura and Nei 1993) + I (invariable sites) + Γ for 28S.

The Cyber Infrastructure for Phylogenetic Research–CIPRES (Miller et al. 2010) computer cluster was used to run the jModelTest and phylogenetic analyses of individual and combined datasets. The ML analyses were computed using RAxML-HCP v.8 (Stamatakis 2014) using the GTR (Tavaré 1986) + I + Γ model for each partition. The robustness of the analyses was assessed by non-parametric bootstrapping. The program halted bootstrapping automatically under the majority-rule criterion.

The BI analysis was conducted using Markov chain Monte Carlo (MCMC) sampling in MrBayes v.3.2.7a (Ronquist et al. 2012) for 20 x 10^6 generations, four parallel chains, sampling a tree every 1000 generations and discarding the first 10% of the sampled trees as burn-in. Chain convergence was monitored by ensuring a standard deviation of the split frequency < 0.01. The robustness of the inferred tree was measured using Bayesian posterior probabilities (BPPs). The tree was edited and the branch supports displayed, using FigTree v.1.4.3 (Rambaut 2012).

In order to proceed with the species delimitation test, an ultrametric tree was first inferred using BEAST v.1.8.4 (Drummond et al. 2012). Clock models and trees were linked while the model for each partition was set according to the jModelTest results. The analysis was conducted in relative divergence time (i.e., all branches evolving with a rate of one substitution per site per unit of time) and the prior for the tree was set to the Yule speciation process (Yule 1925). The MCMC sampling was run with 100 x 10^6 generations, saving a tree every 2000 iterations and discarding the first 10% as burn-in. Stationarity in the posterior distribution was ensured by having an effective sample size (ESSs) above 200, as calculated in Tracer v.1.6 (Rambaut et al. 2013). The maximum clade credibility (MCC) tree was identified from all the sampled trees using TreeAnnotator v.1.8.4. Subsequently, all zero branch lengths were removed using R.

Molecular species delimitation methods

Traditionally, conchological features have been used to delimit species within the Hydrobiidae family and particularly within the genus *Mercuria*. However, due to the high degree of similarities among morphotypes, sometimes species identification or delimitation can be a taxonomic puzzle. For these reasons and to confirm the *Mercuria* species described on the basis of morphology, three molecular species delimitation methods were used that are based on genetic distance, Bayesian and maximum likelihood approaches, respectively. These methods have proven to be useful for the delimitation of molluscan species, including hydrobiids, as the molecular-based results are often congruent with those based on morphology (e.g., Padula et al. 2016, Araujo et al. 2017, Delicado et al. 2019, Strong and Whelan 2019).

The distance-based automatic gap discovery (ABGD) method (Puillandre et al. 2012) based on the detection of a barcode gap between inter- and intraspecific variability was used as a threshold to delimitate species. The COI matrix was uploaded to the ABGD server (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) and a Kimura 2-parameter (Kimura 1980) distance matrix was generated with the parameters of an intraspecific divergence between 0.001 and 0.1 and a relative gap width of 1.0.

The second species delimitation algorithm was the Poisson Tree Processes (PTP) method (Zhang et al. 2013), developed under a Bayesian approach (bPTP), as implemented on the bPTP web server (https://species.h-its.org/ptp/). This method defines species based on the substitutions between and within species independent of time. We used as input tree, in Newick format, the one obtained from MrBayes (i.e., the BI tree based on the combined dataset) collapsing identical haplotypes into a single tip using R package APE (Paradis et al. 2004). The analysis was set to 100,000 generations; default settings were used for the remaining parameters. High Bayesian support (BP) values indicate that all descendants from a node are more likely to be the same species.

Another PTP method used here was the multi-rate PTP (mPTP; Kapli et al. 2017) which incorporates different rates of coalescence within clades and determines the number of species that fits best to the given data by means of Akaike Information Criterion. Because mPTP requires as input file a non-ultrametric and bifurcated tree, we used the best topology inferred by the RAxML analysis after pruning identical haplotypes (done as in the bPTP method). This method was implemented on mPTP (Kapli et al. 2017). The analysis was set to 1,000,000 generations, saving a tree every 10000 iterations and discarding the first 10% as burn-in.

Finally, the single-threshold generalised mixed Yule-coalescent method (ST-GMYC) (Pons et al. 2006), defines either coalescent or speciation processes of ultrametric trees (i.e., calibrated based on the molecular clock approach) by detecting a time threshold on their branching patterns. To do this, the MCC tree previously obtained by BEAST (see the previous section) with unique haplotypes pruned to single tips was used as the input ultrametric tree and the analysis was implemented on the GMYC web server (https://species.h-its.org/gmyc/).

To assess the performance of these species delimitation methods, we calculated the match ratio as in Ahrens et al. (2016), Blair and Bryson (2017) and Hofmann et al. (2019) according to the following formula:

$$Match\ ratio = 2\ x\ \frac{Nmatch}{(Ndelimited + Nmorph)}$$

Where *Nmatch* refers to the number of species delimited that exactly match a defined morphospecies, *Ndelimited* is the total number of species delimited by the method and *Nmorph* refers to the total number of morphospecies. Lower values of match ratio indicate an overestimation of the species delimitation method.

2.2.4 Preparation of specimens for morphological and anatomical studies

Anatomical and morphological characteristics of the phylogenetic clades and potential species obtained through molecular methods were examined next. A total of three to five specimens of each sex (all adults) from the type locality of each species were dissected for the analyses. For the remaining localities, one or two specimens of each sex were dissected to confirm species assignments and to study variability among populations.

For the anatomical studies, specimens were dissected in distilled water in a Petri dish containing a black layer of paraffin, wax and coal (Davis 1967). With the exception of the nervous system, the soft body parts lack pigment. Therefore, the soft body was first immersed in an eosin solution (the solution was prepared using 0.5 ml distilled water + eosin until saturated at room temperature and then diluted to 10%) to stain the reproductive and digestive systems, thus facilitating their dissection. The head of the animal was immersed in Bouin's solution (Galigher and Kozloff 1971) for one minute and then placed in a Petri dish with distilled water to extract the nervous system and the buccal bulb, from which the radula was later extracted.

All images and measurements were taken under a Leica MZ16 stereomicroscope mounted with a Leica DFC550 camera and managed through the software Leica Application Suite (LAS) v4.6. This software was also used to image samples along

the Z axis. The anatomical description of each species is summarised through individual and composite images.

2.2.5 Anatomical and morphological features

Due to the small size of the snails and the complexity of anatomical studies, many authors based their descriptions only on shell features, which can lead to synonyms and wrong combinations. Furthermore, the plethora of terminology used in the study of this group often leads to misunderstandings of the morphological terms, making it difficult for comparisons. To standardise the terminology used in this thesis, the one proposed by Hershler and Ponder (1998) has been applied for most of the anatomical characters, except for those pertaining to the nervous system for which the nomenclature of Davis et al. (1976) has been adopted.

To study the radular teeth, the extracted buccal mass tissue was first dissolved with a 2.5 % solution of sodium hypochlorite (NaClO). The process was carefully monitored under the stereomicroscope and once only the radula remained, it was transferred to a new Petri dish and rinsed with distilled water. To remove the periostracum, shells were immersed in the hypochlorite solution for 5-8 min and then placed in an ultrasonic cleaner for 3-5 s. Uncoated radulas and shells were mounted on stubs and imaged on a FEI INSPECT (FEI Company, Netherlands) environmental scanning electron microscope (ESEM) at low vacuum.

In order to study the details of the columella and inner shell surface, one or two specimens of each species, as well as holotypes, were scanned using a CT-SCAN Nikon XT H-160. The results, a digital 3D model of each specimen, were exported in TIFF format and subsequently imported into and visualised using myVGL v.3.2.5 (Volume Graphics, GmbH, Germany).

The morphological and anatomical characters (and states) examined in this thesis are as described below:

Shell

The shell of hydrobiids is divided into two parts, the protoconch, or embryonic shell and the teleoconch. The protoconch consists of one or several whorls without growth lines. It also presents multiple microsculptural patterns, which are considered to provide relevant taxonomical characters that can be used to delimit and identify species. The teleoconch has a different type of microsculpture (usually axial) and its features are often and primarily used to recognise species (Hershler and Ponder 1998). Shell ornamentations are rare in hydrobiids, although

species living in lakes such as the ancient Lake Ohrid (see Radoman, 1983) have ribs or spines on the teleoconch.

The shell of *Mercuria* species is ovate-conical, small to medium sized (Figure 9), 2-6 mm long, with rounded whorls, deep sutures and a narrow perforate umbilicus. The shell usually has a transparent milky colour (Boeters and Falkner 2017). The colour of the periostracum, which can vary among populations and species, ranges from greyish or yellowish colour to dark brown.

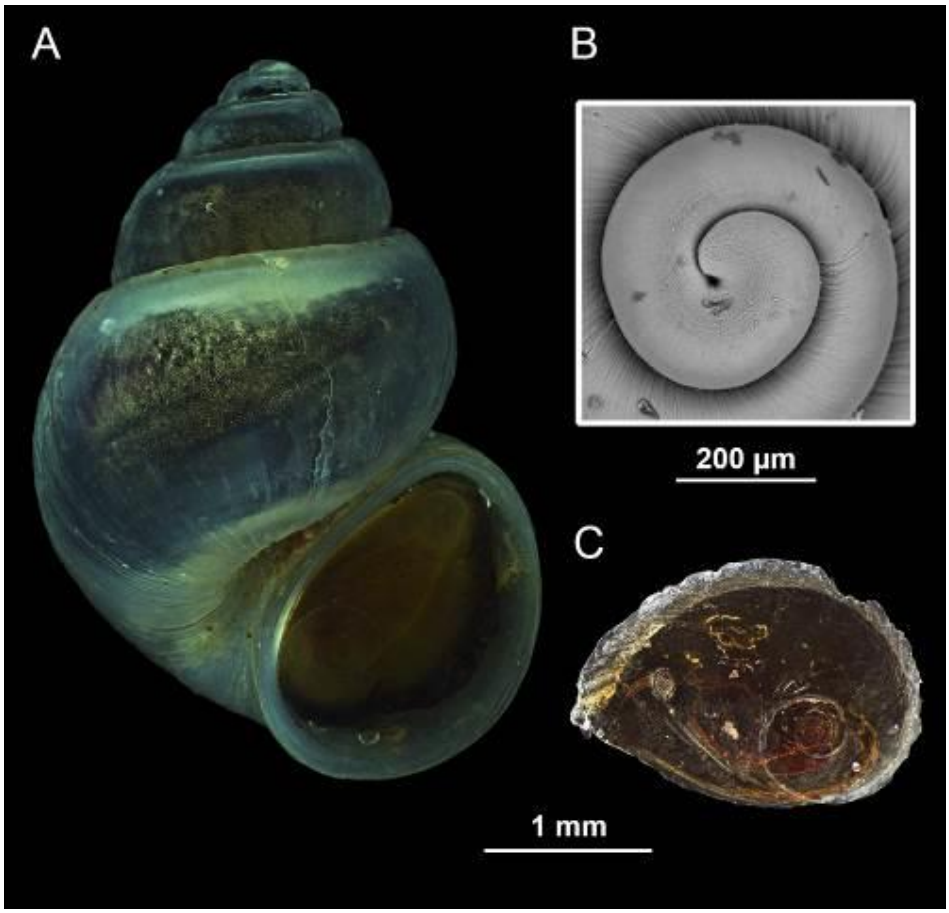


Figure 9. Shell of *Mercuria balearica*. **A** – teleoconch; **B** – protoconch; **C** – operculum.

Operculum

The operculum of all hydrobiids *s. str.* is composed of a corneous, or horny, substance. Depending on the shell aperture, the opercular shape is either recognised as ovate, circular, or elongate-ellipsoidal. Its colour is likely related to its thickness, with thicker opercula seemingly darker. The growth pattern of the

operculum depends on how new material is added, with the vast majority of hydrobiids showing a paucispiral pattern.

In *Mercuria,* the operculum is ovate or elongate-ellipsoidal in shape (Figure 9C), paucispiral (with few whorls) with a submarginal nucleus and reddish in colour. On the inner side of the operculum, rather than a peg, a small callous is observed where the pedal muscle is anchored. On the outer side, very weak axial growth lines are observed (Boeters and Falkner 2017).

Head-Foot

Hydrobiids, in general, have a well-developed head and foot region. The foot is generally broad and has a prominent snout with distal lips. Cephalic tentacles arise from both sides of the head and have a small eyespot within the eye lobe (a small prominence at the base of each tentacle). The foot, which is often thin, contains a mucous gland that discharges into a transverse anterior slit (Hershler and Ponder 1998). Epigean hydrobiids often present dark melanic pigmentation on the head, foot and cephalic tentacles (Hershler and Ponder 1998), while some hypogean species lack pigmentation and even eyespots (see Arconada and Ramos 2006, Delicado 2018).

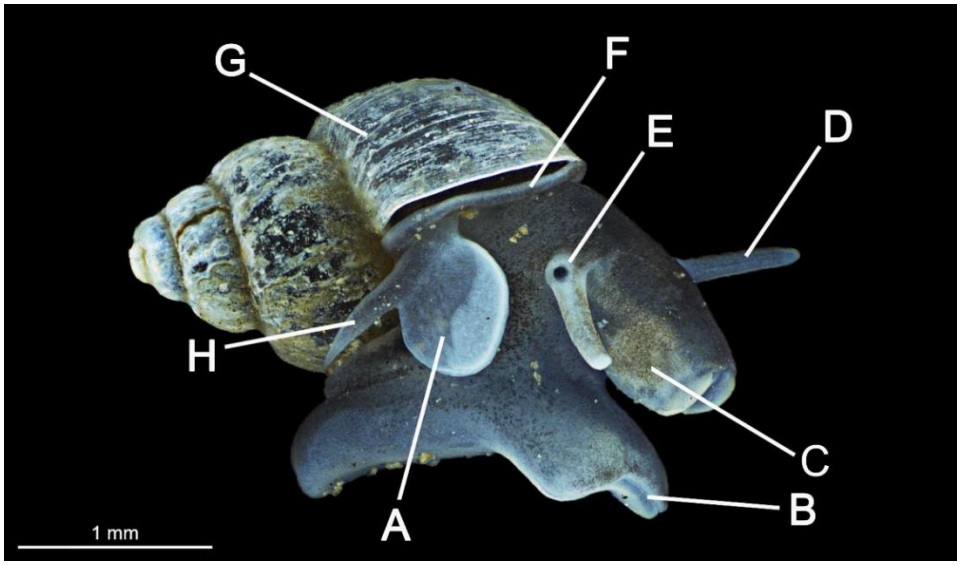


Figure 10. General view of *Mercuria similis* showing details of the head and foot region. **A** – penial appendix; **B** – foot, showing the lateral wings; **C** – snout; **D** – tentacle; **E** – eye; **F** – mantle edge; **G** – shell; **H** – penis.

As in other hydrobiids, *Mercuria* presents a well-developed head and foot region (Figure 10B-D). The head ends in a prominent snout, with long and filiform cephalic tentacles that are scarcely pigmented. The foot presents two well-developed lateral wings (Boeters 1971, Boeters and Falkner 2017).

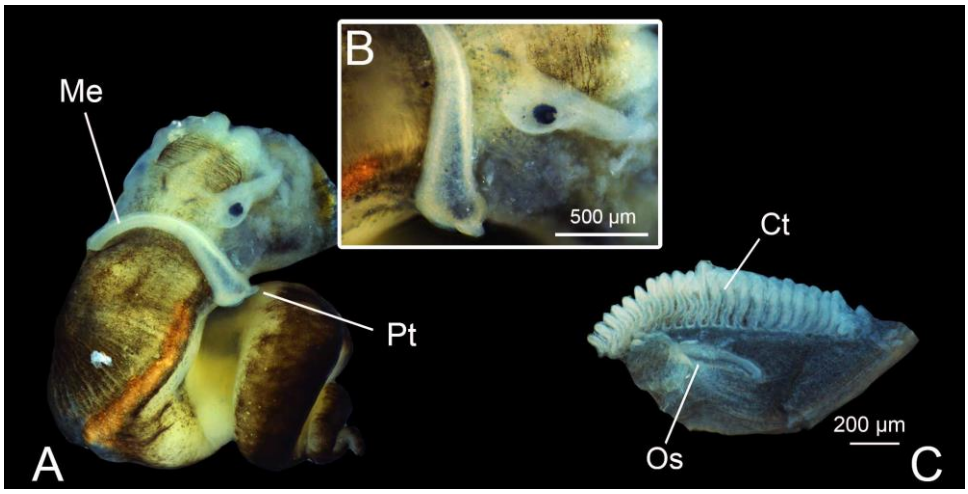


Figure 11. *Mercuria similis* from La Ricarda Pond, Barcelona, Spain. **A** – Pallial tentacle (**Pt**), mantle edge (**Me**); **B** – Higher power view of the pallial tentacle; **C** – A portion of the pallial cavity showing the ctenidium (**Ct**) and the osphradium (**Os**).

Pallial cavity

The pallial cavity of hydrobiids usually contains a ctenidium composed of gill filaments that extend across nearly the entire length of the cavity and functions in gas exchange. Just ventral to the base of the ctenidium is the osphradium, a chemoreceptor organ. Other organs are also located along the pallial cavity including the rectum, which courses most of the cavity length above the ctenidium and terminates in the anus, a portion of the pallial oviduct, the pericardium and the anterior portion of the kidney. The presence of pallial tentacles is rare in hydrobiids: thus far, it has only been described for *Hydrobia truncata* (Vanatta, 1924) by Davis et al. (1988) and Davis et al. (1989) and mentioned for *Mercuria* by Boeters (1971). The pallial tentacle in *Mercuria,* described for the type species *Mercuria confusa*, is small, located on the inner surface of the mantle edge (Figure 11A, B) and in some cases reduced to only a "knob-like protuberance", according to Boeters (1971). More recently, Boeters and Falkner (2017) reported its presence in *Mercuria similis* and *Mercuria meridionalis*.

Ctenidium and osphradium

The ctenidium of *Mercuria* is well developed and occupies most of the length of the pallial cavity. Its filaments are broad and triangular-shaped, with the bases attached to an epithelium and the apexes free to act in gas exchange. Most *Mercuria* species present 20-30 filaments (Boeters and Falkner 2017, Boulaassafer et al. 2018). The osphradium in *Mercuria* is positioned ventral to the ctenidium (Figure 11C), approximately at its middle point (Boulaassafer et al. 2018).

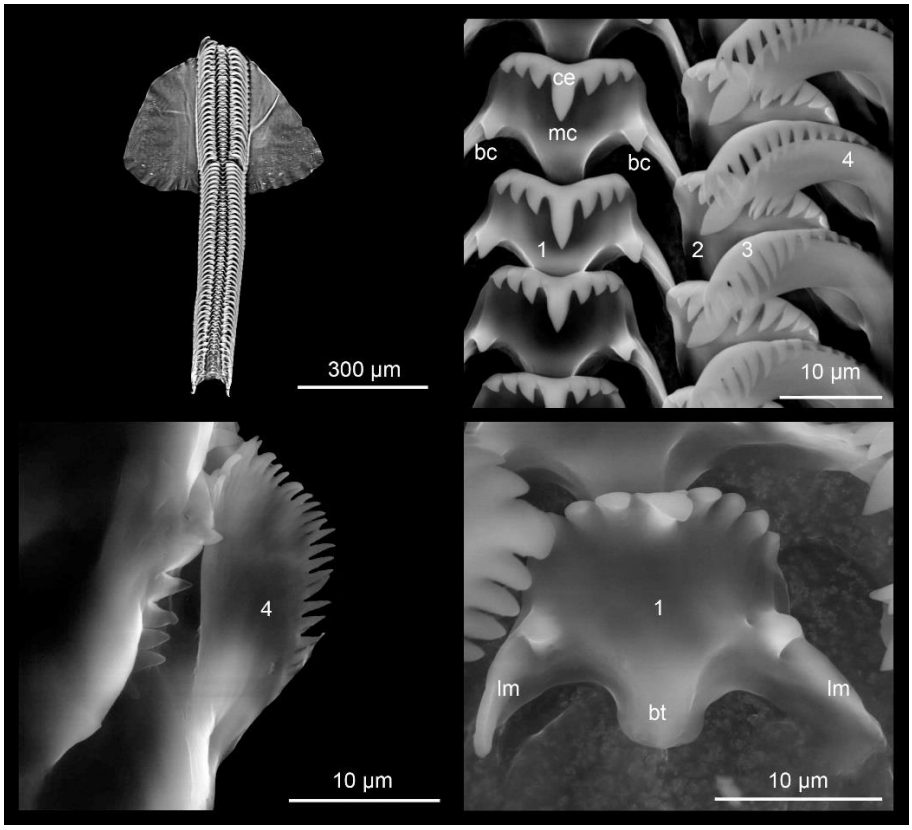


Figure 12. Radula of *Mercuria bayonnensis* showing the details of the central tooth (**1**), lateral teeth (**2**), inner marginal teeth (**3**) and outer marginal teeth (**4**). Abbreviations: **bc** – basal cusps; **bt** – basal tongue; **ce** – cutting edge; **lm** – lateral margin; **mc** – median cusp.

Digestive System

The digestive system in hydrobiids is typical of Caenogastropoda: the mouth opens to a small oral area that contains a pair of simple salivary glands open to the buccal cavity. However, its histology is poorly known. The oesophagus is narrow with no oesophageal gland and ends in the stomach, which is composed

of two chambers containing a crystalline style, the typhlosoles and sometimes a caecum (e.g., *Hydrobia*, *Pyrgula* de Cristofori & Jan, 1832, *Pseudamnicola*, *Corrosella*). The intestine emerges from the anterior edge of the style sac and coils around it. The position of the coil with respect to the style sac is an important taxonomic character used to determine species. The intestine continues into the rectum, which ends near the mantle edge (Hershler and Ponder 1998).

Radula

The radular ribbon in hydrobiids *s. str.* is composed of several rows of teeth and is taenioglossate, i.e., each row comprises a central tooth, two lateral teeth, two inner marginal teeth and two outer marginal teeth (Figure 12). In *Mercuria*, the central tooth has one median cusp, three to four lateral cusps on both sides of the central cusp and one basal cusp on each lateral margin. Lateral teeth usually have three cusps at each side of the central cusp and marginal teeth are very variable in the number of cusps. The radula presents numerous taxonomical characters used to discriminate among species.

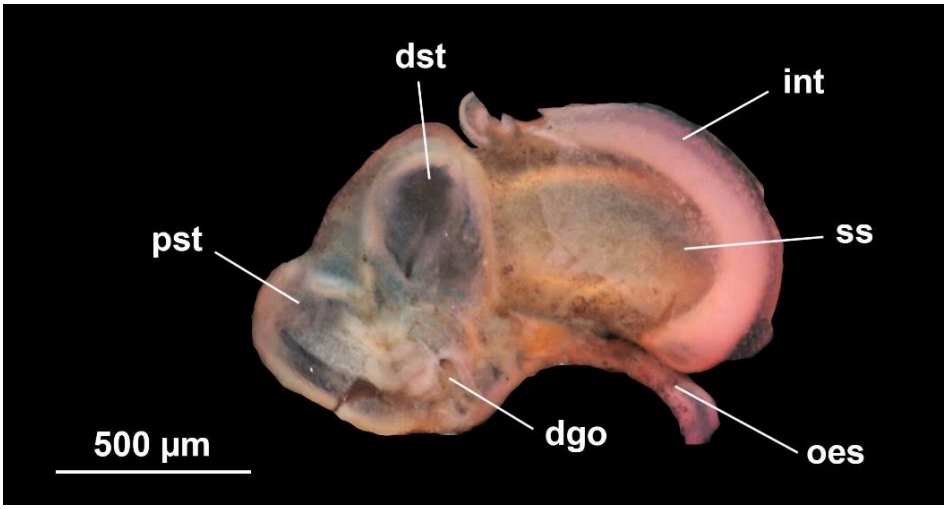


Figure 13. General aspect of the stomach in *Mercuria*. Abbreviations: **dgo** – opening from the stomach to the digestive gland; **dst** – distal stomach chamber; **int** – intestine; **oes** – oesophagus; **pst** – proximal stomach chamber; **ss** – style sac.

Stomach

As in other hydrobiids *s. str.,* the stomach of *Mercuria* is divided into two chambers, with the proximal one connected to the digestive gland by a single opening (Figure 13). The style sac is the distal-most part of the stomach leading towards the intestine, which tightly coils around it. The distal part of the intestine is straight and passes through the pallial cavity (Boeters and Falkner 2017). In

other hydrobiids (e.g. *Corrosella* and *Pyrgulopsis*), the presence/absence of a caecum can be used to discriminate between genera or even between subfamilies as in Hydrobiinae Stimpson, 1865; Horatiinae D. W. Taylor, 1966; and Pseudamnicolinae Radoman, 1977 (see Hershler 1998, Arconada 2000, Delicado and Ramos 2012), highlighting the importance of these characters in making taxonomical decisions.

Female reproductive system

The female reproductive system, due to its complexity, has been extensively used to define higher taxa of Hydrobiidae *s.l.*, providing an important suite of discriminant characters. It is composed of the ovary, the oviduct and the female distal genitalia.

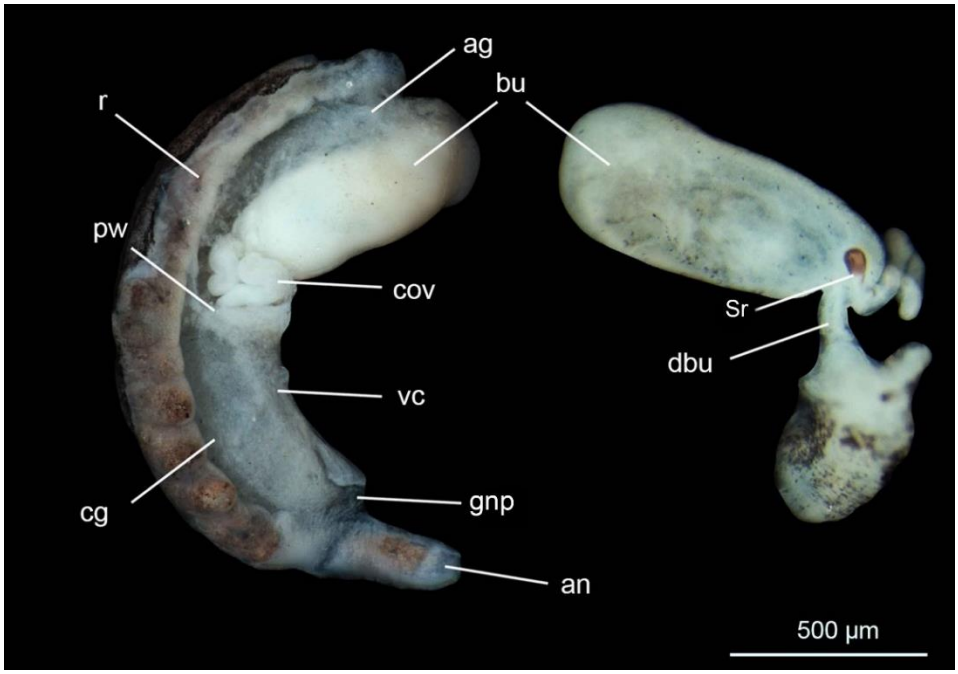


Figure 14. Female pallial oviduct of *Mercuria similis* showing all the different anatomical parts. Abbreviations: **ag** – albumen gland; **an** – anus; **bu** – bursa copulatrix; **cg** – capsule gland; **cov** – coiled renal oviduct; **dbu** – bursal duct; **gnp** – gonopore; **pw** – pallial wall; **r** – rectum; **Sr** – seminal receptacle; **vc** – ventral channel.

The ovary is behind the stomach and dorsal to the digestive gland and it occupies close to 66% of the visceral coil (although in *Pyrgulopsis*, it occupies 50% or less of the coil). Characters specific to the female distal genitalia can be used to discriminate between hydrobiid genera and species. The distal genital system is

composed mainly of the pallial oviduct (albumen gland + the capsule gland), bursa copulatrix, coiled renal oviduct and seminal receptacles (0, 1, 2). These structures are all interconnected by ducts that open at the end of the pallial cavity. The bursa copulatrix, when present, is found behind the pallial wall and the albumen gland and it is directly connected to the coiled renal oviduct. The seminal receptacle (some genera have two, or occasionally it is absent), which stores sperm, is located at the junction of the bursa duct with the renal oviduct at its first loop. The function of the pallial oviduct glands is to nourish and provide external cover for the eggs (Hershler and Ponder 1998).

In *Mercuria*, the female reproductive system (Figure 14) is composed of a coiled renal oviduct and a gonopericardial duct. There is only one seminal receptacle positioned distal to the renal oviduct. The bursa copulatrix lies distal to the renal oviduct and against the albumen gland (Boeters and Falkner 2017).

Male reproductive system

The male reproductive system is comprised mainly of the testis, the prostate gland and the penis. The testis resembles the ovary in its extension and position. As in *Mercuria*, the prostate gland of hydrobiids has a bean shape and is located near the pallial cavity, with its posterior portion inside the cavity. In its posterior region, the posterior vas deferens joins a duct from the seminal vesicle, while in the anterior region, the pallial (anterior) vas deferens emerges and ends at the penis tip (Hershler and Ponder 1998).

Penis shape and position vary among hydrobiid genera. In some taxa, it is located closer to the central axis of the head, while in others, it is displaced to the right. In *Mercuria*, the penis is located on the head, behind the right eye. In some genera, the penis is simple, but *Mercuria* has a bulbous penial appendix (Figure 15), without distinct glandular structures (Boeters and Falkner 2017). The presence of glandular structures is not common; however, when present, they are arranged in one or more glandular papillae or glandular fields (e.g., *Cincinnatia* Pilsbry, 1891; *Fissuria* Boeters, 1981; and *Pyrgulopsis*).

Nervous system

The structure of the nervous system and the shape of its ganglia are highly conserved in hydrobioids (Figure 16). Davis et al. (1976) provided an index to determine the RPG ratio, or the degree of concentration of the ganglia and connectives in the dorsal nerve ring.

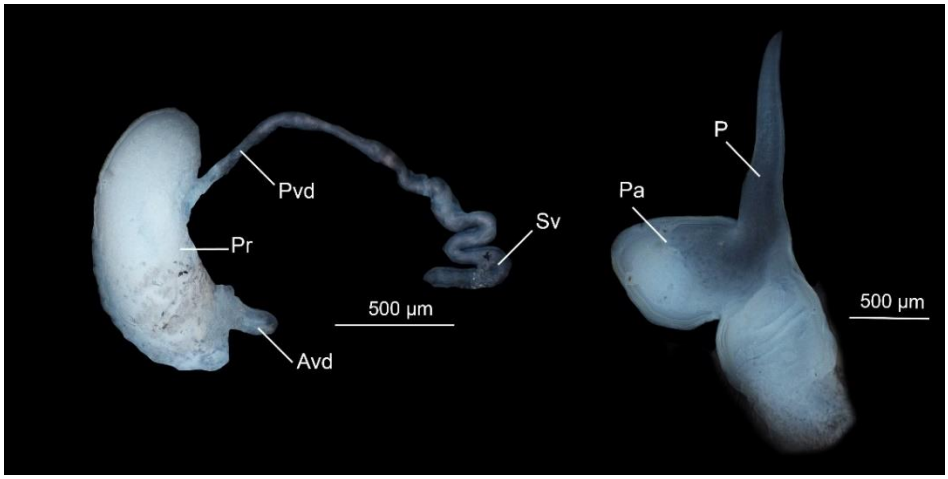


Figure 15. Male reproductive system of *Mercuria similis*, showing the different anatomical parts. Abbreviations: **Avd** – anterior vas deferens; **P** – penis; **Pa** – penial appendix; **Pr** – prostate gland; **Pvd** – posterior vas deferens; **Sv** – seminal vesicle.

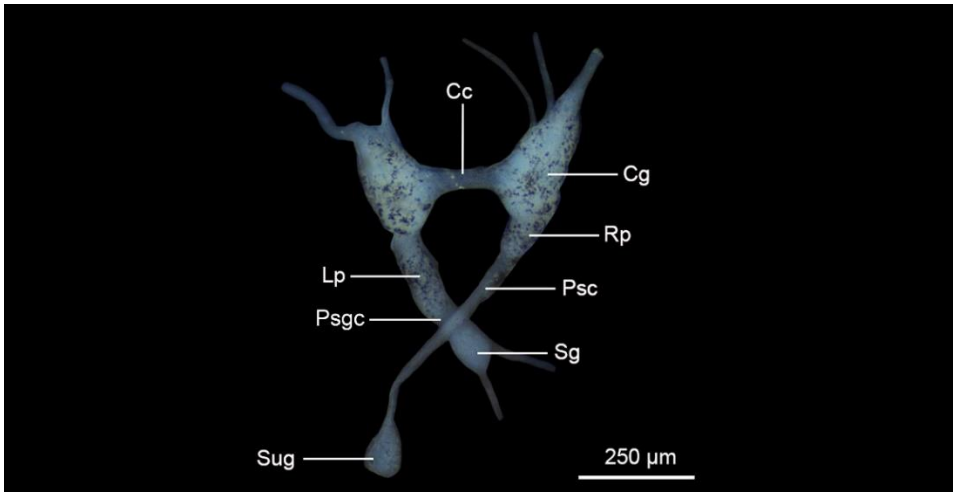


Figure 16. *Mercuria* circumoesophageal ring of ganglia. Abbreviations: **Cc** – cerebral commissure; **Cg** – cerebral ganglion; **Lp** – left pleural ganglion; **Rp** – right pleural ganglion; **Psgc** – pleuro-suboesophageal connective; **Psc** – pleuro-supraoesophageal connective; **Sug** – supraoesophageal ganglion; **Sg** – suboesophageal ganglion.

Prior to the application of more modern techniques (e.g., molecular systematic, non-invasive imaging, or geometric morphometric), this ratio was used and continues to be used, to define species or genera. It is defined by the following formula:

$$RPG_{ratio} = \frac{L.Psc}{L.Rp + L.Psc + L.Sug}$$

L.Psc – Length of the pleuro-supraoesophageal connective

L.Rp – Length of the right pleural ganglion

L.Sug – Length of the supraoesophageal ganglion

Accordingly, the dorsal nerve ring can be concentrated (RPG ≤ 0.29), moderately concentrated (0.30-0.49), elongated (0.50-0.67), or extremely elongated (≥ 0.68) (Davis and Pons da Silva 1984, Davis et al. 1986, Davis et al. 1992).

2.2.6 Histology

Previous histological studies have revealed the non-glandular nature of penial lobes or papillae for many taxa within Hydrobiidae (e.g., Islamiinae and Belgrandiinae de Stefani, 1877) (Arconada and Ramos 2006). However, Hershler and Ponder (1998) have suggested that, in the case of *Mercuria*, the penial appendix may have a glandular function, although more recently, Boeters and Falkner (2017) mentioned that they found no glandular structures on the penial appendix of species of this genus. In addition, many species of the subfamily Nymphophilinae, which has been suggested to have a close phylogenetic relationship with the subfamily Mercuriinae (Wilke et al. 2013), often present penial glands (see Thompson 1969, Davis and Mazurkiewicz 1985, Thompson and Hershler 2002), suggesting the penis of *Mercuria* may also have a glandular component. However, none of the aforementioned authors have examined the histological nature of the penial tissues and, when present, the glandular structures within them. Therefore, a histological analysis was conducted to determine the possible glandular condition of the penial appendix in *Mercuria*.

As described in section 2.2.2, collected live specimens (a few animals per species), were first relaxed in a solution containing menthol crystals. Specimens showing maximal relaxation were fixed in Bouin's solution for one minute, then dehydrated through a graded ethanol series (40% to 100%). The shells were removed using a 5% aqueous dilution of EDTA. Specimens were then cleared in benzyl benzoate prior to being embedded in Paraplast Plus. The samples were incubated at 59ºC for 14 h to allow the wax to fully penetrate the tissue. Serial sections 4-6 µm thick were cut using a Leica RM 2045 microtome. Carazzi's hematoxylin-eosin and Azan staining of the slides were performed as described by Ramos et al. (2000b) and Arconada and Ramos (2002).

2.2.7 Morphometric Studies

Geometric morphometrics

To study shell shape variation, a total of 1014 images, representing 44 populations of *Mercuria* spp., was analysed. Images were taken in frontal view, with the spiral axis on the y-axis. Specimens were positioned accordingly in a petri dish using a little ball of black Blu-Tack, which helped to keep the animal in the desired position. In order to select homologous points, only specimens with a similar number of whorls were analysed. Digital images of the shells were captured under a Leica MZ16 stereomicroscope mounted with a Leica DFC550 camera.

The thin plate splines (TPS) technique (Bookstein 1989) for n-dimensional interpolation of scattered data, representing variation among shapes as a continuum deformation, was used to analyse shell variation among *Mercuria* spp.

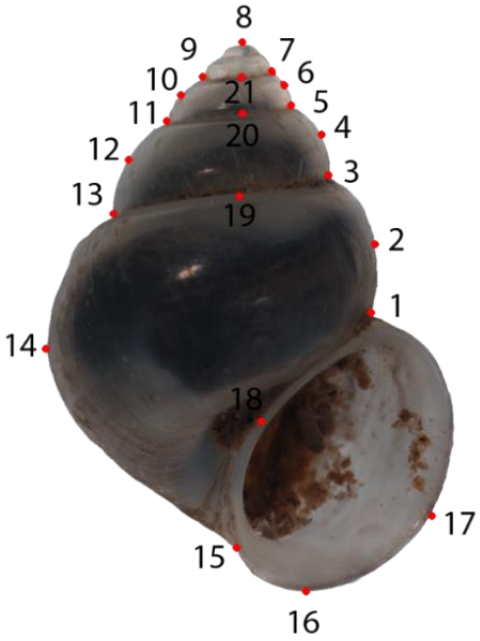


Figure 17. Landmarks and semilandmarks used for the geometric morphometric analysis.

The shell variables, a total of 21 landmarks and semilandmarks per specimen (Figure 17), were scored using TPSdig v.2.31 (Rohlf 2006), which provides coordinate data for each point. The number of landmarks needed for robust results has been disputed: some authors have argued that the most conservative approach is to use more landmarks, while others have suggested that fewer ones can provide equally robust results. (e.g. (Dillon et al. 2013, Tamburi and Martín 2013, Vergara et al. 2017, Verhaegen et al. 2018). The positioning of semilandmarks appears to account for the main difference among these studies. Yet for landmark positioning, homologous structures were used to account for shell variation (e.g., sutures, apex, base, among others). Thus, similar landmark positions were also used in our analyses.

During the imaging process, a small variation in position may occur; thus, all data were subjected to a Procrustes superposition analysis in PAST v.3.0 (Hammer et al. 2001). This analysis eliminates size differences and the potential impact of rotation, thus minimizing errors. The consensus of each population and each species was obtained using TPSRelw v.1.45 (Rohlf 2007). Variables were analysed individually at both levels (i.e., population and species) using a principal component analysis (Jolliffe 2002). A warp analysis was conducted in order to obtain the thin plate splines plot per species used to visualise shell variation among species.

Classic morphometric

Although the use of geometric morphometrics has been proven to be a better method to describe variation in shell shape in gastropods (Van Bocxlaer and Schultheiß 2010, Smith and Hendricks 2013, Márquez and Averbuj 2017), in this thesis, some traditional shell measurements were also made in order to follow the scheme used in other taxonomical papers. Measurements were taken of the same populations and images as in the geometric morphometric study. Shell data included counts of the total number of whorls, following the method described by Ramos et al. (2000) and measurements of the ten continuous variables depicted in Figure 18. Descriptive statistics such as mean, standard deviation and minimum and maximum values were used to summarise intra- and interspecific variation. The results of these analyses are presented in Appendix 6.

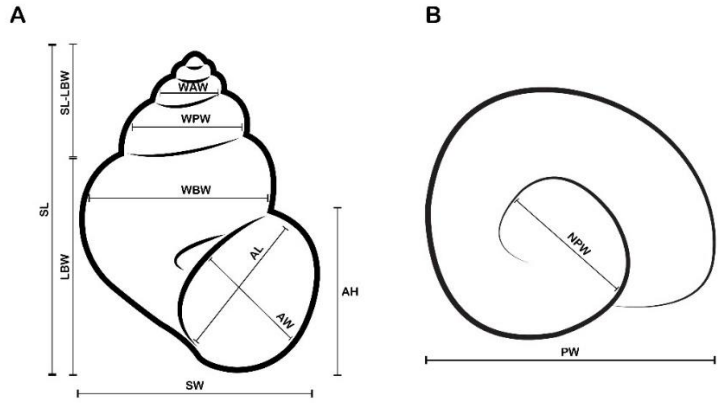


Figure 18. Measured morphometric variables; **A** – shell; **B** – protoconch.

Table 3. List of the studied morphological variables and their abbreviations.

Shell		Nervous System	
AH	Aperture Height	**LRCG**	Length right cerebral ganglion
AL	Aperture Length	**LLCG**	Length left cerebral ganglion
AW	Aperture Width	**LCC**	Length cerebral commissure
SL	Shell Length	**LRPG**	Length right pleural ganglion
SW	Shell Width	**LLPG**	Length left pleural ganglion
LBW	Length of the body whorl	**LSug**	Length supraoesophageal ganglion
WAW	Width of the antepenultimate whorl	**LSg**	Length suboesophageal ganglion
WBW	Width of the body whorl	**LPSuC**	Length supraoesophageal connective
WPW	Width of the penultimate whorl	**LPsc**	Length suboesophageal connective
NPW	Nucleus of the protoconch width		
PW	Protoconch Width		
Male reproductive system		Other structures	
Pr	Prostate	**Ct**	Ctenidium
P	Penis	**Os**	Osphradium
Pa	Penial appendix	**Ss**	Style sac
		St	Stomach
Female reproductive system		Statistical parameters	
Ag	Albumen gland	**Max**	Maximum
Bc	Bursa copulatrix	**Min**	Minimum
Cg	Capsule gland	**SD**	Standard deviation
Dbc	Duct of the bursa copulatrix	**CV**	Coefficient of variation
Po	Pallial oviduct		
Sr	Seminal receptacle		

2.3 Historical biogeography of selected hydrobiid genera

To compare the biogeographic history and dispersal abilities of several hydrobiid genera, the phylogenetic relationships and divergence times of the species were first inferred and then their ancestral biogeography modelled using the obtained calibrated phylogenies. The selected genera included *Mercuria*, *Pseudamnicola*, *Islamia* and *Corrosella*. These groups were selected based on their different species richnesses and elevational occurrences. *Mercuria*, the primary genus studied in the present thesis, *Islamia* and the co-occurring *Pseudamnicola*, mostly live in the low reaches of rivers, while *Corrosella* is found mainly in headwaters. Moreover, *Mercuria* and *Corrosella* present lower number of species than the other two groups. Species tree estimations and molecular dating of *Mercuria* were conducted using the sequences and species delimited in section 2.2.3. For

Pseudamnicola and *Islamia*, previously published genetic sequences along with newly generated ones were used for these analyses. Delicado et al. (2013, 2015) previously reported a calibrated species tree and ancestral area estimation for *Corrosella* species and therefore analyses of this species were not repeated.

Species tree estimation and calibration of speciation events, inferred using *BEAST v.1.8.4 (Heled and Drummond 2009), were based on the combined data sets (COI + 16S + 28S) of the three selected genera without outgroups. This extension of BEAST combines data sets from several gene fragments and multiple individuals per species, clustered according to a grouping file, to generate a species tree. Despite recent discoveries of *Mercuria* and *Islamia* fossils (Sesé et al. 2004, Esu and Girotti 2015b, a, 2019), an external COI substitution rate was used to calibrate molecular phylogenies as the evolutionary convergence of shell shape among hydrobiid genera (e.g., Bodon et al. 2001, Delicado Iglesias et al. 2016) makes it difficult to accurately place fossil assignments at the genus and species levels. Hence, a COI substitution rate of 0.81 ± 0.24% Myr^{-1} (the percentage of substitutions per lineage per million years) that was previously calculated for *Corrosella* (Delicado et al. 2013) was used. This rate represents an average of substitution rates calculated for other hydrobiid genera using different biogeographic events (e.g., 0.91% by Wilke (2003); 0.81% by Hershler and Liu (2008); 0.72% (mean rate) by Wilke et al. (2009)).

Parameters used to run the *BEAST analyses are shown in Table 4. The results of the analyses were evaluated for convergence (ESS > 200) using TRACER. The MCC tree was selected using TreeAnnotator v.1.8.4 with a 10% burn-in and visualised in FigTree. Node ages and 95% highest posterior density (HPD) intervals were also displayed. Bayes factor (BF) was used to assess the applicability of strict vs. relaxed clock models (i.e., substitution rate constancy among branches) by comparing their marginal likelihoods (mL). Marginal likelihoods were calculated in BEAST v.1.8.4. by stepping-stone sampling using 75 paths and 2,666,666 iterations. To place the tree into a geological context, the tree was visualised using the R packages strap (Bell and Lloyd 2015) and phytools (Revell 2012).

Once the MCC tree was obtained for each genus, the species ancestral area was inferred with the R package BioGeoBEARS v.0.2 (Matzke 2013), assigning the actual distribution of the species according to the freshwater ecoregions (Abell et al. 2008) in 29 categories. The applied algorithm estimates the ancestral area of distribution and tests possible models of evolutionary processes such as dispersal, vicariance and founder-event

speciation. The following models were tested: Dispersal–Extinction–Cladogenesis (DEC; Ree et al. 2005), Dispersal–Vicariance (DIVA; Ronquist 1997) and BayArea (Landis et al. 2013). The likelihood versions of the last two models are known as DIVALIKE and ByAreaLIKE, respectively. These models have two free parameters estimated along phylogenetic branches: *d* = dispersion or range extension and *e* = extinction or range contraction. Additionally, model improvement was tested using the founder effect parameter *j* (occurring at cladogenetic events) for each of the tested models, resulting in three additional models: DEC+*j*, DIVA+*j* and ByArea+*j*. The AICc was used to select the best model. The most likely ancestral range was calculated for each node in agreement with the most likely model and later plotted on the MCC tree.

Table 4. Parameters used to run the *BEAST analyses.

	Mercuria	*Pseudamnicola*	*Islamia*
Site Models	COI: TPM3uf+ Γ 16S: K80+ Γ 28S: TrN+I+ Γ	COI: HKY+ I+ Γ 16S: HKY+ Γ 28S: TrN+I+ Γ	COI: GTR I+ Γ 16S: HKY+ Γ 28S: TrN+I+ Γ
Substitution rate	COI: 0.81±0.24% Myr^{-1}	COI: 0.81±0.24% Myr^{-1}	COI: 0.81±0.24% Myr^{-1}
Tree model	Birth and Death	Birth and Death	Birth and Death
Clock model	Lognormal relaxed Uncorrelated	Lognormal relaxed Uncorrelated	Lognormal relaxed Uncorrelated
Generations	120 x 10^6 sampling every 2,000 generations	120 x 10^6 sampling every 2,000 generations	120 x 10^6 sampling every 2,000 generations

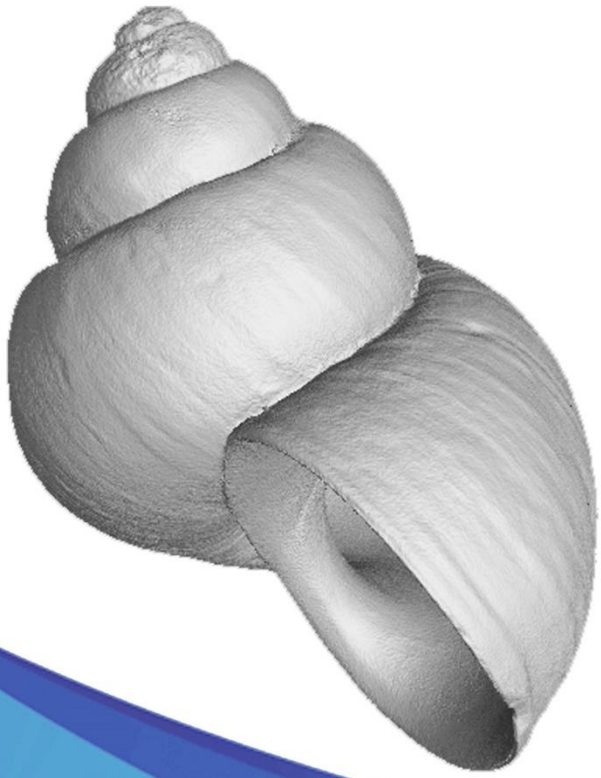
A novel approach has been developed to identify significant differences in the *d* and *j* parameters between genera, which may reflect different dispersal abilities. This consisted in calculating the likelihood of an adapted model in BioGeoBEARS that explains the range evolution along the MCC tree of one genus with the help of the dispersal parameters (i.e., *d* and *j*) of a second genus. This model should show a lower fit than the best-fit model of the focal genus because the dispersal parameters are the maximum likelihood estimates of a different genus. The question is whether the difference in fit is significant, which would indicate a significant difference in *d* and/or *j*. This happened when the difference between the likelihoods (ΔlnL) of the best-fit model for a particular genus and the adapted one was greater than 1.

To assess the potential relation between altitudinal range and dispersal (*d*) and founder-event jump dispersal (*j*) parameters, we calculated the dispersal ratio (d_{ratio}) of each of the studied genera according to the following formula:

$$d_{ratio} = \frac{(N_d + N_j)}{N_{lin}}$$

Where N_d represents the number of dispersal events and N_j the number jump dispersal events both obtained in BioGeoBEARS and N_{lin} the number of lineages. The distribution of the species included in the BioGeoBEARS were compiled according to available sources (i.e., literature, GBIF (GBIF.org 2020) and unpublished data). The values of the altitude where obtained using a global digital elevation model (DEM) of 30´resolution obtained from the WorldClim platform (Hijmans et al. 2006) using the previously compiled dataset and the function Add Surface Information in ArcGIS v. 10.5 (ESRI 2011). The plot of d_{ratio} vs elevational range was done using R (Core-Team 2017).

RESULTS



3.1 Species richness distribution and hotspots

Our dataset comprised a total of 906 extant species classified in 157 genera of Hydrobiidae *s. str.* distributed in 115 freshwater ecoregions (Appendix 1). The taxonomic status of a few records of the brackish-water genus *Hydrobia* reported from the equatorial zone of Africa and South America should be verified. Figure 1a-c shows distribution maps of hydrobiid species richness, endemics and threatened species. We found the highest species richness values in ecoregions from Southern Europe, Northern Africa, Northwestern United States of America and the Caspian Sea (see Figure 19A). The Dniester-Lower Danube region was the richest ecoregion, with over 14% of the global hydrobiid species richness (Table 5). In our dataset, 83% of the species were endemic to a single ecoregion, of which 39% are characterized by a type locality at high elevation (> 500 meters above sea level [m.a.s.l.]).

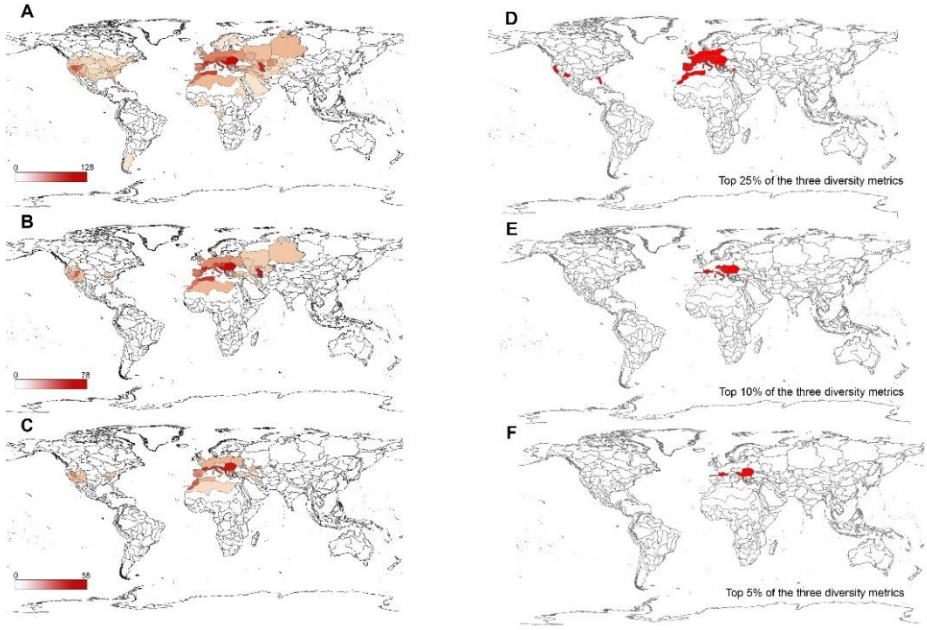


Figure 19. Distribution maps for three measures of hydrobiid diversity: **A**, species richness; **B**, endemic species; **C**, threatened species. The inferred hotspots sensu Myers (1988) at: **D**, top 25%; **E**, top 10%; **F**, top 5% of these metrics are shown in red. All maps use an equal-area projection.

Table 5 shows ecoregions that contain the top quartile of species richness, endemics and threatened species. Congruence among these diversity measures were found in 19 ecoregions, qualifying them as biodiversity hotspots sensu

Myers (1988). These were mainly found across the Mediterranean basin (Figure 19D) and only in three ecoregions from North America.

Table 5. List of ecoregions representing hotspots of hydrobiid diversity, including their species richness, number of threatened and endemic species and the robustness of hotspot selection against species misidentification.

ECOREGION	Species richness	Threatened species	Endemic species	Robustness
Dniester–Lower Danube	128	37	78	1.000
Southeast Adriatic Drainages	90	58	67	1.000
Dalmatia	68	27	32	1.000
Cantabric Coast–Languedoc	59	26	42	1.000
Italian Peninsula & Islands	49	17	27	1.000
Eastern Iberia	37	7	24	0.998
Gulf of Venice Drainages	35	8	8	0.995
Mediterranean Northwest Africa	35	4	28	0.995
Upper Danube	31	20	21	0.990
Thrace	27	10	18	0.997
Southern Iberia	22	9	18	0.992
Central & Western Europe	22	5	14	0.944
Atlantic Northwest Africa	21	13	18	1.000
Ionian Drainages	20	7	16	0.931
Southern Anatolia	18	8	17	0.982
Western Iberia	17	5	9	0.745
Gila	13	5	10	0.698
Florida Peninsula	12	7	10	0.305
Sacramento - San Joaquin	12	5	7	0.287

This number decreases when applying lower thresholds than 25%, with identified hotspots occurring mainly in southern Europe (Figure 19D–F). The analysis of robustness of hotspot selection against a species misidentification showed that ecoregions characterized by a biodiversity close to the lower end of the top 25% were not always classified as hotspots, whereas the most biodiverse regions were robustly classified as hotspot (Table 5).

3.1.1 Risk distribution

According to our dataset, of the 906 species of Hydrobiidae *s. str.*, 284 (31.4%) are classified within the threatened categories (CR, EN, VU) of the IUCN and 104 (11.5%) are of least concern or near threatened species (Figure 20A). The remaining 518 (57.1%) species in our dataset are either listed as data deficient by the IUCN or have not yet been assessed.

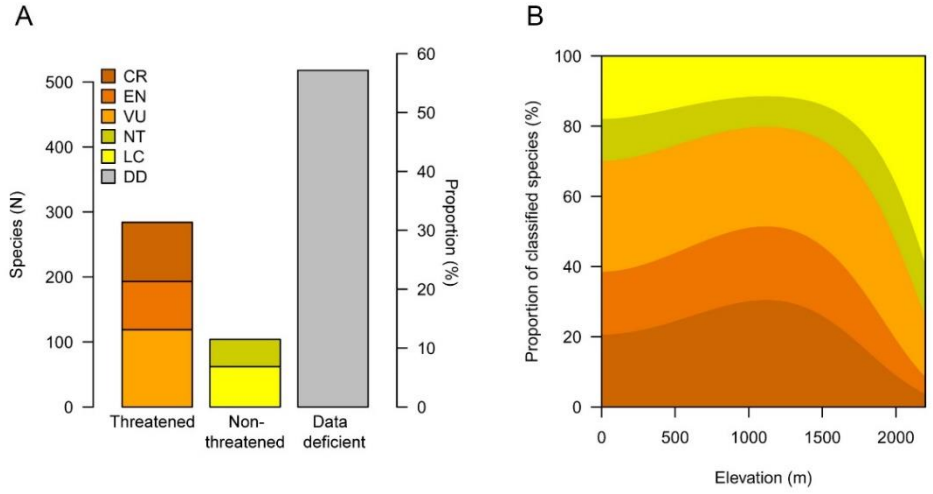


Figure 20. Hydrobiid extinction risk. **A**, Number and proportion of species according to the IUCN Red List categories. We classified all species not listed by the IUCN as data deficient. **B**, Proportion of IUCN Red List categories along the elevation gradient predicted by our non linear ordinal regression model.

The highest number of threatened species was found in the Southeast Adriatic Drainages ecoregion, with a total of 54 threatened species listed by the IUCN. With the lowest AIC, our model selection identified a combination of quadratic and cubic terms of elevation as the best predictor of IUCN Red List category (Table 6). We could only robustly test the proportional odds assumption for a simple linear and quadratic relationship with elevation. More complex models did not converge because of the high number of coefficients. However, for the two

converging models, the simpler proportional odds model was preferred ($\chi^2 = 1.9$, df = 3, p = 0.6; quadratic: $\chi^2 = 0.5$, df = 3, p = 0.9). Using the coefficients of the best fit model to predict of the proportion of IUCN Red List categories along the elevational gradient showed an increase in threatened categories until 1,500 m.a.s.l., after which, threat risk decreases (Figure 20B).

Table 6. Risk models for hydrobiid extinction. Different models represent increasing complexity in the non linear relationship between the IUCN Red List category of a species and the elevation of its type locality. Log likelihood indicates absolute model fit and ΔAIC the complexity penalized difference in model fit in comparison to the best fit model. ΔAIC < 2 indicates no substantial difference in fit.

Model	Log-likelihood	Parameters	ΔAIC
Constant	-602.3	1	2.6
Linear	-602.2	2	4.3
Quadratic	-602.3	2	4.5
Cubic	-601.8	2	3.6
Linear + quadratic	-600.5	3	2.9
Linear + cubic	-599.8	3	1.6
Quadratic + cubic	-599.0	3	0.0
Linear + quadratic + cubic	-598.7	4	1.3

3.1.2 Determinant of species richness distribution

With the lowest AIC, the best fit non-spatial regression model included as predictors of species richness latitude, connectivity, biogeographical realm, precipitation seasonality, annual precipitation and annual temperature range (Table 7). However, this model was potentially biased due to the low but significant spatial autocorrelation (see Methods). The spatial regression model did not indicate autocorrelation. This analysis revealed that annual precipitation was a false positive selected predictor of hydrobiid species richness. Comparison of the estimated coefficients of the non-spatial and spatial regression models

signified no major differences (Table 7), indicating no further bias in estimated regression coefficients.

Table 7. Drivers of hydrobiid richness. Influence (β ± standard error) of ecological and evolutionary predictors on hydrobiid species richness estimated with a non-spatial and a spatial generalized linear model (GLM). All effects are in comparison with the Afrotropical realm (i.e., the intercept level). A superscript of 2 indicates a non linear influence of the respective predictor. We evaluated the significance (p) of individual predictors by the Wald test statistic z. Note that we used an information theory approach for predictor selection through maximizing model fit by penalizing for additional predictors. Therefore, not all predictors were significant at α = 0.05.

	Non-spatial GLM			**Spatial GLM**		
	β	***z***	***p***	**β**	***z***	***p***
Intercept	2.27±0.54	4.23	< 0.001	2.18±0.52	4.19	< 0.001
Latitude^2	-0.33±0.08	-4.00	< 0.001	-0.37±0.08	-4.55	< 0.001
Connectivity	0.31±0.11	2.87	0.004	0.25±0.10	2.43	0.015
Biogeographic realm						
Nearctic	-0.42±0.58	-0.73	0.468	-0.35±0.56	-0.61	0.540
Neotropic	-2.72±1.41	-1.94	0.053	-2.61±1.38	-1.90	0.057
Palearctic	0.52±0.52	1.01	0.314	0.67±0.50	1.33	0.182
Precipitation seasonality	-0.53±0.13	-3.99	< 0.001	-0.42±0.13	-3.25	<0.001
Precipitation seasonality^2	-0.19±0.10	-2.00	0.046	-0.22±0.10	-2.24	0.025
Annual precipitation	-0.33±0.14	-2.37	0.018	-0.22±0.14	-1.56	0.118
Annual temperature range	-0.78±0.16	-4.76	< 0.001	-0.68±0.16	-4.26	< 0.001
Residual autocovariate				0.31±0.10	3.13	0.002

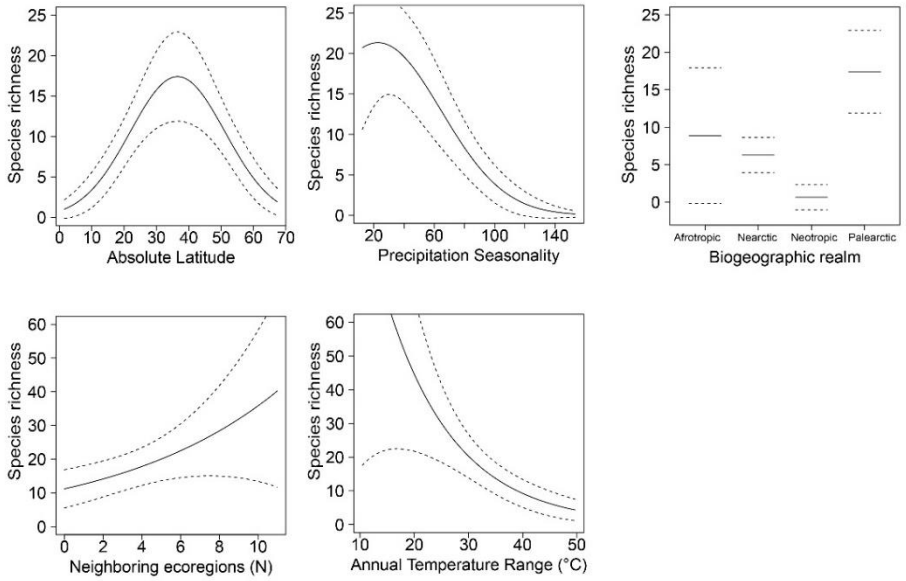


Figure 21. Response plots. Predicted hydrobiid species richness (solid line) and the 95% prediction interval (dashed lines) according to the best-fit spatial generalized linear model along ecological and evolutionary predictors. We fixed all continuous predictors, except the focal one, to their mean and the biogeographic realm to the Palearctic, showing the sole response of richness to the predictor of interest. The GLM specification of the negative binomial model errors caused the non-linear impression of the richness relationship with annual temperature range and connectivity; however, both correlations are monotonic.

As shown by the coefficients of the spatial regression model (Table 7), there was a positive relationship between species richness and connectivity, whereas annual temperature range showed a negative influence. Latitude and precipitation seasonality showed a non-linear relationship with hydrobiid species richness (Figure 21).

With an R^2=0.53, our non-spatial regression model explained more than 50% of the variance in hydrobiid species richness.

3.2 Genetic variation and sequence divergence

The three analysed loci (COI, 16S and 28S) were amplified from a total of 183 specimens of *Mercuria*. Another 26 COI sequences available from GenBank were included in the analyses as were any 16S and 28S sequences that were derived

from the same genomic DNA as COI (i.e. from the same study). The concatenated alignment consisted of 209 sequences and was 2,088 base pairs (bp) in length. From these sequences, we observed 80 identical haplotypes in our search for unique haplotypes to include in the bPTP analysis. Average base frequencies for the complete dataset were 25.2% A, 20.3% C, 25.1% G and 29.4% T. Of the sites, 1,579 (75.51%) were conserved, 512 (24.48%) were variable and 336 (16.07%) were parsimony-informative. Substitution saturation of the analysed gene fragments has not been previously evidenced within Hydrobiidae (Wilke et al. 2013, Falniowski et al. 2016, Delicado et al. 2019); therefore, we did not perform a saturation test.

On the bases of our species delimitation results and morphological characterizations (described below), we identified 14 putative species in our dataset, 9 of which correspond with their traditional taxonomic classification. Interspecific mean sequence divergences (measured as uncorrected pairwise distances) ranged between 1.3% *(M. egarensis* sp. nov. vs. *M. carrillorum* sp. nov.) and 9.3% (*M. midarensis* vs. *M. lupiaensis* sp. nov.) for COI, 0.7% (*M. melitensis* vs. *M. rolani*) and 4.8% (*M. balearica* vs. *M. veronicae* sp. nov.) for 16S and 0.2% (*M. felixi* sp. nov. vs. *M. egarensis* sp. nov. and *M. carrillorum* sp. nov., respectively) and 1.8% (*M. rolani* vs. *M. midarensis*) for 28S. A summary of uncorrected pairwise distances are shown in Appendix 5.

3.3 Molecular phylogeny of *Mercuria* species

3.3.1 Mitochondrial dataset

An alignment of 1,165 bp was generated for the mitochondrial genes COI (658 bp) and 16S (507 bp). For both genes, all phylogenetic inferences recovered 13 species-level clades within *Mercuria* (Figure 23) with high supports (BS > 75, BPP > 0.95). Eight of these clades individually represent seven formally recognized morphospecies and the species *M. melitensis* and *M. rolani*, which were collapsed into a single species-level clade. For both genes, BI analyses recovered four well-supported (BPP > 0.95) groups of clades: (i) *M. similis* + *M. egarensis* sp. nov. + *M. carrillorum* sp. nov.; (ii) *M. balearica* + *M. tensiftensis* + *M. felixi* sp. nov.; (iii) *M. midarensis* + *M. targouasensis* and (iv) *M. lupiaensis* sp. nov. + *M. veronicae* sp. nov. The remaining species grouped with one of four aforementioned clade groups with low support (BPP < 0.95). The ML analyses recovered the (i) and (ii) clade groups with high support (BS > 75), however the rest of the species grouped with low support (BS < 75).

3.3.2 Nuclear dataset

The final alignment of the partial nuclear 28S sequences consisted of 923 bp. The BI analyses yielded four supported species-level clades: *M. lupiaensis* sp. nov., *M. saharica*, *M. targouasensis* and *M. melitensis* (BPP > 0.95). These clades were also recovered in the ML analyses with high support (BS > 75), except for *M. saharica*. The rest of the species grouped with low support (BS < 75).

3.3.3 Combined dataset

Despite the low support of most branches in the 28S phylogeny, the combined nuclear and mitochondrial dataset increased the support of all species-level nodes. The concatenated dataset of COI, 16S and 28S consisted of an alignment with 2,088 bp. Phylogenetic analyses of this dataset using both BI and ML approaches recovered a total of 13 species-level clades with high support (BS > 75, BPP > 0.95). Of these clades, seven correspond to previously described morphospecies, one clusters *M. melitensis* and *M. rolani* and five are newly described ones.

The BI analyses recovered four well-supported (BPP > 0.95) groups of clades: (i) *M. similis* + *M. egarensis* sp. nov. + *M. carrillorum* sp. nov.; (ii) *M. tachoensis*; (iii) *M. balearica* + *M. tensiftensis* + *M. felixi* sp. nov.; (iv) *M. lupiaensis* sp. nov. + *M. veronicae* sp. nov. + *M. saharica* + *M. melitensis* + *M. rolani*; (v) *M. midarensis* + *M. targouasensis*. The same groups of clades were recovered with high support in the ML analyses (BS > 75). Although all the species were well supported, the relationships among them differed in the different analyses and the basal phylogenetic relationships between groups of clades were not well supported.

3.4 Molecular species delimitation methods

Species delimitation methods applied to our dataset (209 sequences) differed in the number of species inferred (Figure 23), ranging between 12 (ABGD) and 24 (bPTP) species. *Mercuria balearica*, *M. tensiftensis* and *M. saharica* were consistently recovered as independent units in all methods, as were three potentially new species, which we describe below as *M. felixi* sp. nov., *M. veronicae* sp. nov. and *M. lupiaensis* sp. nov. The putative morphospecies that were recognized here for the first time, *M. egarensis* sp. nov. and *M. carrillorum* sp. nov., were considered as a single group by all of the methods. Delimitation of the remaining species varied among methods.

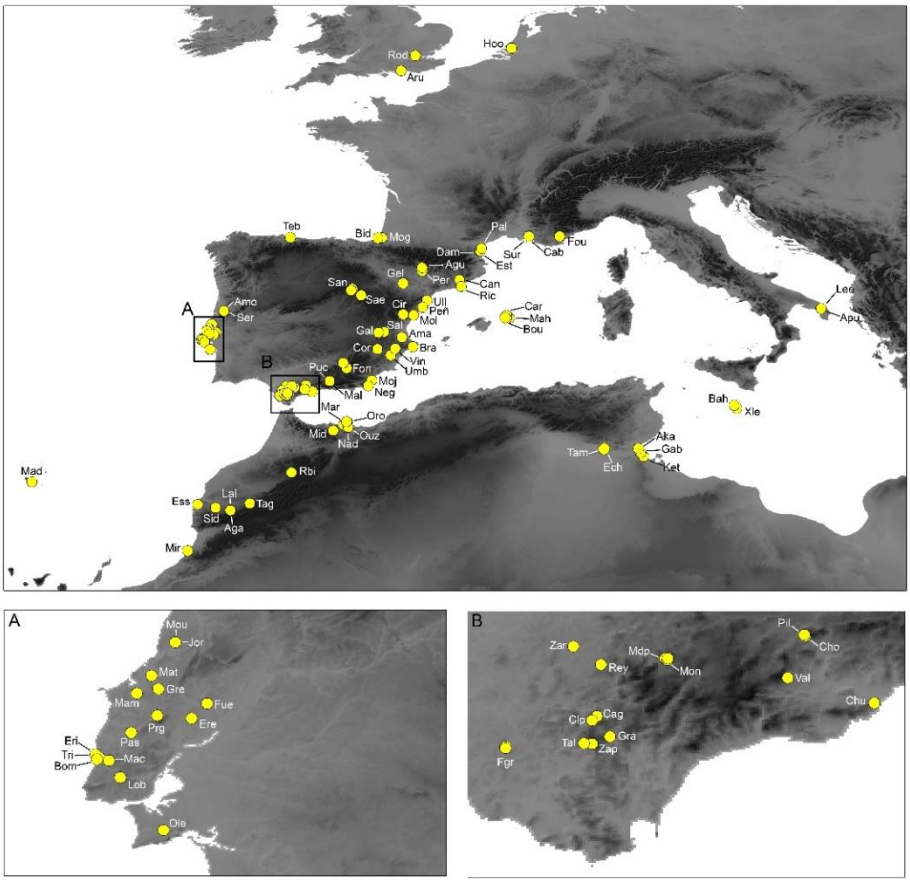


Figure 22. Map showing the location of the *Mercuria* populations included in the molecular study. Code names, species assignment and other information are provided in Table 8.

The ABGD method detected 12 species at a prior maximal distance of 0.007. The number of species inferred by this method is nearly congruent with the number recovered by the phylogenetic analyses and by morphology-based taxonomic classifications, except for the clustering of two different species pairs: *M. egarensis* sp. nov. and *M. carrillorum* sp. nov. and *M. rolani* and *M. melitensis*. The most likely ST-GMYC model suggested 21 putative species [confidence interval (CI) = 2–37], splitting the taxonomy-based species *M. similis*, *M. tachoensis*, *M. midarensis* and *M. targouasensis* into 5, 2, 3 and 2 groups, respectively. The bPTP analysis inferred 24 species and was similar to the ST-GMYC analysis, except that *M. similis* was split into only 3 groups and the other three groups were likely oversplit, considering the low support (P<0.9) obtained

for these groups. The mPTP analysis delimited 17 putative species, which was closer to the number of morphological species (14); however, it split the taxonomy-based species *M. similis* into 3 groups and *M. tachoensis*, *M. veronicae* sp. nov. and *M. targouasensis* into 2 groups each.

Overall, ABGD presented the highest match ratio of all the methods (0.85) and mPTP presented the highest ratio among the tree-based methods (0.45). The bPTP and ST-GMYC methods presented the lowest values (0.32 and 0.34, respectively) (Table 9).

Table 8. Species assignment, coding and locality names for the *Mercuria* specimens sequenced in this study.

Species	***Loc. Code***	***Locality***
M. balearica	**Cho**	Spring in El Chorro, Ardales, Málaga, Spain
M. balearica	**Chu**	Spring in Churriana, Málaga, Spain
M. balearica	**Pil**	Venta El Pilar Spring, Málaga, Spain
M. balearica	**Gra**	Los Granados Trough, Málaga, Spain
M. balearica	**Mon**	Montecorto Stream, Málaga, Spain
M. balearica	**Val**	Fuente Valentín Spring, Alozaina, Spain
M. balearica	**Fon**	Caño de la Rambla Stream, Fontanar, Jaén, Spain
M. balearica	**Moj**	Arabic Spring in Mojácar, Almería, Spain
M. balearica	**Mah**	Colársega, Mahón, Minorca, Spain
M. balearica	**Car**	Spring near Sant Joan de Carbonell, Minorca, Spain
M. balearica	**Bou**	River in Son Bou, Minorca, Spain
M. carrillorum sp. nov.	**Mdp**	Doce Pilares Spring, Málaga, Spain
M. carrillorum sp. nov.	**Cag**	River at Montes de Propio, Canuto de la Gallina, Cádiz, Spain
M. carrillorum sp. nov.	**Clp**	Stream in Canuto de Las Palas, Cádiz, Spain
M. egarensis sp. nov.	**Can**	Spring Font des Canyes, Terrassa, Spain
M. felixi sp. nov.	**Tal**	Stream in Canuto de la Tala, Cádiz, Spain
M. felixi sp. nov.	**Zap**	Stream in Canuto del Zapato, Cádiz, Spain
M. lupiaensis sp. nov.	**Lee**	Giammatteo Creek, Frigole, Leece, Italy
M. lupiaensis sp. nov.	**Apu**	Palude del Capitano, Sant´Isidoro, Apulia, Italy
M. melitensis	**Bah**	Wied-tal Bahrija Spring, Il-Bahrija, Malta
M. melitensis	**Xle**	Spring in Xlendi Valley, Fontana, Gozo, Malta

Species	*Loc. Code*	*Locality*
M. midarensis	**Mid**	Two parallel irrigation channels, Midar, Morocco
M. midarensis	**Nad**	Selouane River, Nador, Morocco
M. midarensis	**Oro**	Rio de Oro River, Melilla, Spain
M. midarensis	**Ouz**	Ouzej River, Morocco
M. midarensis	**Mar**	Mariouari River, Morocco
M. rolani	**Mad**	Spring in Arco da Calheta, Madeira, Portugal
M. saharica	**Gab**	Gabés Stream, Gabés, Tunisia
M. saharica	**Aka**	Al Akarit Stream, El Akarit, Gabés, Tunisia
M. saharica	**Ket**	Stream in Ketana Oasis, Gabés, Tunisia
M. similis	**Dam**	Fonte Dame Spring, Salses-le-Château, France
M. similis	**Est**	Font d'Estramar Spring, Salses-le-Château, France
M. similis	**Fou**	La Foux-de-Draguignan Spring, Var, France
M. similis	**Mal**	Spring in La Malahá, Granada, Spain
M. similis	**Puc**	Fuente la Pucha Spring, Granada, Spain
M. similis	**Rey**	Spring Pilar de los Playeros, Villamartín, Cádiz, Spain
M. similis	**Zar**	Fuente la Zarza Spring, Villamartín, Cádiz, Spain
M. similis	**San**	Salado river, Santamera, Guadalajara, Spain
M. similis	**Sae**	La Vega Stream, Saelices de la Sal, Guadalajara, Spain
M. similis	**Neg**	Las Negras ravine, Almería, Spain
M. similis	**Cor**	Cordovilla Saltings, Albacete, Spain
M. similis	**Saa**	La Salaboreja Spring, Casa de Ves, Albacete, Spain
M. similis	**Sab**	La Cañada Stream, Casa de Ves, Albacete, Spain
M. similis	**Gal**	Stream feeding Galayo's Pond, Fuentalbilla, Albacete, Spain
M. similis	**Gel**	Stream in Barranco del Agua Salada, Gelsa, Zaragoza, Spain
M. similis	**Agu**	Saltwater stream near Aguinaliu, Huesca, Spain
M. similis	**Per**	Stream in Peralta de la Sal, Huesca, Spain
M. similis	**Per**	Sosa River in Peralta de la Sal, Huesca, Spain
M. similis	**Ric**	La Ricarda Pond, Barcelona, Spain
M. similis	**Ull**	Ullals de Baltasar, Amposta, Tarragona, Spain

Species	*Loc. Code*	*Locality*
M. similis	**Umb**	Stream in La Umbría, Murcia, Spain
M. similis	**Ama**	Fuente Amarga Spring, Castellón, Spain
M. similis	**Vin**	Vinalopó River, Sax, Alicante, Spain
M. similis	**Bra**	Braña's ravine, near L'Olla Beach, Alicante, Spain
M. similis	**Peñ**	Marsh in Peñíscola, Castellón, Spain
M. similis	**Mol**	Irrigation ditch in Moli de la Font, Castellón, Spain
M. similis	**Cir**	Irrigation ditch in Cirat, Castellón, Spain
M. similis	**Fgr**	Fuente Grande Spring, Medina Sidonia, Cádiz, Spain
M. similis	**Pal**	Buddle in La Palme, Aude, Languedoc-Roussillon, France
M. similis	**Sur**	Stream near La Suriane, Bouches-du-Rhône, Languedoc-Roussillon, France
M. similis	**Cab**	Arc River near Les Cabanes, Bouches-du-Rhône, Languedoc-Roussillon, France
M. tachoensis	**Hoa**	Small stream, Hoogvliet, The Netherlands
M. tachoensis	**Hob**	Junction of the running surface in the tidal area, Hoogvliet, The Netherlands
M. tachoensis	**Mog**	Stream in Chemin d'Elizaberry, Mouguerre, Pyrénées-Atlantiques, France
M. tachoensis	**Bid**	Stream in Bidart, Pyrénées-Atlantiques, France
M. tachoensis	**Aru**	Stream outflowing from Swanbourne Lake, Arundel, UK
M. tachoensis	**Rod**	Reed belt of Hand Trough Creek, Barking, UK
M. tachoensis	**Teb**	Fuente Tebia Spring, Asturias, Spain
M. tachoensis	**Pas**	Fonte dos Passarinhos Spring, Matacães, Portugal
M. tachoensis	**Amo**	Fonte dos Amores Spring, Quinta das Lágrimas, Coimbra, Portugal
M. tachoensis	**Ser**	Fonte da Nogueira Spring, Coimbra, Portugal
M. tachoensis	**Jor**	Nascente Sr. Jordao Spring, Alpedriz, Portugal
M. tachoensis	**Mou**	Nascente da Moura Spring, Alpedriz, Portugal
M. tachoensis	**Mat**	Spring in Freguesia de Salir de Matos, Caldas de Rainha, Portugal
M. tachoensis	**Gre**	Padre Antonio Spring at São Gregorio de Fanadia, Caldas de Rainha, Portugal
M. tachoensis	**Fue**	Spring beside the road N114 at Vila Nova da Babeca, Santarém, Portugal
M. tachoensis	**Ere**	Spring in Ereira, Santarém, Portugal

Species	*Loc. Code*	*Locality*
M. tachoensis	**Mam**	Spring in São Mamede, Leiria, Portugal
M. tachoensis	**Prg**	Buddle in Pragança, Lisboa, Portugal
M. tachoensis	**Eri**	Fonte do Cabo Spring, Ericeira, Portugal
M. tachoensis	**Tri**	Fonte dos Tritões Spring, Lisboa, Portugal
M. tachoensis	**Bom**	Spring in Valbom, Carvoeira, Portugal
M. tachoensis	**Mac**	Fonte da Ribeira Spring, Ribeira de Maciel, Portugal
M. tachoensis	**Lob**	Spring in Vale de Lobos, Lisboa, Portugal
M. tachoensis	**Ole**	Fonte de Oleiros Spring, Setúbal, Portugal
M. targouasensis	**Rbi**	Oum Rbii Spring, Khenifra, Morocco
M. targouasensis	**Rbi**	Spring flowing into Oued Oum Rbii, Morocco
M. targouasensis	**Ask**	Oued Assaka spring, Oued Noun, Morocco
M. targouasensis	**Mir**	Irrigation channel in North Mirheleft, Morocco
M. tensiftensis	**Ess**	Pond near Lahjar Spring, Essaouira, Morocco
M. tensiftensis	**Ess**	Ditch in Haddada Bouzerktoun, Essaouira, Morocco
M. tensiftensis	**Sid**	Ditch in Sidi Bouzid, near Chichaoua, Morocco
M. tensiftensis	**Aga**	Ditch in Agadir N'tachraft, S of Marrakech, Morocco
M. tensiftensis	**Lal**	Spring near Lalla Takerkoust Dam, Marrakech, Morocco
M. tensiftensis	**Tag**	Ditch in Talkount, NE of Marrakech, Morocco
M. veronicae sp. nov.	**Tam**	El Waha Spring, Oasis Waterfall, Tamerza, Tunisia
M. veronicae sp. nov.	**Ech**	Echbicka Spring, Echbika, Tozeur, Tunisia

Table 9. Match ratio of each of the species delimitation methods.

Delimitation Method	*Nmatch*	*Ndelimited*	*Nmorph*	*MR*
ABGD	11	12	14	**0.85**
GMYC	6	21	14	**0.34**
bPTP	6	24	14	**0.32**
mPTP	7	17	14	**0.45**

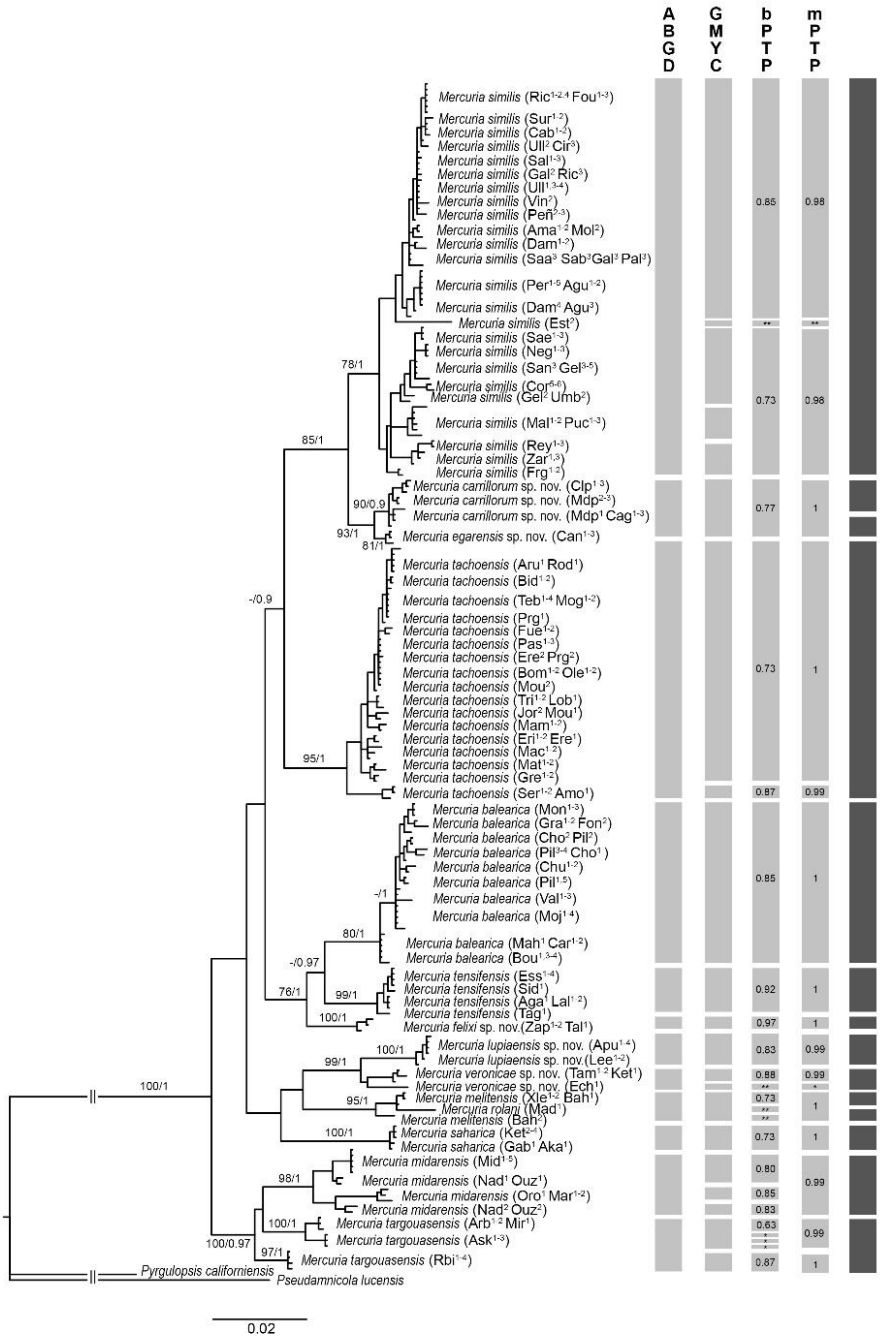


Figure 23. Bayesian phylogenetic tree of *Mercuria* species based on the combined dataset (COI, 16S and 28S). Bootstrap values and Bayesian posterior probabilities are provided above the branches when greater than 75% and 0.95, respectively.

3.5 Geometric morphometrics of shell shape

A total of 1,148 shells from 46 localities were analysed by GM. Morphological similarity among *Mercuria* species was then scored on the basis of this shell shape analysis. In the PCA (Figure 24), the first two components accounted for 75.3% of the variation in shell shape; inclusion of the third component increased the variation to 81.5%.

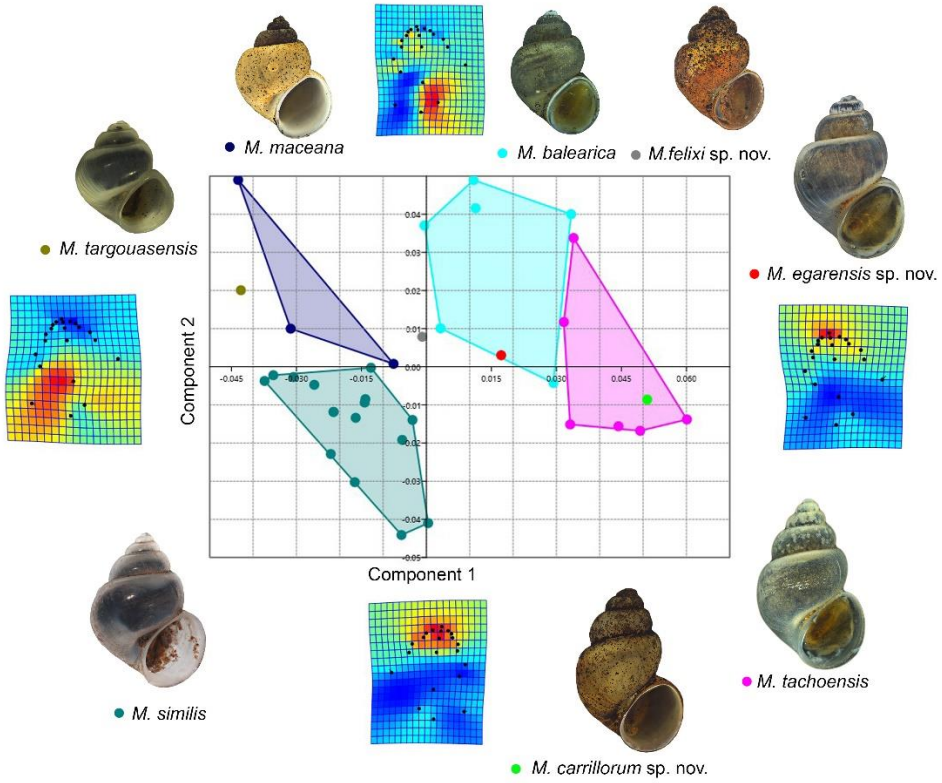


Figure 24. Principal components analysis plot depicting morphological similarity among *Mercuria* species based on 21 coordinates (8 landmarks and 13 semilandmarks). Each dot on the plot represents the consensus of the shape of each of the sampled localities.

Figure 23. Continuation. Codes next to the specimen names refer to the geographic location described in Table 8. Vertical bars on the right represent clusters identified by the molecular species delimitation methods ABGD, GMYC, bPTP and mPTP. * supported putative species; ** unsupported putative species. The dark vertical bars represent the identified morphospecies. Scale bar: expected change per site.

Although the first two components differentiated the species, similar shell shape patterns were observed for the species pairs formed by *M. balearica* and *M. egarensis* sp. nov. and *M. tachoensis* and *M. carrillorum* sp. nov. *Mercuria similis* and *M. maceana* populations formed distinct clusters and could be easily distinguished from the rest of the species.

In general, we can describe three clear shell shape patterns according to the components. The first component describes variation in the body whorl and the aperture against variation of the apex. In this sense, species vary from having a slender to sub-globose shape with a wider aperture and a compact body whorl to being more elongated towards the apex and with a narrower aperture (compare specimens of the left versus right side of Figure 24). The second component also describes variables that represent variations in the body whorl and aperture against the apex: specimens at the top of the plot have a wider body whorl and aperture and a shorter apex than those at the bottom of the plot.

3.6 Taxonomy of the genus *Mercuria*

Class Gastropoda Cuvier, 1795

Subclass Caenogastropoda Cox, 1960

Order Littorinimorpha Golikov & Starobogatov, 1975

Superfamily Truncatelloidea Gray, 1840

Family Hydrobiidae Stimpson, 1865

Subfamily Mercuriinae Boeters & Falkner, 2017

Genus *Mercuria* Boeters, 1971

Cyrniacana Fagot (1892): 23

Similiana Fagot (1892): 24

Anatiniana Fagot (1892): 24

Type species: *Amnicola confusa* Frauenfeld, 1863

Revised diagnosis:

Shell small to medium (2–6 mm), ovate-conic with a pointed apex; protoconch sculpture generally granulated; whorls convex with deep sutures; umbilicus narrow and perforated. Operculum corneous orange, thin, pliable, ellipsoidal, spiral, paucispiral, with a submarginal nucleus and no peg present. Animals

usually darkly pigmented. Snout prominent, with distal lobation. Cephalic tentacles with little pigmentation, eye lobe developed at the base. Foot intermediate, with lateral wings, anterior edge indented and posterior edge tapered. Well-developed ctenidium with 20–30 broad triangular filaments. Osphradium elongate, opposite to approximately the middle of the ctenidium. Radular central tooth with a "V" shaped central cusp and one pair of basal cusps. Renal oviduct coiled with three or more tight loops, unpigmented; one seminal receptacle positioned at the basal part of the bursa; bursa copulatrix pyriform to elongate, lying parallel to the albumen gland; bursal duct shorter than bursa. Penis gradually tapering, with a penial appendix. Prostate gland bean-shaped, connecting with the testis through a coiled duct.

Ecology: This genus occurs mainly in streams, ponds, rivers and springs with high conductivities, although some species present wide ecological plasticity, tolerating from very low to extremely high conductivity. In terms of elevation, *Mercuria* species can be found from sea level to ca. 1000 m.a.s.l.

Mercuria similis (Draparnaud, 1805)

(Figs 25–30)

Cyclostoma simile Draparnaud, 1805: 34, pl. 1, fig 15. Type locality: "*Galia meridionalis*"

Bythinia meridionalis Risso, 1826: 100. Type locality: "*de l'Europe méridionale et particulièrement de celles des environs de Nice et des Alpes Maritimes*" "*Fossés aquatiques. App. Printemps*"

Paludina similis (Draparnaud, 1805) – Turton & Gray, 1840

Hydrobia similis (Draparnaud, 1805) – Dupuy, 1851: 553. Type locality: "*les eaux tranquilles de la France méditerranéenne, les environs de Grasse, marais de la Népoule (Mouton), les environs de Montpellier (de Boissy)*"

Amnicola confusa Frauenfeld, 1863: 1029. Type locality: "*Galia meridionalis*"

Amnicola emiliana Paladilhe 1869: 229, pl. 19, figs 22, 23; 106, pl. 5. Type locality: "*Cette espèce se trouve dans un ruisseau d'eaux douces des environs de Balaruc (Hérault) […] des environs de Salces (Pyrénées-Orientales), de Vendrelle (Catalogne) et des San Giuliano près de Gènes.*" (Italie)." Syntype UM.PDL.003 (Breure and Audibert 2017)

Amnicola compacta Paladilhe, 1869. Type locality: "*vit aux environs d'Alicante (Espagne)*." Syntype UM.PLD.001 (Breure and Audibert 2017)

Paludina cerulea Massot, 1872: 128. Type locality: "*dans des eaux trés limpides qui coulent dans des rigoles, près l´étang de Salces, sur le point où la route nationale passe sous le chemin de fer du Midi*"

Amnicola roigiana Salvañá 1887: 141

Amnicola monjoi Chia, 1887: 14

Amnicola vallensana Almera & Bofill, 1898: 83; pl. III, figure 23

Pseudamnicola confusa (Frauenfeld, 1863) – Adam, 1940

Mercuria confusa (Frauenfeld, 1863) – Boeters, 1971: 178-179, figure 10. Type locality: "*Foux-de-Draguignan*"

Mercuria emiliana (Paladilhe, 1869) – Boeters, 1988: 208, figure 92, 93; 210, figure 118, 125; 211, pl. 3, figure 34.

Pseudamnicola similis (Draparnaud, 1805) – Gasull,1971: 45; pl. II, figure 8

Mercuria meridionalis (Risso, 1826) – Girardi, 2003(4): 83

Pseudamnicola emilianus (Paladilhe, 1869) – Boeters & Falkner, 2017

Type Material: NHMW 14715, designated as lectotype of C*yclostoma simile* by Boeters (1971) and posteriorly designated as neotype (NMW 92596) by Boeters and Falkner (2000).

Type locality: Not precisely specified in the original description by Draparnaud (1805). South France [*Galia Meridionalis*] is assumed to be the type locality of the species.

Material Examined:

France: Font Dame Spring, Salses-le-Château, Languedoc-Roussillon (FW2434); Font d'Estramar Spring, Salses-le-Château, Languedoc-Roussillon (FW2435); buddle in La Palme, Languedoc-Roussillon (FW2436); Stream near La Suriane, Étang de Berre, Provence-Alpes-Côte d'Azur (FW2473); Arc River near Les Cabanes, Étang de Berre, Provence-Alpes-Côte d'Azur (FW2474); La Foux-de-Draguignan Spring, Var, Provence-Alpes-Côte d'Azur (FW2575).

Spain: La Malahá Spring, Granada (FW2347); La Pucha Spring, Granada (FW2348); Spring Pilar de los Playeros, Villamartín, Cádiz (FW2352); La Zarza Spring, Villamartín, Cádiz (FW2353); El Berrueco Spring, Medina-Sidonia, Cádiz (FW2583); Platero Spring, Arcos de la Frontera, Cádiz (FW2516); Pozo

Amargo Spring, Puerto Serrano, Cádiz (FW2521); Alcalá de los Gazules, Cádiz (FW2522); El Algarrobo Spring, Algodonales, Cádiz (FW2540); La Salá Spring, Medina-Sidonia, Cádiz (FW2545-2546); Las Presillas Spring, Alcalá de los Gazules, Cádiz (FW2549); saltings of Riba de Santiuste, Guadalajara (FW2366); Alcolea Stream, tributary of Salado River, road from Imon to Santamera, Guadalajara (FW2368); Salado River at Santamera, Guadalajara (FW2369; FW2370); La Vega Stream at Saelices de la Sal, Guadalajara (FW2382); Las Negras ravine, Almería (FW2392); Cordovilla Saltings, Albacete (FW2398); La Salaboreja Spring, Casas de Vez, Albacete (FW2404); La Cañada Stream near La Salaboreja Spring, Casas de Ves, Albacete (FW2405); Stream feeding Galayo's Pond, Fuentealbilla, Albacete (FW2406); Stream in Barranco del Agua Salada, Gelsa, Zaragoza (FW2411); Saltwater stream near Aguinaliu, Huesca (FW2412); stream crossing the town of Peralta de la Sal, Huesca (FW2413); Ullals de Baltasar, Amposta, Tarragona (FW2440); Chícamo stream near La Umbría (FW2459); La Mula River, Murcia (FW2460); Fuente Amarga, Castellón (FW2463); Vinalopó River, Sax, Valencia (FW2464); Braña's Ravine, near L'Olla Beach, Alicante (FW2466); marsh at Peñíscola, Castellón, Valencia (FW2467); Irrigation ditch in Moli de la Font, Castellón, Valencia (FW2468); irrigation ditch in Cirat, Castellón, Valencia (FW2469); river in Sierra de la Muela, Murcia (FW2503); Puerto de la Cadena, Murcia (FW2504); Torres del Obispo Spring, Huesca (FW2512); Albufera of Majorca, Siquia de Son Senyor, Majorca (FW2598); Albufera of Majorca, Siquia d´en Moix, Majorca (FW2599); Font de Son Sant Joan, Muro, Majorca, Spain (FW2597); Estany de la Ricarda Pond, El Prat de Llobregat, Barcelona (FW2437).

Revised diagnosis: Shell ovate-conic; aperture obliquely broad ovate; periostracum whitish to grey; protoconch microsculpture granulated; central radular tooth formula (2)3–C–3(2)/1–1; female genitalia with bursa copulatrix pyriform to elongate, ca. 3 times longer than wide; seminal receptacle elongate with a short duct; penis darkly pigmented, gradually tapering; penial appendix triangular, variable in length (equal to or shorter/longer than the distal end of the penis), strongly pigmented at the junction with the penis; distal end of the penis triangular; nervous system pigmented, elongate (mean RPG ratio = 0.63); cerebral ganglia approximately equal in size.

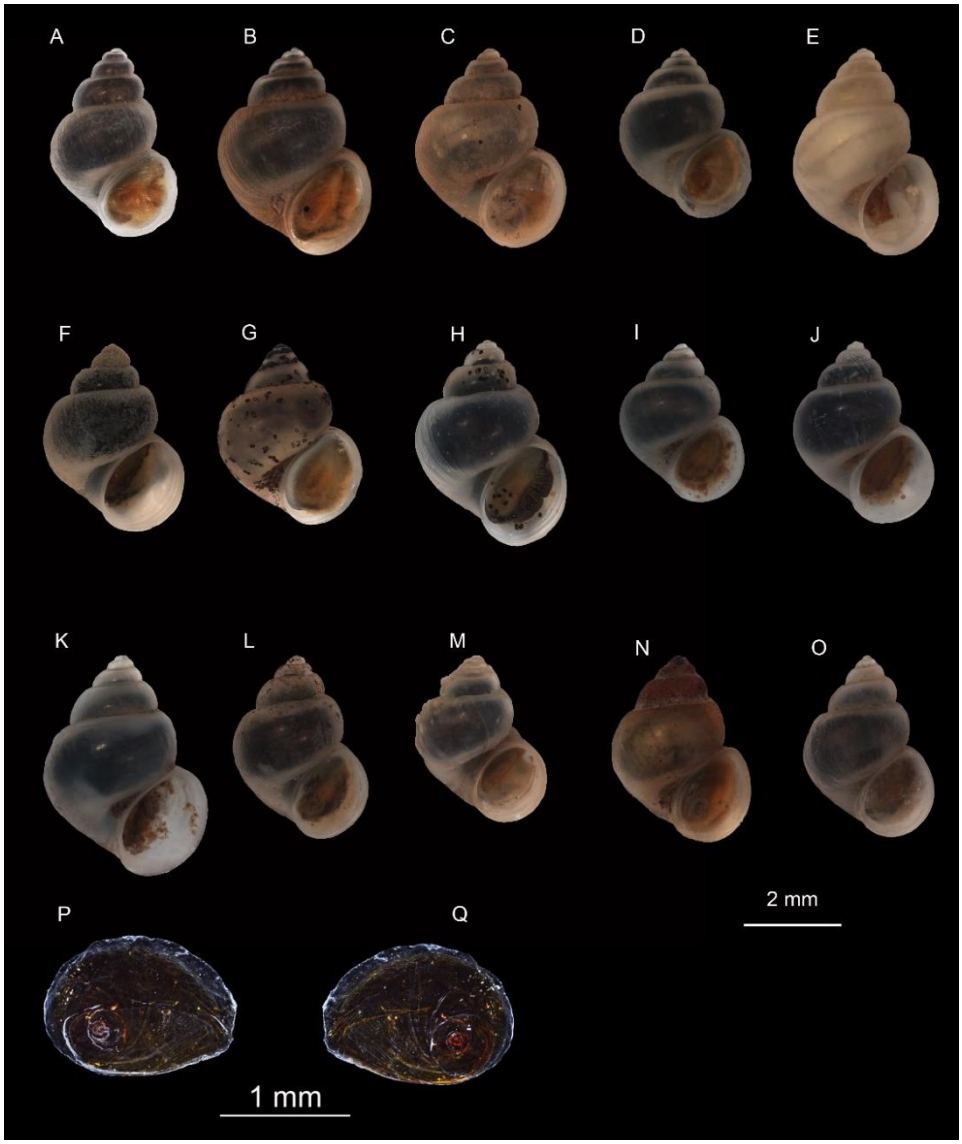


Figure 25. Intraspecific variation in the shell shape and colour of *M. similis*. **A**, FW2404 – Salaboreja Spring, Casa de Ves, Albacete, Spain; **B**, FW2405 – La Cañada Stream, Casa de Ves, Albacete, Spain; **C**, FW2406 – Stream feeding Galayo's Pond, Fuentalbilla, Albacete, Spain; **D**, FW2411 – Barranco del Agua Salada, Gelsa, Zaragoza, Spain (pigmented animal); **E**, FW2411 – Barranco del Agua Salada, Gelsa, Zaragoza, Spain (unpigmented animal); **F**, FW2412 – Saltwater stream near Aguinaliu, Huesca, Spain; **G**, FW2413 – stream in Peralta de la Sal, Huesca, Spain; **H**, FW2414 – Sosa River in Peralta de la Sal, Huesca, Spain; **I**, FW2434 – Fonte Dame, Salses-le-Château, Aude, France; **J**, FW2435 – Font d'Estramar, Salses-le-Château, Aude, France.

Description: Shell ovate-conic, whorls 4–5, height 3.5–5.7 mm, width 1.5–3.27 mm (Figures 25, 28A–O); periostracum whitish to grey; protoconch of 1.5 whorls, ca. 400 µm wide, nucleus ca. 200 µm wide; protoconch microsculpture granulated (Figure 26); teleoconch whorls very convex, separated by a deep suture; body whorl large, convex, occupying about two-thirds of the total shell length; aperture obliquely broad ovate, complete; inner lip thicker than outer lip; aperture margin straight, inner lip touching the shell wall; umbilicus narrow, not covered by the inner lip.

Operculum as for the genus, orange to brown, sometimes yellowish, about two whorls; muscle attachment oval, located near the nucleus (Figure 25P–Q).

Radula length medium, ca. 800 µm long (35% of total shell length), containing about 65 rows of teeth. Central tooth formula (2)3–C–3(2)/1–1, central cusp "V" shaped, cutting edge slightly concave (Figure 27B). Lateral tooth formula (3)2–C–2(3), central cusp "V" shaped and slightly longer than the central tooth one. Inner marginal teeth with 11–15 cusps (Figure 27C); outer marginal teeth with 12–25 cusps (Figure 27D). Radular data were collected from the following specimens: FW2352 – Spring Pilar de los Playeros, Villamartín, Cádiz, Spain; FW2392 – Las Negras ravine, Almería, Spain; FW2398 – Cordovilla Saltings, Albacete, Spain; FW2434 – Fonte Dame Spring, Salses–le–Château, Aude, France; FW2435 – Estramar Spring, Salses–le–Château, Aude, France; FW2436 – buddle in La Palme, Aude, France; FW2475 – La Foux–de–Draguignan Spring, France and FW2697 – Font de Son Sant Joan, Muro, Majorca, Spain.

Pigmentation and anatomy

Animal darkly pigmented, although unpigmented specimens were also found (Figure 25E); head and tentacles black, pigmentation lighter on eye lobes, snout and neck; snout about as long as wide, approximately parallel-sided, with medium distal lobation (Figure 29F). Ctenidium occupying almost the total length of the pallial cavity; 22–27 gill filaments; filaments broad, triangular, fused at the base by an epithelium (Figure 28E).

Figure 25. Continuation. **K**, FW2436 – Buddle in La Palme, Aude, France; **L**, FW2437 – Estany de la Ricarda, El Prat de Llobregat, Barcelona, Spain; **M**, FW2440 – Ullals de Baltasar, Amposta, Tarragona, Spain; **N**, FW2474 – Arc River near Les Cabanes, Bouches-du-Rhône, France; **O**, FW2475 – La Foux-de-Draguignan, France; **P**, operculum inner side; **Q**, operculum outer side.

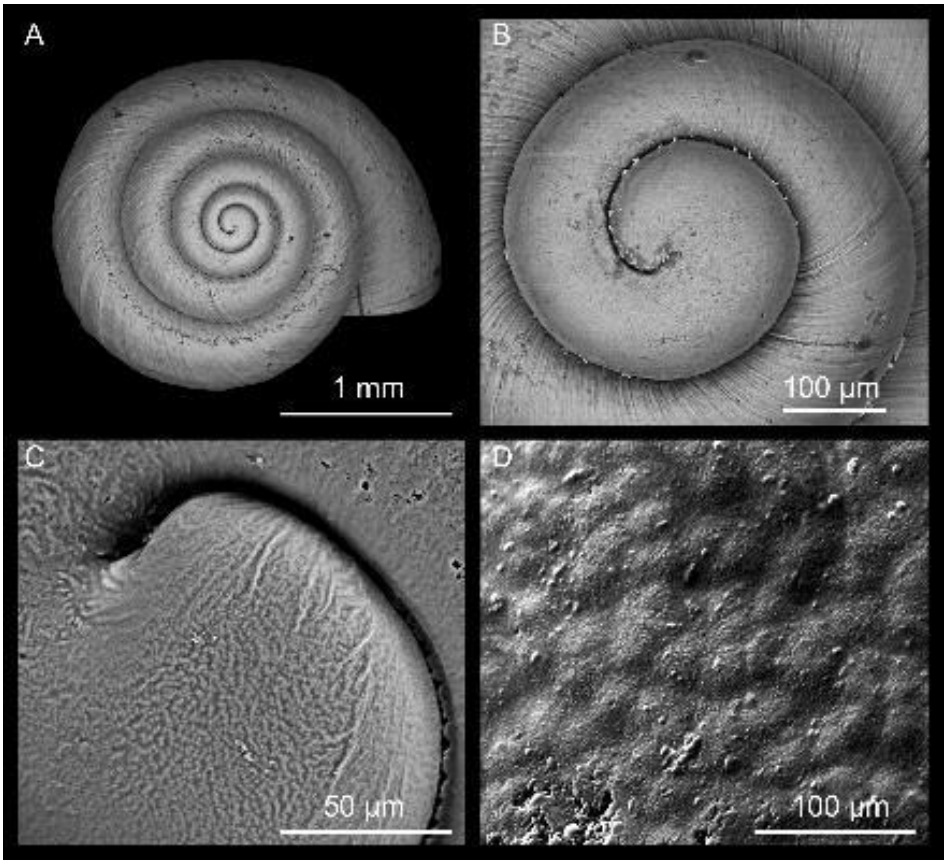


Figure 26. Shell of the *M. similis* specimen from Étang de Berre, Bouches-du-Rhone, France. **A**, apical view; **B**, details of the protoconch; **C**, protoconch nucleus; **D**, protoconch microsculpture.

Pallial tentacle present. Osphradium elongate, more than 3 times longer than wide, positioned opposite to the middle of the ctenidium. Stomach almost as long as wide with two chambers almost equal in size; style sac longer than wide, with the unpigmented intestine surrounding its distal end before continuing on as a straight rectum (Figure 28F).

Female genitalia with a glandular oviduct 2.5 times longer than wide; albumen gland longer than capsule gland (Figure 28); bursa copulatrix pyriform to elongate, ca. 3 times longer than wide; bursal duct shorter than bursa copulatrix; renal oviduct unpigmented, highly coiled with three loops; seminal receptacle elongate, with a short duct, positioned at the distal end of the renal oviduct just above the junction with the bursal duct (Figure 28A–D).

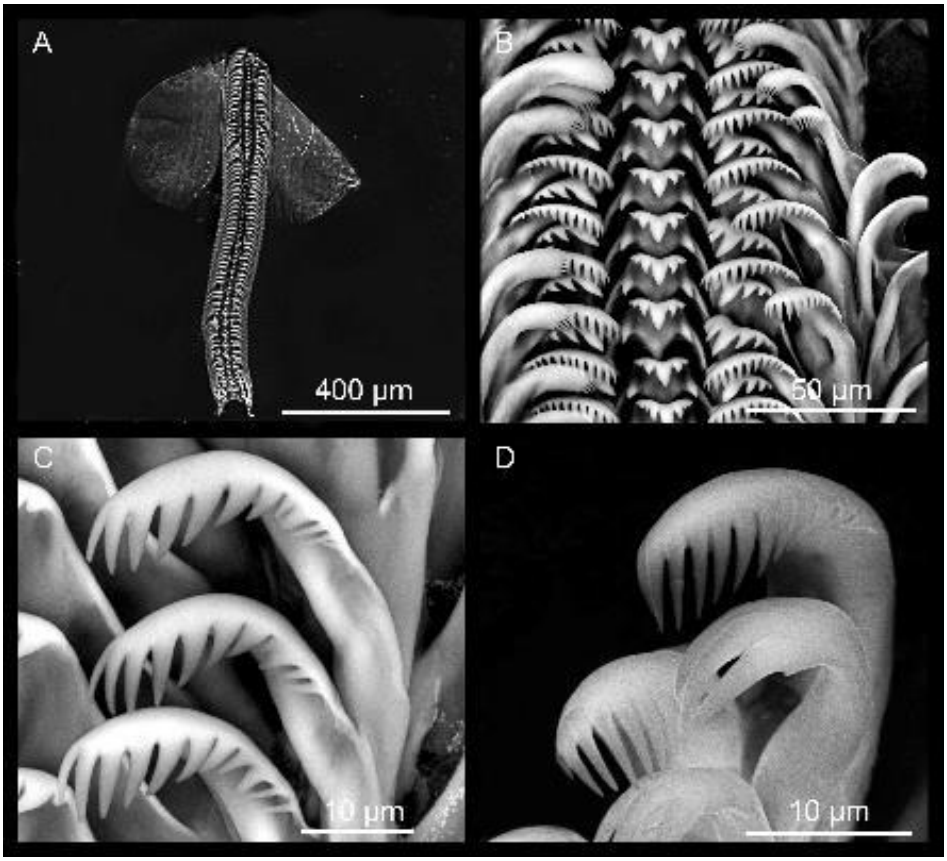


Figure 27. Radula of the *M. similis* specimen from Étang de Berre, Bouches-du-Rhone, France. **A**, general view of the radular ribbon; **B**, detailed view of the central, lateral, inner marginal and outer marginal teeth; **C** and **D**, detailed view of the inner and outer marginal teeth, respectively.

Male genitalia with a penis darkly pigmented, gradually tapering, attached to the neck behind the right eye; penial appendix longer (Figure 29A–C) or shorter (Figure 29D–F) than the distal end of the penis, triangular, strongly pigmented at the junction with the penis, pigmentation gradually weakens from the junction to the middle of the penial appendix where it is very weak. Penial appendix base narrow, medially positioned on the inner edge of the penis. Prostate gland bean-shaped, about 2 times longer than wide, connected by the posterior vas deferens to a convoluted seminal vesicle and the testis (Figure 29G–H).

Nervous system pigmented, elongate (mean RPG ratio = 0.63; see Table 23); cerebral ganglia approximately equal in size; pleuro-supraoesophageal connective ca. 9 times longer than the pleuro-suboesophageal one (Figure 28G).

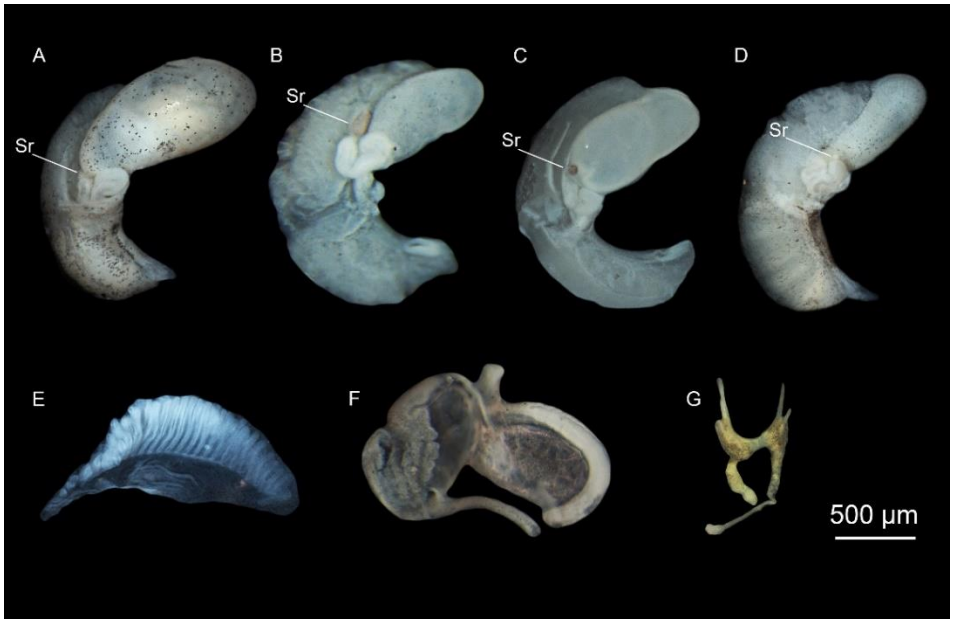


Figure 28. Anatomy of *M. similis*. A–D, female genitalia. A, FW2434 – Font Dame Spring; **B** and **C**, FW2406 – Stream feeding Galayo's Pond, Fuentalbilla, Albacete, Spain; **D**, FW2436 – buddle in La Palme, Aude, France; **E**, ctenidium and osphradium; **F**, stomach; **G**, perioesophageal ring. **Sr**, seminal receptacle.

Distribution: The species is distributed in springs, streams, small rivers, coastal lakes (étangs) and ditches near the Mediterranean coast of southern France, the Iberian Peninsula and North Africa and on the island of Majorca (Figure 30). It is also found near the Atlantic coast of the Iberian Peninsula, northern France, Great Britain and various Atlantic islands. We were unable to confirm previous records of the species for Great Britain (Baker et al. 1999, Abrehart and Forster 2012), the Azores islands (Wollaston 1878) and North Africa (Taybi et al. 2017). Some of these citations may have been incorrectly attributed to *M. similis* instead of *M. tachoensis*, which is found in, at least, Great Britain (see below).

Remarks: The material herein referred to as *M. similis* from southern France (Figure 25I–K, N, O) closely conform to the types of this species in terms of shell shape, size and colouration (for comparison with the type material, see figure 9 of Boeters and Falkner, 2017). On the basis of our morphological and phylogenetic results, we suggest that several previously described *Mercuria* species should now be considered as synonyms of the variable species *M. similis* (see discussion in Chapter 4), which increases the known geographic range and intraspecific variation of this species.

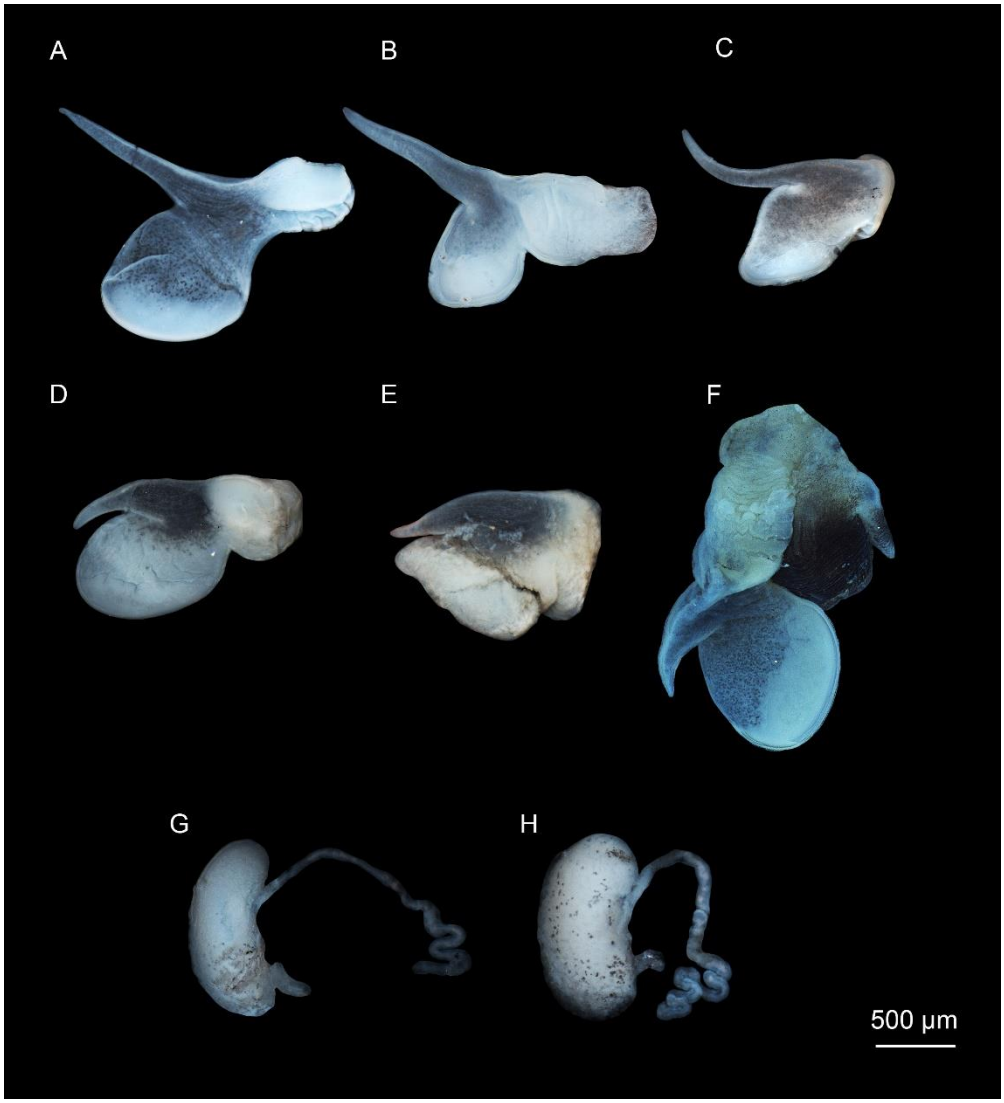


Figure 29. Male genitalia of *M. similis*. Penis: **A**, FW2405 – La Cañada Stream, Casa de Ves, Albacete, Spain; **B**, FW2435 – Font d'Estramar Spring, Salses-le-Château, Aude, France; **C,** FW2434 – Fonte Dame Spring, Salses-le-Château, Aude, France; **D**, FW2392 – Las Negras ravine, Almería, Spain; **E**, FW2413 – stream crossing the town of Peralta de la Sal. Huesca, Spain; **F**, FW2366 – saltings in Riba de Santiuste, Guadalajara, Spain; Prostate gland: **G**, FW2436 – buddle in La Palma, Aude, France; **H**, FW2435 – Font d'Estramar Spring, Salses-le-Château, Aude, France.

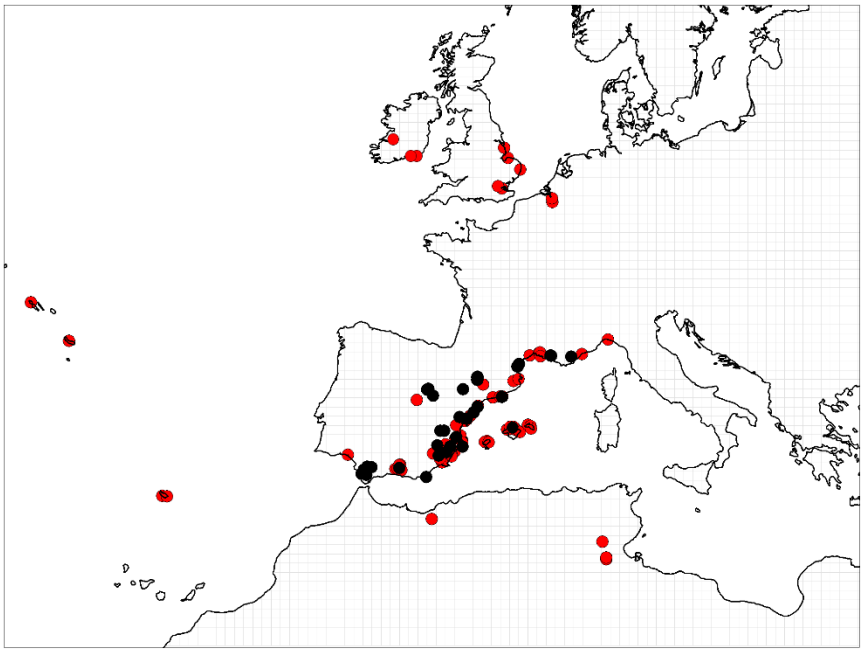


Figure 30. Distribution map of *M. similis* according to the localities from which *Mercuria* specimens were collected in this study (black dots) and those mentioned in the literature (red dots).

The species' moderate shell variation can be observed across its geographic range (Figure 24). However, the PCA assigned all populations identified as *M. similis* to this species, suggesting a low level of intraspecific variation. Most of the variation is attributed to differences in colour pattern and shell size. Although some individuals are orangish-brown [e.g., those from the La Cañada Stream (FW2405), Stream feeding Galayo's Pond (FW2406), the stream in Peralta de la Sal (FW2413) and the Arc River near Les Cabanes (FW2474), Figure 25B, C, G, N] the majority of the specimens have a whitish-grey periostracum.

Another character that affects the perception of shell colour is the colouration of the epithelium. Most specimens have a darkly pigmented epithelium, though a few have an unpigmented epithelium [i.e., individuals from Barranco del Agua Salada (FW2411), Figure 25E].

Table 10. Shell dimensions (mm) of *M. similis*: **1**, FW2434 – Fonte Dame, Salses-le-Château, Aude, France; **2**, FW2435 – Font d'Estramar, Salses-le-Château, Aude, France; **3**, FW2436 – buddle in La Palme, Aude, France; **4**, FW2474 – Arc River near Les Cabanes, Bouches-du-Rhône, France; **5**, FW2673 – Marsh of Pineda de Can Camins, El Prat de Llobregat, Barcelona, Spain; **6**, FW2404 – Salaboreja Spring, Casa de Ves, Albacete, Spain; **7**, FW2412 – Saltwater stream near Aguinaliu, Huesca, Spain. See Table 3 in Chapter 2 for a full list of abbreviations.

	1 Mean ± SD; CV (Max – Min) n=37	**2** Mean ± SD; CV (Max – Min) n=22	**3** Mean ± SD; CV (Max – Min) n=35	**4** Mean ± SD; CV (Max – Min) n=20	**5** Mean ± SD; CV (Max – Min) n=8	**6** Mean ± SD; CV (Max – Min) n=38	**7** Mean ± SD; CV (Max – Min) n=30
SL	3.55 ± 0.27; 0.08 (4.18-3.08)	3.71 ± 0.26; 0.07 (4.26-3.26)	3.74 ± 0.37; 0.1 (4.74-3.1)	4.05 ± 0.33; 0.08 (4.7-3.48)	3.76 ± 0.21; 0.06 (3.99-3.39)	3.71 ± 0.3; 0.08 (4.32 - 3.26)	4.01 ± 0.25; 0.06 (4.57-3.6)
SW	2.84 ± 0.2; 0.07 (3.33-2.45)	3.09 ± 0.18; 0.06 (3.41-2.71)	3.01 ± 0.23; 0.08 (3.66-2.63)	3.27 ± 0.26; 0.08 (3.79-2.73)	3.04 ± 0.15; 0.05 (3.23-2.81)	2.87 ± 0.17; 0.06 (3.18 - 2.49)	3.23 ± 0.2; 0.06 (3.57-2.85)
AL	2.65 ± 0.2; 0.08 (3.14-2.3)	2.88 ± 0.16; 0.06 (3.19-2.59)	1.88 ± 0.17; 0.09 (2.23-1.59)	2 ± 0.18; 0.09 (2.39-1.7)	1.91 ± 0.08; 0.04 (2.06-1.81)	1.85 ± 0.12; 0.06 (2.09 - 1.62)	2.07 ± 0.11; 0.05 (2.33-1.78)
AW	2.16 ± 0.16; 0.07 (2.51-1.88)	2.28 ± 0.13; 0.06 (2.52-1.96)	1.93 ± 0.17; 0.09 (2.3-1.62)	2.07 ± 0.19; 0.09 (2.44-1.77)	1.93 ± 0.08; 0.04 (2.04-1.8)	1.31 ± 0.09; 0.07 (1.46 - 1.18)	1.56 ± 0.1; 0.06 (1.7-1.32)
AH	1.83 ± 0.13; 0.07 (2.15-1.53)	1.95 ± 0.11; 0.06 (2.14-1.7)	1.4 ± 0.12; 0.09 (1.69-1.18)	1.48 ± 0.15; 0.1 (1.72-1.15)	1.43 ± 0.12; 0.08 (1.62-1.26)	1.84 ± 0.12; 0.07 (2.07 - 1.55)	2.08 ± 0.11; 0.05 (2.3-1.8)
LBW	1.79 ± 0.13; 0.07 (2.14-1.48)	1.9 ± 0.11; 0.06 (2.11-1.66)	2.79 ± 0.23; 0.08 (3.31-2.42)	3.04 ± 0.25; 0.08 (3.62-2.56)	2.8 ± 0.17; 0.06 (3.1-2.6)	2.75 ± 0.18; 0.07 (3.03 - 2.41)	3.04 ± 0.18; 0.06 (3.41-2.63)
WBW	1.35 ± 0.09; 0.07 (1.62-1.14)	1.48 ± 0.09; 0.06 (1.66-1.36)	2.27 ± 0.17; 0.07 (2.75-2.02)	2.49 ± 0.17; 0.07 (2.86-2.17)	2.26 ± 0.12; 0.05 (2.47-2.09)	2.22 ± 0.14; 0.06 (2.5 - 1.97)	2.46 ± 0.15; 0.06 (2.86-2.22)
WAW	1.23 ± 0.11; 0.09 (1.47-1.04)	1.28 ± 0.1; 0.08 (1.54-1.13)	1.34 ± 0.13; 0.1 (1.67-1.16)	1.43 ± 0.11; 0.08 (1.64-1.22)	1.32 ± 0.1; 0.08 (1.46-1.18)	1.29 ± 0.13; 0.1 (1.62 - 1.07)	1.35 ± 0.13; 0.1 (1.67-1.07)
WPW	0.67 ± 0.06; 0.09 (0.82-0.53)	0.65 ± 0.09; 0.14 (0.85-0.49)	0.71 ± 0.08; 0.11 (0.86-0.56)	0.76 ± 0.08; 0.11 (0.91-0.6)	0.69 ± 0.08; 0.12 (0.81-0.6)	0.69 ± 0.09; 0.13 (0.84 - 0.47)	0.72 ± 0.09; 0.13 (0.99-0.56)

Table 11. Dimensions of the osphradium, ctenidium and anterior digestive system (mm) in *M. similis*: **1**, FW2348 – Spring Fuente la Pucha, Granada, Spain; **2**, FW2405 – La Cañada Stream, Casa de Ves, Albacete, Spain; **3**, FW2406 – Stream feeding Galayo's Pond, Fuentalbilla, Albacete, Spain; **4**, FW2412 – Saltwater stream near Aguinaliu, Huesca, Spain; **5**, FW2434 – Fonte Dame, Salses-le-Château, Aude, France; **6**, FW2435 – Font d'Estramar, Salses-le-Château, Aude, France; **7**, FW2436 – buddle at La Palme, Aude, France; **8**, FW2369 – Salado Stream, Santamera, Guadalajara, Spain; **9**, FW2474 – Arc River near Les Cabanes, Bouches-du-Rhône, France; **10**, FW2475 – La Foux-de-Draguignan, France. See Table 3 in Chapter 2 for a full list of abbreviations

	1	**2** Mean ± SD; CV (Max – Min)	**3**	**4**	**5** Mean ± SD; CV (Max – Min)	**6**	**7** Mean ± SD; CV (Max – Min)	**8**	**9** Mean ± SD; CV (Max – Min)	**10** Mean ± SD; CV (Max – Min)	**11**
	n=1	n=3	n=1	n=1	n=2	n=1	n=2	n=1	n=2	n=3	n=1
CtL	1.196	1.48 ± 0.1; 0.07 (1.59-1.41)	1.624	0.881	1.42 ± 0.23; 0.16 (1.59-1.26)	0.975	1.42 ± 0.23; 0.16 (1.59-1.26)	1.087	1.21 ± 0.86; 0.71 (1.21-1.21)	1.49 ± 0.52; 0.35 (1.86-1.13)	0.765
OsL	0.308	0.66 ± 0.08; 0.12 (0.74-0.58)	0.536	0.241	0.59 ± 0.09; 0.15 (0.65-0.53)	0.313	0.59 ± 0.09; 0.15 (0.65-0.53)	0.231	0.38 ± 0.27; 0.71 (0.38-0.38)	0.49 ± 0.09; 0.18 (0.56-0.42)	0.287
OsW	0.082	0.1 ± 0.05; 0.5 (0.14-0.04)	0.159	0.048	0.12 ± 0.03; 0.25 (0.14-0.1)	0.061	0.12 ± 0.03; 0.25 (0.14-0.1)	0.067	0.06 ± 0.04; 0.67 (0.06-0.06)	0.08 ± 0.02; 0.25 (0.09-0.06)	0.076
StL		0.65 ± 0.04; 0.06 (0.67-0.62)		0.744	0.415	0.435		0.643	0.66 ± 0.07; 0.11 (0.71-0.61)	0.6 ± 0.02; 0.03 (0.61-0.58)	
StW		0.6 ± 0.07; 0.12 (0.65-0.55)		0.615	0.335	0.43		0.56	0.54 ± 0.01; 0.02 (0.55-0.53)	0.61 ± 0.09; 0.15 (0.67-0.54)	
SsL		0.64 ± 0.05; 0.08 (0.68-0.61)		0.717	0.402	0.5		0.606	0.63 ± 0.02; 0.03 (0.64-0.61)	0.66 ± 0.17; 0.26 (0.78-0.53)	
SsW		0.47 ± 0.06; 0.13 (0.52-0.43)		0.393	0.212	0.26		0.335	0.36 ± 0.05; 0.14 (0.39-0.33)	0.38 ± 0.07; 0.18 (0.43-0.34)	

Table 12. Female genitalia measurements (mm) recorded in *M. similis*: **1**, FW2352 – Spring Pilar de los Playeros, Villamartín, Cádiz, Spain; **2**, FW2405 – La Cañada Stream, Casa de Ves, Albacete, Spain; **3**, FW2406 – Stream feeding Galayo's Pond, Fuentalbilla, Albacete, Spain; **4**, FW2434 – Fonte Dame, Salses-le-Château, Aude, France; **5**, FW2435 – Font d'Estramar, Salses-le-Château, Aude, France; **6**, FW2436 – buddle in La Palme, Aude, France; **7**, FW2437 – Estany de la Ricarda, El Prat de Llobregat, Barcelona, Spain; **8**, FW2474 – Arc River near Les Cabanes, Bouches-du-Rhône, France; **9**, FW2475 – La Foux-de-Draguignan, France; **10**, FW2522 – Alcalá de los Gazules, Cádiz, Spain; **11**, FW2537 – Font d'Estramar, Salses-le-Château, Aude, France. See Table 3 in Chapter 2 for a full list of abbreviations.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
	Mean ± SD; CV (Max – Min) n=2	Mean ± SD; CV (Max – Min) n=2	Mean ± SD; CV (Max – Min) n=2	Mean ± SD; CV (Max – Min) n=2	n=1	Mean ± SD; CV (Max – Min) n=3	n=1	Mean ± SD; CV (Max – Min) n=3	n=1	n=1	n=1
AgL	1.3 ± 0.41; 0.32 (1.59-1.01)	1.05 ± 0.09; 0.09 (1.12-0.99)	1.2 ± 0.1; 0.08 (1.27-1.13)	0.88 ± 0.39; 0.44 (1.16-0.61)	0.903	1.06 ± 0.23; 0.22 (1.29-0.83)	0.813	0.85 ± 0.6; 0.71 (1.28-0.43)	0.606	0.465	1.418
CgL	1.05 ± 0.01; 0.01 (1.06-1.04)	0.71 ± 0.06; 0.08 (0.75-0.67)	0.87 ± 0.09; 0.1 (0.93-0.8)	0.61 ± 0.26; 0.43 (0.79-0.42)	0.889	1.24 ± 0.35; 0.28 (1.65-1)	0.795	0.97 ± 0.49; 0.51 (1.32-0.63)	0.941	0.604	1.239
Sr	0.15 ± 0.03; 0.2 (0.18-0.13)	0.13 ± 0.02; 0.15 (0.14-0.11)	0.13 ± 0.07; 0.54 (0.18-0.08)	0.15 ± 0.02; 0.13 (0.17-0.14)	0.086	0.18 ± 0.04; 0.22 (0.21-0.14)	0.141	0.15 ± 0.08; 0.53 (0.20-0.09)	0.111	0.063	0.206
BCL	0.98 ± 0.18; 0.18 (1.11-0.86)	0.85 ± 0.09; 0.11 (0.91-0.79)	1.06 ± 0.42; 0.4 (1.36-0.76)	1.16 ± 0.56; 0.48 (1.56-0.77)	0.638	1.05 ± 0.08; 0.08 (1.1-0.96)	0.885	0.84 ± 0.38; 0.45 (1.21-0.44)	0.773	0.498	1.215
BCW	0.35 ± 0.06; 0.17 (0.4-0.31)	0.37 ± 0.04; 0.11 (0.4-0.34)	0.43 ± 0.16; 0.37 (0.54-0.32)	0.54 ± 0.28; 0.52 (0.74-0.34)	0.18	0.63 ± 0.2; 0.32 (0.76-0.4)	0.472	0.51 ± 0.23; 0.45 (0.74-0.29)	0.482	0.203	0.582

Table 13. Male genitalia measurements (mm) recorded in *M. similis*: **1**, FW2352 – Spring Pilar de los Playeros, Villamartín, Cádiz, Spain; **2**, FW2392 – Las Negras ravine, Almería, Spain; **3**, FW2405 – La Cañada Stream, Casa de Ves, Albacete, Spain; **4**, FW2413 – stream in Peralta de la Sal, Huesca, Spain; **5**, FW2434 – Fonte Dame, Salses-le-Château, Aude, France; **6**, FW2435 – Font d'Estramar, Salses-le-Château, Aude, France; **7**, FW2436 – buddle in La Palme, Aude, France; **8**, FW2474 – Arc River near Les Cabanes, Bouches-du-Rhône, France; **9**, FW2475 – La Foux-de-Draguignan, France; **10**, FW2522 – spring in Alcalá de los Gazules, Cádiz, Spain; **11**, FW2537 – Font d'Estramar, Salses-le-Château, Aude, France; **12**, FW2597 –Font de Son Sant Joan, Muro, Majorca, Spain. See Table 3 in Chapter 2 for a full list of abbreviations.

	1 Mean ± SD; CV (Max – Min) n=2	**2** Mean ± SD; CV (Max – Min) n=2	**3** Mean ± SD; CV (Max – Min) n=3	**4** Mean ± SD; CV (Max – Min) n=2	**5** Mean ± SD; CV (Max – Min) n=2	**6** Mean ± SD; CV (Max – Min) n=2	**7** Mean ± SD; CV (Max – Min) n=2	**8** n=1	**9** n=1	**10** n=1	**11** n=1	**12** n=1
PrL	1.3 ± 0.11; 0.08 (1.38-1.22)	1.21 ± 0.05; 0.04 (1.25-1.18)	1.18 ± 0.02; 0.02 (1.19-1.17)	1.13 ± 0.8; 0.71 (1.13-1.13)	0.75 ± 0.15; 0.2 (0.85-0.64)	1.08 ± 0; 0 (1.08-1.08)	1.43 ± 1.01; 0.71 (1.43-1.43)	1.07	0.673	0.459	1.27	1.514
PrW	0.65 ± 0.04; 0.06 (0.67-0.62)	0.51 ± 0.06; 0.12 (0.56-0.47)	0.47 ± 0.04; 0.09 (0.5-0.44)	0.38 ± 0.27; 0.71 (0.38-0.38)	0.32 ± 0.07; 0.22 (0.38-0.27)	0.54 ± 0.01; 0.02 (0.55-0.54)	0.69 ± 0.49; 0.71 (0.69-0.69)	0.563	0.382	0.243	0.553	0.643
PL	1.03 ± 0.25; 0.24 (1.2-0.85)	0.88 ± 0.26; 0.3 (1.06-0.7)	1.19 ± 0.14; 0.12 (1.31-1.04)	1.09 ± 0.59; 0.54 (1.51-0.68)	0.99 ± 0.01; 0.01 (1-0.98)	1.16 ± 0.05; 0.04 (1.19-1.12)	0.91 ± 0.17; 0.19 (1.03-0.79)	0.824	0.604	0.519	1.205	0.96
PW	0.18 ± 0.04; 0.22 (0.21-0.15)	0.23 ± 0.02; 0.09 (0.24-0.21)	0.27 ± 0.04; 0.15 (0.32-0.25)	0.23 ± 0.04; 0.17 (0.25-0.2)	0.26 ± 0.01; 0.04 (0.26-0.25)	0.17 ± 0.02; 0.12 (0.18-0.15)	0.19 ± 0.03; 0.16 (0.21-0.17)	0.244	0.192	0.121	0.29	0.261
PaL	1.01 ± 0.04; 0.04 (1.03-0.98)	0.87 ± 0.05; 0.06 (0.9-0.83)	0.86 ± 0.39; 0.45 (1.31-0.62)	0.87 ± 0.27; 0.31 (1.06-0.68)	0.89 ± 0.01; 0.01 (0.9-0.89)	0.78 ± 0.08; 0.1 (0.84-0.72)	0.97 ± 0.08; 0.08 (1.02-0.91)	0.987	0.579	0.559	0.954	0.954
PaW	0.78 ± 0.19; 0.24 (0.91-0.64)	0.85 ± 0.11; 0.13 (0.92-0.77)	0.73 ± 0.12; 0.16 (0.83-0.59)	0.92 ± 0.43; 0.47 (1.22-0.62)	0.82 ± 0.14; 0.17 (0.92-0.71)	0.57 ± 0.07; 0.12 (0.62-0.52)	0.66 ± 0.05; 0.08 (0.7-0.62)	0.759	0.403	0.237	0.739	0.618

Unpigmented individuals were found cohabiting with pigmented ones. Additionally, the species presents low variability in shell size both within and among populations (Table 10), varying from 3.55–4.05 mm, although larger specimens (5.4–5.7 mm) were found in the saltings in Riba de Santiuste, Guadalajara (FW2366). This variability is likely influenced by environmental factors, such as flow rate and turbidity (Verhaegen et al. 2018) and by the relative age of the animal. Hydrobiids are generally univoltine (have one generation per year) and semelparous (breed only once in a lifetime). In the case of the population from Riba de Santiuste, we found animals from a previous generation living together with adults from the current one. The older specimens (i.e., those with a greater number of whorls) collected from this population were indeed the larger-sized adults.

Major anatomical differences are observed in penis features, with three penis morphotypes found in the populations examined: 1–distal end of the penis longer than the penial appendix (Figure 29A, B), 2–distal end of the penis shorter than the penial appendix (Figure 29E, F), 3–distal end of the penis and penial appendix about the same length (Figure 29C). This variation was observed within individual populations and thus does not correspond with any geographic distribution pattern. Holyoak et al. (2017) described the same phenomenon for *M. tachoensis*. These authors attributed the variation to allometric changes in the sexual maturity of the animals that occur during the reproductive season. Boulaassafer et al. (2018) found high variability of penis features in parasitized *M. tensiftensis* specimens. Nevertheless, in our case, none of the *M. similis* specimens collected were parasitized.

Mercuria similis can be distinguished from the phylogenetically closest species *M. egarensis* sp. nov. and *M. carrillorum* sp. nov. by its larger, more globose shell (Figure 24). It can be distinguished from the geographically closest species *M. balearica* by its larger, more globose shell and also its slightly smaller, often pigmented, penial appendix and from *M. tachoensis* by its distant geographic distribution (*M. tachoensis* lives in Atlantic coastal zones, whereas *M. similis* inhabits Mediterranean areas), granulated protoconch microsculpture and radula, which present one cusp less on the central and lateral teeth. *Mercuria similis* is closely related to all of the Iberian congeners: genetic divergence of COI ranged between 3.7% (with *M. egarensis* sp. nov.) and 7.5% (with *M. balearica*). It differs most with the Moroccan species *M. midarensis* with a divergence of 8.8%.

Ecology: Most of the examined specimens were found in waters with very high conductivities (592–28,900 µS/cm, average 6364 µS/cm). These localities are affected by the Keuper facies, Mesozoic evaporitic deposits that contain high

levels of NaCl and $CaSO_4$, among others, that dissolve into the superficial waters inhabited by the species. Specimens were most often found in the mud among the lower parts of the shoreline vegetation. Co-occurring gastropod species are *Melanopsis* spp., *Theodoxus* spp., *Belgrandia gibba*, *Hydrobia acuta*, *Pseudamnicola subproducta*, *Diegus gasulli* and *Potamopyrgus antipodarum.*

Mercuria tachoensis (Frauenfeld, 1865)

(Figs 31–35)

Amnicola tachoensis Frauenfeld, 1865: 529. Type locality: "Quellen des Tajo bei Ajuda"

M. tachoensis (Frauenfeld, 1865) – Boeters, 1988: 207, figures 99–103

M. edmundi Boeters, 1986

M. edmundi Boeters, 1986 – Boeters, 1988: 209, figures 96–98, Type locality "*2 km nördlich Burgau und ca. 10 km westsüdwestllich Lagos (Flußbett mit stehenden Wasserlachen)*"

M. edmundi Boeters, 1986 – Holyoak *et al.* 2017

M. bayonnensis (Locard, 1894) – Boeters and Falkner (2017) [in part]: 230, figure 6P "*Bidart, Pyrénées-Atlantiques"*

M. anatina (Poiret, 1801) – Boeters and Falkner (2017) [in part]: 239, figure 2 "*Oude Maas at Hoogvliet"*

Type Material: Syntypes: NHMW 113526–113531

Type locality: According to the original description, the type locality is a spring close to the Tagus River [Quellen des Tajo bei Ajuda] near Ajuda, Lisbon, Portugal.

Material Examined:

Portugal: Fonte dos Passarinhos Spring, Matacães (FW2420); Fonte dos Amores Spring, Coimbra (FW2477); Fonte da Nogueira Spring, Coimbra (FW2478); Nascente Sr. Jordão, Alpedriz Spring, Leiria (FW2480); Nascente da Moura Spring, Alpedriz, Leiria (FW2481); spring in Salir de Matos, Leiria (FW2482); Padre Antonio Spring, São Gregorio de Fanadia, Leiria (FW2483); spring along road N114 near the crossing to Moçarria and Vila Nova de Babeca, Santarém (FW2484); spring in Rua da Fonte, Ereira, Santarém (FW2485); spring in São Mamede, Leiria (FW2486); buddle in Pragança, Lisboa (FW2487); Fonte do Cabo

Spring, Ericeira, Lisboa (FW2488); Fonte dos Tritões Spring, Mafra, Lisboa (FW2489); spring in Valbom, Carvoeira, Lisboa (FW2490); Fonte da Ribera Spring, Ribera da Maciel Forro, Mafra (FW2491); spring in Vale de Lobos, Almargem do Bispo, Lisboa (FW2492); Fonte do Oleiros Spring, Oleiros, Setúbal (FW2493); Guadiana River, Baixo Alentejo near Pomarão (FW2502).

United Kingdom: Swanbourne Lake, Arundel (UGSB21168); Cuckold's Haven Wetland, reed belt of Hand Trough Creek, Barking (UGSB21193); Arun Banks, Burpharm, West Sussex (FW2669).

Spain: Fuente Tebia Spring, Camoca, Asturias (FW2449).

France: Stream near Chemin d'Elizaberry, Mouguerre (FW2593); stream near the beach in Bidart (FW2596).

The Netherlands: Small stream, Hoogvliet (UGSB23178); junction of the running surface in the tidal area, Hoogvliet (UGSB23179); wet reed land, Oude Maas River, Ruigeplaatbosch Park, Hoogvliet (UGSB23177).

Revised diagnosis: Shell ovate-conic; aperture obliquely and broadly ovate; protoconch microsculpture granulated; periostracum whitish to pale grey; central radular tooth formula (3)4–C–4(3)/1–1; female genitalia with bursa copulatrix elongate, ca. 4 times longer than wide; seminal receptacle pyriform; penis darkly pigmented; penial appendix longer than the distal end of the penis, strongly pigmented at the junction with the penis, pigmentation gradually weakens from the junction until two-thirds of the appendix where it is weak; penial appendix base narrow, distally positioned on the inner edge of the penis; nervous system pigmented, elongate (mean RPG ratio = 0.61); cerebral ganglia approximately equal in size.

Description: Shell ovate to ovate-conic, whorls 4–5, height 2–4 mm, width 2.3–2.8 mm (Figure 31A–D); periostracum yellowish to pale grey, occasionally dark brown; protoconch of 1.5 whorls, ca. 350 µm wide, nucleus ca. 180 µm wide; protoconch microsculpture pitted (Figure 32D); teleoconch whorls convex, separated by a deep suture; body whorl large, convex, occupying about two-thirds of the total shell length; aperture obliquely broad ovate, complete; inner lip thicker than outer lip; aperture margin slightly reflexed; inner lip attached to the body whorl; umbilicus narrow, not covered by the inner lip (Figure 31A, C, D).

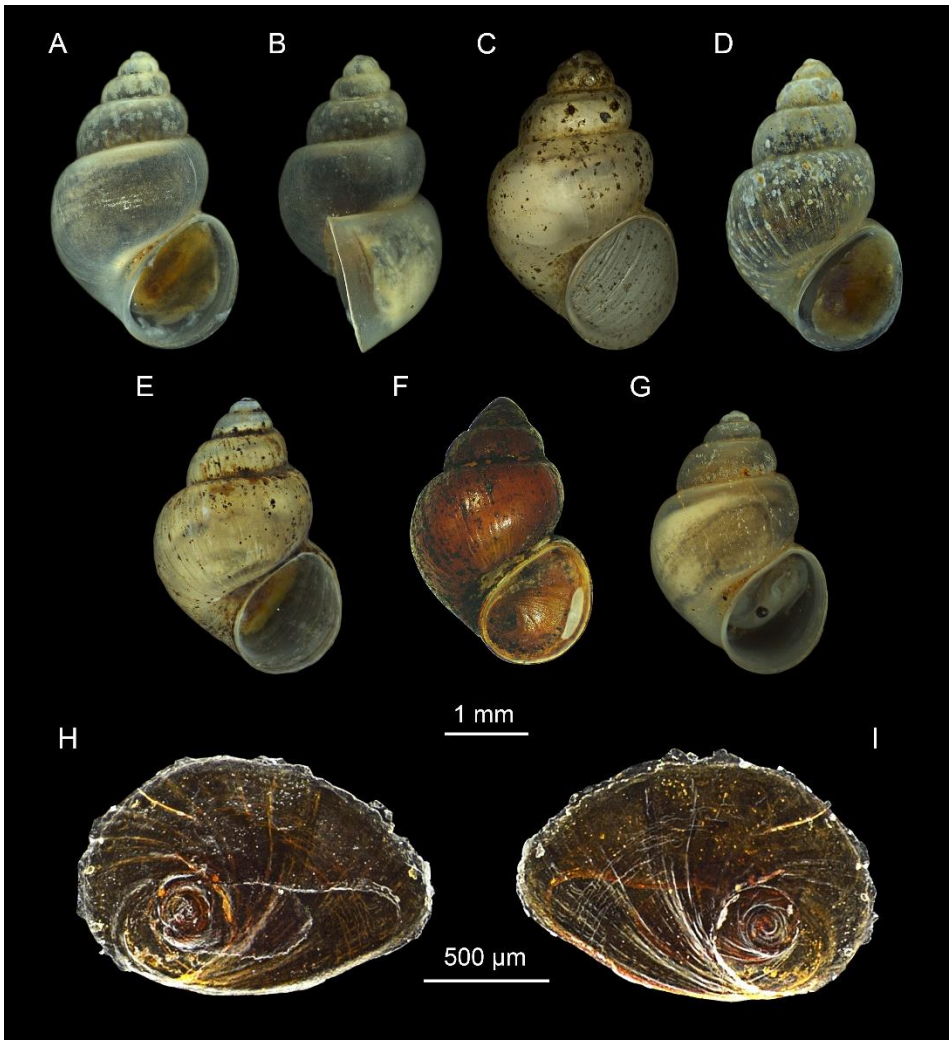


Figure 31. Shells and opercula of *M. tachoensis.* **A** and **B**, FW2485 – spring in São Mamede, Leiria, Portugal; **C**, UGSB23177 – wet reed land, Oude Maas River, Netherlands; **D,** FW2481 – Nascente da Moura Spring, Alpedriz, Leiria, Portugal; **E**, FW2669 – Arun Banks Burpharm, West Sussex, United Kingdom; **F**, FW2492 – spring in Vale de Lobos, Almargem do Bispo, Lisboa, Portugal; **G,** FW2478 – Fonte da Nogueira Spring, Coimbra, Portugal; **H**, operculum inner side; **I**, operculum outer side.

Operculum as for the genus, orange to brown, sometimes yellowish, about two whorls; muscle attachment oval, located near the nucleus (Figure 31E, F).

Radula length medium, ca. 800 µm long (35% of total shell length), containing about 65 rows of teeth (Figure 33A). Central tooth formula (3)4–C–4(3)/1–1, central cusp "V" shaped, cutting edge slightly concave (Figure 33B). Lateral tooth

formula (3)4–C–4(3), central cusp "V" shaped and slightly longer than the central tooth one. Inner marginal teeth with 12–17 cusps; outer marginal teeth with 17–27 cusps (Figure 33C, D).

Radular data were collected from the following specimens: FW2449 – Fuente Tebia Spring, Camoca, Asturias, Spain; FW2489 – Dos Tritões Spring, Mafra, Lisboa, Portugal; FW2490 – spring in Valbom, Carvoeira, Lisboa, Portugal; FW2493 – Do Oleiros Spring, Oleiros, Setúbal, Portugal; FW2593 – stream near Chemin d'Elizaberry, Mouguerre, France; FW2596 – stream near the beach in Bidart, France; FW2669 – Arun Banks Burpharm, West Sussex, United Kingdom.

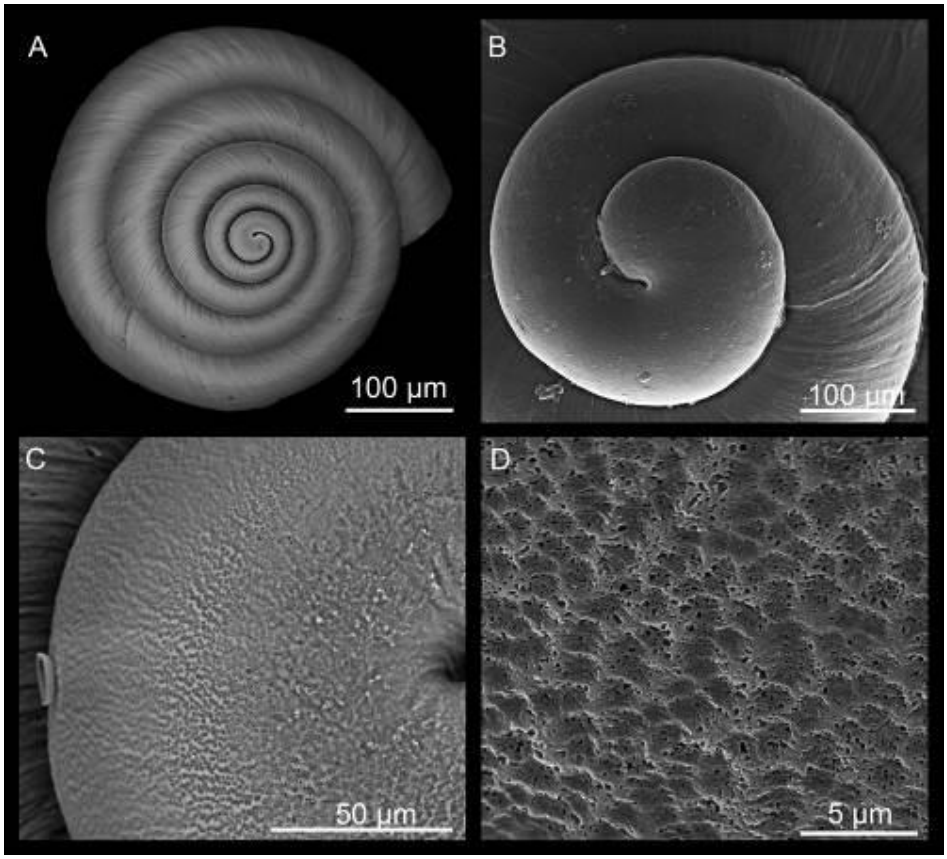


Figure 32. Shell of the *M. tachoensis* specimen from Spring Fonte dos Amores, Coimbra, Portugal. **A**, apical view; **B**, protoconch nucleus; **C**, details of the protoconch; **D**, protoconch microsculpture.

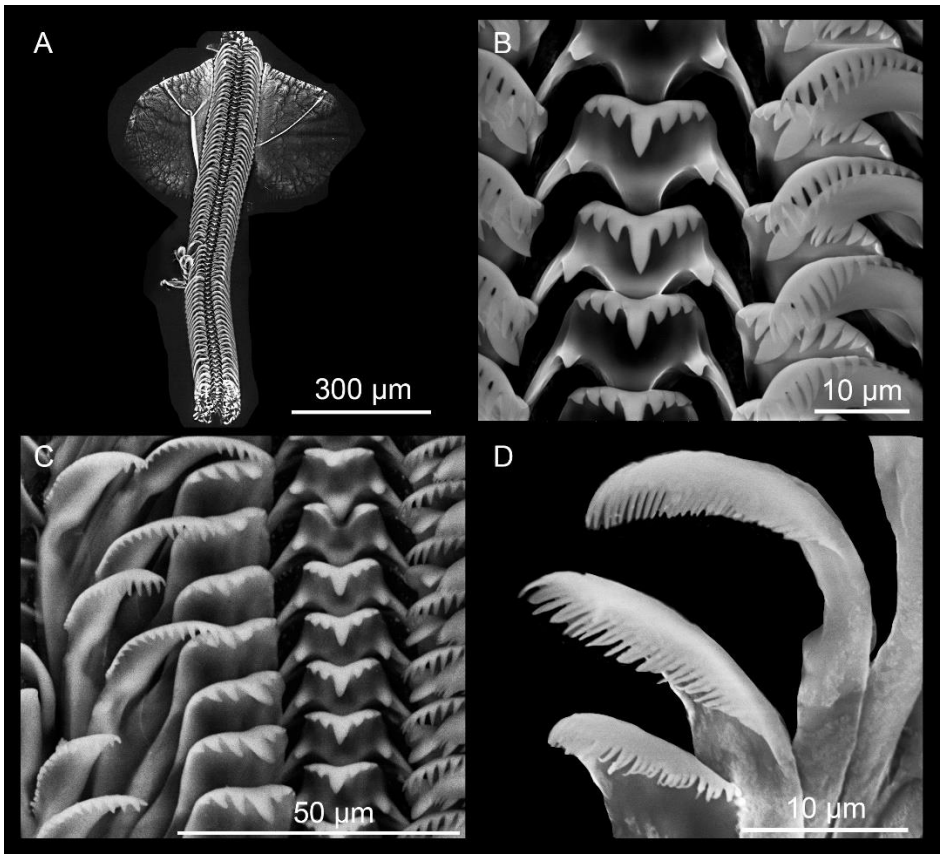


Figure 33. Radula of the *M. tachoensis* specimen from the stream near the beach in Bidart, France (FW2596). **A**, general view of the radular ribbon; **B**, detailed view of the central and lateral teeth; **C**, rows of radular teeth; **D**, detailed view of the outer marginal teeth.

Pigmentation and anatomy

Animal usually darkly pigmented, head and tentacles black to brown, pigmentation lighter on eye lobes and snout; snout about as long as wide, approximately parallel-sided, with medium distal lobation. Ctenidium occupying almost the total length of the pallial cavity; 20–26 gill filaments; filaments broad, triangular, fused at the base by an epithelium (Figure 34D). Pallial tentacle present. Osphradium elongate, more than 3 times longer than broad, positioned opposite to the middle of ctenidium.

Stomach almost as long as wide with two chambers almost equal in size; style sac longer than wide, with the unpigmented intestine surrounding its distal end before continuing on as a straight rectum (Figure 34E).

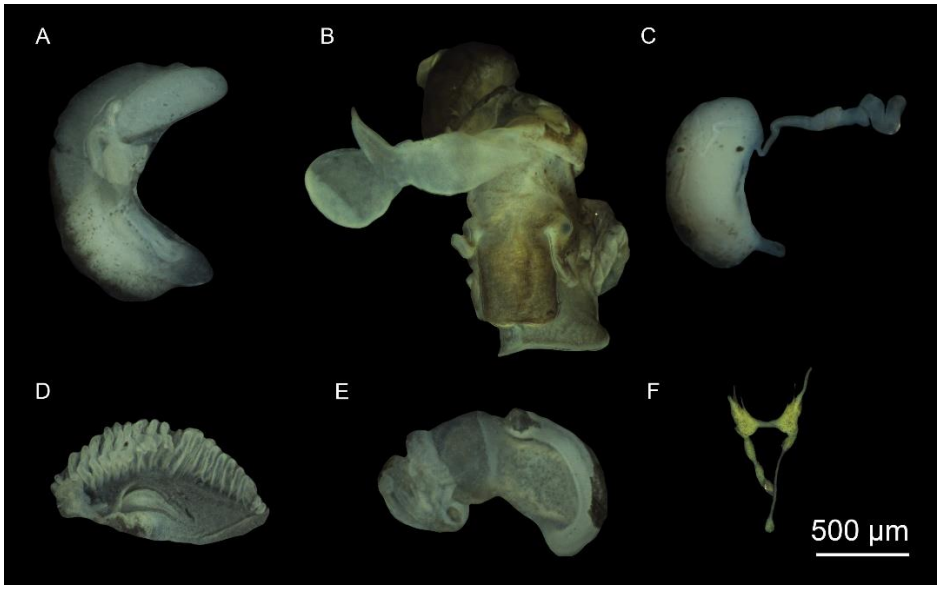


Figure 34. Anatomy of the *M. tachoensis* specimen from Nascente Sr. Jordão Spring, Alpedriz, Leiria, Portugal; **A**, female genitalia; **B**, penis; **C**, prostate gland; **D**, ctenidium and osphradium; **E**, stomach; **F**, perioesophageal ring.

Female genitalia with a glandular oviduct 2.5 times longer than wide; albumen gland and capsule gland about the same length (Figure 34A); bursa copulatrix elongate, ca. 4 times longer than wide; bursal duct shorter than bursa copulatrix; renal oviduct unpigmented, highly coiled with three loops; seminal receptacle pyriform, with a short duct, positioned on the distal part of the renal oviduct just above the junction with the bursal duct (Figure 34A).

Male genitalia with a bean-shaped prostate gland, about 2 times longer than wide, connected by the posterior vas deferens to a convoluted seminal vesicle and the testis. Pallial vas deferens emerge near both the anterior end of the prostate gland and the external margin of the penis (Figure 34C). Penis darkly pigmented, gradually tapering, attached to the neck behind the right eye; penial appendix longer than the distal end of the penis, strongly pigmented at the junction with the penis, pigmentation gradually weakens from the junction until two-thirds of the appendix where it is weak. Penial appendix base narrow, distally positioned on the inner edge of the penis (Figure 34B).

Table 14. Shell dimensions (mm) of *M. tachoensis*: **1**, FW2420 – Spring Fonte dos Passarinhos, Matacães, Portugal ; **2**, FW2477 – Spring Fonte dos amores, Coimbra, Portugal; **3**, FW2478 – Fonte da Nogueira Spring, Coimbra, Portugal; **4**, FW2481 – Fonte das Mouras, Alpedriz, Leiria, Portugal; **5**, FW2482 – spring in Sair de Matos, Leiria, Portugal ; **6**, FW2483 – Spring Fonte Padre Antonio, São Gregorio de Fanadia, Leiria, Portugal. See Table 3 in Chapter 2 for a full list of abbreviations.

	1 Mean ± SD; CV (Max – Min) n=30	**2** Mean ± SD; CV (Max – Min) n=30	**3** Mean ± SD; CV (Max – Min) n=18	**4** Mean ± SD; CV (Max – Min) n=31	**5** Mean ± SD; CV (Max – Min) n=7	**6** Mean ± SD; CV (Max – Min) n=24
SL	3.15 ± 0.16; 0.05 (3.43-2.82)	2.9 ± 0.19; 0.07 (3.35-2.48)	2.88 ± 0.26; 0.09 (3.3-2.3)	3.19 ± 0.2; 0.06 (3.58-2.8)	2.86 ± 0.23; 0.08 (3.21-2.55)	3.18 ± 0.26; 0.08 (3.69-2.84)
SW	2.29 ± 0.09; 0.04 (2.5-2.12)	2.08 ± 0.12; 0.06 (2.38-1.86)	2.1 ± 0.16; 0.08 (2.36-1.79)	2.25 ± 0.13; 0.06 (2.51-1.95)	2.13 ± 0.14; 0.07 (2.36-1.94)	2.46 ± 0.17; 0.07 (2.78-2.22)
AL	2.24 ± 0.09; 0.04 (2.44-2.09)	2.04 ± 0.13; 0.06 (2.37-1.71)	2.06 ± 0.15; 0.07 (2.31-1.77)	2.21 ± 0.13; 0.06 (2.48-1.9)	2.1 ± 0.14; 0.07 (2.35-1.94)	2.37 ± 0.18; 0.08 (2.71-2.09)
AW	1.77 ± 0.09; 0.05 (1.98-1.57)	1.61 ± 0.09; 0.06 (1.82-1.47)	1.62 ± 0.11; 0.07 (1.78-1.37)	1.71 ± 0.1; 0.06 (1.91-1.53)	1.71 ± 0.12; 0.07 (1.89-1.53)	1.92 ± 0.13; 0.07 (2.15-1.64)
AH	1.54 ± 0.07; 0.05 (1.67-1.37)	1.36 ± 0.09; 0.06 (1.55-1.11)	1.36 ± 0.11; 0.08 (1.55-1.12)	1.48 ± 0.08; 0.06 (1.68-1.34)	1.44 ± 0.08; 0.06 (1.56-1.34)	1.64 ± 0.11; 0.07 (1.83-1.41)
LBW	1.51 ± 0.07; 0.05 (1.64-1.36)	1.34 ± 0.08; 0.06 (1.53-1.1)	1.35 ± 0.12; 0.09 (1.53-1.06)	1.45 ± 0.09; 0.06 (1.63-1.29)	1.42 ± 0.09; 0.06 (1.56-1.31)	1.62 ± 0.12; 0.07 (1.82-1.4)
WBW	1.14 ± 0.05; 0.04 (1.26-1.01)	1.06 ± 0.06; 0.06 (1.19-0.93)	1.05 ± 0.08; 0.07 (1.15-0.87)	1.11 ± 0.06; 0.05 (1.22-0.98)	1.06 ± 0.08; 0.07 (1.18-0.97)	1.18 ± 0.09; 0.08 (1.36-1.04)
WAW	1.08 ± 0.05; 0.05 (1.19-0.97)	0.98 ± 0.07; 0.07 (1.11-0.87)	0.97 ± 0.08; 0.08 (1.1-0.79)	1.06 ± 0.07; 0.06 (1.17-0.93)	1.02 ± 0.08; 0.08 (1.14-0.93)	1.13 ± 0.09; 0.08 (1.33-1.01)
WPW	0.61 ± 0.05; 0.09 (0.75-0.51)	0.61 ± 0.06; 0.1 (0.73-0.51)	0.58 ± 0.08; 0.13 (0.71-0.42)	0.63 ± 0.05; 0.08 (0.73-0.53)	0.55 ± 0.08; 0.14 (0.65-0.39)	0.6 ± 0.06; 0.1 (0.75-0.52)

Table 14. Continuation Shell dimensions (mm) of *M. tachoensis*: **7**, FW2484 – Spring Vila Nova de Babeca, Santarém, Portugal; **8**, FW2485 – spring in Rua da Fonte, Ereira, Santarém, Portugal; **9**, FW2489 – Spring Fonte dos Tritões, Mafra, Lisboa, Portugal; **10**, FW2669 – Arun Banks Burpharm, West Sussex, UK. See Table 3 in Chapter 2 for a full list of abbreviations.

	7 Mean ± SD; CV (Max – Min) n=33	**8** Mean ± SD; CV (Max – Min) n=22	**9** Mean ± SD; CV (Max – Min) n=23	**10** Mean ± SD; CV (Max – Min) n=18
SL	3 ± 0.18; 0.06 (3.28-2.61)	3.32 ± 0.23; 0.07 (3.7-2.87)	2.09 ± 0.48; 0.23 (3.44-1.59)	3.45 ± 0.22; 0.06 (3.95-3.18)
SW	2.18 ± 0.11; 0.05 (2.33-1.9)	2.35 ± 0.15; 0.06 (2.58-2)	1.56 ± 0.3; 0.19 (2.42-1.28)	2.49 ± 0.13; 0.05 (2.84-2.26)
AL	2.11 ± 0.1; 0.05 (2.33-1.85)	2.29 ± 0.15; 0.07 (2.54-2)	0.99 ± 0.19; 0.19 (1.58-0.78)	1.54 ± 0.07; 0.05 (1.68-1.45)
AW	1.71 ± 0.09; 0.05 (1.85-1.52)	1.82 ± 0.12; 0.06 (2.12-1.64)	1.03 ± 0.19; 0.18 (1.63-0.82)	1.59 ± 0.08; 0.05 (1.75-1.49)
AH	1.42 ± 0.06; 0.04 (1.49-1.26)	1.53 ± 0.1; 0.07 (1.71-1.3)	0.72 ± 0.14; 0.2 (1.1-0.54)	1.16 ± 0.07; 0.06 (1.33-0.99)
LBW	1.38 ± 0.06; 0.04 (1.45-1.22)	1.5 ± 0.1; 0.07 (1.67-1.27)	1.55 ± 0.34; 0.22 (2.48-1.21)	2.46 ± 0.13; 0.05 (2.82-2.27)
WBW	1.09 ± 0.07; 0.07 (1.26-0.96)	1.2 ± 0.09; 0.07 (1.42-1.05)	1.17 ± 0.22; 0.18 (1.78-0.93)	1.92 ± 0.12; 0.06 (2.26-1.77)
WAW	0.99 ± 0.05; 0.05 (1.16-0.89)	1.11 ± 0.09; 0.08 (1.29-0.93)	0.74 ± 0.15; 0.21 (1.17-0.53)	1.2 ± 0.11; 0.09 (1.49-1.08)
WPW	0.64 ± 0.06; 0.09 (0.74-0.53)	0.68 ± 0.07; 0.11 (0.82-0.54)	0.4 ± 0.09; 0.23 (0.66-0.28)	0.7 ± 0.07; 0.1 (0.85-0.59)

Table 15. Dimensions of the osphradium, ctenidium and anterior digestive system (mm) in *M. tachoensis*: **1**, FW2420 – Spring Fonte dos Passarinhos, Matacães, Portugal; **2**, FW2485 – spring in Rua da Fonte, Ereira, Santarém, Portugal; **3**, FW2487 – buddle in Pragança, Lisboa, Portugal; **4**, FW2492 – spring in Vale de Lobos, Almargem do Bispo, Lisboa, Portugal; **5**, FW2593 – stream near Chemin d'Elizaberry, Mouguerre, France; **6**, FW2669 – Arun Banks Burpharm, West Sussex, UK. See Table 3 in Chapter 2 for a full list of abbreviations.

	1	**2** Mean ± SD; CV (Max – Min)	**3** Mean ± SD; CV (Max – Min)	**4**	**5**	**6** Mean ± SD; CV (Max – Min)
	n=1	n=2	n=2	n=1	n=1	n=2
CtL	0.811	1.05 ± 0; 0 (1.05-1.05)	1.16 ± 0.18; 0.16 (1.29-1.03)	1.044	0.434	1.16 ± 0.18; 0.16 (1.29-1.03)
OsL	0.35	0.38 ± 0.03; 0.08 (0.4-0.35)	0.37 ± 0.06; 0.16 (0.42-0.33)	0.361	0.215	0.37 ± 0.06; 0.16 (0.42-0.33)
OsW	0.122	0.11 ± 0.04; 0.36 (0.14-0.08)	0.7 ± 0; 0 (0.7-0.7)	0.087	0.063	0.7 ± 0; 0 (0.7-0.7)
StL		0.84 ± 0.1; 0.12 (0.91-0.77)	0.92 ± 0; 0 (0.92-0.92)	0.766		0.92 ± 0; 0 (0.92-0.92)
StW		0.83 ± 0.06; 0.07 (0.88-0.79)	0.87 ± 0.17; 0.2 (1-0.75)	0.685		0.87 ± 0.17; 0.2 (1-0.75)
SsL		0.79 ± 0.01; 0.01 (0.79-0.78)	0.75 ± 0.01; 0.01 (0.76-0.75)	0.582		0.75 ± 0.01; 0.01 (0.76-0.75)
SsW		0.47 ± 0.08; 0.17 (0.52-0.42)	0.54 ± 0.03; 0.06 (0.57-0.52)	0.362		0.54 ± 0.03; 0.06 (0.57-0.52)

Table 16. Female genitalia measurements (mm) recorded in *M. tachoensis*: **1**, FW2478 – Fonte da Nogueira Spring, Coimbra, Portugal; **2**, FW2480 – Nascente Sr. Jordão, Alpedriz, Leiria, Portugal; **3**, FW2485 – spring in Rua da Fonte, Ereira, Santarém, Portugal; **4**, FW2487 – buddle in Pragança, Lisboa, Portugal; **5**, FW2669 – Arun Banks Burpharm, West Sussex, UK. See Table 3 in Chapter 2 for a full list of abbreviations.

	1 Mean ± SD; CV (Max – Min) n=3	**2** Mean ± SD; CV (Max – Min) n=2	**3** Mean ± SD; CV (Max – Min) n=3	**4** Mean ± SD; CV (Max – Min) n=2	**5** Mean ± SD; CV (Max – Min) n=3
AgL	0.85 ± 0.19; 0.22 (1.01-0.64)	0.81 ± 0.19; 0.23 (0.94-0.68)	1.27 ± 0.05; 0.04 (1.3-1.21)	1.03 ± 0.22; 0.21 (1.18-0.87)	0.95 ± 0.16; 0.17 (1.11-0.8)
CgL	0.9 ± 0.13; 0.14 (1.00-0.75)	0.9 ± 0.11; 0.12 (0.98-0.82)	0.81 ± 0.02; 0.02 (0.83-0.79)	0.68 ± 0.02; 0.03 (0.69-0.66)	0.9 ± 0.06; 0.07 (0.96-0.85)
SR1L	0.09 ± 0.01; 0.11 (0.1-0.08)	0.14 ± 0; 0 (0.14-0.14)	0.09 ± 0; 0 (0.1-0.09)	0.11 ± 0; 0 (0.11-0.11)	0.12 ± 0.03; 0.25 (0.15-0.09)
BCL	0.6 ± 0.08; 0.13 (0.69-0.52)	0.61 ± 0.09; 0.15 (0.68-0.55)	0.89 ± 0.02; 0.02 (0.91-0.87)	0.69 ± 0; 0 (0.69-0.69)	0.62 ± 0.09; 0.15 (0.72-0.57)
BCW	0.23 ± 0.03; 0.13 (0.27-0.21)	0.23 ± 0.01; 0.04 (0.24-0.23)	0.26 ± 0.02; 0.08 (0.27-0.24)	0.28 ± 0.07; 0.25 (0.33-0.24)	0.19 ± 0.03; 0.16 (0.23-0.17)

Table 17. Male genitalia measurements (mm) recorded in *M. tachoensis*: **1**, FW2480 – Nascente Sr. Jordão, Alpedriz, Leiria, Portugal; **2**, FW2485 – spring in Rua da Fonte, Ereira, Santarém, Portugal; **3**, FW2487 – buddle in Pragança, Lisboa, Portugal; **4**, FW2492 – spring in Vale de Lobos, Almargem do Bispo, Lisboa, Portugal; **5**, FW2593 – stream near Chemin d'Elizaberry, Mouguerre, France. See Table 3 in Chapter 2 for a full list of abbreviations.

	1 Mean ± SD; CV (Max – Min) n=3	**2** n=1	**3** n=1	**4** n=1	**5** n=1
PrL	0.65 ± 0.13; 0.2 (0.78-0.52)	1.028	1.034	1.138	0.561
PrW	0.3 ± 0.03; 0.1 (0.34-0.27)	0.450	0.45	0.554	0.253
PL	0.42 ± 0.24; 0.57 (0.67-0.18)	0.544	0.545	0.768	0.317
PW	0.13 ± 0.07; 0.54 (0.18-0.05)	0.149	0.192	0.238	0.063
PaL	0.51 ± 0.26; 0.51 (0.68-0.22)	0.859	0.743	1.029	0.442
PaW	0.46 ± 0.2; 0.43 (0.58-0.23)	0.421	0.416	0.767	0.284

Nervous system slightly pigmented, elongate (mean RPG ratio = 0.61; see Table 23); cerebral ganglia approximately equal in size; pleuro-supraoesophageal connective ca. 7 times longer than the pleuro-suboesophageal one (Figure 34F).

Distribution: This species was previously recorded from springs and streams in Portugal. In this study, we report new records, extending its distribution to include northern Spain, northern France, the UK and the Netherlands (Figure 35). At these localities, the species was found in the tidal mud of small rivers near the river mouth. According to this extended distribution, *M. tachoensis* inhabits water bodies that have an Atlantic drainage. The type locality of the species has likely disappeared due to the extensive growth of the city of Lisbon, intensive exploitation of local aquifers and pollution. Recent surveys around Ajuda, Lisbon did not yield any new records (Rui da Costa Mendes, pers. comm.).

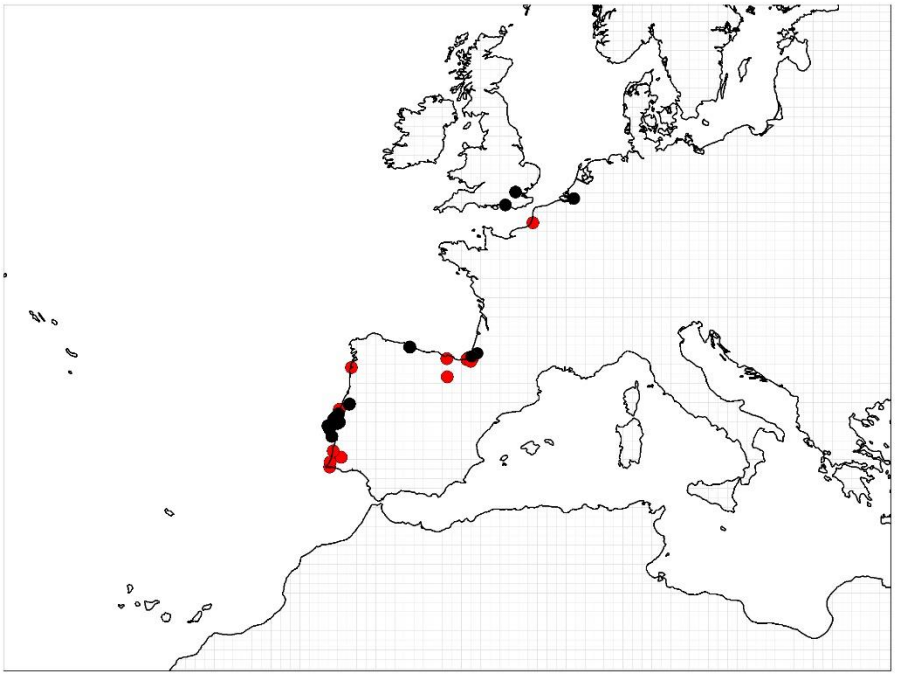


Figure 35. Distribution map of *M. tachoensis*. Black dots, localities from which *M. tachoensis* specimens were collected in this study; red dots, localities mentioned in the literature.

Remarks: The material herein referred to as *M. tachoensis* from Portugal (Figure 31 A, B, D, F, G) closely conform to the types of this species in terms of shell

shape, size and colouration. On the basis of our morphological and phylogenetic results, we suggest several synonymizations of other *Mercuria* species to *M. tachoensis* (see discussion in Chapter 4), which increases the previously known geographic range and intraspecific variation of this species.

The species' moderate shell variation can be observed across its geographic range (Figure 24). However, the PCA attributed all populations identified as *M. tachoensis* to this species, (although it is very closely related to *M. balearica*), suggesting a low level of intraspecific variation. This variability is due to differences in overall shell shape and colour pattern. Regarding shell shape, we found populations with only elongated ovate-conic shells [e.g., Spring Fonte dos Amores (FW2477), Fonte da Nogueira Spring (FW2478), spring in Rua da Fonte, Ereira (FW2485), Arun Banks (FW2669)] or only ovate-conic shells [e.g., spring in Salir de Matos (FW2482), Spring Fonte dos Tritões Spring (FW2489)]. We also observed populations with both shell morphotypes [e.g., Spring Fonte dos Passarinhos (FW2420), spring in Vale de Lobos (FW2492), Spring Fonte do Oleiros (FW2493)]. Colour pattern does not show high intraspecific variation: only the specimens from the spring in Vale de Lobos (FW2492) are remarkable for being particularly darkly coloured with a dark brown thick periostracum (Figure 31F) compared with the rest of the specimens, which have yellowish-brown periostracums.

Anatomically, the bursa copulatrix is about the same size in all of the analysed specimens of the species, except for those from Fonte da Nogueira Spring (FW2378) and Nascente Sr. Jordão (FW2480), which are considerably smaller (Table 16). Males from the stream near Chemin d'Elizaberry (FW2593) and Nascente Sr. Jordão, Alpedriz (FW2480) have the smallest prostate glands and penises. Despite this, all males present the same penis characteristics: a rounded, pigmented penial appendix that is longer than the distal end of the penis. However, Boeters (1988) described the penis as longer than the penial appendix. Based on this discrepancy in the size and shape of the male genitalia, Holyoak et al. (2017) proposed *M. edmundi* as junior synonym of *M. tachoensis* and described an allometric growth of the male genitalia related to the sexual maturity of the animals. Here, we confirmed that only one species occurs in the localities where both species have been cited.

Mercuria tachoensis differs from all the Iberian congeners by its pitted protoconch microsculpture (which is granulated in the rest of the species). In addition to this character, it differs from the phylogenetically closely related species *M. similis* and *M. egarensis* sp. nov. by often having one more cusp in the central radular

teeth and a longer bursa copulatrix (ca. 4 times longer than wide). Although our PCA (Figure 24) was unable to differentiate *M. tachoensis* and *M. carrillorum* sp. nov. by shell shape, both species can be distinguished by differences in the radula (smaller in *M. carrillorum* sp. nov. and often with one cusp less in the lateral teeth), the bursa copulatrix (smaller in *M. carrillorum* sp. nov.) and the male genitalia (the distal end of the penis is longer than the penial appendix, which is flattened, ovate and less pigmented in *M. carrillorum* sp. nov.).

Genetic divergence estimates indicated that *M. tachoensis* is closely related to all the Iberian congeners. Divergence for COI (uncorrected pairwise distance) between *M. tachoensis* and other species was 7.7% with *M. egarensis* sp. nov., 7.9% with *M. carrillorum* sp. nov., 7.4% with *M. felixi* sp. nov. and 8% with *M. similis. Mercuria tachoensis* differed most with *M. saharica* with a divergence of 9.1%.

Ecology: *Mercuria tachoensis* presents a lower conductivity tolerance than *M. similis*. The analysed populations of this species from Spain, Portugal and France inhabit springs, streams and buddles with conductivities from 359 to 1805 µS/cm. Water parameters of the localities in the UK and Netherlands were not available. Specimens were usually found inside the water. Amphibious behaviour has not been commonly observed in this species, except for populations located on the tide banks of the Guadiana River (Dr David T. Holyoak, pers. comm.) and on those of the Arun River (Dr Martin Willing, pers. comm.). The species occurs sympatrically with the gastropod species *Theodoxus* spp., *Belgrandia* spp. and *Potamopyrgus antipodarum.*

Mercuria balearica (Paladilhe, 1869)

(Figs 36–41)

Amnicola balearica Paladilhe, 1869:113. Type locality: “de Port-Mahon (îles Baléares)”

M. balearica (Paladilhe, 1869) – Boeters, 1988:207, figures 106 and 107

Type Material: Syntypes UM.PLD.005 (Breure and Audibert 2017)

Type locality: According to the original description, the type locality is Port of Maho, Minorca, Balearic Islands [Port-Mahon (îles Baléares)].

Material Examined:

Spain: Colarsega River, Port of Maho, Minorca (FW2603); stream near Sant Joan de Carbonell, Minorca (FW2604); Cala en Porter, Minorca (FW2605);

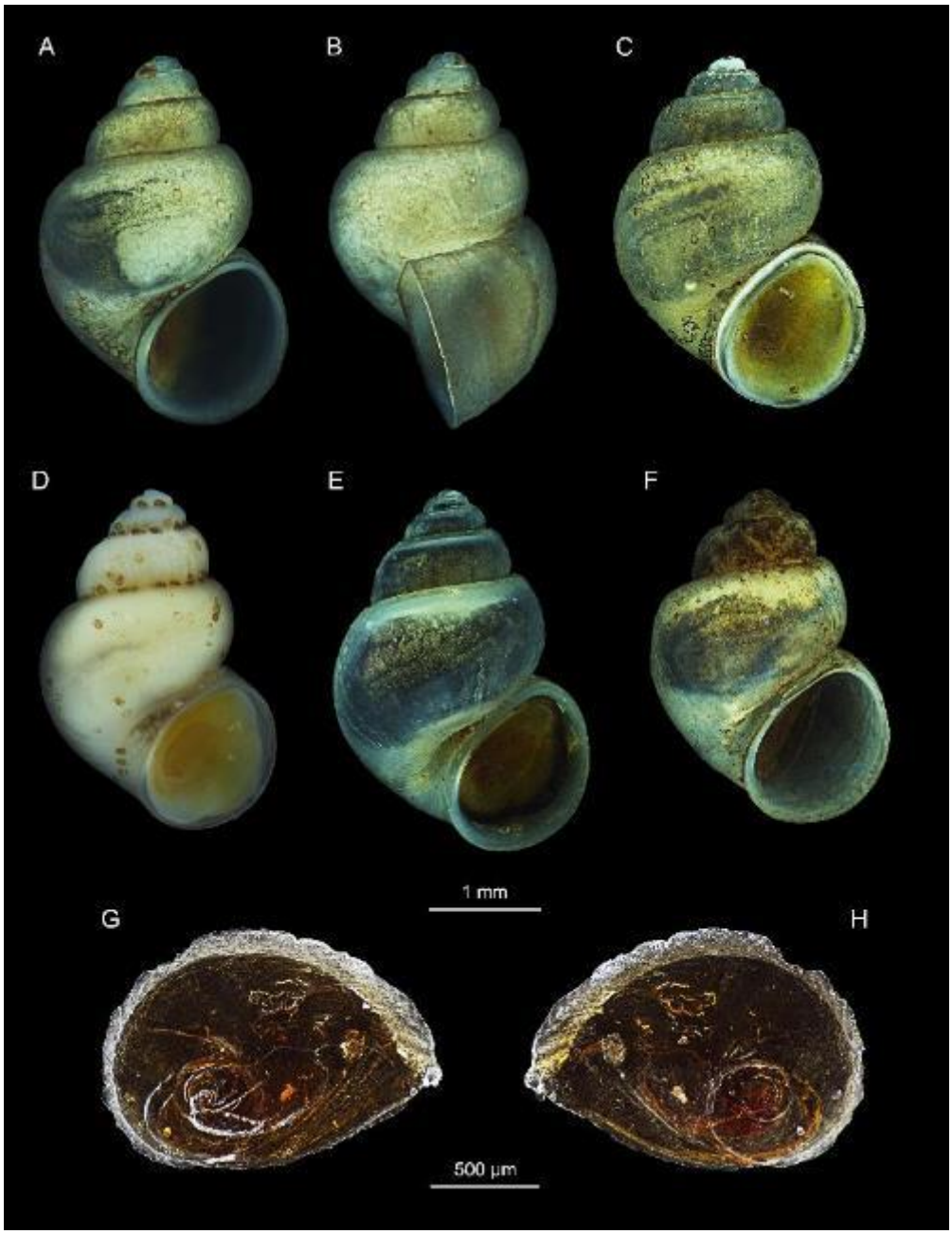


Figure 36. Shells and operculum of *M. balearica*. **A** and **B**, FW2604 – stream near Sant Joan de Carbonell, Minorca, Spain; **C** and **D**, FW2395 – Arabic Spring , Mojácar, Granada, Spain (pigmented and unpigmented specimens); **E**, FW2354 – Venta El Pilar Spring, Málaga, Spain; F, FW2357 – Valentín Spring, Alozaina, Cádiz, Spain; **G**, operculum inner side; **H**, operculum outer side.

Barranc de Bec, Son Bou , Minorca (FW2609); El Chorro Spring, Ardales, Málaga (FW2249); spring in Montecorto, Málaga (FW2236 and FW2351); trough in Churriana, Málaga (FW2309); Venta El Pilar Spring, Málaga (FW2322 and FW2354); trough in Los Granados, Málaga (FW2335); stream in Montecorto, Málaga (FW2350); Valentín Spring, Alozaina, Cádiz (FW2357); stream in Caño de la Rambla, Fontanar, Jaén (FW2386); Arabic Spring , Mojácar, Granada (FW2395); Cinco Caños Spring, Peal del Becerro, Jaén (FW2568); spring in Sierra de la Muela, Murcia (FW2503); Pantaneta de Getares Spring, Algeciras, Cádiz (FW2582).

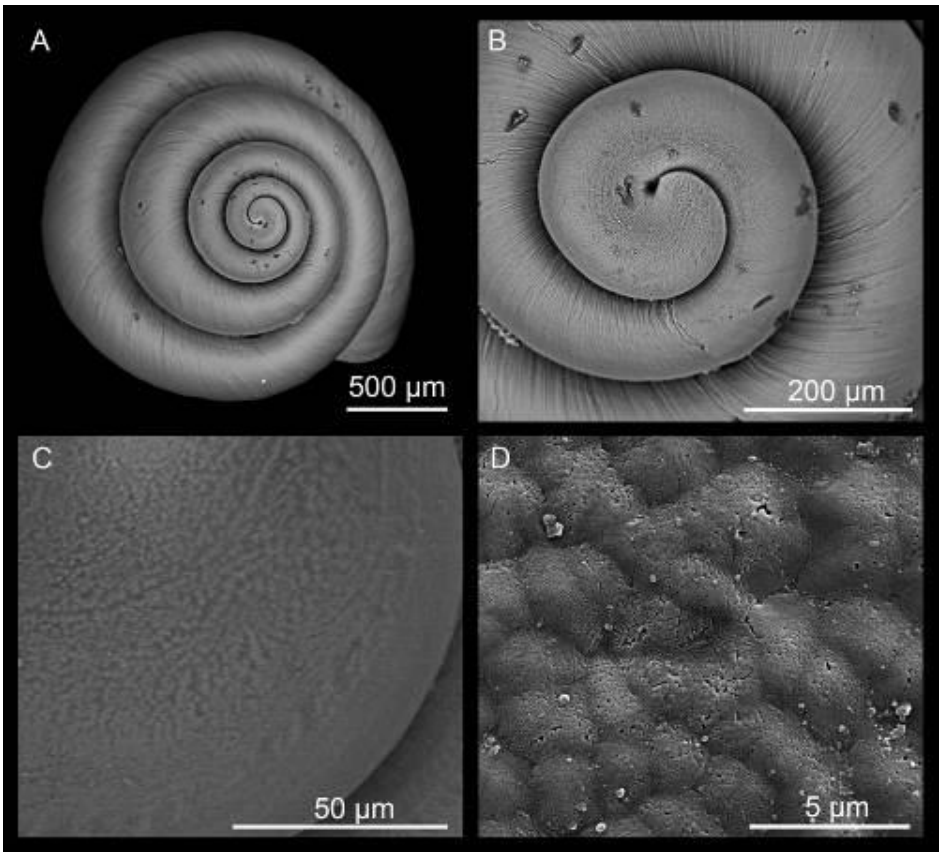


Figure 37. Shell of the *M. balearica* specimen from Venta El Pilar Spring, Málaga, Spain. **A**, apical view; **B**, details of the protoconch; **C**, details of the protoconch nucleus; **D**, protoconch microsculpture.

Revised diagnosis: Shell ovate-conic; aperture obliquely broad ovate; protoconch microsculpture granulated; central radular tooth formula (3)4–C–4(3)/1–1; glandular oviduct 2.5 times longer than wide; albumen gland longer than capsule gland; bursa copulatrix pyriform to elongate; seminal receptacle elongate, with a

short duct; penis darkly pigmented, as long as the penial appendix; penial appendix unpigmented or slightly pigmented at the junction with the penis, rounded, small, base narrow, medially positioned on the inner edge of the penis; nervous system pigmented, elongate (mean RPG ratio = 0.53); cerebral ganglia approximately equal in size.

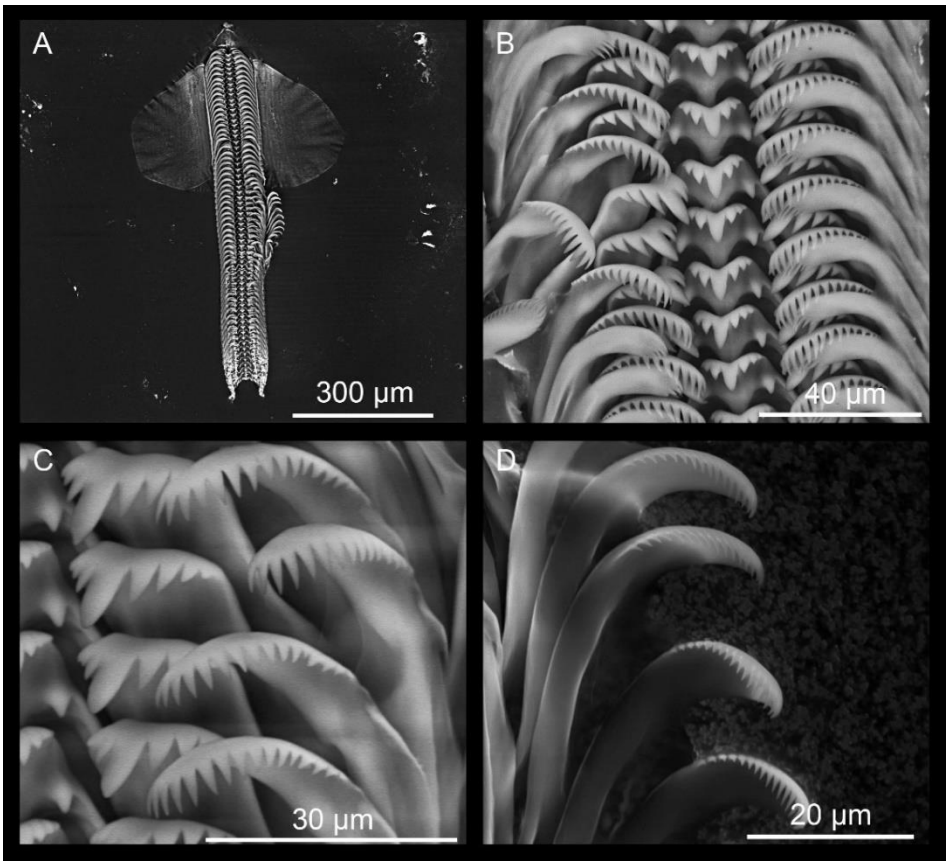


Figure 38. Radula of the *M. balearica* specimen from the stream near Sant Joan de Carbonell, Minorca. **A**, general view of the radular ribbon; **B**, detailed view of the central, lateral and marginal teeth; **C**, detailed view of the lateral and inner marginal radular teeth; **D**, detailed view of the outer marginal teeth.

Description: Shell ovate-conic, whorls 4–5, height 2–3.6 mm, width 1.4–2.6 mm (Figure 36A–F); periostracum yellowish to brown; protoconch of 1.5 whorls, ca. 350 µm wide, nucleus less than 200 µm wide; protoconch microsculpture granulated (Figure 37A–D); teleoconch whorls convex, separated by a deep suture; body whorl large, convex, occupying about two-thirds of the total shell length of the shell; aperture obliquely broad ovate, complete; inner lip thicker than

outer lip; aperture margin straight, inner lip touching the shell's wall; umbilicus narrow, not covered by the inner lip (Figure 36).

Operculum as for the genus, orange to brown, sometimes yellowish, about two whorls; muscle attachment oval, located near the nucleus (Figure 36G, H).

Radula length medium, ca. 800 µm long (35% of total shell length), containing about 65 rows of teeth. Central tooth formula (3)4–C–4(3)/1–1, central cusp "V" shaped, cutting edge slightly concave (Figure 38). Lateral tooth formula (3)4–C–4(3), central cusp "V" shaped and slightly longer than the central tooth one. Inner marginal teeth with 14–18 cusps; outer marginal teeth with 15–17 cusps (Figure 38C, D).

Radular data were collected from the following specimens: FW2604 – stream near Sant Joan de Carbonell, Minorca; FW2609 – Barranc de Bec, Son Bou, Minorca; FW2354 – Venta El Pilar Spring, Málaga; FW2395 – Arabic Spring, Mojácar, Granada; FW2568 – Spring Fuente de los Cinco Caños, Peal del Becerro, Jaén.

Figure 39. Anatomy of *M. balearica.* **A**, **D**, **F**, FW2604 – stream near Sant Joan de Carbonell, Minorca, Spain; **B**, **C**, **E**, FW2354 – Venta El Pilar Spring, Málaga, Spain; **A**, female genitalia; **B**, penis; **C**, prostate gland; **D**, ctenidium and osphradium; **E**, stomach; **F**, perioesophageal ring.

Pigmentation and anatomy

Animal slightly darkly pigmented, although unpigmented specimens were also found (Figure 36D); head and tentacles black, pigmentation lighter on eye lobes, snout and neck; snout about as long as wide, approximately parallel-sided, with medium distal lobation. Ctenidium occupying almost the total length of the pallial cavity; 21–27 gill filaments; filaments broad, triangular, fused at the base by an epithelium (Figure 39D). Osphradium elongate, more than 3 times longer than broad, positioned opposite to the middle of the ctenidium. Stomach almost as long as wide with two chambers almost equal in size; style sac longer than wide, with the unpigmented intestine surrounding its distal part before continuing on as a straight rectum (Figure 39E).

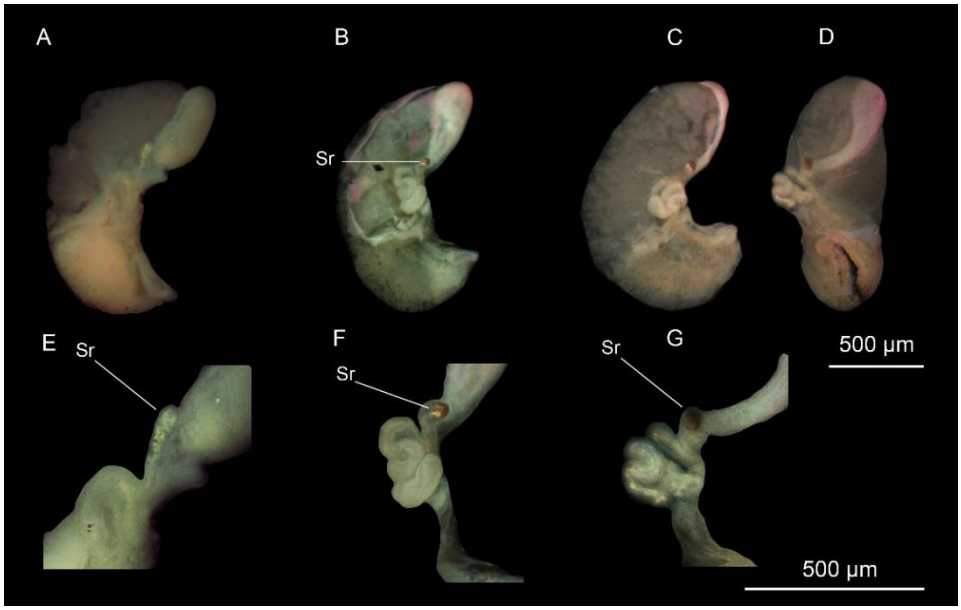


Figure 40. Variation of the bursa copulatrix of *M. balearica*. **A**, elongate, FW2604 – stream near Sant Joan de Carbonell, Minorca, Spain; **B**, pyriform, FW2354 – Venta El Pilar Spring, Málaga, Spain; **C** and **D**, pyriform, FW2357 – Fuente Valentín Spring, Alozaina, Cádiz, Spain; **E**–**G**, detailed views of the seminal receptacle (**Sr**) and renal oviduct loops.

Female genitalia with a glandular oviduct 2.5 times longer than wide; albumen gland longer than capsule gland (Figure 39A); bursa copulatrix pyriform (Figure 40B–D) to elongate (Figure 40A), ca. 2 times longer than wide, lying against the albumen gland; bursal duct shorter than bursa length; renal oviduct unpigmented, coiled, with three loops; coils can be wide open (Figure 40E) or highly coiled (Figure 40F, G); seminal receptacle elongate, with a short duct, positioned on the

distal part of the renal oviduct just above the junction with the bursal duct (Figure 40E–G).

Male genitalia with a bean-shaped prostate gland, about 2 times longer than wide, connected by the posterior vas deferens to a convoluted seminal vesicle and the testis. Penis darkly pigmented, gradually tapering, attached to the neck behind the right eye; penial appendix and distal end of the penis about the same size; penial appendix unpigmented or slightly pigmented at the junction with the penis, rounded, small, base narrow, medially positioned on the inner edge of the penis (Figure 39B).

Nervous system pigmented, elongate (RPG ratio 0.53; see Table 23); cerebral ganglia approximately equal in size; pleuro-supraoesophageal connective ca. 9 times longer than the pleuro-suboesophageal one (Figure 39F).

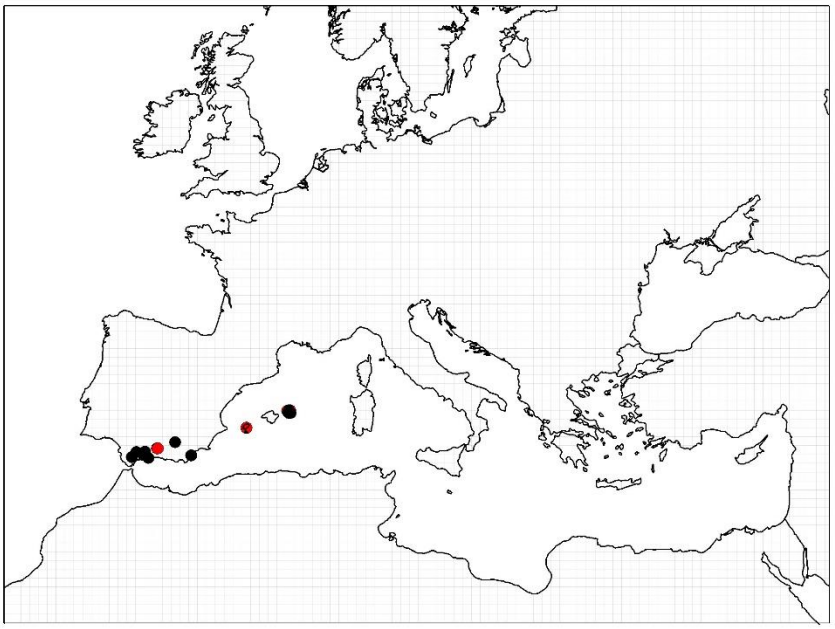


Figure 41. Distribution map of *M. balearica*. Black dots, localities from which *M. balearica* specimens were collected in this study; red dots, localities mentioned in the literature.

Distribution: The species was previously cited from springs, streams, small rivers and ditches in the southern Iberian Peninsula and on the Balearic islands of Ibiza and Minorca (Boeters 1988, Glöer et al. 2015b). We confirmed the species in

Minorca (Ibiza was not included as a field site) and also in several other springs and streams in south-eastern Iberia (Figure 41).

Remarks: Previous assignments of Iberian populations to *M. balearica* (Boeters 1988, Glöer et al. 2015a), whose type locality is on Minorca, have been confirmed by our morphological and genetic results. Populations from the stream near Sant Joan de Carbonell, Minorca (FW2604), Venta El Pilar Spring, Málaga (FW2354) and Fuente Valentín Spring, Cádiz (FW2357) accounted for most of the morphological variation observed between Minorcan and Iberian populations. COI sequence divergence among these populations was low (< 1.2%).

Intraspecific morphological differences for this species are observed mainly in shell, penis and female genitalia features. Specimens from Minorca are generally larger in size and present globose to high-spired shells, whereas the Iberian specimens present only globose shells (Table 18). The specimens from Los Granados Trough in Málaga (FW2335) have the smallest shells. All the specimens present a pigmented epithelium, though some specimens found in the Arabic Spring in Mojácar (FW2395) are unpigmented. Variation in the male reproductive organs is as previously described for the genus: the penis is either slightly longer or shorter than the appendix and the appendix is usually rounded and unpigmented (only the population in Peal del Becerro, Jaén presents slight pigmentation on the appendix). The bursa copulatrix varies from pyriform in most of the contential populations to elongate in the specimens from Venta El Pilar Spring, Málaga (FW2322). Populations from Minorca present both morphotypes. Females from Venta El Pilar Spring (FW2322), stream in Montecorto (FW2350), Fuente Valentín Spring (FW2357) and Arabic Spring (FW2395) have a bursa copulatrix that surrounds the albumen gland, which is located beneath it (see Figure 40C) rather than parallel to it (its typical orientation for other members of the genus).

Mercuria balearica can be distinguished from *M. similis* by its smaller, less globose shell, less convex body whorl, shorter tapering triangular penis with a rounded, unpigmented penial appendix that is slightly larger than or equal in size to the penis, pyriform bursa copulatrix and higher number of cusps on the central and lateral teeth of the radula. *Mercuria balearica* resembles *M. carrillorum* sp. nov. and *M. felixi* sp. nov. in shell shape (ovate-conic) and colour but differs from these congeners by having a shorter, more pigmented penis with a larger rounded penial appendix. Also, their distribution areas do not overlap.

Table 18. Shell dimensions (mm) of *M. balearica*: **1**, FW2335 – Trough Los Granados, Málaga, Spain; **2**, FW2351 – stream in Montecorto, Málaga, Spain; **3**, FW2354 –Venta El Pilar Spring, Málaga, Spain; **4**, FW2357 – Fuente Valentín Spring, Alozaina, Cádiz, Spain; **5**, FW2395 – Arabic Spring , Mojácar, Granada, Spain; **6**, FW2568 – Spring Fuente de los Cinco Caños, Peal del Becerro, Jaén, Spain; **7**, FW2604 – stream near Sant Joan de Carbonell, Minorca, Spain. See Table 3 in Chapter 2 for a full list of abbreviations.

	1 Mean ± SD; CV (Max – Min) n=9	**2** Mean ± SD; CV (Max – Min) n=5	**3** Mean ± SD; CV (Max – Min) n=26	**4** Mean ± SD; CV (Max – Min) n=31	**5** Mean ± SD; CV (Max – Min) n=17	**6** Mean ± SD; CV (Max – Min) n=10	**7** Mean ± SD; CV (Max – Min) n=13
SL	2.45 ± 0.16; 0.07 (2.73-2.22)	2.75 ± 0.06; 0.02 (2.89-2.67)	2.81 ± 0.15; 0.05 (3.29-2.47)	2.82 ± 0.15; 0.05 (3.3-2.43)	2.84 ± 0.17; 0.06 (3.35-2.44)	2.73 ± 0.21; 0.08 (3.08-2.42)	3.1 ± 0.42; 0.14 (3.62-1.82)
SW	1.84 ± 0.1; 0.05 (2.03-1.71)	2.1 ± 0.05; 0.02 (2.16-1.98)	2.17 ± 0.1; 0.05 (2.51-1.92)	2.22 ± 0.1; 0.05 (2.59-2)	2.21 ± 0.12; 0.05 (2.62-1.9)	2.1 ± 0.13; 0.06 (2.32-1.91)	1.64 ± 0.18; 0.11 (1.93-1.45)
AL	1.77 ± 0.09; 0.05 (1.93-1.64)	2.07 ± 0.04; 0.02 (2.12-1.97)	2.11 ± 0.1; 0.05 (2.48-1.87)	2.13 ± 0.09; 0.04 (2.52-1.92)	2.14 ± 0.13; 0.06 (2.54-1.74)	2.05 ± 0.14; 0.07 (2.27-1.88)	1.8 ± 0.46; 0.26 (3.19-1.45)
AW	1.43 ± 0.08; 0.06 (1.58-1.3)	1.71 ± 0.04; 0.02 (1.79-1.62)	1.73 ± 0.09; 0.05 (2-1.5)	1.74 ± 0.08; 0.05 (2.03-1.55)	1.77 ± 0.08; 0.05 (2.02-1.57)	1.6 ± 0.11; 0.07 (1.84-1.46)	2.04 ± 0.44; 0.22 (2.65-1.44)
AH	1.21 ± 0.08; 0.07 (1.32-1.09)	1.28 ± 0.04; 0.03 (1.37-1.22)	1.4 ± 0.06; 0.04 (1.64-1.23)	1.46 ± 0.05; 0.03 (1.69-1.31)	1.46 ± 0.06; 0.04 (1.67-1.28)	1.31 ± 0.08; 0.06 (1.4-1.16)	1.82 ± 0.47; 0.26 (2.51-1.39)
LBW	1.17 ± 0.1; 0.09 (1.32-1.01)	1.24 ± 0.04; 0.03 (1.34-1.18)	1.35 ± 0.06; 0.04 (1.54-1.2)	1.39 ± 0.05; 0.04 (1.6-1.23)	1.4 ± 0.06; 0.04 (1.57-1.24)	1.26 ± 0.09; 0.07 (1.36-1.1)	1.32 ± 0.46; 0.35 (2.43-1.03)
WBW	0.9 ± 0.05; 0.06 (1-0.82)	1.03 ± 0.03; 0.03 (1.09-0.99)	1.04 ± 0.06; 0.06 (1.23-0.89)	1.03 ± 0.05; 0.05 (1.18-0.92)	1.08 ± 0.07; 0.06 (1.25-0.88)	1 ± 0.07; 0.07 (1.1-0.86)	2.21 ± 0.54; 0.24 (2.72-1.02)
WAW	0.83 ± 0.06; 0.07 (0.97-0.76)	0.94 ± 0.02; 0.02 (0.98-0.91)	0.97 ± 0.06; 0.06 (1.16-0.84)	0.98 ± 0.05; 0.05 (1.09-0.86)	0.99 ± 0.07; 0.07 (1.2-0.77)	0.95 ± 0.07; 0.07 (1.08-0.86)	0.6 ± 0.06; 0.1 (0.71-0.48)
WPW	0.47 ± 0.05; 0.11 (0.56-0.42)	0.56 ± 0.04; 0.07 (0.61-0.48)	0.57 ± 0.05; 0.09 (0.69-0.43)	0.51 ± 0.04; 0.08 (0.62-0.4)	0.53 ± 0.07; 0.13 (0.76-0.3)	0.56 ± 0.06; 0.11 (0.69-0.46)	1.14 ± 0.05; 0.04 (1.22-1.07)

Table 19. Dimensions of the osphradium, ctenidium and anterior digestive system (mm) in *M. balearica*: **1**, FW2334 – Venta El Pilar Spring, Málaga, Spain; **2**, FW2335 – Trough Los Granados, Málaga, Spain; **3**, FW2350 – stream in Montecorto, Málaga, Spain; **4**, FW2357 – Fuente Valentín Spring, Alozaina, Cádiz, Spain; **5**, FW2395 – Arabic Spring , Mojácar, Granada, Spain. See Table 3 in Chapter 2 for a full list of abbreviations.

	1	**2**	**3**	**4**	**5**
	Mean ± SD; CV (Max – Min)	Mean ± SD; CV (Max – Min)		Mean ± SD; CV (Max – Min)	
	n=5	n=2	n=1	n=2	n=1
CtL	1.12 ± 0.57; 0.51 (1.23-1.05)	1.24 ± 0.87; 0.7 (1.24-1.24)	0.633		1.026
OsL	0.44 ± 0.22; 0.5 (0.5-0.37)	0.36 ± 0.25; 0.69 (0.36-0.36)	0.248		0.343
OsW	0.09 ± 0.05; 0.56 (0.1-0.08)	0.09 ± 0.06; 0.67 (0.09-0.09)	0.090		0.112
StL	0.5 ± 0.15; 0.3 (0.68-0.32)	0.63 ± 0.06; 0.1 (0.67-0.59)		0.44 ± 0.03; 0.07 (0.47-0.42)	0.518
StW	0.51 ± 0.16; 0.31 (0.69-0.31)	0.63 ± 0; 0 (0.63-0.62)		0.39 ± 0.02; 0.05 (0.4-0.37)	0.438
SsL	0.55 ± 0.16; 0.29 (0.72-0.39)	0.68 ± 0.07; 0.1 (0.73-0.63)		0.38 ± 0.01; 0.03 (0.39-0.38)	0.518
SsW	0.28 ± 0.07; 0.25 (0.34-0.19)	0.42 ± 0.02; 0.05 (0.44-0.4)		0.27 ± 0.01; 0.04 (0.28-0.27)	0.307

Table 20. Female genitalia measurements (mm) recorded in *M. balearica*: **1**, FW2334 – Venta El Pilar Spring, Málaga, Spain; **2**, FW2351 – stream in Montecorto, Málaga, Spain; **3**, FW2357 – Fuente Valentín Spring, Alozaina, Cádiz, Spain; **4**, FW2395 – Arabic Spring , Mojácar, Granada, Spain; **5**, FW2568 – Spring Fuente de los Cinco Caños, Peal del Becerro, Jaén, Spain; **6**, FW2604 – stream near Sant Joan de Carbonell, Minorca, Spain. See Table 3 in Chapter 2 for a full list of abbreviations.

	1 Mean ± SD; CV (Max – Min) n=2	**2** n=1	**3** Mean ± SD; CV (Max – Min) n=2	**4** n=1	**5** Mean ± SD; CV (Max – Min) n=3	**6** Mean ± SD; CV (Max – Min) n=2
AgL	0.76 ± 0.3; 0.39 (0.98-0.55)	0.876	0.45 ± 0.1; 0.22 (0.52-0.37)	0.860	0.796	0.45 ± 0.1; 0.22 (0.52-0.37)
CgL	0.71 ± 0.32; 0.45 (0.93-0.48)	0.651	0.37 ± 0.03; 0.08 (0.39-0.35)	0.650	0.699	0.37 ± 0.03; 0.08 (0.39-0.35)
SR1L	0.12 ± 0.05; 0.42 (0.15-0.09)	0.142	0.11 ± 0.01; 0.09 (0.12-0.1)	0.170	0.121	0.11 ± 0.01; 0.09 (0.12-0.1)
BCL	0.51 ± 0.18; 0.35 (0.64-0.38)	0.475	0.36 ± 0.08; 0.22 (0.41-0.3)	0.458	0.507	0.36 ± 0.08; 0.22 (0.41-0.3)
BCW	0.2 ± 0.1; 0.5 (0.27-0.13)	0.249	0.12 ± 0; 0 (0.13-0.12)	0.091	0.221	0.12 ± 0; 0 (0.13-0.12)

Table 21. Male genitalia measurements (mm) recorded in *M. balearica*: **1**, FW2357 – Fuente Valentín Spring, Alozaina, Cádiz, Spain; **2**, FW2395 – Arabic Spring , Mojácar, Granada, Spain; **3**, FW2334 – Venta El Pilar Spring, Málaga, Spain; **4**, FW2351 – stream in Montecorto, Málaga, Spain; **5**, FW2568 – Spring Fuente de los Cinco Caños, Peal del Becerro, Jaén, Spain. See Table 3 in Chapter 2 for a full list of abbreviations.

	1	**2** Mean ± SD; CV (Max – Min)	**3**	**4**	**5**
	n=1	n=2	n=1	n=1	n=1
PrL		0.78 ± 0.49; 0.63 (1.01-0.55)	0.592	0.781	0.882
PrW		0.26 ± 0.16; 0.62 (0.34-0.19)	0.324	0.329	0.404
PL	0.168	0.38 ± 0.08; 0.21 (0.45-0.27)	0.625	0.66	0.545
PW	0.134	0.23 ± 0.08; 0.35 (0.33-0.14)	0.14	0.199	0.287
PaL	0.356	0.66 ± 0.21; 0.32 (0.86-0.41)	0.51	0.518	0.858
PaW	0.168	0.41 ± 0.06; 0.15 (0.47-0.34)	0.364	0.37	0.71

Genetic divergences for COI indicated that *M. balearica* is most closely related to *M. tensiftensis* and *M. felixi* sp. nov (5.2% mean divergence with both species) and most distantly to *M. midarensis* and *M. melitensis* (8.6% mean divergence with both species).

Ecology: *Mercuria balearica* presents broad ecological plasticity, inhabiting springs, streams and buddles with conductivities ranging 179–2750 µS/cm. Population densities were low. Also, during our field surveys, we found evidence of local extinctions in the region of Andalusia and in Minorca, suggesting that the species was more abundant in the late twentieth century. This species occurs sympatrically with *Theodoxus* spp., *Melanopsis* spp. and *Potamopyrgus antipodarum.*

Mercuria maceana (Paladilhe, 1869)

(Figs 42–45)

> *Amnicola maceana* Paladilhe, 1869:103. Type locality: "*a été récoltée à Antunez, près de Barcelone (Espagne).*"

Type Material: Syntypes: MHNG-MOLL-105438/2; MHNL 45013950; UM.PDL.006 (Breure and Audibert 2017)

Revised diagnosis: Shell ovate-conic; aperture obliquely broad ovate; periostracum yellowish to pale grey; protoconch microsculpture granulated; central radular tooth formula 3–C–3/1–1.

Type locality: C'an Tunis, Barcelona, Spain. The type locality of this species in the original description "*récoltée à Antunez, près de Barcelone"* refers to a series of local geographic names for a private property: C'an Tunis, Casa Antúnez, Camp de la Bota, Llano del Llobregat. These names have been written either conserving the original language, Catalan, sometimes using the Spanish form, or even using other local names for the same place.

Material Examined: C'an Tunis, Barcelona, Spain (MNCN 15.05.7009; MZB 77-8445; MZB 77-8107).

Description: Shell ovate-conic, whorls 4–5, height 3.9–5.2 mm, width 3–3.8 mm (Figure 27A–E); periostracum yellowish to pale grey; protoconch of 1.5 whorls, ca. 350 µm wide, nucleus ca. 200 µm wide; protoconch microsculpture granulated (Figure 43C, D); teleoconch whorls convex, separated by a deep suture; body whorl large, convex, occupying about two-thirds of the total shell length; aperture

obliquely broad ovate, complete; inner lip thicker than outer lip; aperture margin slightly reflexed; umbilicus narrow, not covered by the inner lip (Figure 42A–D).

Radula length medium, ca. 800 µm long (35% of total shell length), containing about 63 rows of teeth (Figure 44A). Central tooth formula 3–C–3/1–1, central cusp "V" shaped, cutting edge slightly concave (Figure 44B). Lateral tooth formula 3–C–3, central cusp "V" shaped and slightly longer than the central tooth one. Inner marginal teeth with 12–14 cusps; outer marginal teeth with 12–14 cusps (Figure 44C, D). Radular data were compiled from a specimen collected from the type locality (MZB 77-8445).

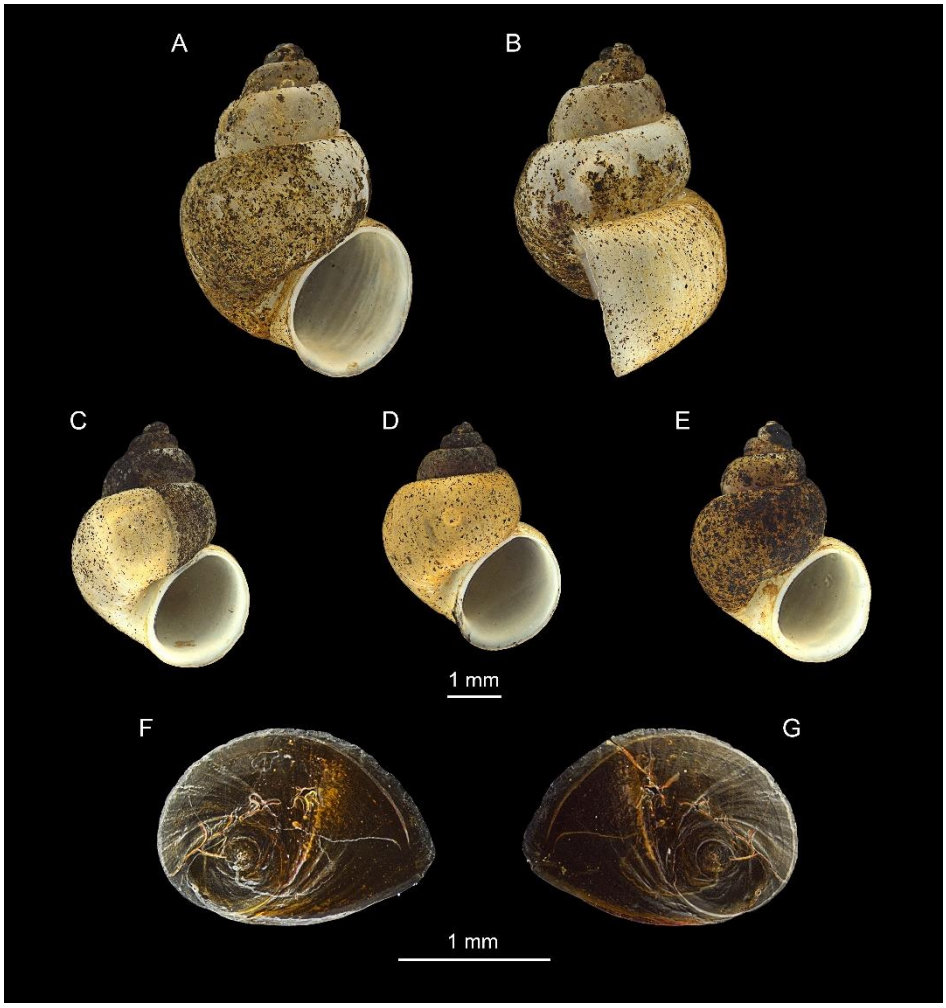


Figure 42. Shells and opercula of *M. maceana* from C'an Tunis, Barcelona, Spain. **A**–**E,** shells; **F**, operculum inner side; **G**, operculum outer side.

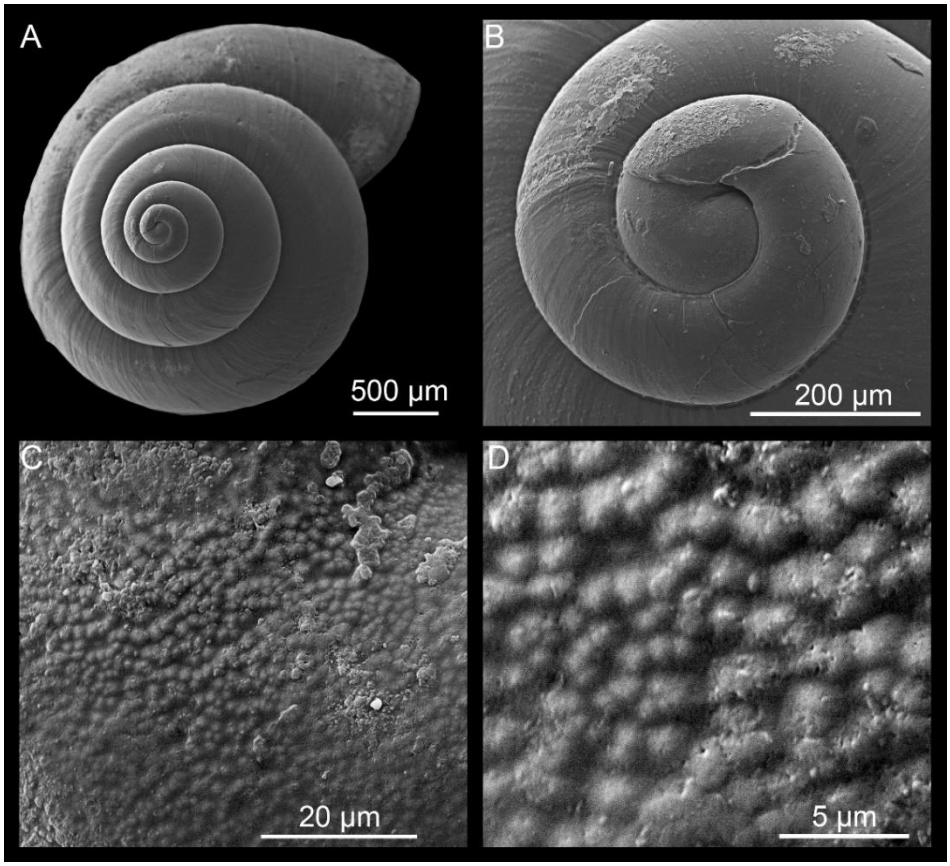


Figure 43. Shell of *M. maceana* specimen from C'an Tunis, Barcelona, Spain. **A**, apical view; **B**, protoconch nucleus; **C**, details of the protoconch; **D**, protoconch microsculpture.

Distribution: This species is only known from the type locality, C'an Tunis (Casa Antúnez), Catalonia, Spain (Figure 45). The type locality has likely disappeared due to extensive alterations (e.g., construction, pollution) of the area as a result of port works conducted in the Barcelona harbour and the industrial zone in the 1980s and the construction work carried out for the 1992 Olympic Games (Jordi Corbella and Francesc Uribe, pers. comm.). Recent surveys conducted around Barcelona did not yield live specimens.

Remarks: *Mercuria maceana* is only known from its type locality, which has been destroyed over the past few decades. Recent populations have not been found in the nearby areas, suggesting that the species is extinct (IUCN category EX). The shells and radulae of *M. maceana* deposited in the Zoological Museum of Barcelona were examined to delineate the morphological variation presented by

the species and to support its recognition. Our PCA based on shell shape clearly differentiated this species (Figure 24). Accordingly, *M. maceana* differs from *M. egarensis* sp. nov. by its shorter and more globose or subglobose shell and is more similar to the Moroccan species *M. targouasensis*. Based on the characters observed on the radula, *M. maceana* cannot be distinguished from *M. similis* or *M. egarensis* sp. nov. We were unable to study other anatomical features due to the lack of ethanol preserved material.

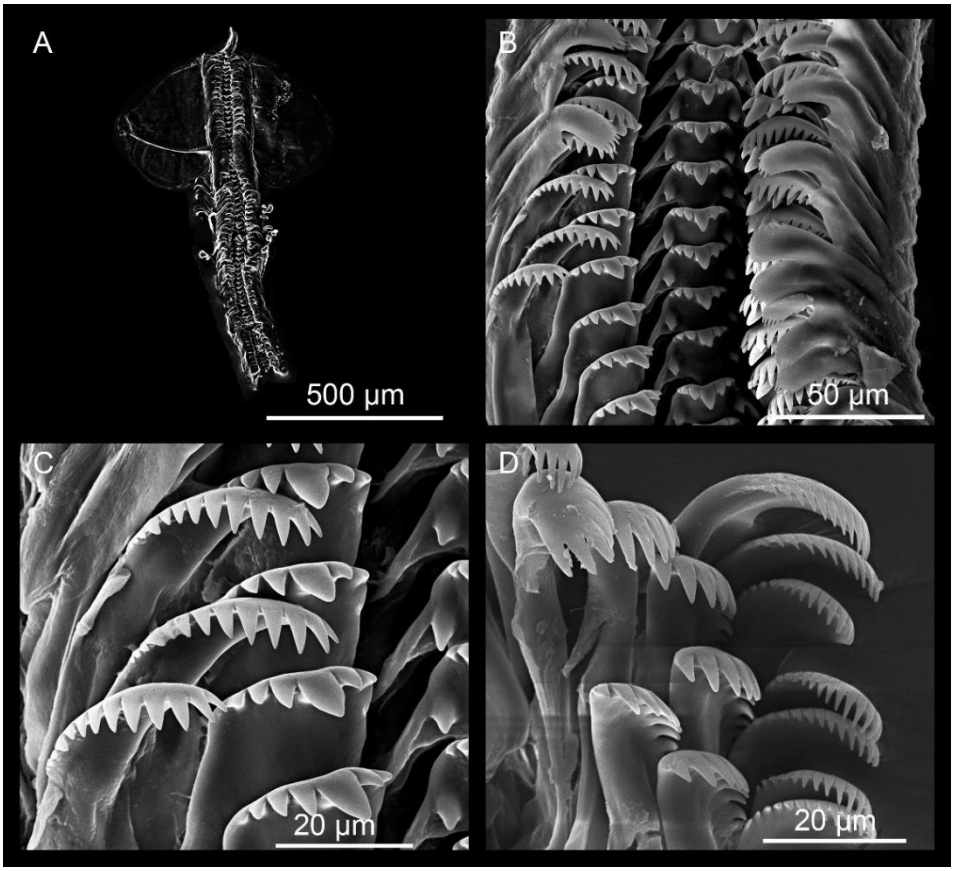


Figure 44. Radula of *M. maceana* specimen from C'an Tunis, Barcelona, Spain. **A**, general view of the radular ribbon; **B**, detailed view of the central, lateral, inner marginal and outer marginal teeth; **C** and **D**, detailed view of the inner and outer marginal teeth, respectively.

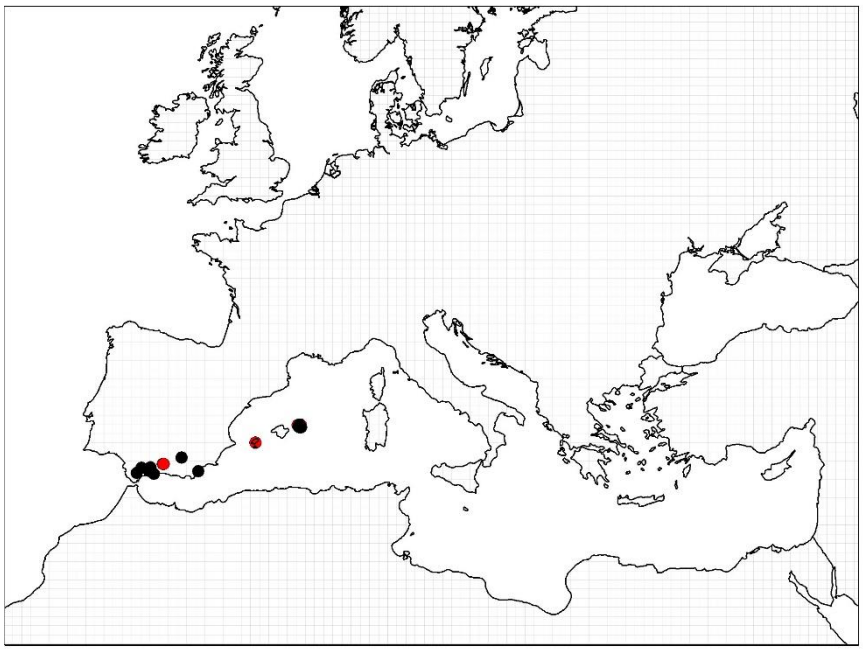


Figure 45. Known distribution of *M. maceana*, C'an Tunis, Barcelona, Spain

***Mercuria egarensis* sp. nov.**

(Figs 46–50)

Type Material: HOLOTYPE: FW2308H; PARATYPES: FW2308, FW2439; (2), Museu Zoologico Barcelona, Spain; (1), Natural History Museum, London, UK; (1), Naturhistorisches Museum Wien, Austria (NHMW 113529); (1) Naturalis, Leiden, The Netherlands; (1), JPM-586 personal collection; (1), MCP personal collection.

Type locality: Font de les Canyes, Catalonia, Spain

Material Examined: Spain: Font de les Canyes, Terrassa, Barcelona (FW2308, FW2439). Additional locality information provided in Appendix 5.

Etymology: The name *egarensis* refers to the ancient Roman city of Egara, present-day Terrassa, where the species occurs.

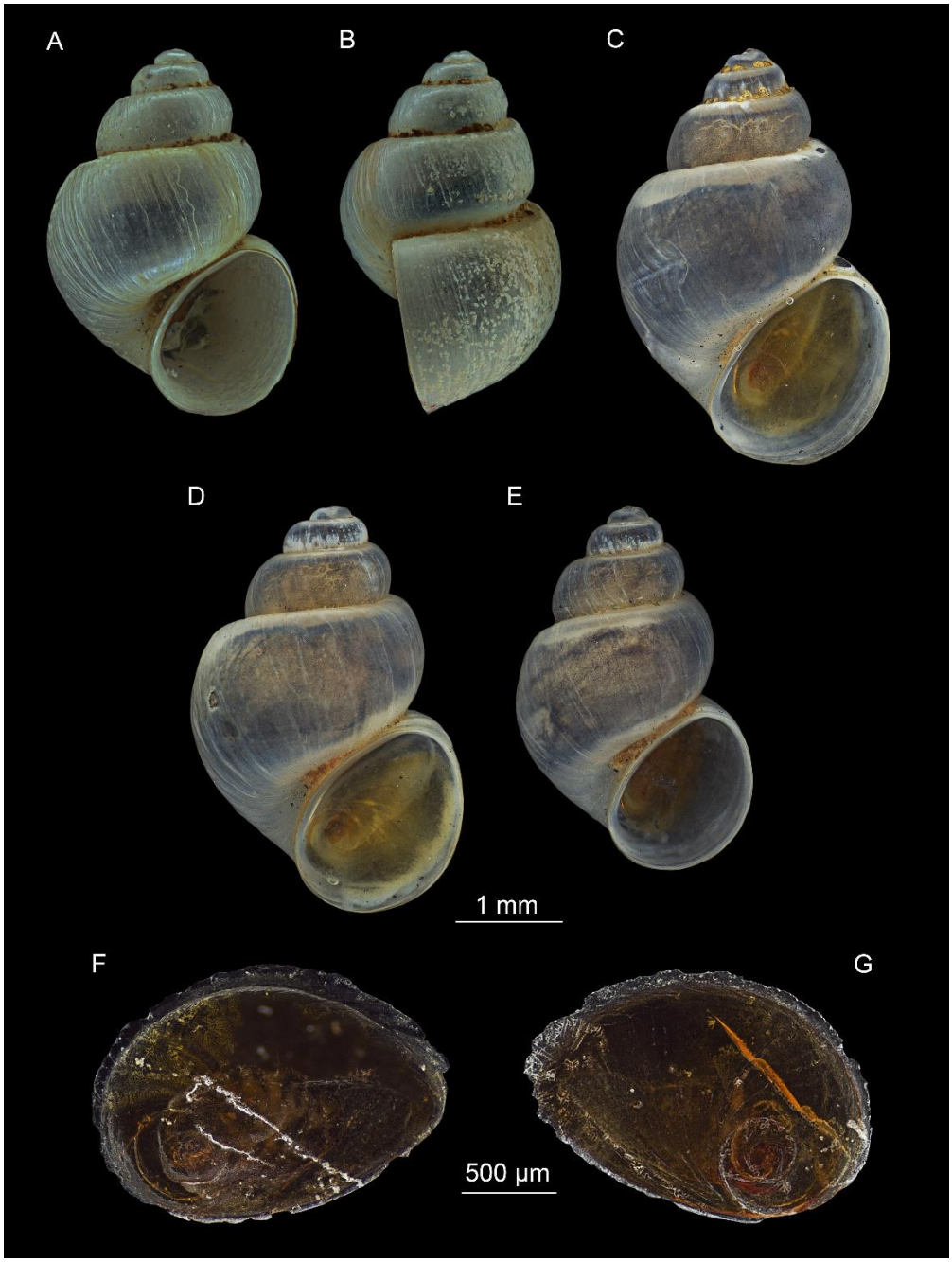


Figure 46. Shells and operculum of *M. egarensis* sp. nov. **A**, holotype FW2308H; **B**–**E**, paratypes; **F**, operculum inner side; **G**, operculum outer side

Diagnosis: Shell ovate-conic; aperture obliquely ovate; protoconch microsculpture granulated; periostracum whitish to pale grey; central radular tooth formula (2)3–C–3(2)/1–1; female genitalia with bursa copulatrix pyriform to elongate, ca. 2.5–3 times longer than wide; seminal receptacle elongate with short peduncle; penis darkly pigmented, gradually tapering; penial appendix unpigmented, ovate, about equal in length or slightly shorter than the distal end of the penis and medially positioned on the inner edge of the penis; nervous system pigmented, elongate (mean RPG ratio = 0.57); cerebral ganglia approximately equal in size.

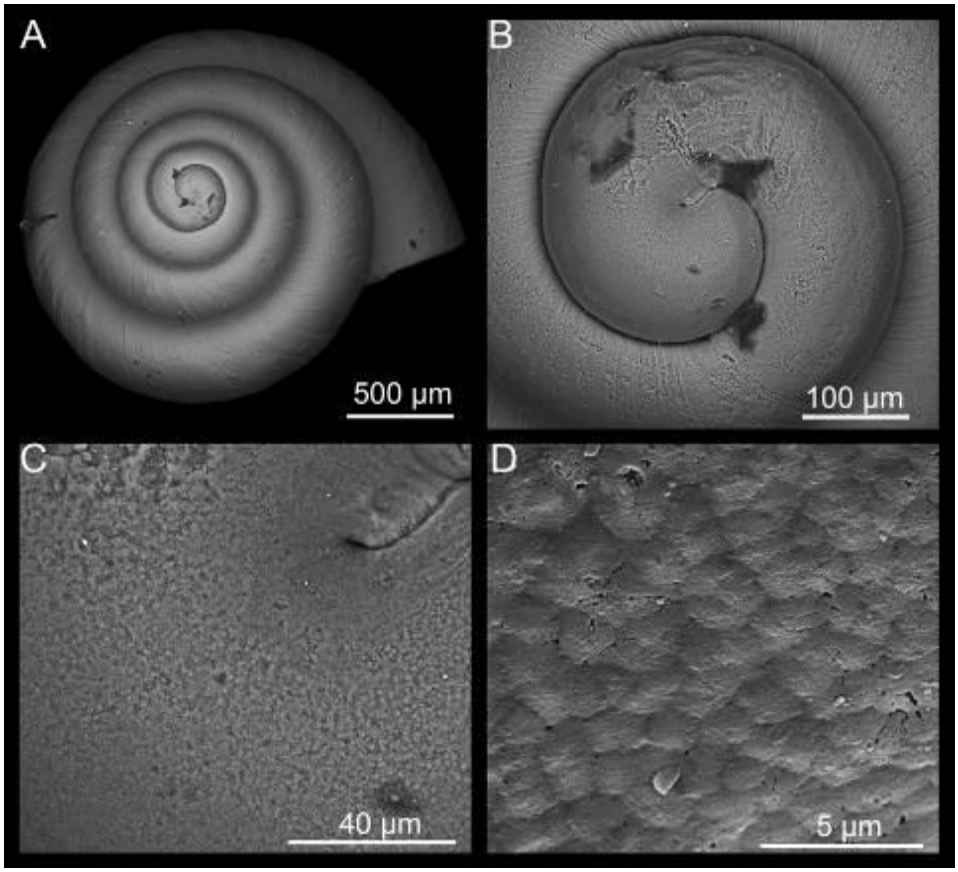


Figure 47. Shell of the *M. egarensis* sp. nov. specimen from Font de les Canyes, Barcelona, Spain. **A**, apical view; **B**, details of the protoconch; **C**, protoconch nucleus; **D**, protoconch microsculpture.

Description: Shell ovate-conic, whorls 4–5, height 3–4.3 mm, width 1.7–2.8 mm (Figure 46A–E); periostracum whitish to pale grey; protoconch of 1.5 whorls, ca. 350 µm wide, nucleus ca. 150 µm wide; protoconch microsculpture granulated

(Figure 47C, D). Teleoconch whorls slightly convex, separated by a deep suture; body whorl large, convex, occupying about two-thirds of the total shell length; aperture obliquely broad ovate, complete; inner lip thicker than outer lip; aperture margin straight; umbilicus narrow, not covered by the inner lip (Figure 46A–E).

Operculum as for the genus, orange to brown, sometimes yellowish, about two whorls; muscle attachment oval, located near the nucleus (Figure 46F, G).

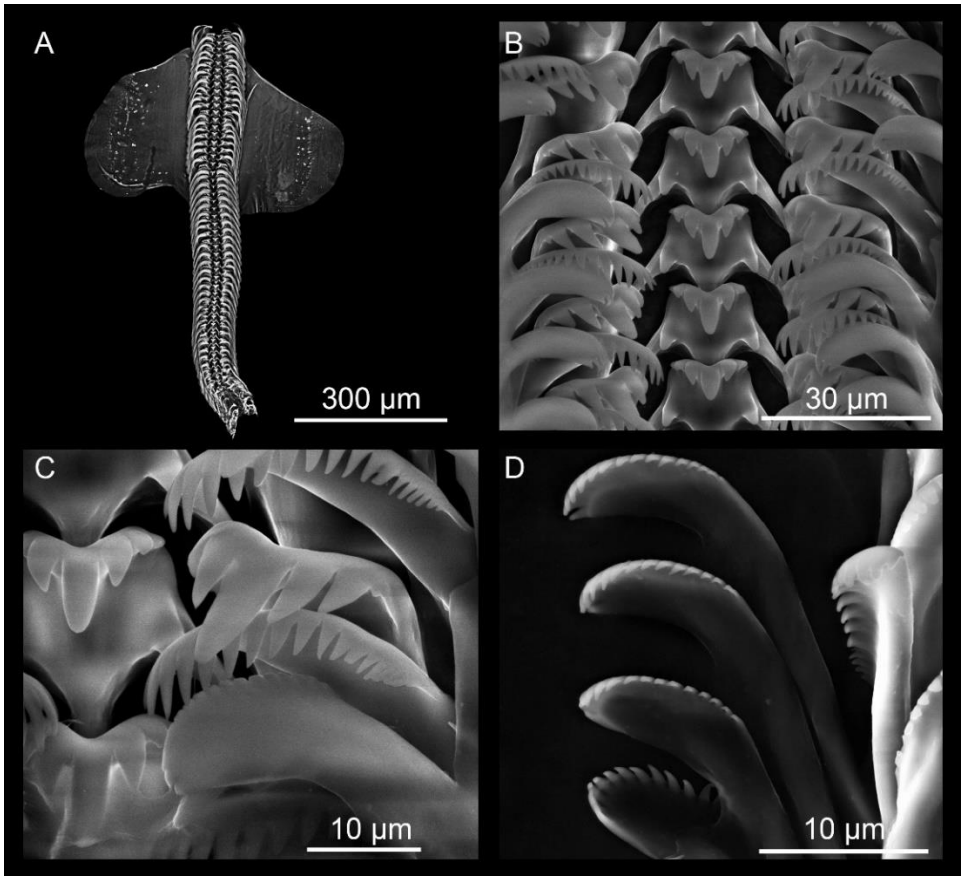


Figure 48. Radula of the *M. egarensis* sp. nov. specimen from Font de les Canyes, Barcelona, Spain. **A**, general view of the radular ribbon; **B**, overview of radular teeth rows; **C** and **D**, detailed view of the inner and outer marginal teeth, respectively.

Radula length medium, ca. 800 µm long (35% of total shell length), containing about 55 rows of teeth. Central tooth formula (2)3–C–3(2)/1–1, central cusp "V" shaped, cutting edge slightly concave (Figure 48B). Lateral tooth formula (3)2–C–2(3), central cusp "V" shaped and slightly longer than the central tooth one. Inner marginal teeth with 11–15 cusps; outer marginal teeth with 15–17 cusps

(Figure 48C, D). Radular data were collected from specimens from the type locality.

Pigmentation and anatomy

Animal slightly brownish to darkly pigmented (Figure 49B); head and tentacles dark brown, pigmentation lighter on eye lobes, snout and neck; snout about as long as wide, approximately parallel-sided, with medium distal lobation. Ctenidium occupying almost the total length of the pallial cavity; 19–23 gill filaments; filaments broad, triangular, fused at the base by an epithelium (Figure 49D). Osphradium elongate, more than 3 times longer than broad, positioned opposite to the middle of the ctenidium. Stomach almost as long as wide with two chambers almost equal in size; style sac longer than wide, protruding anteriorly into the intestinal loop (Figure 49E). Intestine unpigmented, continuing on as a straight rectum.

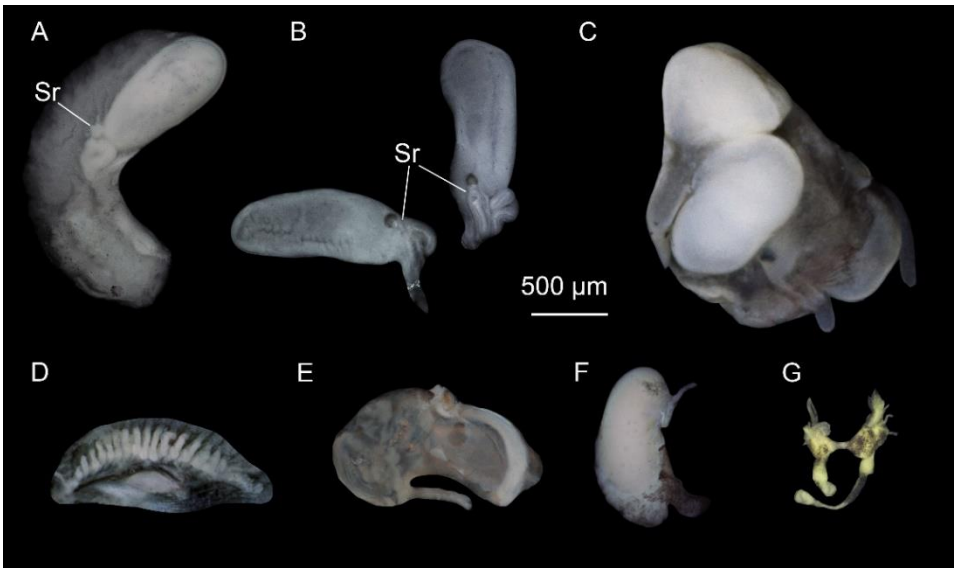


Figure 49. Anatomy of the *M. egarensis* sp. nov. specimen from Font de les Canyes, Barcelona, Spain. **A**, female genitalia; **B**, detailed view of the bursa copulatrix and seminal receptacle; **C**, head and penis; **D**, ctenidium and osphradium; **E**, stomach; **F**, prostate gland; **G**, perioesophageal ring.

Female genitalia with a glandular oviduct 2.5 times longer than wide; albumen gland longer than capsule gland (Figure 49A); bursa copulatrix pyriform to elongate (Figure 49A, B), ca. 2.5–3 times longer than wide; bursal duct shorter than bursa copulatrix; renal oviduct unpigmented, highly coiled with three loops;

seminal receptacle elongate, with a short duct, positioned on the distal part of the renal oviduct just above the junction with the bursal duct (Figure 49B).

Male genitalia with a bean-shaped prostate gland, about 2 times longer than wide, connected by the posterior vas deferens to a convoluted seminal vesicle and the testis. Penis darkly pigmented, gradually tapering, attached to the neck behind the right eye; penial appendix unpigmented, ovate, about equal in length or slightly shorter than the distal end of the penis and medially positioned on the inner edge of the penis (Figure 49C).

Nervous system pigmented, elongate (mean RPG ratio = 0.57; Table 23); cerebral ganglia approximately equal in size and having small black dots of pigment; pleuro-supraoesophageal connective ca. 7 times longer than the pleuro-suboesophageal one (Figure 49G).

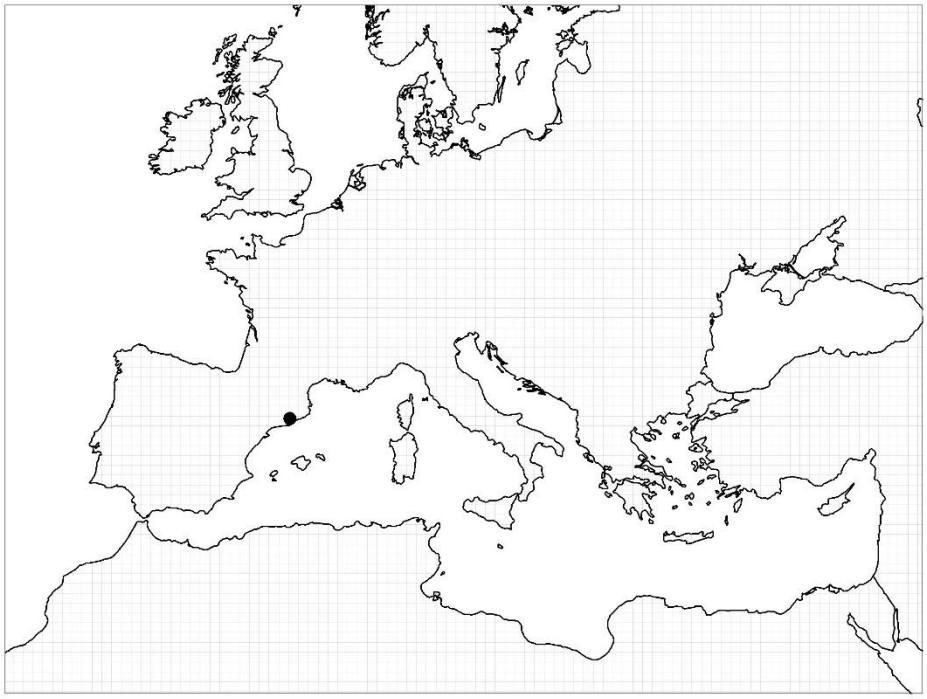


Figure 50. Distribution map of *M. egarensis* sp. nov. The species is represented by only a single, endemic population in the Iberian Peninsula**.**

Distribution: Endemic to the type locality, Font de les Canyes, Barcelona, Spain (Figure 50). A sampling campaign of this and nearby areas was carried out

between 2016 and 2018 and the species was not found elsewhere. The spring, located in a small zone in the eastern part of the municipality of Terrassa, discharges to the Besós river basin. Water courses from the western part of the municipality drain into the Llobregat river basin (Jordi Corbella pers. com.). Bofill and Haas (1920) cited *M. similis* from Terrassa; however, as the drainage of this spring is associated with another hydrological basin, it is unlikely that the Bofill and Haas (1920) citation for *M. similis* corresponds to the species found in Font de les Canyes.

Remarks: *Mercuria egarensis* sp. nov. differs from the closely related species *M. carrillorum* sp. nov. by having a slightly shorter penis, a larger penial appendix, a longer radular ribbon, a larger number of cusps on the central radular tooth [(2)3–C–3(2)/1–1 vs. (3)4–C–4(3)/1–1], a more concentrated perioesophageal ring, a pyriform seminal receptacle positioned on the renal oviduct just above the junction with the bursal duct (in *M. carrillorum* sp. nov., it is positioned at the junction) and the absence of a pallial tentacle. Regarding their ecology, the two species differ in their conductivity preferences, with *M. egarensis* sp. nov. occurring in waters with higher conductivity.

Mercuria egarensis sp. nov. does not resemble any of the congeners found in the Catalonia region. However, according to our PCA results, *M. egarensis* sp. nov. and *M. balearica* have overlapping morphological shell variation (Figure 24), though the latter presents a more globose shell with a wider aperture (Figure 36). Although *M. egarensis* sp. nov. also resembles *M. tachoensis* and *M. similis* in terms of shell shape, its granulated protoconch microsculpture discriminates it from the pitted one in *M. tachoensis*. The shape and colouration of the penial appendix differentiates *M. egarensis* sp. nov. from *M. similis*: it is a highly ovate and unpigmented in the former species and triangular and pigmented in the latter.

Genetic divergence of COI indicated that *M. egarensis* sp. nov. is closely related to *M. carrillorum* sp. nov. with a divergence of only 1.3%. *Mercuria egarensis* sp. nov. diverges 3.7% with *M. similis* and 8.1% with *M. balearica.* The species is most distantly related to *M. tensiftensis* and *M. melitensis*, diverging with both around 9%.

Ecology: The species is endemic to the spring Font de les Canyes, alongside the Besós River. The conductivity of this spring is relatively high (1412 µS/cm). The species was collected from the spring's rocky bottom at a low abundance. No other snail species were found in the spring. Apparently, the water is used for human consumption, which may have had an impact on this and other snail populations.

***Mercuria carrillorum* sp. nov.**

(Figs 51–55)

Type Material. HOLOTYPE: FW2547H PARATYPES: MNCN FW2333, MNCN FW2415, MNCN FW2542, MNCN FW2543, MNCN FW2547, MNCN FW2548, MNCN FW2495; (2) Museu Zoologico Barcelona, Spain; (2), Naturhistorisches Museum Wien, Austria (NHMW 113527); (1), Naturalis, Leiden, the Netherlands; (1), JPM-588 personal collection; (1), MCP personal collection.

Type locality: Stream in Canuto de la Gallina, Montes de Propio, Jerez de la Frontera, Cádiz, Spain.

Material Examined: Spain: Manantial de los Doce Pilares Spring, Málaga (FW2333); Stream in Canuto de la Gallina, Montes de Propio, Jerez de la Frontera, Cádiz (FW2415, FW2542, FW2547, FW2495); Stream in Canuto de las Palas, Montes de Propio, Jerez de la Frontera, Cádiz (FW2543, FW2548). Additional locality information provided in Appendix 5.

Etymology: Named after the family of Miguel Carrillo who instilled his scientific curiosity and passion for malacology and in recognition for his help with the fieldwork conducted across Spain, France and Portugal.

Diagnosis: Shell ovate-conic; periostracum dark brown to blackish; protoconch microsculpture granulated; aperture obliquely ovate; central radular tooth formula (3)4–C–4(3)/1–1; female genitalia with bursa copulatrix elongate, ca. 3 times longer than wide; seminal receptacle elongate; distal end of the penis ca. 1.5 times longer than the penial appendix, darkly pigmented; penial appendix ovate, pigmented to the distal part, medially positioned on the inner edge of the penis; nervous system pigmented, elongate (mean RPG ratio = 0.63); cerebral ganglia approximately equal in size.

Description: Shell ovate-conic, whorls 4–5, height 2.4–3.8 mm, width 1.8–2.7 mm (Figure 51A–F); periostracum dark brown to blackish; protoconch of 1.5 whorls, ca. 350 µm wide, nucleus ca. 150 µm wide; protoconch microsculpture granulated (Figure 52A–D); teleoconch whorls slightly convex, separated by a deep suture; body whorl large and convex, occupying about two-thirds of the total shell length; aperture obliquely broad ovate, complete; inner lip slightly thicker than outer lip; aperture margin straight; outer lip slightly sinuated; umbilicus narrow, not covered by the inner lip (Figure 51A,C–F).

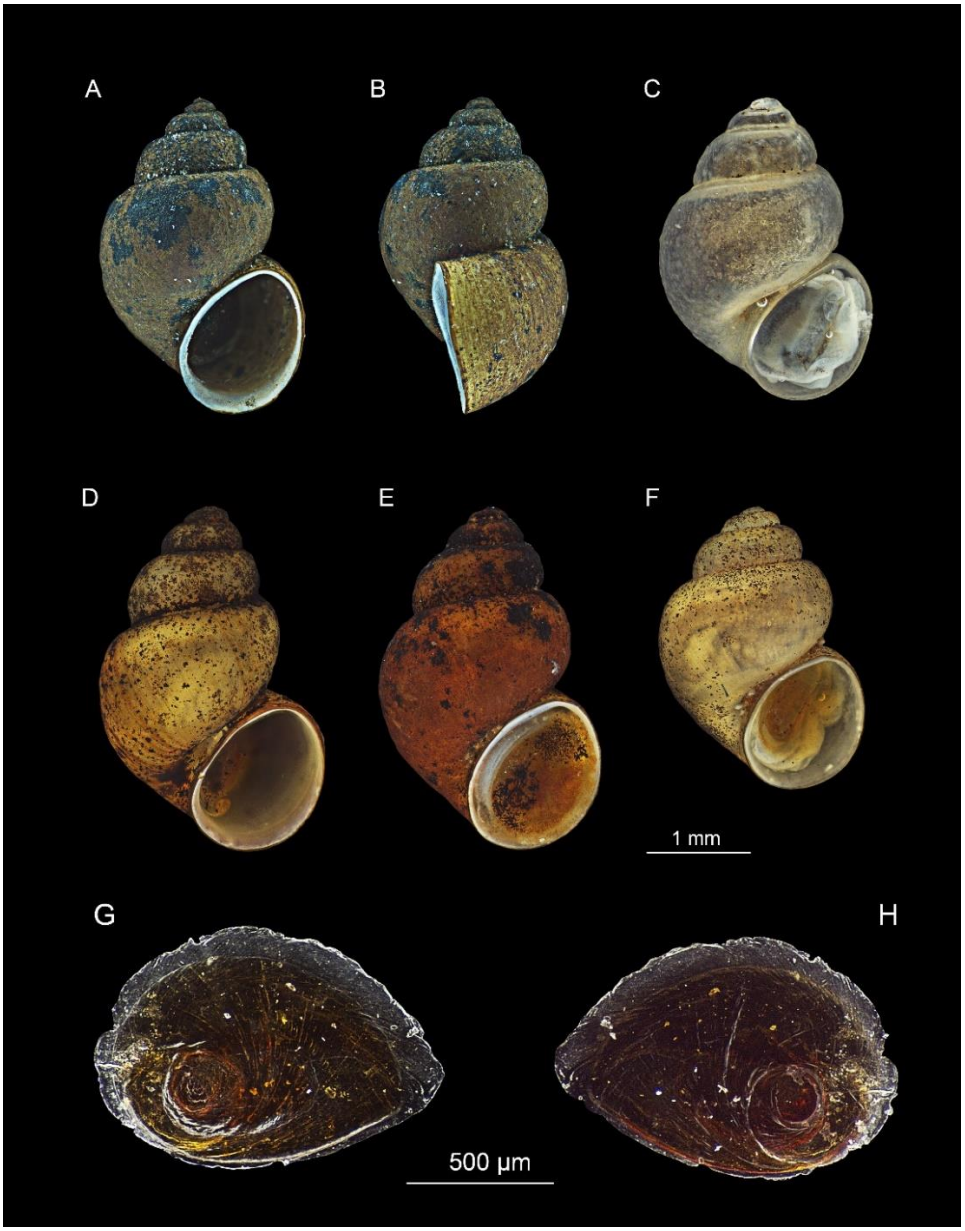


Figure 51. Shells and operculum of *M. carrillorum* sp. nov. **A** and **B**, Holotype FW2547H; **C**–**F** Paratypes; **C**, FW2333 – Manantial de los Doce Pilares Spring, Málaga, Spain; **D**, FW2547 – stream in Canuto de la Gallina, Montes de Propio, Jerez de la Frontera, Cádiz, Spain; **E** and **F**, FW2548, FW2543 – stream in Canuto de las Palas, Montes de Propio, Jerez de la Frontera, Cádiz, Spain; **G**, operculum inner side; **H**, operculum outer side.

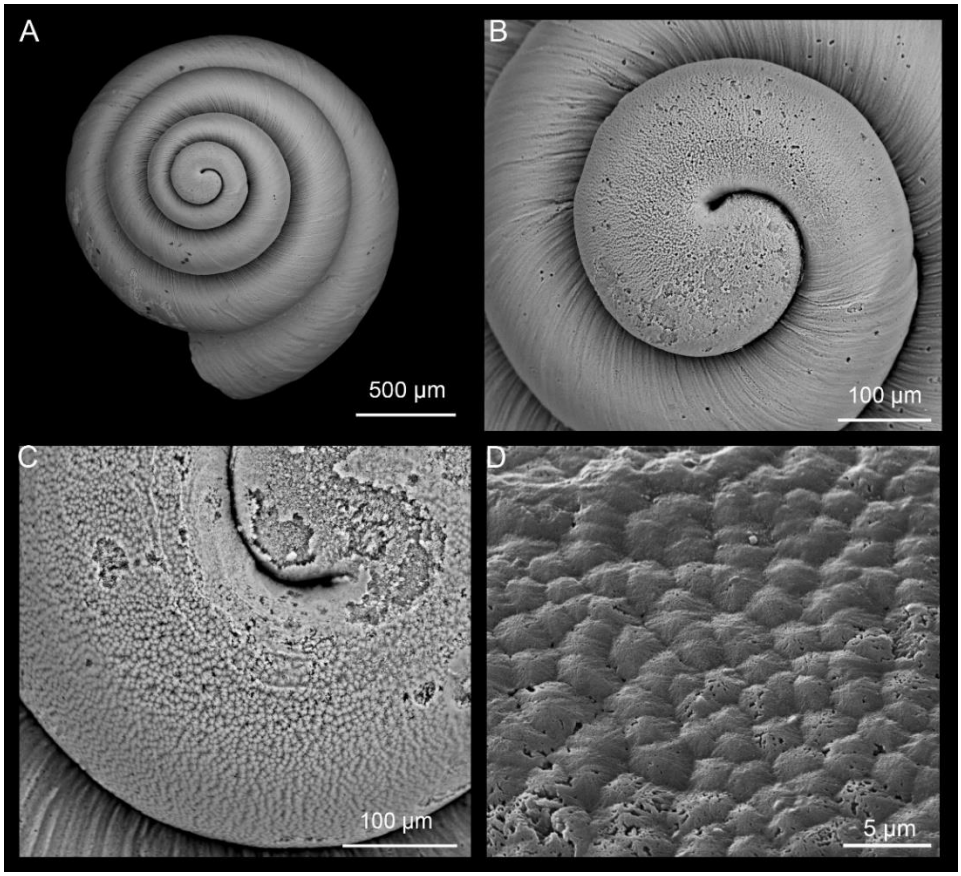


Figure 52. Shell of a *M. carrillorum* sp. nov. specimen from the stream in Canuto de las Palas, Montes de Propio, Jerez de la Frontera, Cádiz, Spain. **A**, apical view; **B**, details of the protoconch; **C**, protoconch nucleus; **D**, details of the protoconch microsculpture.

Operculum as for the genus, orangish to brown, about 2 whorls; muscle attachment oval, located near the nucleus (Figure 51G, H).

Radula length medium, ca. 600 µm long (20% of total shell length), containing about 70 rows of teeth. Central tooth formula (3)4–C–4(3)/1–1, central cusp "V" shaped, cutting edge slightly concave (Figure 53C). Lateral tooth formula (3)2–C–2(3), central cusp "V" shaped and slightly longer than the central tooth one. Inner marginal teeth with 12–16 cusps; outer marginal teeth with 25–27 cusps (Figure 53D). Radular data were collected from the following specimens: FW2542 and FW2547 from Canuto de la Gallina, Cádiz and FW2543 and FW2548 from Canuto de las Palas, Cádiz.

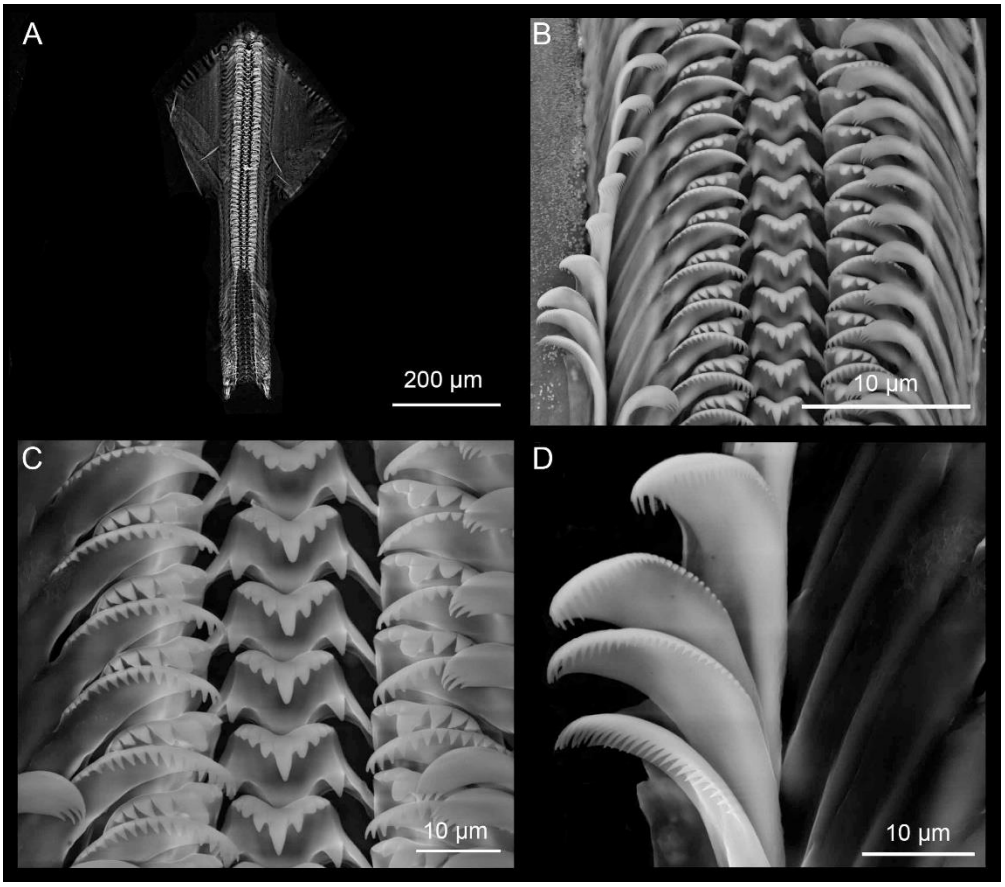


Figure 53. Radula of a *M. carrillorum* sp. nov. specimen from the stream in Canuto de las Palas Cádiz, Spain. **A**, general view of the radular ribbon; **B** and **C**, overviews of the radular teeth rows; **D**, detailed view of the outer marginal teeth.

Pigmentation and anatomy

Animal brownish to pale brown (Figure 54E); head and tentacles brown, eye lobes unpigmented; snout and neck weakly pigmented; snout longer than wide, approximately parallel-sided, with medium distal lobation. Pallial tentacle present. Ctenidium occupying almost the total length of the pallial cavity; 22–26 gill filaments broad, triangular, fused at the base by an epithelium (Figure 54G). Osphradium elongate, more than 3 times longer than wide, positioned opposite to the middle of the ctenidium. Stomach almost as long as wide with two chambers about equal in size; style sac longer than wide, with the unpigmented intestine surrounding its distal part before continuing on as a straight rectum (Figure 54H).

Female genitalia with a glandular oviduct 2.5 times longer than wide; albumen gland longer than capsule gland (Figure 54A); bursa copulatrix elongate, ca. 3 times longer than wide; bursal duct shorter than bursa copulatrix; renal oviduct unpigmented, highly coiled with three loops; seminal receptacle elongate, with a short duct, positioned on the distal part of the renal oviduct at the junction with the bursal duct (Figure 54B).

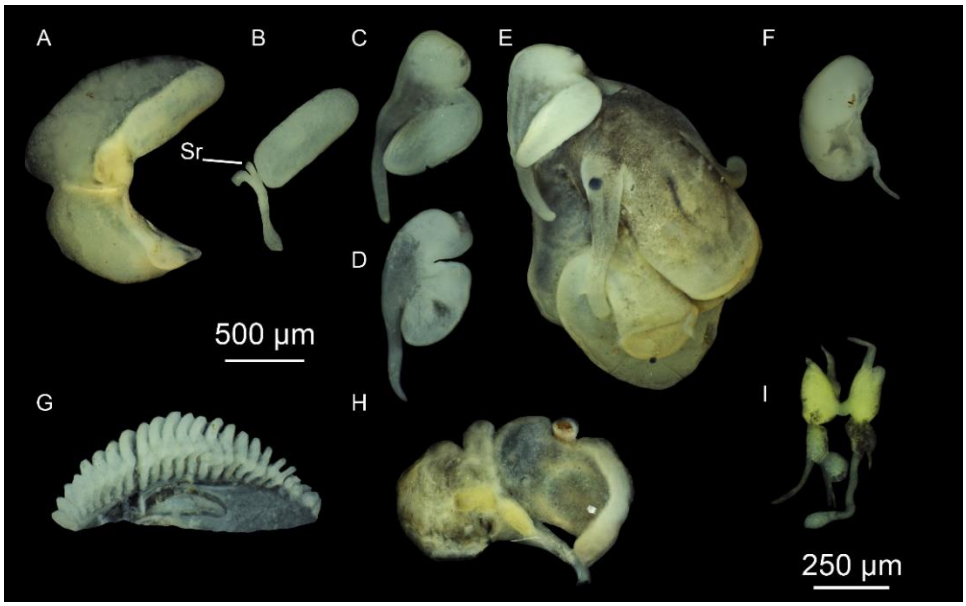


Figure 54. Anatomy of a *M. carrillorum* sp. nov. specimen from the stream in Canuto de las Palas, Cádiz, Spain. **A** and **B**, female genitalia; **C** and **D**, penis; **E**, penis and head; **F**, prostate gland; **G**, ctenidium and osphradium; **H**, stomach; **I**, perioesophageal ring.

Male genitalia with a small bean-shaped prostate gland, about 2 times longer than wide, connected by the posterior vas deferens to a convoluted seminal vesicle and the testis. The distal end of the penis ca. 1.5 times longer than the penial appendix, darkly pigmented, gradually tapering, attached to the neck behind the right eye; penial appendix ovate, pigmented on the distal part, medially positioned on the inner edge of the penis (Figure 54C–F).

Nervous system pigmented, elongate (mean RPG ratio = 0.63; Table 23); cerebral ganglia approximately equal in size; pleuro-supraoesophageal connective ca. 9 times longer than the pleuro-suboesophageal one (Figure 54I).

Distribution: Restricted, as the species has been found at only three localities, all in Cádiz or Málaga, Spain. Very small specimens of the species were collected for the first time at Manantial de los Doce Pilares Spring, Málaga in 2015. However,

we were unable to find any specimens at this locality during a subsequent sampling trip in 2017 (Figure 55).

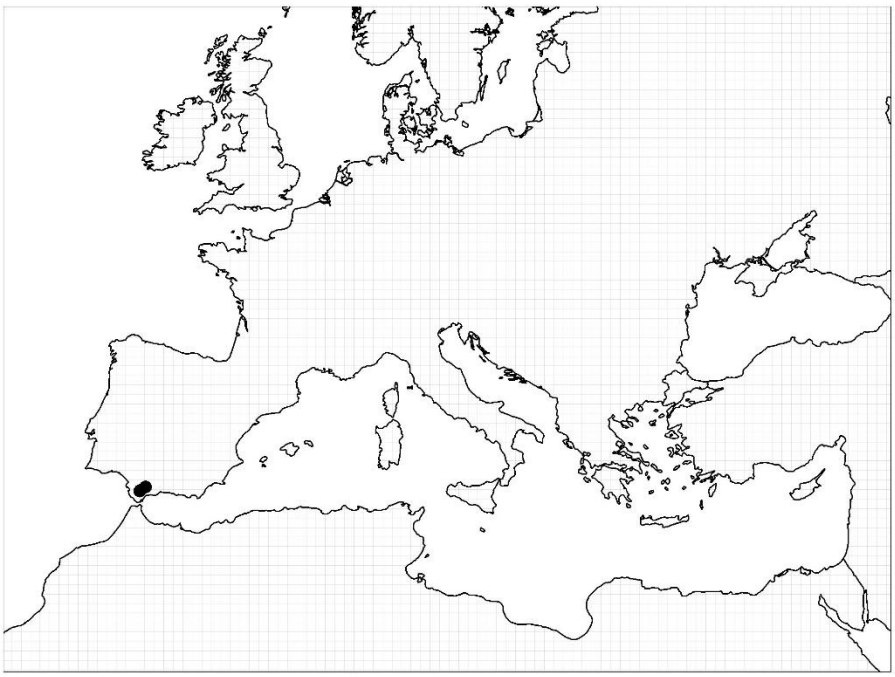


Figure 55. Distribution map of *M. carrillorum* sp. nov. according to the studied populations.

Remarks: *Mercuria carrillorum* sp. nov. differs from the phylogenetically closely related species *M. egarensis* sp. nov. by having a more ovate than high-spired shell, a smaller and more pigmented penial appendix, an elongated bursa copulatrix (from pyriform to elongate in *M. egarensis* sp. nov.), a smaller radular ribbon, a larger number of cusps on the central radular tooth and the presence of a pallial tentacle.

Mercuria carrillorum sp. nov. resembles *M. felixi* sp. nov. and *M. balearica* in terms of shell shape (Figure 24) but differs from them in other features. The new species has an ovate, pigmented penial appendix that is shorter than the distal end of the penis, whereas in *M. felixi* sp. nov., the distal end of the penis and appendix are about equal in length. In *M. balearica*, the penial appendix is longer than the distal end of the penis, which is triangular and extremely pigmented. These species also differ in the lengths of the bursa copulatrix (longer in *Mercuria carrillorum* sp. nov. than in *M. balearica*) and the pleuro-supraoesophageal connective (longer

in *M. felixi* sp. nov.). The major difference in the radula is the number of cusps on the outer marginal teeth: *M. carrillorum* sp. nov. has considerably more cusps (25–27) than *M. balearica* and *M. felixi* sp. nov.

Genetic divergence of COI for *Mercuria carrillorum* sp. nov. and the other Iberian congeners ranged between 1.3% (with *M. egarensis* sp. nov.) and 8% (with *M. felixi* sp. nov.). With a divergence of 8.9%, the most distantly related species was the Tunisian *M. saharica*.

Ecology: Contrary to other *Mercuria* species, *M. carrillorum* sp. nov. lives in streams with very low conductivities, ranging from 80–179 µS/cm. Co-occurring species, though at low abundances, are *Ancylus fluviatilis* Müller, 1774 and *Galba truncatula* (Müller, 1774), except the stream in Canuto de la Gallina in which only *M. carrillorum* sp. nov. is found (Félix Ríos Jiménez pers. comm.).

***Mercuria felixi* sp. nov.**

(Figs 56–50)

Type Material: HOLOTYPE: FW2584H. PARATYPES: MNCN FW2584, MNCN FW2585, MNCN FW2685, MNCN FW2686; (2), Museu Zoologico Barcelona, Spain; (1), Natural History Museum, London, UK; (1), Naturhistorisches Museum Wien, Austria (NHMW 113528); Naturalis, Leiden, The Netherlands; (2), JPM-587 personal collection; (1), MCP personal collection.

Type locality: Stream in Canuto de la Tala, Cádiz, Spain.

Material Examined: Spain: Stream in Canuto de la Tala, Cádiz (FW2584, FW2685); Stream in Canuto del Zapato, Cádiz (FW2585, FW2686). Additional locality information provided in Appendix 5.

Etymology: Named after Félix Ríos Jiménez, who provided us with valuable samples of the new species.

Diagnosis: Shell ovate-conic; aperture obliquely ovate; protoconch microsculpture granulated; periostracum dark brown to blackish; central radular tooth formula 3(4)–C–(4)3/1–1; female genitalia with bursa copulatrix pyriform to elongate, ca. 3 times longer than wide; seminal receptacle elongate; penis darkly pigmented; penial appendix ovate, darkly pigmented at the junction with the penis, about the same length or slightly shorter than the distal end of the penis and medially positioned on the inner edge of the penis; nervous system pigmented, elongate (mean RPG ratio = 0.60). Cerebral ganglia approximately equal in size.

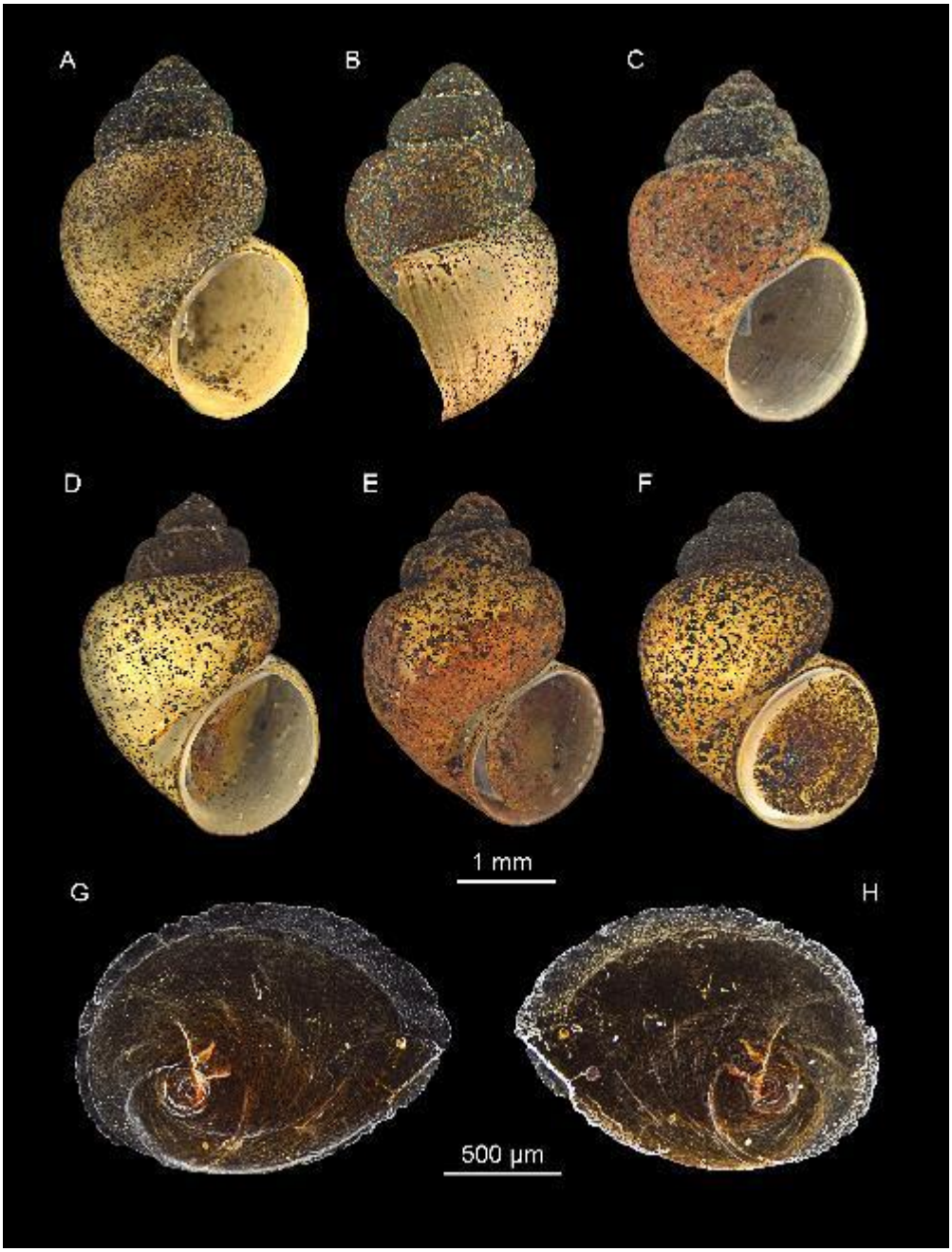


Figure 56. Shells and operculum of *M. felixi* sp. nov from the stream in Canuto de la Tala, Cádiz, Spain. **A** and **B,** holotype FW2584H; **C**–**F**, paratypes; **G**, operculum inner side; **H**, operculum outer side.

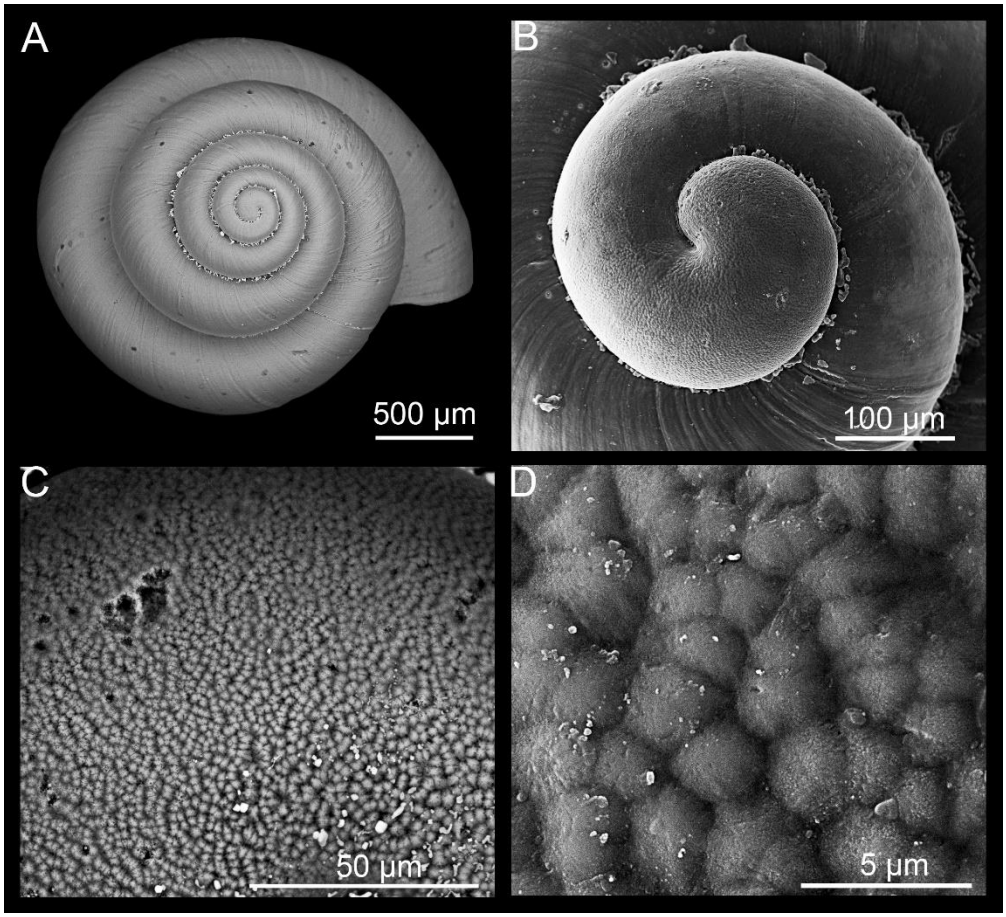


Figure 57. Shell of a *M. felixi* sp. nov. specimen from the stream in Canuto de la Tala, Cádiz, Spain. **A**, apical view; **B**, details of the protoconch; **C**, protoconch nucleus; **D**, protoconch microsculpture.

Description: Shell ovate-conic, whorls 4–5, height 2.4–3.8 mm, width 2–2.9 mm (Figure 56A–F); periostracum dark brown to blackish; protoconch of 1.5 whorls, ca. 270 µm wide, nucleus ca. 125 µm wide; protoconch microsculpture granulated (Figure 57C, D). Teleoconch whorls slightly convex, separated by a deep suture; body whorl large, convex, occupying more than two-thirds of the total shell length; aperture obliquely broad ovate, complete; inner lip thicker than outer lip; outer margin straight, inner lip touching the body whorl; umbilicus narrow, not covered by the inner lip (Figure 56A, C–F).

Operculum as for the genus, orange to brown, sometimes yellowish, with about two whorls; muscle attachment oval, located near the nucleus (Figure 56G, H).

Radula length medium, ca. 700 µm long (35% of total shell length), containing about 60 rows of teeth. Central tooth formula 3(4)–C–(4)3/1–1, central cusp "V" shaped, cutting edge slightly concave (Figure 58A, B). Lateral tooth formula 3–C–3, central cusp "V" shaped and slightly longer than the central tooth one. Inner marginal teeth with 15–18 cusps; outer marginal teeth with 23–27 cusps (Figure 58C, D). Radular data were collected from specimens from the type locality.

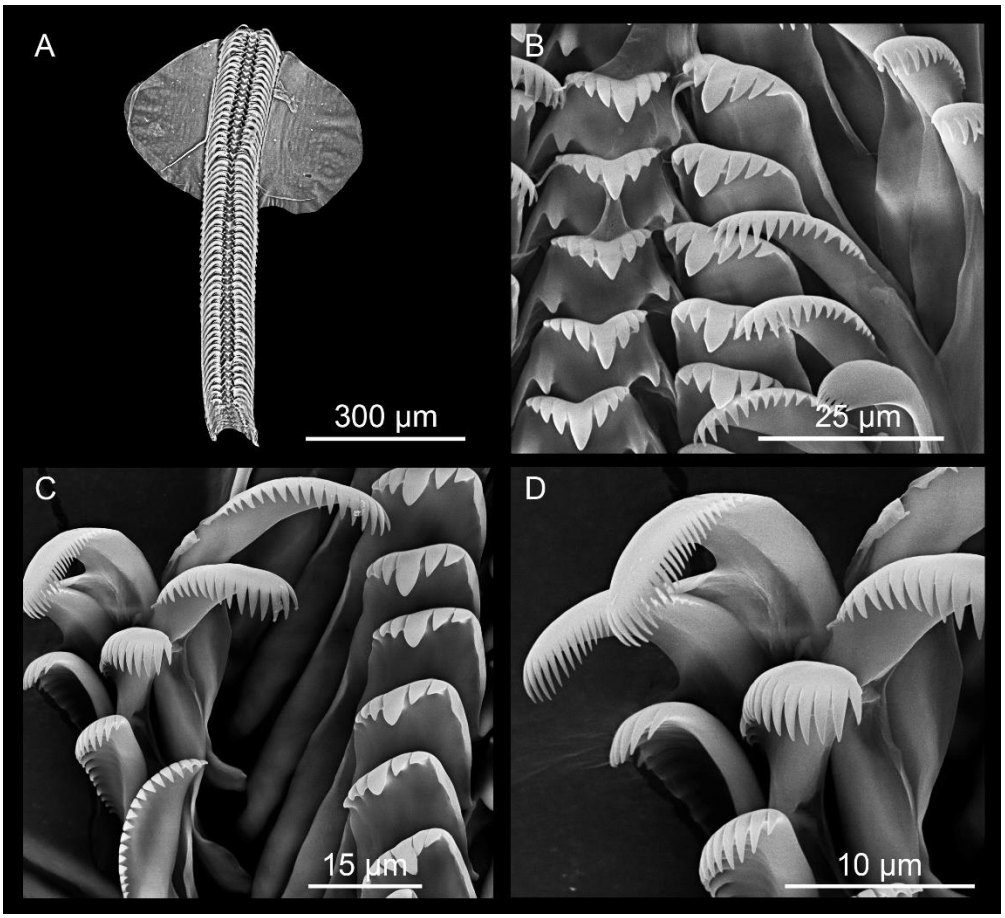


Figure 58. Radula of a *M. felixi* sp. nov. specimen from the stream in Canuto de la Tala, Cádiz, Spain. **A**, general view of the radular ribbon; **B** and **C**, overviews of radular teeth rows; **D**, detailed view of the inner and outer marginal teeth.

Pigmentation and anatomy

Animal weakly pigmented (Figure 59E); head and tentacles unpigmented to weakly pigmented; eye lobe unpigmented; snout and neck weakly pigmented; snout longer than wide, approximately parallel-sided, with medium distal lobation. Ctenidium occupying almost the total length of the pallial cavity; 21–25

gill filaments; filaments broad, triangular, fused at the base by an epithelium (Figure 59F). Osphradium elongate, more than 3 times longer than wide, positioned opposite to the middle of the ctenidium. Stomach almost as long as wide with two chambers almost equal in size; style sac longer than wide, with the unpigmented intestine surrounding its distal part and then continuing on as a straight rectum (Figure 59G).

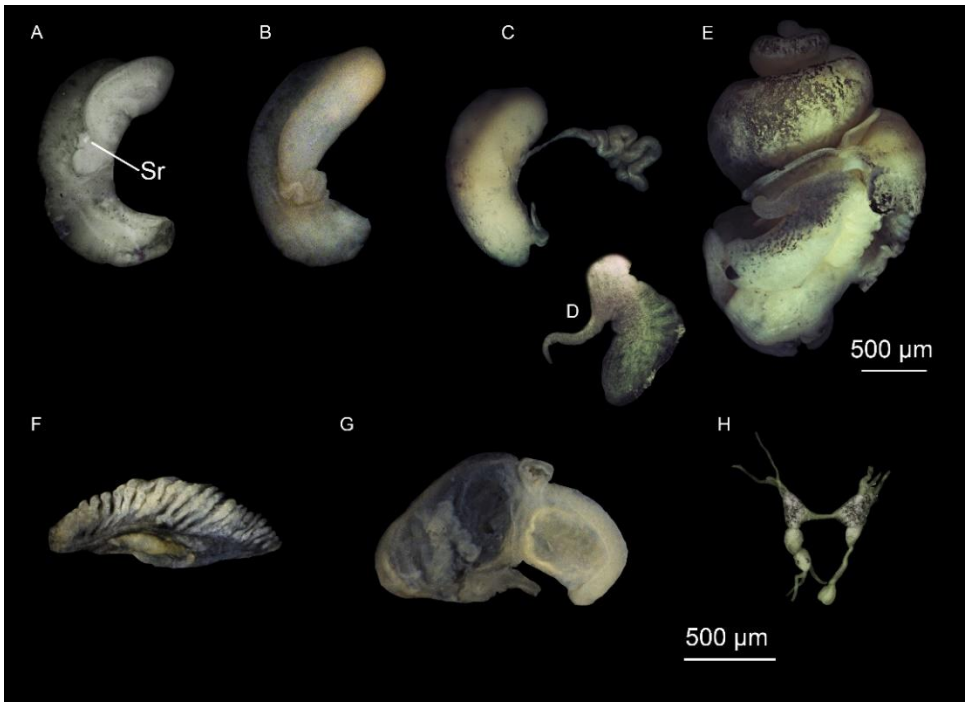


Figure 59. Anatomy of a *M. felixi* sp. nov. specimen from the stream in Canuto de la Tala, Cádiz, Spain. **A** and **B,** female genitalia; **C**, prostate gland; **D**, penis; **E**, animal and penis; **F**, ctenidium and osphradium; **G**, stomach; **H**, perioesophageal ring.

Female genitalia with a glandular oviduct 3 times longer than wide; albumen gland ca. 2 times longer than the capsule gland; bursa copulatrix pyriform to elongate, ca. 3 times longer than wide (Figure 59A,B); bursal duct shorter than bursa copulatrix; renal oviduct unpigmented, highly coiled with three loops; seminal receptacle elongate, with a short duct, positioned on the distal part of the renal oviduct just above the junction with the bursal duct.

Male genitalia with a bean-shaped prostate gland, about 3 times longer than wide, connected by the posterior vas deferens to a convoluted seminal vesicle and the testis. Penis darkly pigmented, gradually tapering, attached to the neck behind the right eye; penial appendix ovate, darkly pigmented at the junction with the penis,

about the same length or slightly shorter than the distal end of the penis and medially positioned on the inner edge of the penis (Figure 59D, E).

Nervous system pigmented, elongate (RPG ratio 0.60; see Table 23); cerebral ganglia approximately equal in size, pleuro-supraoesophageal connective ca. 11 times longer than the pleuro-suboesophageal one (Figure 59H).

Distribution: This species was collected from two streams in southern Spain: the stream in Canuto de las Palas (type locality) and the stream in Canuto del Zapato (Figure 60).

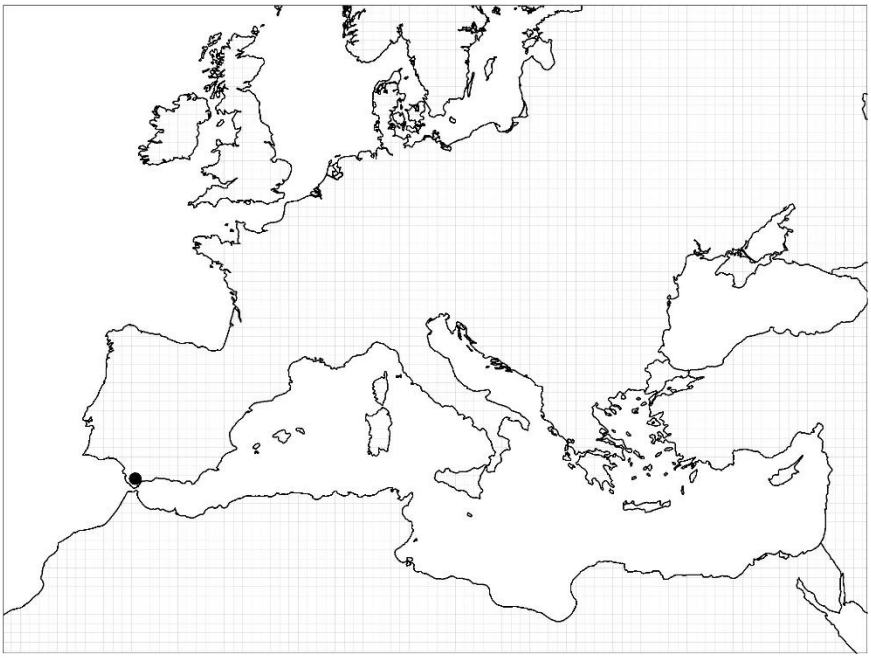


Figure 60. Distribution map of *M. felixi* sp. nov. according to the studied populations.

Remarks: *Mercuria felixi* sp. nov. differs from the phylogenetically closely related species *M. tensiftensis* and *M. balearica* by having a narrower aperture (wider in *M. balearica*); a more globose shell (high-spired in *M. tensiftensis)*; a granulated protoconch microsculpture (grooved in *M. tensiftensis*); an ovate, pigmented penial appendix that is about the same length or slightly shorter than the distal end of the penis (rounded, unpigmented and shorter than the distal end of the penis in *M. balearica* and darkly pigmented and shorter than the distal end of the penis in *M. tensiftensis*); a thin, filiform, pigmented distal end of the penis (broad, triangular and strongly and darkly pigmented in *M. balearica and*

triangular and pigmented in *M. tensiftensis*); a prostate gland that is 3 times longer than wide (2 times longer than wide in *M. tensiftensis* and *M. balearica*) and a smaller radular ribbon. The species also has one more cusp on the central teeth than in *M. tensiftensis.*

The shell shape of *M. felixi* sp. nov. resembles that of *M. carrillorum* sp. nov. and *M. tachoensis*; however, our PCA (Figure 24) showed that *M. felixi* sp. nov. can be differentiated from the rest of the *Mercuria* species. The penile morphology of *M. felixi* sp. nov. further differentiates it from the Iberian congeners: it has a very thin, pigmented penial appendix and a long, filiform and pigmented distal penis.

According to our molecular analyses, this species forms a clade with *M. balearica* (5.3% of COI divergence) and *M. tensiftensis* (5.1% of COI divergence). It is most distantly related to *M. midarensis* and *M. saharica*, diverging from these species by 8% and 8.6%, respectively.

Ecology: The ecology of the species is not well known. The species inhabits streams of very clear running waters with relatively low conductivities (75–292 mS/cm^2). The specimens collected from the stream in Canuto del Zapato were found in very low abundance. Co-occurring species are *Ancylus fluviatilis* and *Galba truncatula* in low abundance and *Psidium* spp. in high abundance (Félix Ríos Jiménez, pers. comm.).

***Mercuria lupiaensis* sp. nov.**

(Figs 61–64)

Type Material: HOLOTYPE: MNCN 15.05/200123H

PARATYPES: (1) MNCN 15.05/200123P; (USGB16236, USGB16237), Justus Liebig University Giessen, Germany.

Type locality: Giammatteo Creek, Frigole, Lecce, Italy.

Material Examined: Italy: Giammatteo Creek, Frigole, Lecce (USGB8015); Palude del Capitano Pond, Sant´Isidoro, Nardò, Apulia (USGB16236); Spring in Torre Castiglione, Ionian Coast, Porto Cesareo, Apulia (USGB16237). Additional locality information provided in Appendix 5.

Etymology: The specific epithet *lupiaensis* refers to Lupiae, the name of the city of Lecce during the time of the ancient Roman empire.

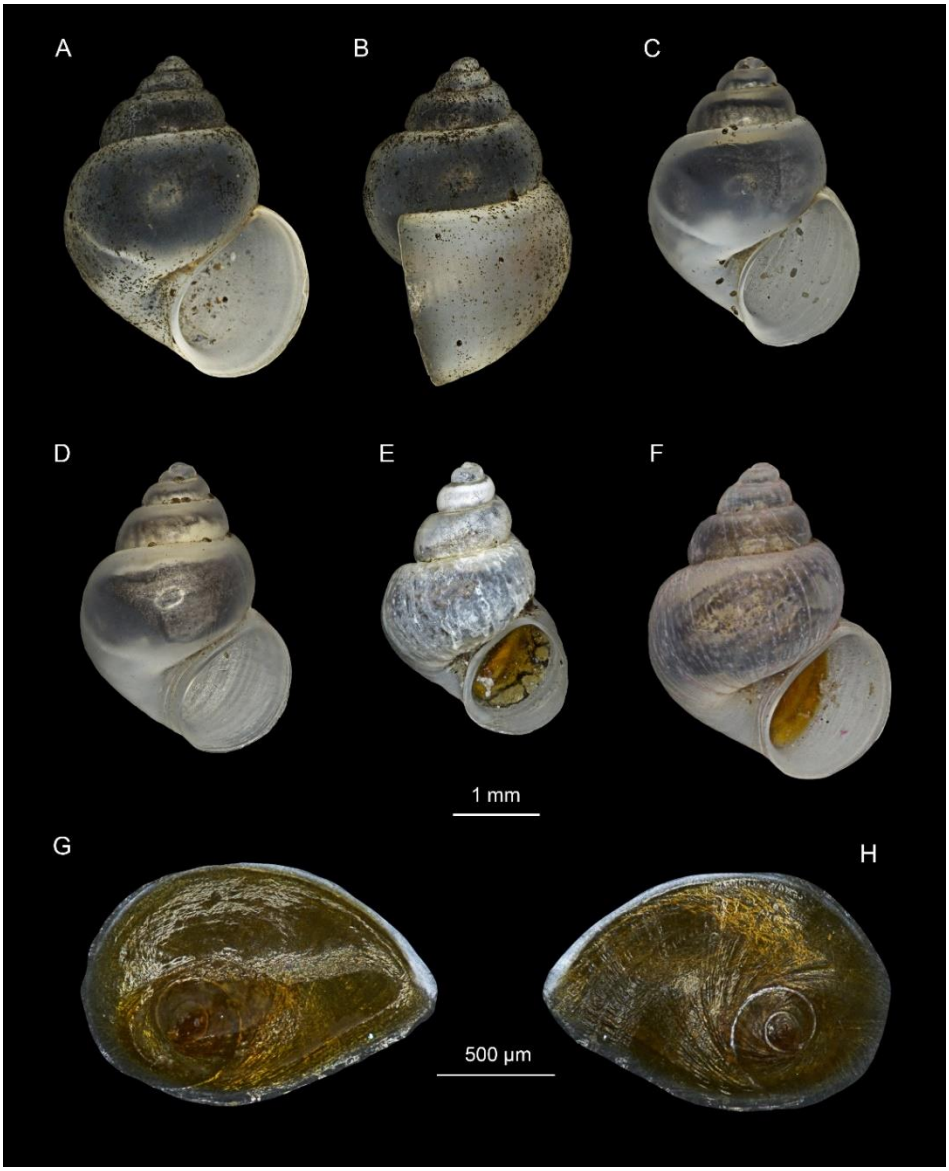


Figure 61. Shells and operculum of *M. lupiaensis* sp. nov. **A** and **B,** holotype USGB8015; **C** and **D,** paratypes; **A**–**D**, specimens from Giammatteo Creek, Frigole, Lecce; **E**, specimen from the spring in Torre Castiglione, Ionian Coast, Porto Cesareo, Apulia; **F**, specimen from Palude del Capitano Pond, Sant´Isidoro, Ionian coast, Nardò, Apulia; **G**, operculum inner side; **H**, operculum outer side. Modified from original pictures of Diana Delicado Iglesias.

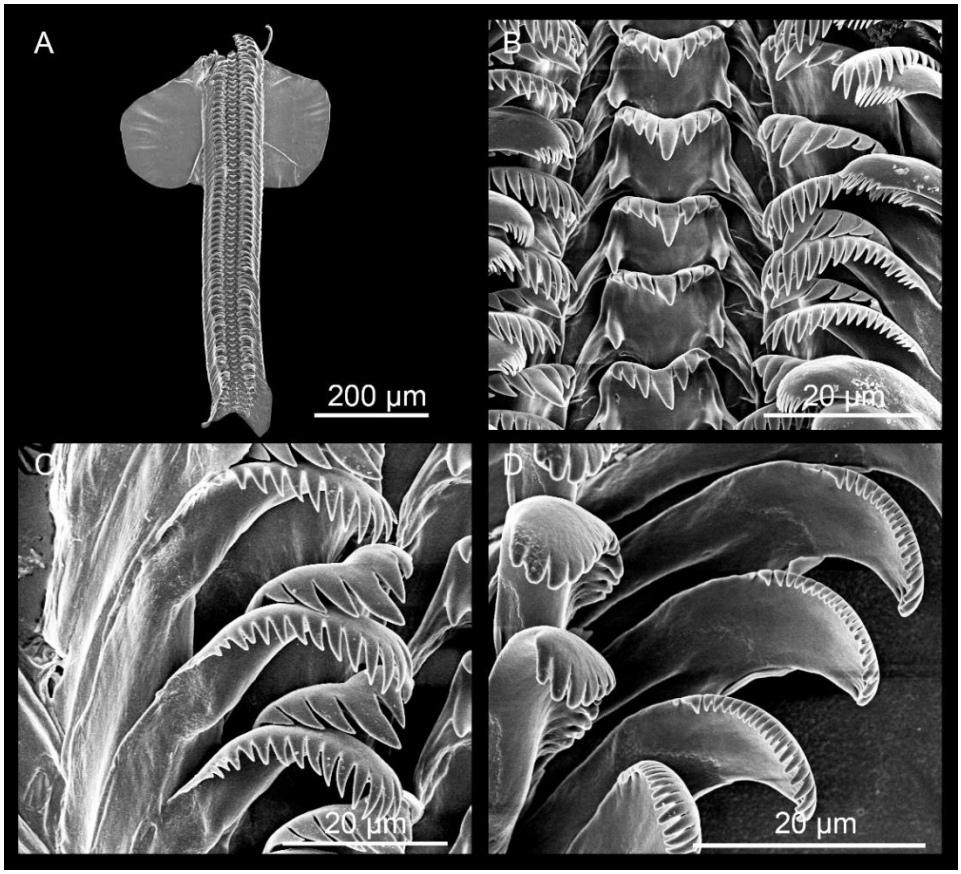


Figure 62. Radula of the *M. lupiaensis* sp. nov. specimen from Giammatteo Creek, Lecce. **A**, general view of the radular ribbon; **B**, overview of radular teeth rows; **C**, detailed view of the central teeth; **D**, detailed view of the outer marginal teeth. Modified from original pictures of Diana Delicado Iglesias.

Diagnosis: Shell ovate-conic; aperture obliquely ovate; periostracum whitish to pale grey; central radular tooth formula 3–C–3/1–1; female genitalia with bursa copulatrix pyriform to elongate, ca. 2 times longer than wide; seminal receptacle pyriform; penis darkly pigmented; penial appendix unpigmented, ovate, smaller than the distal end of the penis and medially positioned on the inner edge of the penis; nervous system pigmented, elongate (mean RPG ratio = 0.53); cerebral ganglia approximately equal in size, with small black spots.

Description: Shell ovate-conic, whorls 4–5, height 2.5–3.2 mm, width 2.4–2.6 mm (Figure 61A–F); periostracum whitish to pale grey; protoconch of 1.5 whorls, ca. 300 µm wide, nucleus ca. 150 µm wide; teleoconch whorls slightly convex, separated by a deep suture; body whorl large, convex, occupying about two-thirds

of the total shell length; aperture obliquely broad ovate, complete; inner lip thicker than outer lip; aperture margin straight, inner lip attached to the body whorl; umbilicus narrow, not covered by the inner lip (Figure 61A, C–F).

Operculum as for the genus, orange to brown, about two whorls; muscle attachment oval, located near the nucleus (Figure 61G, H).

Radula length medium, ca. 800 µm long (35% of total shell length), containing about 60 rows of teeth. Central tooth formula 3–C–3/1–1, central cusp "V" shaped, cutting edge slightly concave (Figure 62A–D). Lateral tooth formula 3–C–3, central cusp "V" shaped and slightly longer than the central tooth one. Inner marginal teeth with 14–17 cusps; outer marginal teeth with 22–25 cusps (Figure 62D). Radular data were collected from specimens from Giammatteo Creek, Frigole, Lecce (USGB8015); Palude del Capitano Pond, Sant´Isidoro, Ionian coast, Nardó, Apulia (USGB16236) and a spring in Torre Castiglione, Ionian Coast, Porto Cesareo, Apulia (USGB16237).

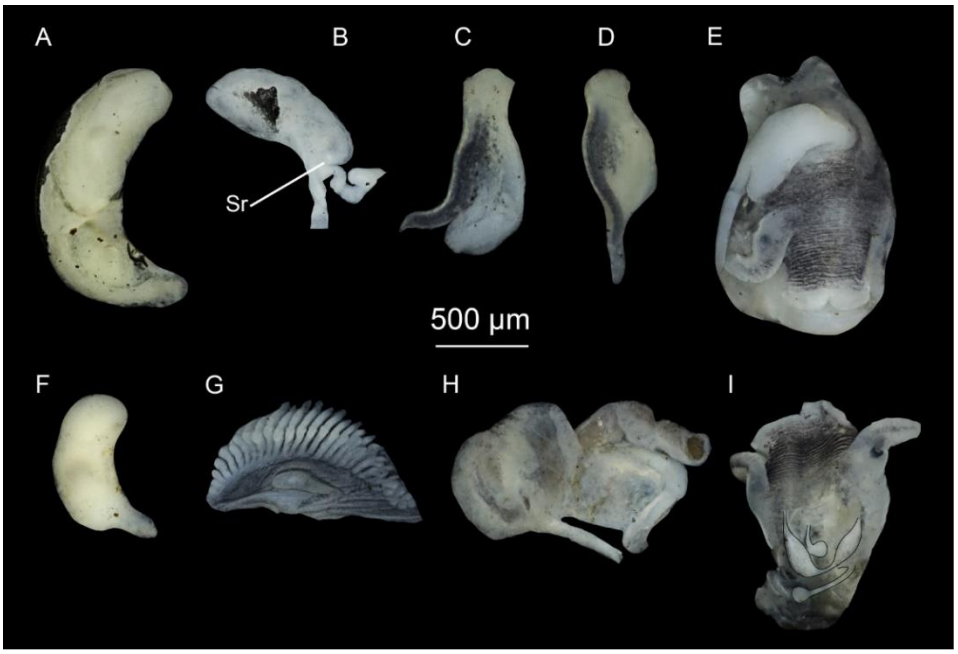


Figure 63. Anatomy of *M. lupiaensis* sp. nov. **A** and **B**, female genitalia; **C** and **D**, penis; **E**, head of a male; **F**, prostate gland; **G**, ctenidium and osphradium; **H**, stomach; **I**, head with perioesophageal ring attached. Modified from original pictures of Diana Delicado Iglesias.

Pigmentation and anatomy

Animal darkly pigmented (Figure 63E, I); head and tentacles black pigmented, pigmentation lighter on eye lobes, snout and neck; snout about as long as wide, approximately parallel-sided, with medium distal lobation. Ctenidium occupying almost the total length of the pallial cavity; 23–27 gill filaments; filaments broad, triangular, fused at the base by an epithelium (Figure 63G). Stomach almost as long as wide with two chambers almost equal in size; style sac longer than wide, with the unpigmented intestine surrounding its distal part and continuing on as a straight rectum (Figure 63H).

Female genitalia with a glandular oviduct 2.5 times longer than wide; albumen gland and capsule gland about the same size (Figure 63A, B); bursa copulatrix elongate, ca. 2 times longer than wide; bursal duct shorter than bursa copulatrix; renal oviduct unpigmented, highly coiled with three loops; seminal receptacle pyriform, with a short duct, positioned on the distal part of the renal oviduct just above the junction with the bursal duct (Figure 63B).

Male genitalia with a bean-shaped prostate gland, about 2 times longer than wide, connected by the posterior vas deferens to a convoluted seminal vesicle and the testis. Penis darkly pigmented, gradually tapering, attached to the central area of the neck; penial appendix unpigmented or slightly pigmented at the junction with the distal end of the penis. Penial appendix ovate, smaller than the penis, base narrow, medially positioned on the inner edge of the penis (Figure 63C–E).

Nervous system pigmented, elongate (mean RPG ratio = 0.53; see Table 23); cerebral ganglia approximately equal in size (Figure 63I).

Distribution: The species is only known from the three nearby localities in the Salento Peninsula in the region of Apulia, Italy (Figure 64).

Remarks: *Mercuria lupiaensis* sp. nov. now represents the easternmost species of the genus and the first *Mercuria* species described from the Apennine Peninsula. According to the PCA, the new species and *M. similis* have a similar shell shape; however, they can be easily distinguished by differences in the penial appendix: in *M. similis*, it is large, triangular and highly pigmented, whereas in *M. lupiaensis* sp. nov., it is small, sometimes very small, ovate and slightly pigmented or unpigmented. In the new species, the albumen and capsule glands are about the same size, whereas, in *M. similis*, the albumen gland is longer than the capsule gland. They also present differences in the bursa copulatrix: elongate and about 2 times longer than wide in *M. lupiaensis* sp. nov. and pyriform to elongate and 3 times longer than wide in *M. similis*.

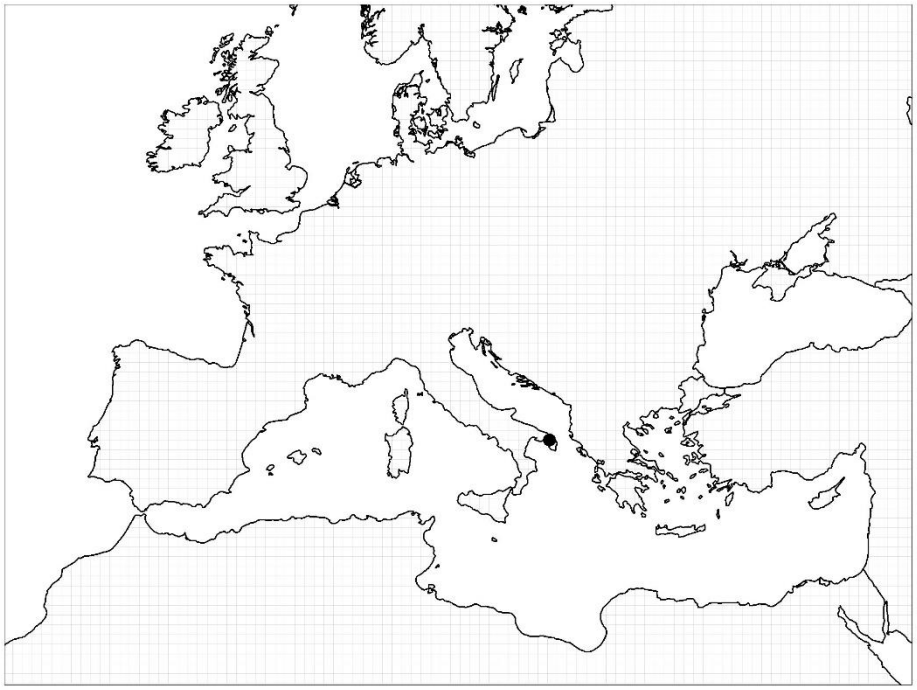


Figure 64. Distribution map of *M. lupiaensis* sp. nov. according to the studied populations.

Mercuria lupiaensis sp. nov. can be further distinguished from the phylogenetically closely related species *M. veronicae* sp. nov. by its smaller size, lighter dorsal pigmentation, similarly sized capsule and albumen glands (albumen gland longer than capsule gland in *M. veronicae* sp. nov.), a penial appendix that is shorter than the distal end of the penis and a smaller number of cusps on the central teeth.

Genetic divergence estimates of COI showed that the species is most closely related to *M. veronicae* sp. nov. (average divergence of 3.9%) and most distantly to *M. midarensis* (with a divergence of 9.3%).

Ecology: The ecology of the species is not well known. The species was found in very clear running waters, close to the coastal margins. Co-occurring species are *Pseudamnicola conovula*, *Heleobia stagnorum* and *Bithynia leachii* (Sheppard, 1823).

***Mercuria veronicae* sp. nov.**

(Figs 65–68)

Type Material. HOLOTYPE: MNCN 15.05/200124H

PARATYPES: (3) MNCN 15.05/200124P; USGB17271, USGB17272, USGB17274, USGB17276, Justus Liebig University Giessen, Germany;

Material Examined: Tunisia: El Waha Spring, Oasis Waterfall, Tamerza, Tozeur (USGB17271, USGB17272); Lekbir Spring, Big Waterfall, Tamerza, Tozeur (USGB17274); Echbicka Spring, Echbika Waterfall, Echbika, Tozeur (USGB17276). Additional locality information provided in Appendix 5.

Type locality: El Waha Spring, Oasis Waterfall, Tamerza, Tozeur, Tunisia.

Etymology: For Veronica Ruiz, my grandmother, who suffered the consequences of having a scientist at home.

Diagnosis: Shell ovate-conic; aperture obliquely ovate; periostracum whitish to pale grey; central radular tooth formula (5)4–C–4(5)/1–1; female genitalia with a bursa copulatrix pyriform to elongate, ca. 2 times longer than wide; seminal receptacle elongate; penis darkly pigmented; penial appendix slightly pigmented, ovate, shorter than or equal in length to the distal end of the penis, medially positioned on the inner edge of the penis; nervous system pigmented, elongate (mean RPG ratio = 0.55); cerebral ganglia approximately equal in size; pleuro-supraoesophageal connective ca. 11 times longer than the pleuro-suboesophageal one.

Description: Shell ovate-conic, whorls 4–5, height 4.6–5.3 mm, width 3.4–4 mm (Figure 65A–F); periostracum whitish to pale grey; protoconch of 1.5 whorls, ca. 250 µm wide, nucleus ca. 150 µm wide; teleoconch whorls slightly convex, separated by a deep suture; body whorl large, convex, occupying about two-thirds of the total shell length; aperture obliquely broad ovate, complete; inner lip thicker than outer lip; aperture margin straight; inner lip touching the body whorl; umbilicus narrow, not covered by the inner lip.

Operculum as for the genus, orange to brown, sometimes yellowish, about two whorls; muscle attachment oval, located near the nucleus (Figure 65G, H).

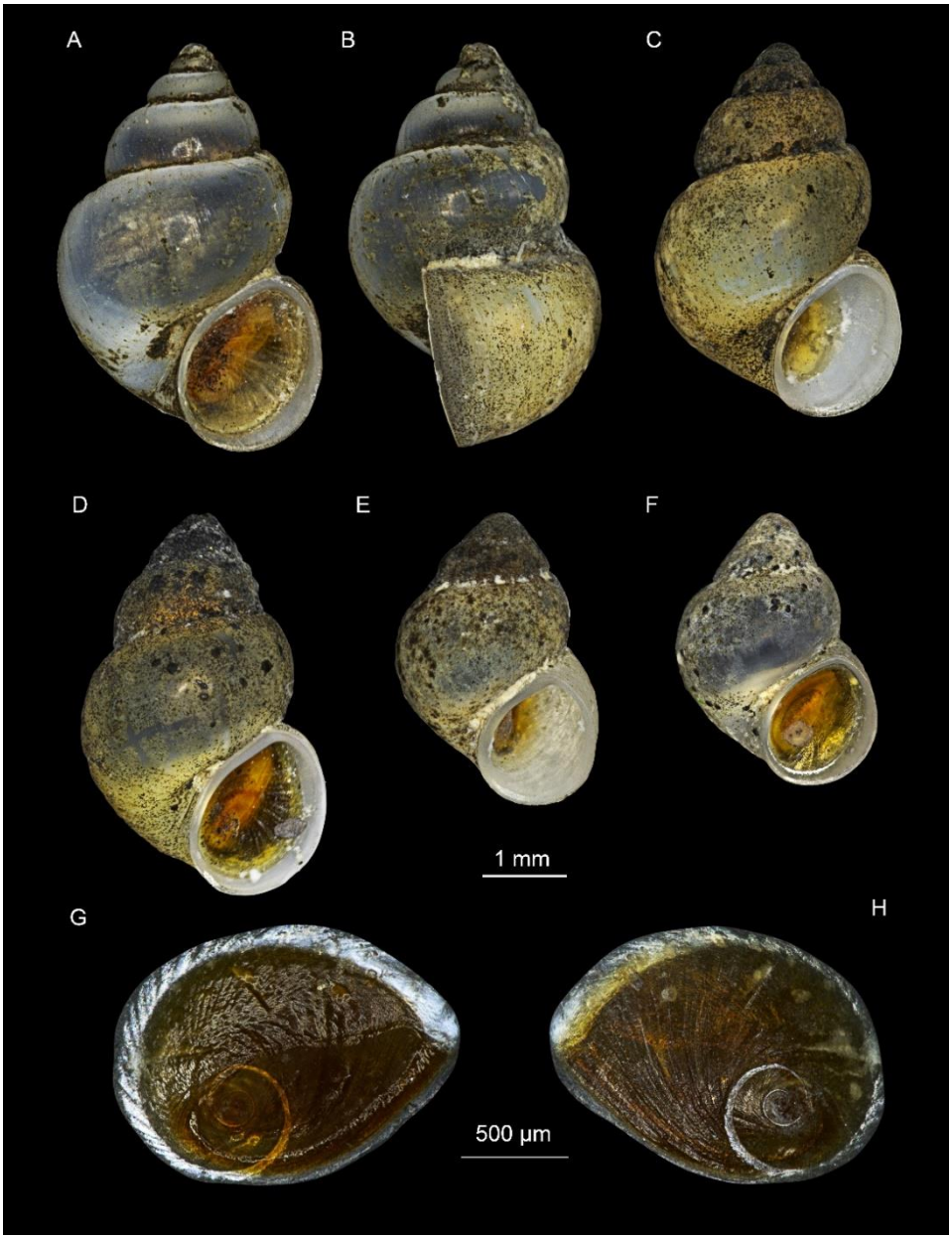


Figure 65. Shells and operculum of *M. veronicae* sp. nov. **A** and **B,** holotype USGB17271; **C**–**F,** paratypes; **A**–**D**, El Waha Spring, Oasis Waterfall, Tamerza, Tozeur; **E** and **F**, Lekbir Spring, Big Waterfall, Tamerza, Tozeur; **G**, operculum inner side; **H**, operculum outer side. Modified from original pictures of Diana Delicado Iglesias.

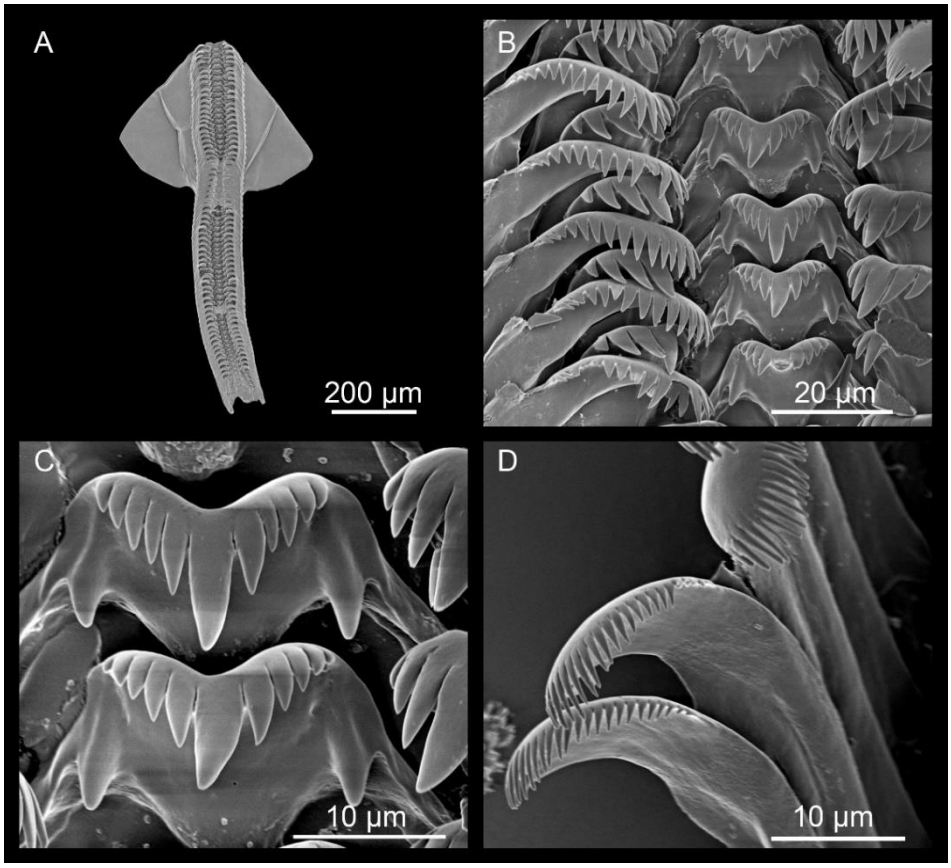


Figure 66. Radula of the *M. veronicae* sp. nov. specimen from El Waha Spring, Tozeur. **A**, general view of the radular ribbon; **B**, overview of radular teeth rows; **C**, detailed view of the central teeth; **D**, detailed view of the outer marginal teeth. Modified from original pictures of Diana Delicado Iglesias.

Radula length medium, ca. 750 µm long (35% of total shell length), containing about 60 rows of teeth. Central tooth formula (5)4–C–4(5)/1–1, central cusp "V" shaped, cutting edge concave (Figure 66A–C). Lateral tooth formula (3)4–C–4(3), central cusp "V" shaped and slightly longer than the central tooth one. Inner marginal teeth with 11–15 cusps; outer marginal teeth with 21–25 cusps (Figure 66D). Radular data were collected from the following specimens: USGB17271 – El Waha Spring, Oasis Waterfall, Tamerza, Tozeur, Tunisia; USGB17274 – Lekbir Spring, Big Waterfall, Tamerza, Tozeur, Tunisia; USGB17276 – Echbicka Spring, Echbika Waterfall, Echbika, Tozeur, Tunisia.

Pigmentation and anatomy

Animal darkly pigmented (Figure 67A, D); head and tentacles dark brown, pigmentation lighter on eye lobes, snout and neck; snout about as long as wide, approximately parallel-sided, with medium distal lobation. Ctenidium occupying almost the total length of the pallial cavity; 28–33 gill filaments; filaments broad, triangular, fused at the base by an epithelium (Figure 67B). Osphradium elongate, more than 3 times longer than wide, positioned opposite to the middle of the ctenidium.

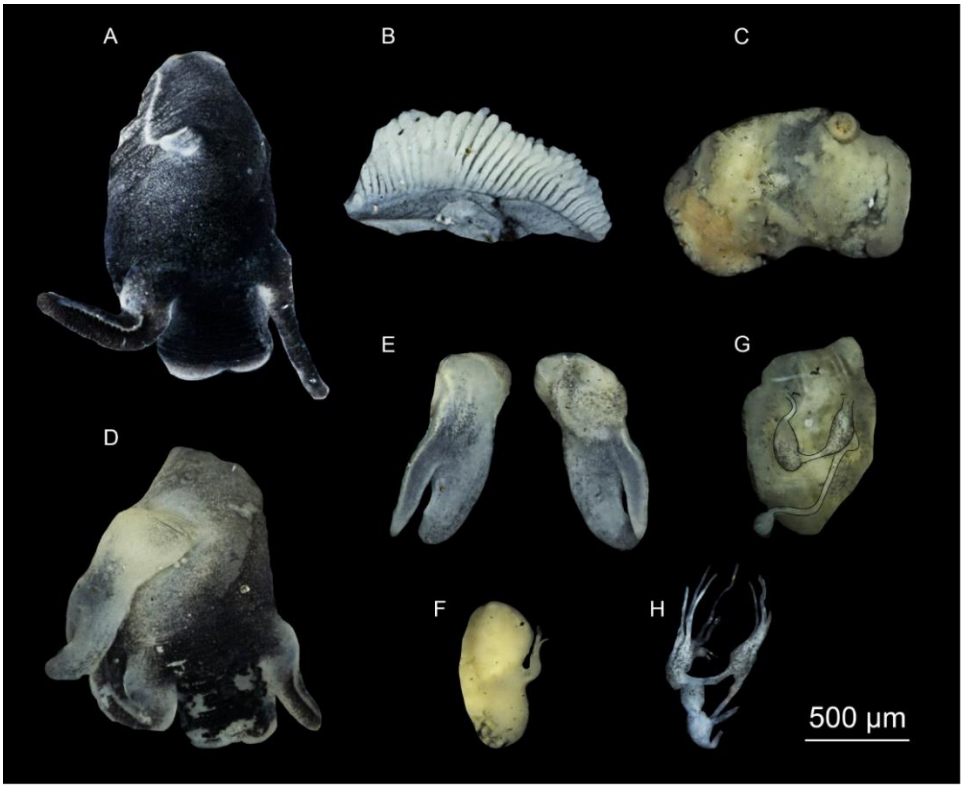


Figure 67. Anatomy of the *M. veronicae* sp. nov. specimen from Spring El Waha, Tozeur, Tunisia. **A**, head; **B**, ctenidium and osphradium; **C**, stomach; **D**, head of a male; **E**, penises; **F**, prostate gland; **G,** head with perioesophageal ring attached and **H**, perioesophageal ring. Modified from original pictures of Diana Delicado Iglesias.

Stomach almost as long as wide with two chambers almost equal in size; style sac longer than wide, with the unpigmented intestine surrounding its distal part and continuing on as a straight rectum (Figure 67C).

Female genitalia with a glandular oviduct 2.5 times longer than wide, albumen gland longer than capsule gland; bursa copulatrix elongate, ca. 2 times longer than

wide; bursal duct shorter than bursa copulatrix; renal oviduct unpigmented, highly coiled with three loops; seminal receptacle elongate, with a short duct, positioned on the distal part of the renal oviduct just above the junction with the bursal duct.

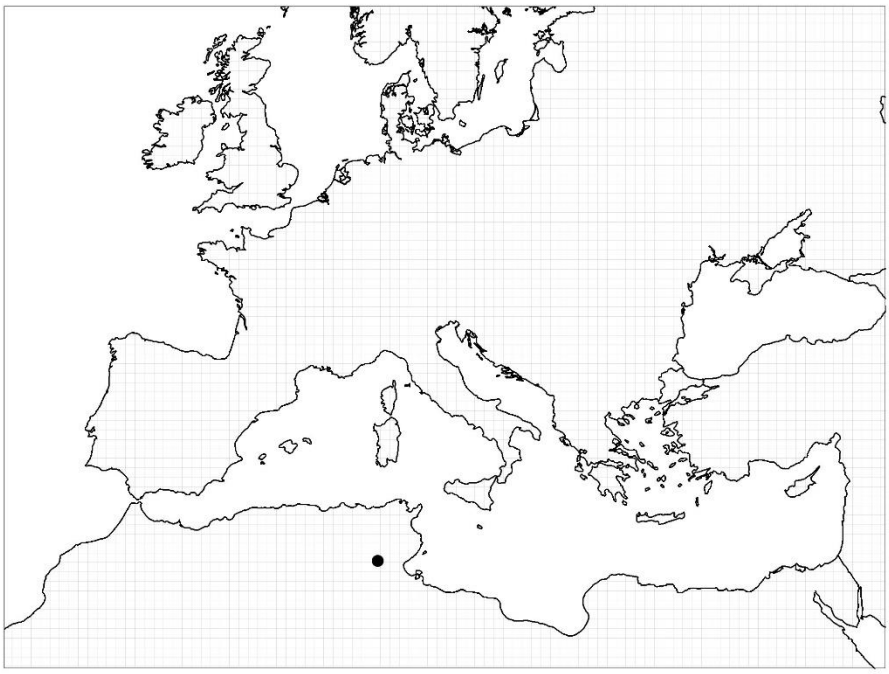


Figure 68. Distribution map of *M. veronicae* sp. nov. according to the studied populations.

Male genitalia with a bean-shaped prostate gland, about 2 times longer than wide. Pallial vas deferens emerge close to both the anterior edge of the prostate and the external margin of the penis (Figure 67F). Penis darkly pigmented, gradually tapering, attached to the neck behind the right eye, distal end of the penis broad, triangular; penial appendix pyriform, shorter than or about to the same length as the distal end of the penis, slightly pigmented at the junction with the penis, medially positioned on the inner edge of the penis (Figure 67D, E).

Nervous system pigmented, elongate (mean RPG ratio = 0.55; see Table 23); cerebral ganglia approximately equal in size; pleuro-supraoesophageal connective ca. 11 times longer than the pleuro-suboesophageal one (Figure 67G, H).

Distribution: The species is only known from the three nearby localities in Tozeur, Tunisia (Figure 68).

Remarks: Intraspecies morphological differences were observed mainly in the penial features of individuals from the same population (Fig. 67D, E). Such variation may be parasite-induced, similar to the case reported in another *Mercuria* species (Boulaassafer *et al*., 2018). Parasitized females also presented an atrophied pallial oviduct, making it difficult to conduct a complete anatomical study.

Mercuria veronicae sp. nov. differs from the phylogenetically closely related species *M. lupiaensis* sp. nov. and *M. melitensis* by having a high-spired shell and a more elongated bursa copulatrix compared with *M. lupiaensis* sp. nov. and thinner inner and outer shell lips compared with the characteristically thick ones of *M. melitensis* (Glöer et al. 2015a). *Mercuria veronicae* sp. nov. also has a longer, triangular and more pigmented penial appendix compared with the short (to very short), ovate and slightly pigmented or unpigmented one in *M. lupiaensis* sp. nov. In *M. melitensis*, the penial appendix is only as long as the distal end of the penis. Compared with other geographically proximate species, *M. veronicae* sp. nov. resembles *M. pycnocheilia* in shell shape, although the latter presents a thicker inner shell lip (see Glöer et al. 2010) and and the former, smaller-sized shells. In addition, Glöer (2019) and Glöer et al. (2010) noted the acute apex of *M. pycnocheilia*, which is not present in *M. veronicae* sp. nov. Shell shape features also distinguish the new species from *M. globulina*, which has a larger, more slender shell and *M. bourguignati*, which has a larger shell and smaller aperture (about half the shell length in *M. bourguignati* vs. about three quarters the shell length in *M. veronicae* sp. nov.). The anatomy of these geographically proximate species is unknown.

According to our genetic divergence estimates of COI, *M. veronicae* sp. nov. is most similar to *M. lupiaensis* sp. nov. (average divergence of 3.9%) and diverges most with *M. tensiftensis* (by 8.4%).

Ecology: The ecology of the species is not well known. It inhabits water bodies within the Sahara Desert in the region of Tozeur, Tunisia. Co-occurring species are *Heleobia* sp., *Melanoides* sp. and *Melanopsis* sp.

Table 22. Shell dimensions (mm) of *Mercuria* spp.: **1**, *M. egarensis* sp. nov., Font de les Canyes, Terrassa, Catalonia, Spain; **2**, *M. carrillorum* sp. nov., stream in Canuto de la Gallina, Cadiz, Spain and **3**, stream in Canuto de las Palas, Cadiz, Spain; **4**, *M. lupiaensis* sp. nov., Palude del Capitano Pond, Sant´Isidoro, Ionian coast, Nardó, Apulia, Italy and **5**, Giammatteo Creek, Frigole, Lecce, Italy; **6**, *M. felixi* sp. nov., Canuto de la Tala, Cadiz, Spain; **7**, *M. veronicae* sp. nov., El Waha Spring, Oasis Waterfall, Tamerza, Tozeur, Tunisia. See Table 3 in Chapter 2 for a full list of abbreviations.

	1 Mean ± SD; CV (Max – Min) n=32	**2** Mean ± SD; CV (Max – Min) n=18	**3** Mean ± SD; CV (Max – Min) n=33	**4** Mean ± SD; CV (Max – Min) n=5	**5** Mean ± SD; CV (Max – Min) n=5	**6** Mean ± SD; CV (Max – Min) n=34	**7** Mean ± SD; CV (Max – Min) n=6
SL	3.58 ± 0.28; 0.08 (4.31-2.95)	3.33 ± 0.21; 0.06 (3.82-2.84)	2.87 ± 0.28; 0.1 (3.54-2.4)	3.46 ± 0.15; 0.04 (3.71-3.3)	3.24 ± 0.12; 0.04 (3.39-3.11)	3.21 ± 0.31; 0.1 (3.83-2.57)	4.19 ± 0.43; 0.09 (5.27-4.62)
SW	2.5 ± 0.19; 0.08 (2.8-1.78)	2.42 ± 0.18; 0.07 (2.77-2)	2.18 ± 0.17; 0.08 (2.5-1.84)	2.64 ± 0.06; 0.02 (2.7-2.56)	2.56 ± 0.1; 0.04 (2.65-2.46)	2.48 ± 0.2; 0.08 (2.9-2.05)	2.94 ± 0.43; 0.13 (4.06-3.37)
AH	1.71 ± 0.15; 0.09 (1.93-1.28)	1.5 ± 0.11; 0.07 (1.66-1.23)	1.36 ± 0.1; 0.07 (1.55-1.17)	1.6 ± 0.07; 0.04 (1.71-1.54)	1.52 ± 0.17; 0.11 (1.79-1.37)	1.59 ± 0.12; 0.08 (2-1.38)	1.77 ± 0.2; 0.1 (2.3-1.98)
AL	1.46 ± 0.39; 0.27 (2.68-0.92)	1.47 ± 0.12; 0.08 (1.65-1.2)	1.4 ± 0.09; 0.06 (1.59-1.2)	1.62 ± 0.09; 0.06 (1.77-1.52)	1.6 ± 0.14; 0.09 (1.81-1.43)	1.62 ± 0.09; 0.06 (1.85-1.45)	1.87 ± 0.19; 0.09 (2.34-2.03)
AW	1.44 ± 0.24; 0.17 (1.85-1.09)	1.08 ± 0.08; 0.07 (1.25-0.86)	1.05 ± 0.1; 0.1 (1.24-0.89)	1.25 ± 0.05; 0.04 (1.32-1.19)	1.19 ± 0.1; 0.08 (1.29-1.08)	1.15 ± 0.12; 0.1 (1.42-0.94)	1.42 ± 0.16; 0.1 (1.81-1.57)
LBW	2.75 ± 0.21; 0.08 (3.33-2.4)	2.33 ± 0.17; 0.07 (2.67-1.94)	2.11 ± 0.19; 0.09 (2.48-1.78)	2.48 ± 0.12; 0.05 (2.65-2.35)	2.44 ± 0.1; 0.04 (2.56-2.33)	2.4 ± 0.25; 0.1 (2.82-1.41)	2.95 ± 0.42; 0.12 (4.13-3.39)
WBW	2.13 ± 0.16; 0.07 (2.46-1.8)	1.8 ± 0.11; 0.06 (2.05-1.57)	1.68 ± 0.13; 0.08 (1.94-1.42)	2.07 ± 0.09; 0.04 (2.21-1.98)	2.04 ± 0.08; 0.04 (2.11-1.93)	1.85 ± 0.19; 0.1 (2.11-0.95)	2.38 ± 0.33; 0.12 (3.25-2.72)
WAW	1.35 ± 0.19; 0.14 (2.19-1.12)	1.19 ± 0.08; 0.07 (1.36-1.02)	0.98 ± 0.08; 0.08 (1.16-0.85)	1.27 ± 0.1; 0.08 (1.39-1.13)	1.19 ± 0.04; 0.03 (1.23-1.14)	1.1 ± 0.12; 0.11 (1.38-0.83)	1.56 ± 0.14; 0.08 (1.92-1.72)
WPW	0.69 ± 0.08; 0.12 (0.9-0.54)	0.66 ± 0.06; 0.09 (0.78-0.57)	0.55 ± 0.07; 0.13 (0.69-0.39)	0.71 ± 0.06; 0.08 (0.78-0.61)	0.64 ± 0.04; 0.06 (0.7-0.6)	0.61 ± 0.08; 0.13 (0.76-0.46)	0.73 ± 0.11; 0.13 (1.02-0.88)

Table 23. Nervous system measurements (mm) and RPG ratios of *Mercuria* spp.: **1**, *M. tachoensis*; **2**, *M. balearica*; **3**, *M. similis*; **4**, *M. egarensis* sp. nov.; **5**, *M. carrillorum* sp. nov.; **6**, *M. lupiaensis* sp. nov.; **7**, *M. felixi* sp. nov.; **8**, *M. veronicae* sp. nov. See Table 3 in Chapter 2 for a full list of abbreviations

	1 Mean ± SD; CV (Max – Min) n=13	**2** Mean ± SD; CV (Max – Min) n=4	**3** Mean ± SD; CV (Max – Min) n=10	**4** Mean ± SD; CV (Max – Min) n=6	**5** Mean ± SD; CV (Max – Min) n=6	**6** n=1	**7** Mean ± SD; CV (Max – Min) n=9	**8** Mean ± SD; CV (Max – Min) n=3
LRCG	0.22 ± 0.04; 0.18 (0.3-0.13)	0.18 ± 0.04; 0.22 (0.22-0.13)	0.19 ± 0.05; 0.26 (0.32-0.15)	0.24 ± 0.07; 0.29 (0.33-0.13)	0.19 ± 0.07; 0.37 (0.29-0.12)	0.176	0.22 ± 0.03; 0.14 (0.16-0.24)	0.21 ± 0.016; 0.08 (0.22-0.19)
LLCG	0.19 ± 0.03; 0.16 (0.28-0.15)	0.16 ± 0.05; 0.31 (0.23-0.12)	0.19 ± 0.08; 0.42 (0.39-0.1)	0.21 ± 0.05; 0.24 (0.27-0.13)	0.18 ± 0.06; 0.33 (0.26-0.11)	0.216	0.21 ± 0.02; 0.1 (0.19-0.24)	0.22 ± 0.02; 0.09 (0.26-0.2)
LCC	0.14 ± 0.03; 0.21 (0.21-0.1)	0.15 ± 0.1; 0.67 (0.27-0.06)	0.18 ± 0.28; 1.56 (0.96-0.07)	0.14 ± 0.05; 0.36 (0.21-0.07)	0.18 ± 0.21; 1.17 (0.61-0.06)	0.159	0.14 ± 0.04; 0.29 (0.08-0.19)	0.18 ± 0.02; 0.11 (0.21-0.17)
LRPG	0.15 ± 0.03; 0.2 (0.18-0.08)	0.11 ± 0.04; 0.36 (0.15-0.08)	0.12 ± 0.04; 0.33 (0.22-0.08)	0.15 ± 0.05; 0.33 (0.22-0.07)	0.09 ± 0.05; 0.56 (0.12-0)	0.104	0.13 ± 0.01; 0.08 (0.12-0.14)	0.15 ± 0.016; 0.11 (0.17-0.14)
LLPG	0.14 ± 0.03; 0.21 (0.21-0.07)	0.12 ± 0.04; 0.33 (0.16-0.09)	0.16 ± 0.07; 0.44 (0.32-0.09)	0.14 ± 0.03; 0.21 (0.16-0.09)	0.15 ± 0.04; 0.27 (0.23-0.11)		0.11 ± 0.02; 0.18 (0.09-0.15)	0.16 ± 0.04; 0.25 (0.21-0.14)
LSug	0.09 ± 0.04; 0.44 (0.13-0)	0.09 ± 0.03; 0.33 (0.11-0.06)	0.1 ± 0.03; 0.3 (0.16-0.07)	0.11 ± 0.03; 0.27 (0.15-0.08)	0.07 ± 0.04; 0.57 (0.11-0)	0.111	0.1 ± 0.02; 0.2 (0.07-0.12)	0.12 ± 0.01; 0.08 (0.13-0.11)
LSg	0.1 ± 0.02; 0.2 (0.16-0.07)	0.1 ± 0.04; 0.4 (0.13-0.06)	0.09 ± 0.03; 0.33 (0.16-0.05)	0.09 ± 0.03; 0.33 (0.12-0.04)	0.1 ± 0.01; 0.1 (0.11-0.08)		0.09 ± 0.01; 0.11 (0.07-0.11)	0.1 ± 0.03; 0.3 (0.13-0.06)
LPSuC	0.38 ± 0.03; 0.08 (0.51-0.33)	0.27 ± 0.04; 0.15 (0.29-0.24)	0.37 ± 0.12; 0.32 (0.65-0.26)	0.38 ± 0.1; 0.26 (0.48-0.28)	0.22 ± 0.17; 0.77 (0.38-0)	0.248	0.35 ± 0.09; 0.26 (0.24-0.49)	0.35 ± 0.1; 0.29 (0.46-0.26)
LPsc	0.05 ± 0.01; 0.2 (0.08-0.02)	0.03 ± 0.00; 0.00 (0.04-0.03)	0.04 ± 0.02; 0.5 (0.07-0.02)	0.05 ± 0.01; 0.2 (0.06-0.03)	0.04 ± 0.02; 0.5 (0.07-0.02)		0.03 ± 0.01; 0.33 (0.03-0.05)	0.13 ± 0.17; 1.31 (0.33-0.03)
RPG	0.61 ± 0.07; 0.11 (0.74-0.52)	0.53 ± 0.00; 0.01 (0.53-0.53)	0.63 ± 0.04; 0.06 (0.72-0.58)	0.57 ± 0.07; 0.12 (0.67-0.51)	0.63 ± 0.03; 0.05 (0.67-0.6)	0.53	0.6 ± 0.07; 0.12 (0.51-0.7)	0.55 ± 0.08; 0.15 (0.62-0.46)

Table 24. Dimensions of the osphradium, ctenidium and anterior digestive system (mm) in *Mercuria* spp.: **1**, *M. tachoensis*; **2**, *M. balearica*; **3**, *M. similis*; **4**, *M. egarensis* sp. nov.; **5**, *M. carrillorum* sp. nov.; **6**, *M. lupiaensis* sp. nov.; **7**, *M. felixi* sp. nov.; **8**, *M. veronicae* sp. nov. See Table 3 in Chapter 2 for a full list of abbreviations.

	1 Mean ± SD; CV (Max – Min) n=18	**2** Mean ± SD; CV (Max – Min) n=9	**3** Mean ± SD; CV (Max – Min) n=12	**4** Mean ± SD; CV (Max – Min) n=6	**5** Mean ± SD; CV (Max – Min) n=4	**6** Mean ± SD; CV (Max – Min) n=10	**7** Mean ± SD; CV (Max – Min) n=9	**8** Mean ± SD; CV (Max – Min) n=10
CtL	1 ± 0.26; 0.26 (1.33-0.43)	1.09 ± 0.5; 0.46 (1.35-0.63)	1.27 ± 0.45; 0.35 (1.86-0.67)	1.2 ± 0.39; 0.33 (1.77-0.77)	0.92 ± 0.17; 0.18 (1.1-0.7)	1.04 ± 0.13; 0.13 (1.24-0.88)	1.04 ± 0.13; 0.13 (1.25-0.86)	1.54 ± 0.17; 0.11 (1.77-1.32)
OsL	0.36 ± 0.07; 0.19 (0.45-0.22)	0.38 ± 0.18; 0.47 (0.5-0.25)	0.45 ± 0.19; 0.42 (0.74-0.23)	0.41 ± 0.18; 0.44 (0.64-0.2)	0.33 ± 0.08; 0.24 (0.44-0.25)	0.42 ± 0.05; 0.12 (0.49-0.34)	0.36 ± 0.03; 0.08 (0.41-0.31)	0.57 ± 0.05; 0.09 (0.64-0.5)
OsW	0.23 ± 0.27; 1.17 (0.7-0.06)	0.1 ± 0.05; 0.5 (0.14-0.08)	0.09 ± 0.04; 0.44 (0.16-0.04)	0.2 ± 0.2; 1 (0.6-0.06)	0.11 ± 0.03; 0.27 (0.14-0.08)	0.14 ± 0.02; 0.14 (0.17-0.11)	0.08 ± 0.01; 0.13 (0.11-0.07)	0.15 ± 0.03; 0.2 (0.19-0.12)
StL	0.84 ± 0.33; 0.39 (0.92-0.76)	0.51 ± 0.11; 0.22 (0.68-0.32)	0.61 ± 0.11; 0.18 (0.74-0.42)	0.74 ± 0.18; 0.24 (0.99-0.47)	0.61 ± 0.32; 0.52 (1.08-0.4)	1.64 ± 0.19; 0.12 (1.94-1.38)	0.95 ± 0.06; 0.06 (1.03-0.83)	1.8 ± 0.46; 0.26 (2.16-1.03)
StW	0.82 ± 0.33; 0.4 (1-0.69)	0.49 ± 0.13; 0.27 (0.69-0.31)	0.54 ± 0.1; 0.19 (0.67-0.34)	0.68 ± 0.2; 0.29 (0.92-0.38)	0.5 ± 0.18; 0.36 (0.75-0.33)	0.87 ± 0.15; 0.17 (1.16-0.64)	0.8 ± 0.1; 0.13 (0.92-0.63)	1.03 ± 0.25; 0.24 (1.38-0.71)
SsL	0.73 ± 0.29; 0.4 (0.79-0.58)	0.53 ± 0.14; 0.26 (0.73-0.38)	0.61 ± 0.11; 0.18 (0.78-0.4)	0.69 ± 0.13; 0.19 (0.86-0.5)	0.48 ± 0.2; 0.42 (0.77-0.37)	0.85 ± 0.15; 0.18 (1.08-0.67)	0.73 ± 0.08; 0.11 (0.8-0.58)	1.06 ± 0.11; 0.1 (1.17-0.88)
SsW	0.47 ± 0.19; 0.4 (0.57-0.36)	0.3 ± 0.08; 0.27 (0.44-0.19)	0.36 ± 0.09; 0.25 (0.52-0.21)	0.38 ± 0.07; 0.18 (0.48-0.28)	0.29 ± 0.13; 0.45 (0.47-0.2)	0.57 ± 0.08; 0.14 (0.67-0.44)	0.46 ± 0.07; 0.15 (0.57-0.37)	0.74 ± 0.13; 0.18 (0.97-0.64)

Table 25. Female genitalia measurements (mm) recorded in *Mercuria* spp.: **1**, *M. tachoensis*; **2**, *M. balearica*; **3**, *M. similis*; **4**, *M. egarensis* sp. nov.; **5**, *M. carrillorum* sp. nov.; **6**, *M. lupiaensis* sp. nov.; **7**, *M. felixi* sp. nov.; **8**, *M. veronicae* sp. nov. See Table 3 in Chapter 2 for a full list of abbreviations.

	1 Mean ± SD; CV (Max – Min) n=13	**2** Mean ± SD; CV (Max – Min) n=10	**3** Mean ± SD; CV (Max – Min) n=20	**4** Mean ± SD; CV (Max – Min) n=6	**5** Mean ± SD; CV (Max – Min) n=5	**6** Mean ± SD; CV (Max – Min) n=6	**7** Mean ± SD; CV (Max – Min) n=6	**8** Mean ± SD; CV (Max – Min) n=1
AgL	0.99 ± 0.22; 0.22 (1.3-0.64)	0.71 ± 0.21; 0.3 (0.98-0.37)	1 ± 0.33; 0.33 (1.59-0.43)	1.29 ± 0.15; 0.12 (1.43-1.01)	1.1 ± 0.26; 0.24 (1.36-0.69)	1.18 ± 0.31; 0.26 (1.67-0.85)	1.05 ± 0.21; 0.2 (1.32-0.84)	1.32
CgL	0.84 ± 0.11; 0.13 (1-0.66)	0.56 ± 0.18; 0.32 (0.93-0.35)	0.92 ± 0.29; 0.32 (1.65-0.42)	0.85 ± 0.08; 0.09 (0.92-0.7)	0.85 ± 0.07; 0.08 (0.92-0.74)	0.66 ± 0.15; 0.23 (0.82-0.42)	0.78 ± 0.12; 0.15 (0.93-0.63)	0.889
SR1L	0.11 ± 0.02; 0.18 (0.15-0.08)	0.12 ± 0.03; 0.25 (0.17-0.08)	0.15 ± 0.05; 0.33 (0.21-0.06)	0.16 ± 0.03; 0.19 (0.2-0.12)	0.27 ± 0.34; 1.26 (0.88-0.1)	0.14 ± 0.04; 0.29 (0.19-0.1)	0.12 ± 0.02; 0.17 (0.15-0.1)	0.11
BCL	0.69 ± 0.13; 0.19 (0.91-0.52)	0.44 ± 0.09; 0.2 (0.64-0.3)	0.97 ± 0.28; 0.29 (1.56-0.44)	1.01 ± 0.09; 0.09 (1.16-0.89)	0.86 ± 0.2; 0.23 (1.04-0.57)	0.98 ± 0.28; 0.29 (1.31-0.69)	0.86 ± 0.22; 0.26 (1.18-0.6)	0.996
BCW	0.24 ± 0.04; 0.17 (0.33-0.17)	0.16 ± 0.06; 0.38 (0.27-0.09)	0.47 ± 0.17; 0.36 (0.76-0.18)	0.42 ± 0.06; 0.14 (0.52-0.36)	0.38 ± 0.1; 0.26 (0.49-0.25)	0.43 ± 0.12; 0.28 (0.61-0.33)	0.42 ± 0.09; 0.21 (0.56-0.32)	0.501

Table 26. Male genitalia measurements (mm) recorded in *Mercuria* spp.: **1**, *M. tachoensis*; **2**, *M. balearica*; **3**, *M. similis*; **4**, *M. egarensis* sp. nov.; **5**, *M. carrillorum* sp. nov.; **6**, *M. lupiaensis* sp. nov.; **7**, *M. felixi* sp. nov.; **8**, *M. veronicae* sp. nov. See Table 3 in Chapter 2 for a full list of abbreviations.

	1 Mean ± SD; CV (Max – Min) n=7	**2** Mean ± SD; CV (Max – Min) n=8	**3** Mean ± SD; CV (Max – Min) n=9	**4** Mean ± SD; CV (Max – Min) n=3	**5** Mean ± SD; CV (Max – Min) n=4	**6** Mean ± SD; CV (Max – Min) n=3	**7** Mean ± SD; CV (Max – Min) n=5	**8** Mean ± SD; CV (Max – Min) n=1
PrL	0.82 ± 0.25; 0.3 (1.14-0.52)	0.76 ± 0.4; 0.53 (1.01-0.55)	1.09 ± 0.44; 0.4 (1.51-0.46)	1.2 ± 0.23; 0.19 (1.49-1.02)	0.83 ± 0.29; 0.35 (1.14-0.58)	0.77 ± 0.17; 0.22 (0.96-0.62)	1.03 ± 0.26; 0.25 (1.42-0.71)	1.038
PrW	0.37 ± 0.11; 0.3 (0.55-0.25)	0.32 ± 0.17; 0.53 (0.4-0.19)	0.5 ± 0.2; 0.4 (0.69-0.24)	0.53 ± 0.05; 0.09 (0.57-0.47)	0.38 ± 0.14; 0.37 (0.52-0.23)	0.36 ± 0.06; 0.17 (0.43-0.32)	0.46 ± 0.11; 0.24 (0.58-0.34)	0.476
PL	0.49 ± 0.2; 0.41 (0.77-0.18)	0.44 ± 0.17; 0.39 (0.66-0.17)	0.99 ± 0.25; 0.25 (1.51-0.52)	1.06 ± 0.3; 0.28 (1.41-0.83)	0.72 ± 0.14; 0.19 (0.89-0.55)	0.91 ± 0.15; 0.16 (1.09-0.8)	0.58 ± 0.17; 0.29 (0.76-0.31)	0.564
PW	0.15 ± 0.07; 0.47 (0.24-0.05)	0.21 ± 0.07; 0.33 (0.33-0.13)	0.22 ± 0.05; 0.23 (0.32-0.12)	0.19 ± 0.08; 0.42 (0.28-0.09)	0.17 ± 0.06; 0.35 (0.24-0.11)	0.14 ± 0.02; 0.14 (0.16-0.12)	0.14 ± 0.03; 0.21 (0.18-0.09)	0.129
PaL	0.66 ± 0.27; 0.41 (1.03-0.22)	0.61 ± 0.2; 0.33 (0.86-0.36)	0.87 ± 0.19; 0.22 (1.31-0.56)	0.89 ± 0.19; 0.21 (1.06-0.62)	0.75 ± 0.27; 0.36 (1.01-0.43)	0.54 ± 0.12; 0.22 (0.65-0.41)	0.86 ± 0.15; 0.17 (1.01-0.7)	0.25
PaW	0.47 ± 0.19; 0.4 (0.77-0.23)	0.41 ± 0.15; 0.37 (0.71-0.17)	0.73 ± 0.18; 0.25 (1.22-0.4)	0.64 ± 0.13; 0.2 (0.81-0.49)	0.43 ± 0.17; 0.4 (0.6-0.25)	0.29 ± 0.04; 0.14 (0.31-0.24)	0.5 ± 0.12; 0.24 (0.62-0.34)	0.63

3.7 Clock model and substitution rate estimations

Using the levels of evidence suggested by Kass and Raftery (1995), the Bayes factor estimation favoured the relaxed lognormal clock approach for all studied genera (Table 27). Substitution rates and absolute ages were estimated for the 16S and 28S partitions (Table 28) on the basis of a prior COI rate of 0.81± 0.24% (Delicado et al. 2013b). For 16S, *Mercuria* presented the highest substitution rate (0.36 ± 0.09%) and *Islamia*, the lowest one (0.29 ± 0.08%). For 28S, substitution rates were low for all of the genera: *Pseudamnicola* presented the highest rate (0.09 ± 0.02%) and *Corrosella*, the lowest one (0.03 ± 0.001%).

Table 27. Maximum Likelihood Estimation (MLE, expressed in -*lnL*) of the strict and relaxed molecular clock approaches of the studied genera and the Bayes factor (BF) for the best clock model selection.

Clock Model	NSequences	MLE	BF
Islamia strict clock	18	-6382.62	-
Islamia relaxed clock	18	-6359.10	-47.04**
Mercuria strict clock	191	-8120.12	-
Mercuria relaxed clock	191	-8119.66	-0.92**
Pseudamnicola strict clock	123	-9721.21	-
Pseudamnicola relaxed clock	123	-9702.69	-18.52**
Corrosella strict clock*	102	-6865.59	-
Corrosella relaxed clock*	102	-6860.90	-4.69**

* values from Delicado et al. (2013b)
** selected model

Table 28. Estimated substitution rates per gene and genus obtained with *BEAST. Rates were estimated on the basis of an external COI rate.

	Mercuria	*Islamia*	*Pseudamnicola*	*Corrosella**
16S	0.36 ± 0.09%	0.29 ± 0.08%	0.29 ± 0.06%	0.3 ± 0.001%
28S	0.07 ± 0.02%	0.05 ± 0.01%	0.09 ± 0.02%	0.03 ± 0.0001%

* values from Delicado et al. (2013b)

3.8 Divergence times and ancestral range estimation

3.8.1 Reconstruction of *Mercuria* biogeographic history

According to the BioGeoBEARS analysis, the best-fit model was DEC+J, with an AICc = 79.64327 (Table 29). The earliest divergence in *Mercuria* occurred around 7.5 Mya (HPD: 12.81−4.11 Mya), most likely in the Iberian Peninsula (Figure 72A). This event separated the western European species and the African *M. tensiftensis* from the remaining species from North Africa, Italy and Malta (Figure 69).

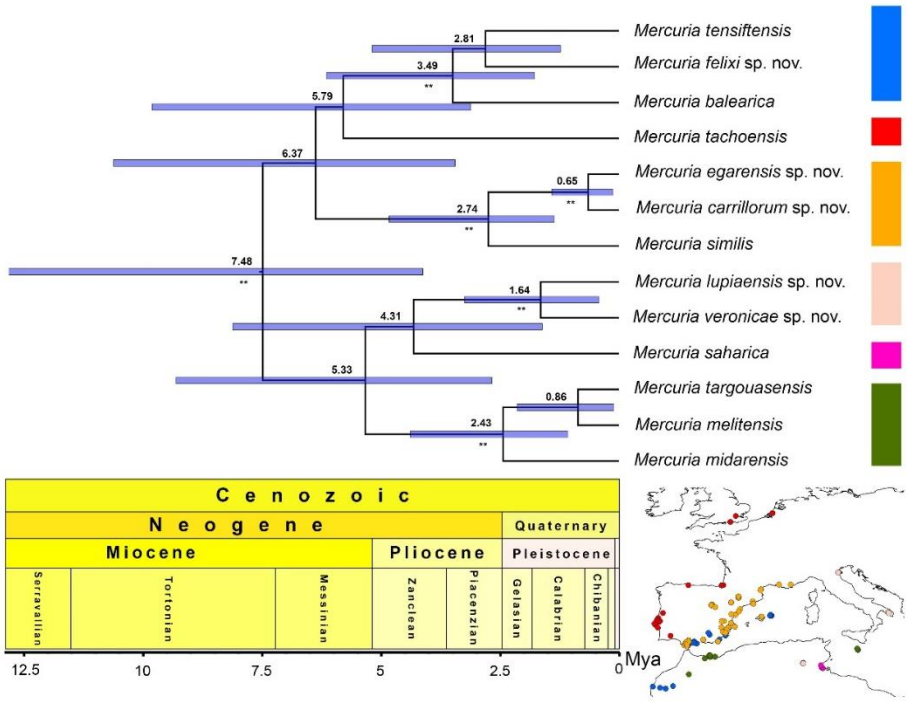


Figure 69. Divergence times of *Mercuria* species based on the lognormal uncorrelated relaxed clock approach and the concatenated dataset (COI + 16S + 28S). An external COI substitution rate calculated from other hydrobiid substitution rates on the basis of biogeographic events was used for the estimation. Coloured bars correspond to well-supported species clades. BPP > 0.95 are indicated with **.

The ancestral range estimation analysis in BioGeoBEARS indicated three major range expansion events (via anagenesis and jump dispersal) in the evolutionary history of *Mercuria*. One event was the expansion of the most recent common ancestor (MRCA) of clade *iii* (comprising *M. tensiftensis* + *M. felixi* n. sp. + *M. balearica*) and *M. tachoensis*, which involved, first, a cladogenetic (jump) dispersal 5.79 Mya (HPD: 9.80–3.11 Mya) from southern to northern Iberia and

then expansion by anagenesis to northern Europe. Another cladogenetic (jump) dispersal event occurred 1.64 Mya (HPD: 3.24–0.42 Mya), resulting in the divergence of the Tunisian species *M. veronicae* sp. nov. and the Italian species *M. lupiaensis* sp. nov. The expansion of the MRCA of the Moroccan species *M. targouasensis* + *M. midarensis* and the Maltese species *M. melitensis* occurred 2.43 Mya (HPD: 4.38–1.08 Mya), corresponding to the Galasiann stage.

The remaining *Mercuria* species grouped in clade *i* (*M. similis*, *M. egarensis* sp. nov. and *M. carrillorum* sp. nov.) diverged within a region of the Iberian ancestral area around 2.74 Mya (HPD: 4.83–1.36 Mya), with the subsequent divergence of *M. carrillorum* sp. nov. and *M. egarensis* sp. nov. occurring within a portion of that ancestral region.

Table 29. Comparison of the models estimated in BioGeoBEARS for *Mercuria* species. The best-fit model was selected on the basis of the AICc.

	-*ln*L	*d*	*e*	*j*	AICc
DEC	-43.95061	0.03198747	0.03668831	0	93.10121
DEC+J	-35.4883	0.01312812	0	0.2689386	**79.64327**
DIVA	-40.10455	0.03160558	0	0	85.40909
DIVA+J	-36.10551	0.01616918	0	0.1566708	80.87768
BAYAREA	-40.31066	0.02662296	0.02204008	0	85.82133
BAYAREA+J	-35.50138	0.01363714	0	0.1315414	79.66944

d, dispersal; *e*, expansion; *j*, founder-event speciation

In total, the ancestral range estimation indicated 11 dispersal events for 24 lineages (dispersal ratio of 0.42), of which four were anagenetic range expansions and seven were range changes causing speciation (founder-event speciation).

3.8.2 Reconstruction of *Islamia* biogeographic history

According to the ancestral range estimation under the best-fit model DEC+J (AICc = 96.32685; Table 30), *Islamia* likely originated in the Italian Peninsula around 12.93 Mya (HPD: 21.98–7.04 Mya) (Figures 70, 72B). Descendant lineages formed five well-supported species clades, each containing species from the same biogeographic area (Figure 72B) and the species *I. cianensis* (see coloured bars in Figure 70). These clades occur in Eurasian territories over the entire Mediterranean region. The BioGeoBEARS analysis showed a remarkable geographic expansion from central to eastern and western Mediterranean areas in the Miocene.

The founder event from Anatolia (Figure 70, pink clade) to Iberia (Figure 70, blue clade) was estimated to have occurred 10.68 Mya (HPD: 17.92–6.01 Mya),

although with low support. Other founder events occurring during the early evolution of the genus included one from southern continental European territories (Figure 70, red clade) to the Balkan Peninsula (Figure 70, brown clade) around 10.28 Mya (HPD: 17.70–5.52 Mya) and, within the Iberian Peninsula, from eastern to western Iberia around 9.48 Mya (HPD: 16.19–5.11 Mya). Another early biogeographic event was the subset sympatry of the species *I. cianensis* (Figure 55, orange lineage) around 9.98 Mya (HPD: 17.24–5.18 Mya).

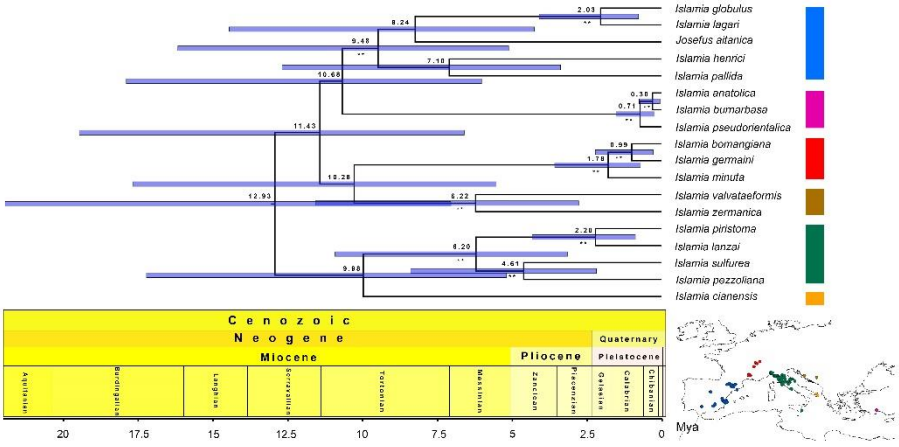


Figure 70. Divergence times of *Islamia* species based on the Bayesian relaxed clock approach (*BEAST) on the concatenated dataset (COI + 16S + 28S). An external COI substitution rate calculated from other hydrobiid substitution rates on the basis of biogeographic events was used for the estimation. Coloured bars correspond to species clades according to well supported groups. BPP > 0.95 are indicated with **.

Three cladogenetic events, corresponding to two founder events and one subset sympatry speciation, were estimated to have occurred during the Messinian: within the Balkan Peninsula, the divergence of *I. valvataeformis* and *I. zermanica* approximately 6.22 Mya (HPD: 11.58–2.76 Mya); within the Iberian Peninsula, the divergence of *I. henrici* and *I. pallida* around 7.10 Mya (HPD: 12.70–3.37 Mya) and, within the Italian Peninsula, the divergence of the clade *I. piristoma* + *I. lanzai* from the clade *I. sulfurea* + *I. pezzoliana* by subset sympatry around 6.20 Mya (HPD: 10.93–3.14 Mya). Also, three geographic range changes were estimated to have occurred at the Pliocene to Pleistocene transition: the subset sympatry of *I. lagari* 2.03 Mya (HPD: 4.06–0.76 Mya) and of *I. lanzai* 2.20 Mya (HPD: 4.32–0.86 Mya) and the founder event involving the southern European species *I. germaini* and *I. bomangiana* at 0.99 Mya.

Table 30. Comparison of the models estimated in BioGeoBEARS for *Islamia* species. The best-fit model was selected on the basis of the AICc.

	-*ln*L	*D*	*e*	*j*	AICc
DEC	-51.60847	0.0186975	0.048527	0	108.01694
DEC+J	-44.30628	0.0029697	1^{-12}	0.077535	**96.32685**
DIVA	-52.62985	0.0155877	0.032244	0	110.05969
DIVA+J	-46.15335	0.0039091	1^{-12}	0.046024	100.02099
BAYAREA	-52.32797	0.0190984	0.058556	0	109.45594
BAYAREA+J	-47.37179	0.0032171	0.0033971	0.032940	102.45786

d, dispersal; *e*, expansion; *j*, founder-event speciation

In total, the ancestral range estimation indicated 13 dispersal events for 34 lineages (dispersal ratio of 0.38), of which five were anagenetic range expansions and eight were founder-speciation events (i.e., jump dispersals).

3.8.3 Reconstruction of *Pseudamnicola* biogeographic history

According to the BioGeoBEARS analysis, the best-fit model was DEC+J (AICc = 119.5205; Table 31). Under this model, the MRCA of *Pseudamnicola* species occurred in the joint area comprising the Italian Peninsula and Islands and Mediterranean Northwest Africa but diverged 6.99 Mya (HPD: 10.65–4.34 Mya) during the Upper Miocene (Figures 71, 72C). The first range expansion, from the Italian Peninsula and Islands to western North Africa 6.12 Mya (HPD: 9.35–3.86 Mya), accounted for the geographic origin of the Iberian–western North African clade (Figure 71, orange clade). Two subsequent jump dispersals were estimated to have occurred within this clade: the divergence of the Majorcan species (*P. subproducta* + *P. meloussensis*) and the Moroccan *P. ouarzazatensis* around 1.26 Mya (HPD: 2.88–0.98 Mya) and the divergence of the North African species *P. dupotetiana* and *P. leprevieri* around 3.34 Mya (HPD: 5.77–1.22 Mya). However, the relationship between the Majorcan species and *P. ouarzazatensis* was not well supported. As such, the divergence of the MRCA of the lineage comprising the Majorcan species and the Moroccan *P. ouarzazatensis* + P. *ramosae*, estimated to have occurred 1.72 Mya (2.88–0.92 Mya), serves as a better reference of trans-Mediterranean speciation events.

Three other jump dispersal events were estimated to have occurred from the Italian Peninsula and Islands to (1) the Balkan Peninsula (involving *P. negropontina* and *Pseudamnicola* sp.10) 2.94 Mya (HPD: 5.37–0.98 Mya), (2) eastern Iberia and the Balearic Islands (at the origin of the clade of *P. subproducta* and *P. meloussensis*) 3.20 Mya (HPD: 5.31–1.69 Mya) and (3) eastern Northwest Africa (inferred for the split between *P. conovula* and *Pseudamnicola* sp.2 1.88 Mya

(HPD: 3.13–0.98 Mya). Minorcan and Iberian species diverged 3.38 Mya (HPD: 5.59–1.74 Mya).

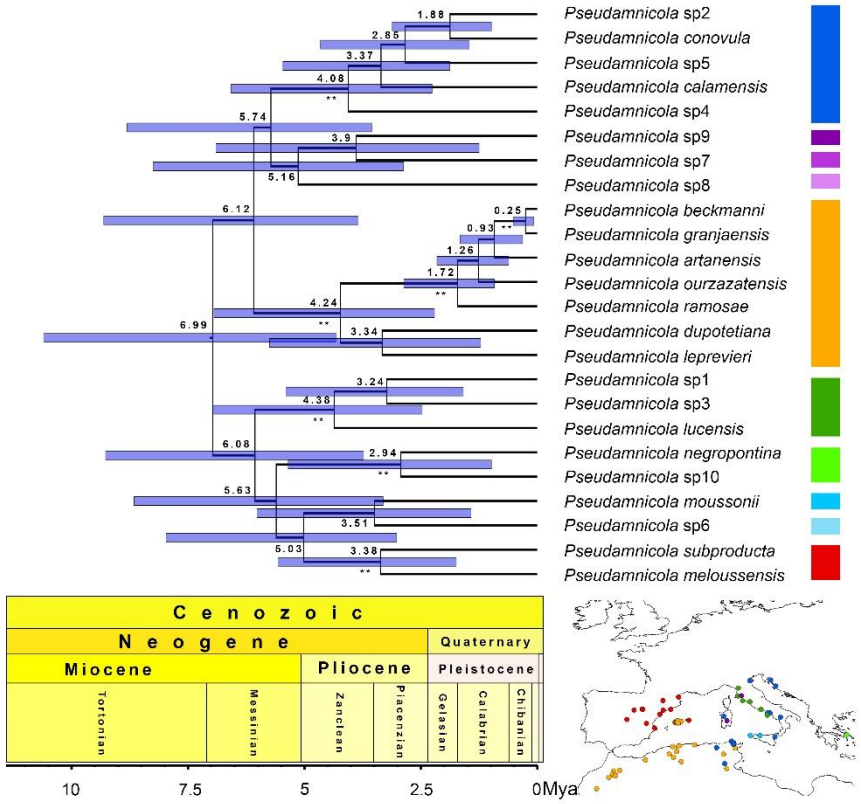


Figure 71. Divergence times of *Pseudamnicola* species based on the Bayesian relaxed clock approach (*BEAST) on the concatenated dataset (COI + 16S + 28S). An external COI substitution rate calculated from other hydrobiid substitution rates on the basis of biogeographic events was used in the estimation. Coloured bars correspond to well-supported clades or single species clades. BPP > 0.95 are indicated with **.

The anagenetic expansion of the MRCA of the Italian species *P. calamensis* to northern Africa was estimated to have occurred 3.37 Mya (HPD: 5.49–1.89 Mya). Another anagenetic expansion event, this time involving *P. conovula*, occurred from the Italian Peninsula to North Africa and Dalmatia around 1.88 Mya (HPD: 3.13–0.98 Mya).

The MRCA of the Iberian species *P. subproducta* expanded its range via anagenesis from eastern to western Iberia and southern France around 3.38 Mya (HPD: 5.59–1.74 Mya).

The most recent speciation event within *Pseudamnicola* was the divergence of *P. beckmanni* and *P. granjaensis* 0.25 Mya (HPD: 0.52–0.08 Mya). The most recent dispersal events involved founder events and anagenetic range expansions during the Calabrian to Chibanian stages.

Table 31. Comparison of the models estimated in BioGeoBEARS for *Pseudamnicola* species. The best-fit model was selected on the basis of the AICc.

	-*ln*L	*d*	*e*	*j*	AICc
DEC	-65.24993	0.01430216	0.02041124	0	135.0713
DEC+J	-56.16025	0.006503166	0	0.03995882	**119.5205**
DIVA	-66.64973	0.01773152	0.004199578	0	137.8709
DIVA+J	-58.35277	0.008049132	0	0.0385345	123.9055
BAYAREA	-65.60857	0.01323405	0.0444098	0	135.7886
BAYAREA+J	-58.24484	0.007525647	0	0.03779423	123.6897

d, dispersal; *e*, expansion; *j*, founder-event speciation

In total, the ancestral range estimation indicated nine dispersal events for 46 lineages (dispersal ratio of 0.24), of which two were anagenetic range expansions and seven were range changes causing speciation (founder-event speciation).

3.8.4 Reconstruction of *Corrosella* biogeographic history

For this genus, the BAYAREA model was selected as the best-fit model (AICc = 37.49505) (Table 32). Under this scenario, the MRCA of the *Corrosella* species occurred in the Southern and the Eastern Iberia ecoregions (Figure 72D). Its descendants diverged around 10.2 Mya and occupied different subsets of the ancestral distribution area.

Table 32. Comparison of the models estimated in BioGeoBEARS for *Corrosella* species. The best-fit model was selected on the basis of the AICc.

	-*ln*L	*d*	*e*	*j*	AICc
DEC	-16.86205	0.01408651	0.008741437	0	39.05742
DEC+J	-15.23098	0.008155047	1^{-12}	0.03373291	39.46195
DIVA	-17.71871	0.01987018	1^{-12}	0	40.77076
DIVA+J	-16.5299	0.01225272	1^{-12}	0.04483717	42.05979
BAYAREA	-16.08086	0.01429506	1^{-12}	0	**37.49505**
BAYAREA+J	-16.01547	0.012588	1^{-12}	0.01127465	41.03095

d, dispersal; *e*, expansion; *j*, founder-event speciation

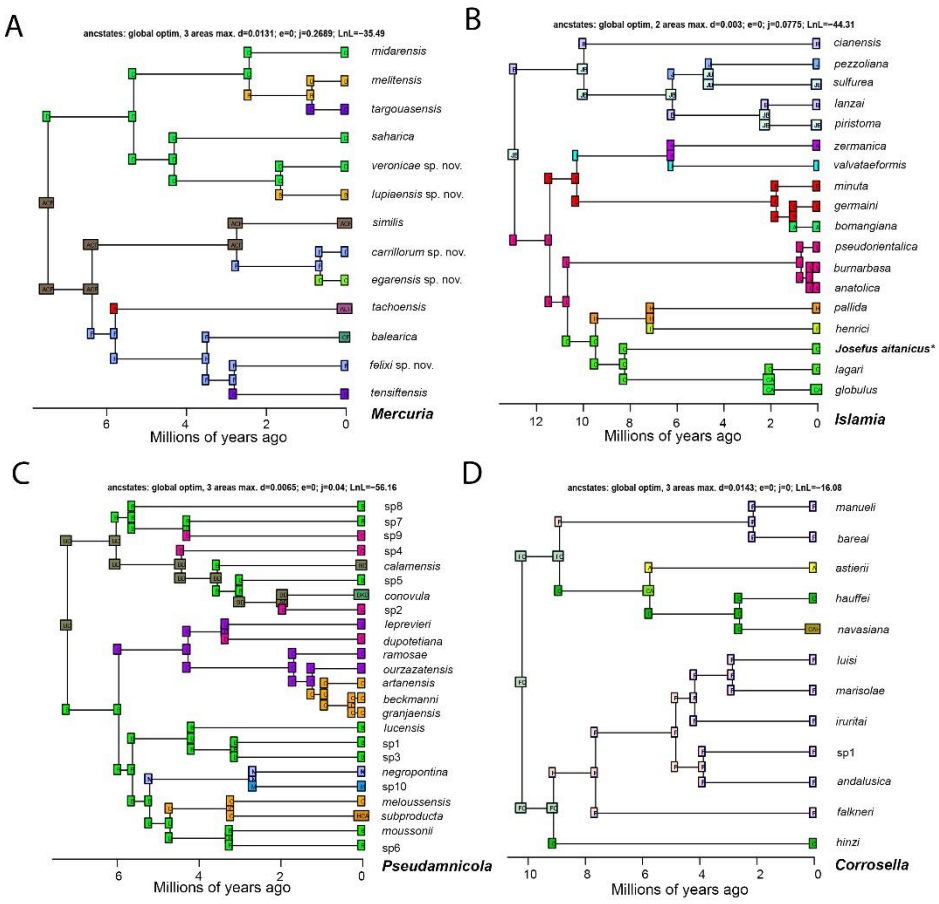


Figure 72. Ancestral range reconstructions estimated using the R package BioGeoBEARS for the hydrobiid genera (A) *Mercuria*, (B) *Islamia*, (C) *Pseudamnicola*, (D) *Corrosella* and. Letters within the squares represent freshwater ecoregions described by Abell et al. (2008): **A**, Cantabric Coast – Languedoc; **B**, Italian Peninsula & Islands; **C**, Eastern Iberia; **D**, Mediterranean Northwest Africa; **E**, Central & Western Europe; **F**, Southern Iberia; **G**, Atlantic Northwest Africa; **H**, Western Iberia; **I**, Dniester – Lower Danube; **J**, Gulf of Venice Drainages; **K**, Dalmatia; **L**, Southern Anatolia; **M**, Ionian Drainages; **N**, Aegean Drainages.

Two anagenetic expansion events were also estimated to have occurred, the first with the MRCA of the well-supported clade I (*C. navasiana* + *C. hauffei* + *C. astieri*) around 5.7 Mya and the second involving a subset of this clade (*C. navasiana* + *C. hauffei*) around 2.6 Mya (Figure 72D). In total, the ancestral range estimation indicated three dispersal events for 22 lineages (dispersal ratio of 0.14), of which two were anagenetic range expansions and one was a range change associated with founder-event speciation.

3.9 Comparison of dispersal ability among genera

The BioGeoBEARS analyses showed that dispersal rates (*d*) were higher in species that occur at high (*Corrosella*) and low (*Mercuria*) elevations, whereas those that occur at medium elevations (*Pseudamnicola* and *Islamia*) tended to have low dispersal rates. According to the founder-event (jump dispersal) speciation parameter (*j*), species that occur at high elevation (i.e., *Corrosella*) presented the lowest *j*, while species that occur at lower elevations (i.e., *Mercuria* and *Islamia*), showed a propensity for jump dispersals (Figure 73A).

A

parameters

Genus	Model	*d*	*j*
Pseudamnicola (P)	DecJ	0.006503	0.039959
Corrosella (C)	BayArea	0.014295	0
Mercuria (M)	DecJ	0.016800	0.150703
Islamia (I)	DecJ	0.002970	0.077535

B

d comparison

	P	C	M	I
P	NA	-0.726	-0.712	-0.720
C	**-1.752**	NA	-0.014	**-4.221**
M	**-1.345**	-0.011	NA	**-3.787**
I	-0.954	**-2.288**	**-2.433**	NA

j comparison

	P	C	M	I
P	NA	-0.140	**-2.076**	-0.407
C	**-9.090**	NA	**-8.462**	**-7.302**
M	**-6.825**	**-4.217**	NA	**-2.661**
I	-0.836	-0.684	-0.830	NA

d+j comparison

	P	C	M	I
P	NA	-0.726	**-3.382**	**-1.053**
C	**-9.090**	NA	**-10.557**	**-7.534**
M	**-8.600**	**-4.291**	NA	**-7.218**
I	**-1.652**	**-1.809**	**-4.052**	NA

C

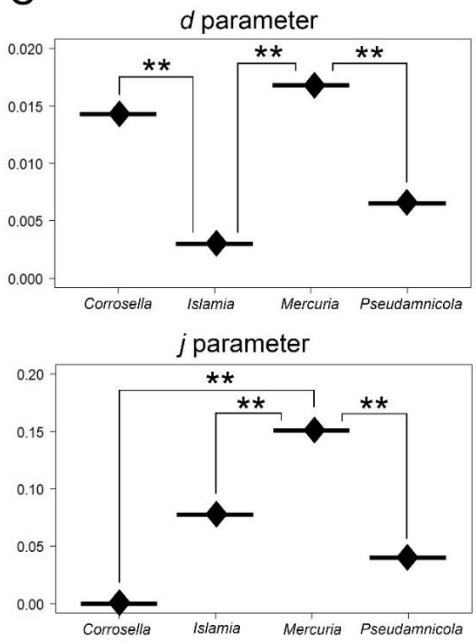


Figure 73. Comparison of dispersal abilities among hydrobiid genera living at different elevations. **A**, biogeographic model selected by AICc for each genus; **B**, pair-wise comparisons of genera based on the Δ*ln*L of each model according to the *d*, *j* and *d*+*j* parameters, significant values indicated in bold; **C**, comparison among genera based on the values of the *d* and *j* parameters. ** indicates significantly different values according to the Δ*ln*L depicted in B.

According to a difference in log-likelihood greater than 1 (Δ*ln*L > 1), *d* was significantly higher in *Corrosella* and *Mercuria* compared with *Islamia* and in *Mercuria* compared with *Pseudamnicola* (Figure 73B, C). The parameter *j* was significantly higher in *Mercuria* compared with the other three genera.

Comparison among genera according to the dispersal + founder-event jump dispersal parameters (*d+j*) revealed significant differences in both parameters for all genera pairs except *Corrosella* vs *Pseudamnicola* (Figure 73).

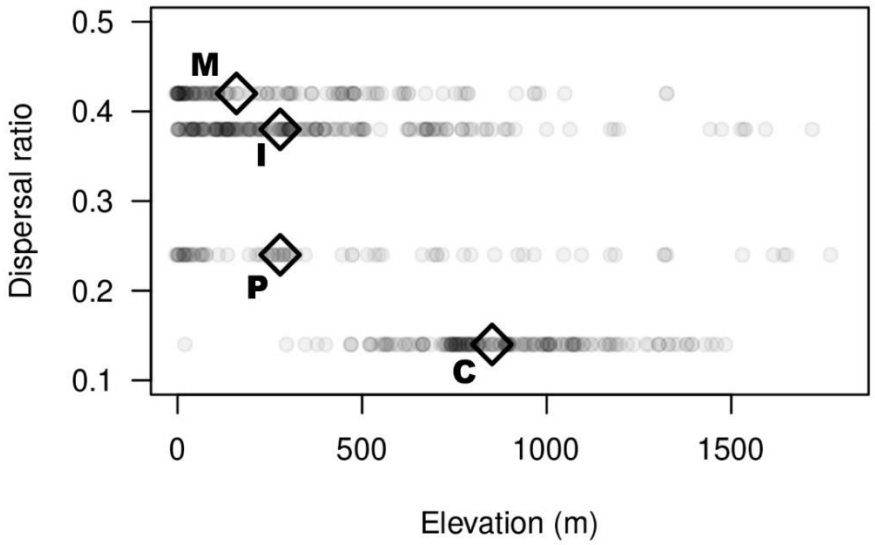


Figure 74. Comparison of the dispersal ratio *vs* median elevation of the studied genera. **M**, *Mercuria*; **I**, *Islamia*; **P**, *Pseudamnicola*; **C**, *Corrosella*.

Comparison of the dispersal ratio with the elevational range of the four genera showed that dispersal ratio decreased with elevation, from 0.42 for the lowland genus *Mercuria* to 0.14 for the headwater genus *Corrosella* (Figure 74).

DISCUSSION

4.1 Effects of climatic, geographic and evolutionary variables on the global species richness distribution of the family Hydrobiidae *s.str.*

4.1.1 Spatial variation in richness, endemism and threat

With an estimated 1,000–1,250 described species (Kabat and Hershler 1993, Strong et al. 2008), hydrobioids represent one of the most speciose groups of non-marine aquatic molluscs. Molecular and morphological studies have shown that hydrobioids actually comprise several microgastropod families (Wilke et al. 2001, Wilke et al. 2013) and have defined the sensu stricto character of the family Hydrobiidae. However, the total number of known species comprising this family was unknown, until now. Using the molecular definition of Hydrobiidae *s. str.* as a criterion and following a comprehensive literature and database review, we found that the family is comprised of 906 valid hydrobiid species described thus far. This estimate nearly equals previous observations of species richness for Hydrobiidae *s. l.*, suggesting that either other hydrobioid families are extremely species poor or we know very little about the actual diversity of the other hydrobioids. According to Strong et al. (2008), only 25% of these species are known. Given that, for instance, Australian hydrobioid genera alone comprise ~300 species (Ponder and Colgan 2002), it is more likely that the global species richness of hydrobioids has vastly been underestimated rather than other hydrobioid families being species poor. Moreover, spring systems in some regions, such as Africa and South America, have not yet been extensively surveyed, implying that, with more research, the number of hydrobiids species will increase even more.

We found congruence among the distribution of high species richness, endemicity and number of threatened hydrobiid species in 19 freshwater ecoregions, mainly in Europe and North Africa, qualifying them as biodiversity hotspots (see Figure 19D). Other ecoregions, such as in the Caspian Sea or in the western part of North America, also present high species richness (Kantor et al. 2010, Hershler et al. 2014a, Vinarski and Kantor 2017) but a low number of assessed species according to the IUCN criteria. The lack of conservation attention invalidates these areas as hydrobiid hotspots (sensu Myers, 1988); however, their status may change with future conservation assessments. In fact, in our simulation evaluating the robustness of hotspots against species misidentification, the IUCN status seems to be a crucial factor. By re-assigning 50% of all species names, species richness generally remained the same, except in those cases in which a randomly sampled species is already present in the focal ecoregion. Endemism, the second hotspot criteria, was slightly lower in the simulated data than in the observed data given the high share of endemic species. These findings support the idea that the IUCN

status determines hotspot robustness. Also, having a high number of threatened species in a biogeographic realm likely results in the replacement of an endangered species by another one. Consequently, the limited assessment of Nearctic species may explain the low hotspot robustness of North American ecoregions (Table 5).

In general, our results support the findings that the Mediterranean basin is a global hotspot of biodiversity for various taxa (Myers et al. 2000, Cuttelod et al. 2009, De Figueroa et al. 2013) and that it contains the highest proportion of threatened freshwater species in Europe (Skoulikidis et al. 2017). Some previous studies have already identified the importance of some of these ecoregions in relation to hydrobiid species richness. For instance, Arconada and Ramos (2003) and Strong et al. (2008) identified the mountainous regions in southern France and Spain, the southern Alps, the Balkan Peninsula and the southwestern USA as biodiversity hotspots. Other authors found a very high number of endemic hydrobiid species in the Balkan region, such as in the Zrmanja River in Croatia and in the ancient lakes Ohrid, Prespa and Skadar (Hadžišče 1956, Radoman 1963, Pešić and Glöer 2013, Falniowski and Beran 2015, Beran et al. 2016). In North America, Howard et al. (2015) considered the Sacramento river basin and the Lahontan ecoregion as zones with high species richness and vulnerability for molluscs and crustaceans. Likewise, Brown et al. (2008) highlighted the Great Basin, the Rio Grande, the Colorado River and Florida drainages as zones in which the families Pleuroceridae and Hydrobiidae show a high level of diversity, consistent with our results. Notably, we scored the North American genus *Pyrgulopsis*, with its 134 species (but see Hershler et al. (2013), who estimated 137 species), as the richest one within Hydrobiidae, with the highest diversity occurring in the Great Basin (Hershler and Sada 2002).

The high number of endemic hydrobiid species listed in this work (i.e., 754 out of 906 species) supports the results of previous taxonomic and ecological studies of the family Hydrobiidae. In general, hydrobiid species present high endemicity and very restricted distributions, with some species occurring in only one or two localities. Habitat type, which in most cases are isolated springs and the low dispersal capacity of most of the species may account for the observed distribution patterns (Hershler 1998, Arconada and Ramos 2003, Strong et al. 2008, Delicado et al. 2013b). The high endemicity observed in our study may also be related to the fact that many of the descriptions of included species were based only on a single population. Therefore, the actual distribution of these species may not be completely known and thus may have been underestimated.

We applied the monophyly criterion of the family Hydrobiidae (sensu (Wilke et al. 2013) to calculate the number of threatened species assessed by the IUCN. We found 284 Hydrobiidae *s. str.* species catalogued under a threatened category, representing ~54% of the total number of threatened hydrobioid species listed in the IUCN Red List. The number of threatened species is likely even higher as more than 50% of Hydrobiidae *s. str.* species are classified as data deficient. These findings indicate that hydrobiids face a high degree of threat, mainly caused by habitat degradation and/or the intrinsic characteristics of the species (Strong et al. 2008). By comparing the threat category assigned by the IUCN with the elevational zone of the type localities, we found that a relatively high proportion of the threatened species occur at elevations up to 1,000 m.a.s.l., with a peak at 1,500 m.a.s.l., after which, the number of threatened species decreases with higher elevation. This pattern is unexpected as most of the high-elevation species are narrow range endemics, which should be more vulnerable to, for instance, habitat loss compared with lowland species. However, the relatively lower risk at higher elevations could be related to the fact that high-elevation populations are less accessible and therefore more protected from human activities than those living at lower elevations (Angeli et al. 2010). Regardless, Vörösmarty et al. (2010) found that 65% of global river discharges and the ecosystems supported by these waters, were under a moderate to high threat, with an estimated 10,000–20,000 species considered at risk or already extinct. This estimation supports the idea that freshwaters are the most endangered ecosystems in the world (Camargo and Alonso 2006, Dudgeon et al. 2006, Markovic et al. 2017).

4.1.2 Predictors of hydrobiid species richness distribution

Climate and geographic variables are often strong descriptors of broad-scale species richness patterns (Hawkins et al. 2003), reflecting the sorting of species along ecological gradients (Gaston 2000). Evolutionary and dispersal processes also play an important role in richness distribution (Wiens and Donoghue 2004). Our regression model included, as predictors of hydrobiid richness, variables that reflect all three mechanisms, as observed in other freshwater animal groups (Tisseuil et al. 2013).

Freshwater gastropods present an overall pattern of high species richness in tropical zones that generally decreases with increasing latitude, such that lower richness and endemicity are observed at higher latitudes (Strong et al. 2008). This pattern has also been found for other groups of freshwater organisms, such as fishes, amphibians and dragonflies (Heino 2011, Tisseuil et al. 2013). Nevertheless, incongruences with this general pattern are common in freshwater ecosystems: for instance, mayflies and stoneflies show a reversed latitudinal

gradient with higher species richness at high latitudes, whereas caddisflies and salamanders lack latitudinal richness gradients (Heino 2011). The latitudinal gradient of hydrobiids follows a hump-shaped pattern, peaking at mid latitudes (30º–40º), with minimums at warm tropical areas and boreal latitudes. Like most latitudinal richness gradients (Mittelbach et al. 2007), this distribution likely reflects the interaction of many factors, such as geography, climate, evolutionary history and water availability, all of which facilitate the coexistence of species. In fact, this mid-latitudinal zone, which harbours the highest number of hydrobiid species, features relatively low temperature seasonality and intermediate values of precipitation seasonality (see Figure 21). These findings suggest that hydrobiids thrive in areas with more stable climatic conditions, thus supporting the climatic-stability theory (Klopfer 1959, Klopfer and MacArthur 1960). Indeed, similar observations have been reported for other freshwater organisms (Oberdoff et al. 1995, Oberdorff et al. 2011b, Whitton et al. 2012).

Climatic stability in Europe during certain Quaternary periods promoted the geographic expansion and increase in species richness of lacustrine gastropod assemblages (Georgopoulou et al. 2016). Similarly, in our study, past temperatures (i.e., temperature of the Last Glacial Maximum) were strongly correlated with latitude and present-day temperatures; therefore, they may have also influenced the current distribution of hydrobiid species richness. In fact, the regions showing a great share of the richness in the southern Palearctic and Nearctic realms coincide with the permafrost line in these realms during the Last Glacial Maximum (Frenzel 1992). However, glacial refugia do not always correspond to regions of high gastropod diversity, as demonstrated in the case of the European spring snail genus *Bythinella* Moquin-Tandon, 1856 (Benke et al. 2011), suggesting that climate may differentially affect the evolutionary history of lentic and lotic species (Dehling et al. 2010). However, testing of this hypothesis is beyond the scope of this study and requires more information about the environment and the ecology of the different species.

Areas of high elevation have served as evolutionary centres of endemism, which are additionally driven by complex topographies that generate heterogeneous environments (Allouche et al. 2012). Moreover, these areas feature strong climatic changes along elevational gradients, which may have buffered against extinction events during periods of climate oscillations in the past by facilitating short distance dispersal (Jetz et al. 2004, Ohlemüller et al. 2008). In this study and contrary to what Pérez-Quintero (2015) showed for European freshwater gastropods, the elevation range of ecoregions did not contribute to explain the distribution of hydrobiid species richness. Differences in taxonomic and spatial

scale may account for this disparity: Pérez-Quintero (2015) used point data, whereas we used the mean value and range of elevation per ecoregion as a proxy for the elevation in which a species lives, due to limitations in determining the exact geographic range of all 906 species. Similarly, we did not detect the influence of geological heterogeneity on hydrobiid species richness, suggesting that either the geographic unit of our study (i.e., ecoregions) is too large to detect such a relationship or habitat heterogeneity does not influence the distribution of species richness in the family Hydrobiidae. Although habitat heterogeneity was shown to play an important role in the species richness distribution of freshwater African molluscs (Hauffe et al. 2014), we found no significant correlation with our ecoregion binning. Notably, we also did not find substantial support for the species-area relationship (i.e., no effect of ecoregion size on species richness was found), which is thought to be associated with habitat heterogeneity (Ricklefs and Lovette 1999). This lack of correlation may indicate that hydrobiid species richness is more likely attributable to past and present climatic conditions affecting a particular ecoregion, which contribute to create more potential habitats for hydrobiid species, rather than to the geographic extent of species.

Factors influencing dispersal, including some analysed here, such as connectivity among ecoregions, peninsula effect and biogeographic affiliation, can influence certain evolutionary processes and shape species richness distributions. Geographic connectivity favours dispersal processes by having common boundaries and creating corridors for movement (Taylor et al. 1993). Despite the low dispersal ability of most hydrobiid species, which are, in many cases, restricted to a single ecoregion (but see Wilke et al. (2000b) and Haase et al. (2010), who showed a wider species distribution for the brackish genera *Hydrobia* and *Ecrobia*), we found a positive relationship between species richness and connectivity. This pattern has also been observed in other molluscs and in other groups, such as fishes and odonates (Ward et al. 1999, Amoros and Bornette 2002, Wepfer et al. 2016). However, our findings also indicate that peninsular ecoregions, which are supposedly geographically isolated and species poor, are actually as diverse as non-peninsular ecoregions. The peninsula effect may have been counteracted by past colonisation of the southern peninsulas during glacial episodes (as mentioned above) and by long-distance dispersal via birds, which has been suggested to play an important role in the geographic distribution of certain hydrobiid species (Haase et al. 2010, Delicado et al. 2014, Szarowska et al. 2016). The importance of historical factors is also reflected by the variation observed in species richness among biogeographic realms (see Figure 21). The inclusion of biogeographic realm adds explanatory power to richness models by accounting for the common ancestry of species and the diversification dynamics of a region

(e.g., Tedesco et al. 2005, Griffiths et al. 2014). The findings presented here demonstrate that the species richness distribution of Hydrobiidae *s. str.* can be explained by the joint effect of global and regional factors.

We conclude that stable conditions, in terms of temperature and precipitation and habitat connectivity have contributed to increase hydrobiid species richness. Moreover, our study has revealed a lower proportion of threatened species in highlands than in lowlands. This result, however, must be taken with caution as conservation assessments are lacking for more than half of the known hydrobiid species. This scenario may change as global climate change models predict perturbations in most of the world's ecosystems, mainly due to changes in temperature and precipitation seasonality (Carpenter et al. 1992, Walther et al. 2002). Therefore, hydrobiid species and their ecosystems may be at elevated risk in the future. Although our study included a large portion of the known extant species, a fine-scale geographic study of hydrobiid gastropods would likely identify additional predictors of diversity hotspots.

4.2 Systematics and geographic distribution of selected hydrobiid genera

Some extant hydrobiid genera are species rich and widely distributed (e.g., *Pseudamnicola*, *Mercuria* and *Islamia*) and others (e.g., *Corrosella*) are not. A potential driver of such differences may be dispersal strategy, determined by the habitat and elevation in which species occur. The rich genera typically inhabit the lower reaches of rivers, while poor ones such as *Corrosella* live mostly in headwater springs (Delicado et al. 2013b). Our selected model excluded elevation as a predictor of hydrobiid richness (Table 7) but accounted for other variables related to dispersal abilities such as connectivity among ecoregions and peninsula effect (Miller et al. 2018). Given that accurate elevational ranges have not been recorded for most hydrobiid species, which likely accounts for this variable not being a predictor in our model, we had to use the elevation of the type locality as a proxy for this factor in the global analysis presented in section 3.9. To overcome this bias and to provide sufficient statistical power to the subsequent biogeographic analyses, we first studied the phylogenetic relationships and elevation/geographic distribution of four hydrobiid genera that present distinct habitat preferences.

4.2.1 Multilocus phylogeny and molecular species delimitation methods reveal extensive diversity in *Mercuria*

The species richness of *Mercuria* had been previously based only on conchological characteristics (e.g., Glöer et al. 2010, Gloer et al. 2015, Boeters

and Falkner 2017). Prior to the present study, the genus was comprised of 29 recognised species (including four fossil species) and for 25 of these species, their description was based only on shell characters. A major problem associated with establishing the taxonomy of hydrobiids on such characters is the high level of convergence and similarities found in shell features (Hershler and Ponder 1998, Bodon et al. 2001), which may conceal the presence of cryptic species (i.e., species that are not morphologically distinguishable from each other).

Among the applied molecular delimitation methods, ABGD represented the most efficient one for recognising *Mercuria* species (match ratio value of 0.85) and confirmed 12 of the 14 putative morphospecies (nine described species plus five new ones). Among the methods used, the tree-based methods (i.e., GMYC and PTP) both presented a relatively low match ratio value and an overestimation in the number of species. Inconsistencies between the number of morphospecies versus putative species have also been found in other studies. Several authors have recognised that results derived from these methods should be interpreted with caution for groups with low vagility and poor dispersal capacities, such as gastropods (e.g., Sauer and Hausdorf 2012, Razkin et al. 2017, Delicado et al. 2019, Strong and Whelan 2019), thereby contrasting the validity of the delimited species with morphological data under an integrative taxonomic approach (Padial and De La Riva 2010).

The main problem with using methods based on the generation of partitions (GMYC and PTP) is that they use the information obtained through the modelling of speciation and coalescent processes. These processes can be affected by inter- and intraspecific variation (Ross et al. 2008, Zhang et al. 2013), leading to, in many cases, the delimitation of entities within species with a strong population structure, as is frequently found for gastropods. Consistent with this, the GMYC and bPTP method, respectively, inferred 21 and 24 putative species. These numbers reflect structure between populations rather than speciation events. Only the species with little genetic structure, such as *M. balearica* or *M. tensiftensis*, were supported by all of the delimitation methods. The presence of cryptic species could also lead to a discrepancy in species numbers. For example, in the GM analysis, overlap in shell shape was inferred between *M. egarensis* sp. nov. and *M. balearica* and between *M. carrillorum* sp. nov. and *M. tachoensis*, however substantial genetic distances were observed among these species.

Despite its higher performance, the barcode gap method (ABGD) may underperform among closely related species (Puillandre et al. 2012) and should therefore not be used as a single source of evidence. For instance, a lower mean

COI divergence was observed between the morphologically described species *M. egarensis* sp. nov. and *M. carrillorum* sp. nov. (1.3%, Appendix 5) than between other hydrobiid species (e.g., 8% between *Corrosella* species; Delicado et al. (2012), or 3–5.5% between *Hydrobia* species; Wilke et al. (2000b)). Despite this low genetic divergence, the morphological differences between *M. egarensis* sp. nov. and *M. carrillorum* sp. nov. sufficiently support their delineation as two distinctiveness species. Low genetic distance in conjunction with high morphological divergence has been detected between other molecularly closely related species of hydrobiids as a result of evolutionary processes such as adaptive radiation, dispersal to a new environment or recent speciation (Delicado et al. 2014, Falniowski et al. 2016, Stelbrink et al. 2020). In the case of these two *Mercuria* species, the inferred patterns could be explained by recent speciation promoted by long distance dispersal and subsequent isolation (further discussed below in section 4.3).

After refining the topology of the phylogeny and the ABGD approach using additional information on the morphology and geographic distribution of the recovered groups, we estimated a total of 14 *Mercuria* species from our data. Using these methods, species known since the mid-nineteenth century, such as *M. melitensis* and *M. saharica*, were recovered. Three of the species, *M. tensiftensis*, *M. midarensis* and *M. targouasensis*, correspond to the three clades inferred by Boulaassafer et al. (2018), though with different phylogenetic positions.

In our phylogeny, a sister taxa relationship between *M. midarensis* and *M. targouasensis* was recovered, in agreement with the findings of Boulaassafer et al. (2018). By contrast, in our BI analysis, *M. tensiftensis* clustered as the sister taxon to *M. balearica*. Our study, however, did not include the species *M. bakeri* and *M. tingitana*, which may account for the difference in the phylogenetic relationship of the aforementioned species. Also, as in Boulaasafer et al. (2018), neither of our phylogenetic inferences recovered the species *M. targouasensis* as a monophyletic group, despite the incorporation of a greater number of species and gene fragments, which provided more global coverage of species diversity and distribution. Furthermore, the COI and 16S sequences from individuals collected in northern Italy that were previously classified as *M. similis* by Wilke et al. (2001) and (Wilke 2003) do not correspond to this species (mean COI divergence 8.5%; unpublished data) but to a new species yet to be described.

4.2.2 Diagnostic characters for *Mercuria* species

As seen in other hydrobiids (Arconada López 2000, Arconada and Ramos 2006, Bodon and Cianfanelli 2012, Delicado et al. 2012, Delicado et al. 2019), our

morphological observations highlight the problematic nature of identifying *Mercuria* species using only shell features.

In this study, we used geometric morphometrics to describe the shell shape of over 1,100 individuals from 46 populations and predicted that differences in shell shape may be congruent with the delimited morphospecies. From a total of eight species, two pairs of species could not be resolved using this methodology (*M. carrillorum* sp. nov. from *M. tachoensis* and *M. egarensis* sp. nov. from *M. balearica*); however, the remaining four species were recovered as independent clusters (see Figure 24). According to these findings, only 50% of the species were successfully delimited on the basis of the morphometry of the shell. Therefore, we conclude that this structure, whether studied through geometric or classic morphometry, cannot be used to achieve a complete understanding of species diversity in *Mercuria*. Giusti et al. (1995) emphasised that the taxonomy of this genus should be taken with caution, given the high morphological variability of the shell and our data corroborate this assertion. In our study, some species (i.e., *M. tachoensis*, *M. similis* and *M. balearica*) presented a high level of interpopulation variability in the shell form (Figure 24), which was not observed in the remaining species, in part, due to their poorly known distribution. However, all of the species studied here do have, to some degree, high variability in shell dimensions (Table 19). We cannot rule out the possibility that this phenomenon is due to sexual dimorphism, which is generally common in Caenogastropoda (Davis and McKee 1989, Bichain et al. 2007, Falniowski et al. 2007, Reichenbach et al. 2012, Páll-Gergely et al. 2020), or to parasitism (Boulaassafer et al. 2018). Further studies would be necessary to test which underlying mechanism is responsible for the variability in shell dimensions.

In *Mercuria*, the colour of the operculum, thought to be dark brown, has been used as a taxonomic criterion to assign species to this group (Boeters and Falkner, 2017); however, this character status can vary among species. For instance, we found that, for some species presenting an orange to brown operculum, some specimens had a yellowish operculum (i.e., in *M. tachoensis*, *M. balearica*, *M. egarensis* sp. nov., *M. felixi* sp. nov. and *M. veronicae* sp. nov.). In other species, the orange to brown coloured operculum was observed for all specimens (i.e., *M. similis*, *M. carrillorum* sp. nov. and *M. lupiaensis* sp. nov.).

Substantial variability was also observed in characters related to the reproductive system (e.g., penis, penial appendix and bursa copulatrix). In particular, we observed intraspecific differences in the shape and size of these characters. The use of these characters has been indiscriminate, leading to misclassifications and

an overestimation of the species richness of *Mercuria* (Giusti et al. 1995). Interestingly, Boulaassafer et al. (2018) observed specimens of *M. tensiftensis* with marked atrophy of the penis or with prostatic hyperplasia and concluded that the size variability of the penis and prostate gland in this species may be caused by parasitism. In our study, however, we did not find any evidence of parasitism in any of the populations studied from the Iberian Peninsula, France, the Balearic Islands, Italy or Tunisia.

Holyoak et al. (2017) found that allometric growth of the penis and the penial appendix is a source of variability in the reproductive system of *M. tachoensis* and highlighted how this system varies according to the maturation time of the specimens. We observed a similar phenomenon in *M. similis* (see Figure 29 A – F), however, their growth could not be correlated with different maturation periods since we did not sequentially sample the population over time.

4.2.3 Taxonomic reappraisal of the studied *Mercuria* species

Hydrobiids, in general, present high levels of homoplasy due to convergence in shell characters and the simplicity of their structures, which makes the recognition of species boundaries difficult (Hershler 1994, Bodon et al. 2001, Falniowski 2018). The assignment of hydrobiid species to a genus should follow an integrative taxonomic approach (Dayrat 2005) as morphological studies alone cannot resolve the systematics of the group, particularly given that many of these studies have been based on shell characters (colour and measurements) proven to be highly variable within populations (Wilke et al. 2000b, Wilke and Falniowski 2001, Barszcz 2004) and therefore insufficient for proper species delimitation.

On the basis of our observations, we have identified characters previously considered diagnostic as insufficient to distinguish between *Mercuria* species; therefore, we used integrative taxonomy to address some unresolved taxonomic questions about species of this genus:

a) Mercuria confusa *as a synonym of* M. similis.

Assessing the taxonomic status of *Mercuria* populations is further hampered by the inability to accurately identify the type material of the widespread species. Several species of *Mercuria* were described in the Mediterranean region in the nineteenth century (Poiret 1801, Draparnaud 1805, Risso 1826, Frauenfeld 1863, Paladilhe 1869, Bourguignat 1876), however, the taxonomic status and location of their type materials remain elusive. One such case involves the taxonomic uncertainty surrounding the type species of the genus, *M. confusa* and *M. similis*. These two species were synonymised by Boeters and Falkner (2000) when these

authors assigned the lectotype of *M. confusa* (currently deposited under lot number NHMW-MO 92596 at the Natural History Museum in Vienna; Eschner et al., 2020) as the neotype of *M. similis*. The justification for this synonymisation, which reduced the number of recognised species of *Mercuria*, was based on the similarity in height of two shells (i.e., the lectotype of *M. confusa* and the shell illustrated by Draparnaud (1802) in the original description). As supported by our geometric morphometric analysis and shell measurements, shell features cannot be used to reliably distinguish *Mercuria* species, diminishing confidence in this synonymy. Despite this, our morphological and molecular data have delimited only one species, a finding that, without more data, would support the hypothesis that only a single species of *Mercuria* is present across southern France (i.e., "*Gallia Meridionalis*"). These findings dispute past interpretations about the presence of *M. meridionalis* and *M. similis* in southern France and their geographic distributions being separated by the Rhone River (Boeters and Falkner, 2017).

b) *Genus assignment for* A. emiliana

Our study confirms that the *Mercuria* populations sampled by Boeters (1988) from southern France, southern Spain and the Balearic island of Majorca are conspecific and likely all correspond to *M. similis*. Boeters (1988) initially assigned the specimens from these populations to *M. emiliana* but later identified them as belonging to *M. similis* (Boeters and Falkner, 2017). The species *M. emiliana* was recently combined with the genus *Pseudamnicola* and the species re-named as *P. emilianus* by Boeters and Falkner (2017). This taxonomic change was based on differences found in the coloration and size of the shell between the neotype assigned by these authors (i.e., MNHN-IM-2000-32541; Boeters and Falkner, 2017) and other specimens of *M. similis* sampled from four proximal localities (see Table 6 in Boeters and Falkner, 2017).

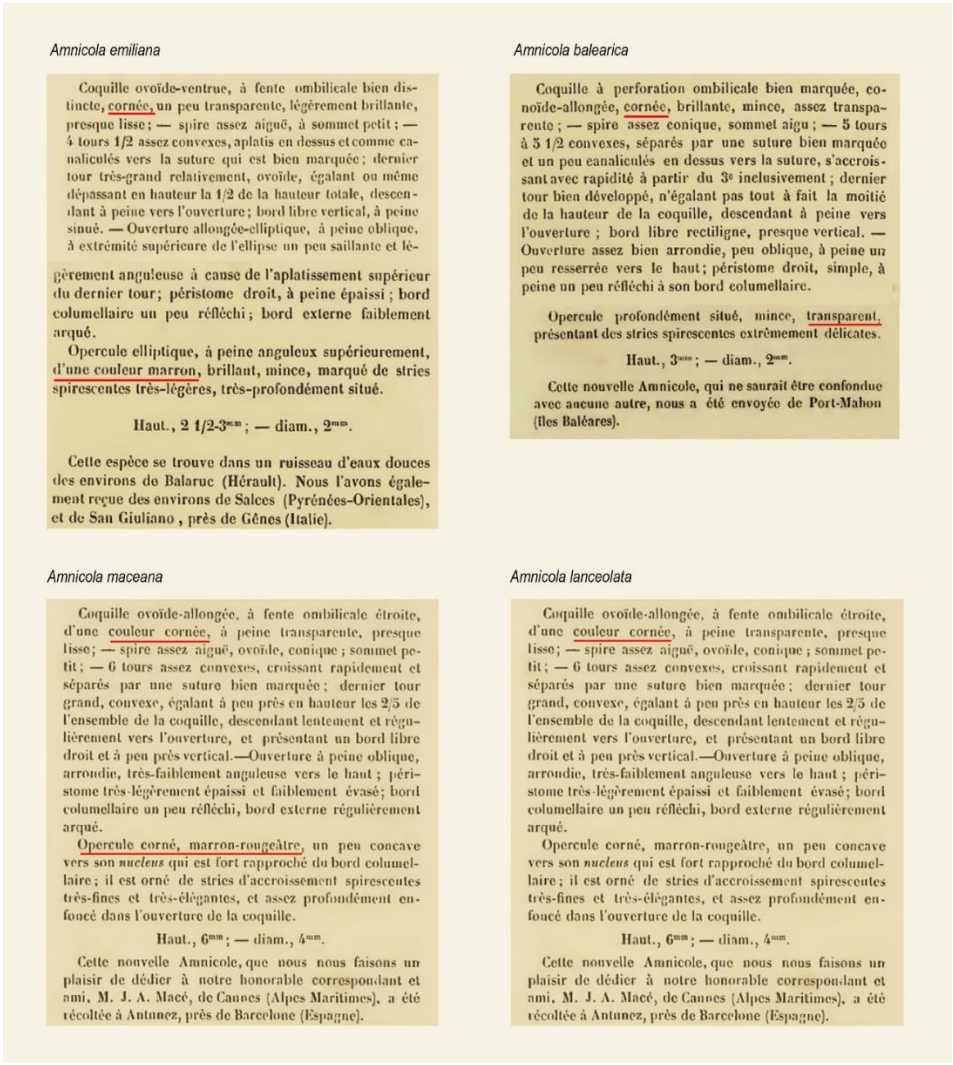
The assignment of *M. emiliana* to *Pseudamnicola* may be erroneous for several reasons. First, Boeters and Falkner (2017) designated a neotype for *Amnicola emiliana*, stating that syntypes of this species might be lost. However, Breume and Audibert (2017) declared this designation as invalid as the original syntypes of *A. emiliana* can be found at the University of Montpellier under the lot number UM.PDL.003. Second, the decision for the species combination was based on only two shell measurements of three shells (which are probably not adults) and shell coloration, an insufficient character for genus assignment. According to Boeters and Falkner (2017), the neotype is too small (2.75 mm) to be a member of *Mercuria*, although in Table 6 of their study, they reported shells of *M. similis* from Port-La Nouvelle ranging in size from 2.75 to 3.85 mm. We also found

numerous individuals of *Mercuria* that are 2.75 mm high (Appendix 6). Boeters and Falkner (2017) also claimed that Paladilhe (1869), in his original description of the species, stated the colour of the shell as *cornée* [corneous] and not milky as in *Mercuria* and the operculum as corneous instead of chestnut brown. However, it is worth mentioning that all *Amnicola* species described by Paladilhe (1869) presented corneous shells and similar shell dimensions (Figure 75). Moreover, we observed both types of shell and operculum colorations among the studied populations of *Mercuria* (see Figure 25 for shell morphotypes and differences in operculum coloration). Therefore, based on our observations, we consider these particular shell features insufficient to assign the species *A. emiliana* to *Pseudamnicola*. In fact, Boeters and Falkner (2017) acknowledged the vagueness of their proposed taxonomic act: "The fact that *Amnicola emiliana* belongs to *Pseudamnicola* confronts us with a lot of questions which can only be mentioned in this context but will not be solved here" (p. 252). Given the difficulty of determining the precise taxonomic status of *A. emiliana* based on the shell characteristics of a designated neotype from a lot other than the type material, we consider it more appropriate to base the assignment of the taxonomic status of this species on populations found later in the region of the type locality. We found specimens of *M. similis* in areas close to the type locality and in Font de Estramar, another locality mentioned in the original description of the species. Therefore, we consider that *A. emiliana* is more likely a representative of *Mercuria*.

a) *Taxonomy and geographic distribution of* M. tachoensis

The *Mercuria* populations sampled along the Atlantic coast of Europe and the British Islands clustered together in a single clade (see Figure 23). This phylogenetic clustering along with the morphological similarities found among these populations suggest the need for a taxonomic re-evaluation of some previously published records of *Mercuria*. Moreover, topotypes or near topotypes of the oldest known species, *M. tachoensis*, collected from localities surrounding the city of Lisbon in Portugal also grouped within this clade, suggesting that all of these populations may be of *M. tachoensis*. Despite exhaustive sampling, the species *M. edmundi* was not found in either of the two locations mentioned by Boeters (1987, 1988) and the localities mentioned for both *M. edmundi* and *M. tachoensis* in Lisbon have disappeared.

Subsequent sampling campaigns carried out in other parts of the city, including the civil parish of Ajuda and the Jardim Botânico da Ajuda, did not yield any evidence confirming the presence of these species in these localities [Rui M. da Costa Mendes pers. comm.].



Amnicola emiliana

Coquille ovoïde-ventrue, à fente ombilicale bien distincte, cornée, un peu transparente, légèrement brillante, presque lisse ; — spire assez aiguë, à sommet petit ; — 4 tours 1/2 assez convexes, aplatis en dessus et comme canaliculés vers la suture qui est bien marquée ; dernier tour très-grand relativement, ovoïde, égalant ou même dépassant en hauteur la 1/2 de la hauteur totale, descendant à peine vers l'ouverture ; bord libre vertical, à peine sinué. — Ouverture allongée-elliptique, à peine oblique, à extrémité supérieure de l'ellipse un peu saillante et légèrement anguleuse à cause de l'aplatissement supérieur du dernier tour ; péristome droit, à peine épaissi ; bord columellaire un peu réfléchi ; bord externe faiblement arqué.

Opercule elliptique, à peine anguleux supérieurement, d'une couleur marron, brillant, mince, marqué de stries spirescentes très-légères, très-profondément situé.

Haut., 2 1/2-3^{mm} ; — diam., 2^{mm}.

Cette espèce se trouve dans un ruisseau d'eaux douces des environs de Balaruc (Hérault). Nous l'avons également reçue des environs de Salces (Pyrénées-Orientales), et de San Giuliano, près de Gênes (Italie).

Amnicola balearica

Coquille à perforation ombilicale bien marquée, conoïde-allongée, cornée, brillante, mince, assez transparente ; — spire assez conique, sommet aigu ; — 5 tours à 5 1/2 convexes, séparés par une suture bien marquée et un peu canaliculés en dessus vers la suture, s'accroissant avec rapidité à partir du 3^{e} inclusivement ; dernier tour bien développé, n'égalant pas tout à fait la moitié de la hauteur de la coquille, descendant à peine vers l'ouverture ; bord libre rectiligne, presque vertical. — Ouverture assez bien arrondie, peu oblique, à peine un peu resserrée vers le haut ; péristome droit, simple, à peine un peu réfléchi à son bord columellaire.

Opercule profondément situé, mince, transparent, présentant des stries spirescentes extrêmement délicates.

Haut., 3^{mm} ; — diam., 2^{mm}.

Cette nouvelle Amnicole, qui ne saurait être confondue avec aucune autre, nous a été envoyée de Port-Mahon (îles Baléares).

Amnicola maceana

Coquille ovoïde-allongée, à fente ombilicale étroite, d'une couleur cornée, à peine transparente, presque lisse ; — spire assez aiguë, ovoïde, conique ; sommet petit ; — 6 tours assez convexes, croissant rapidement et séparés par une suture bien marquée ; dernier tour grand, convexe, égalant à peu près en hauteur les 2/3 de l'ensemble de la coquille, descendant lentement et régulièrement vers l'ouverture, et présentant un bord libre droit et à peu près vertical. — Ouverture à peine oblique, arrondie, très-faiblement anguleuse vers le haut ; péristome très-légèrement épaissi et faiblement évasé ; bord columellaire un peu réfléchi, bord externe régulièrement arqué.

Opercule corné, marron-rougeâtre, un peu concave vers son *nucleus* qui est fort rapproché du bord columellaire ; il est orné de stries d'accroissement spirescentes très-fines et très-élégantes, et assez profondément enfoncé dans l'ouverture de la coquille.

Haut., 6^{mm} ; — diam., 4^{mm}.

Cette nouvelle Amnicole, que nous nous faisons un plaisir de dédier à notre honorable correspondant et ami, M. J. A. Macé, de Cannes (Alpes Maritimes), a été récoltée à Antunez, près de Barcelone (Espagne).

Amnicola lanceolata

Coquille ovoïde-allongée, à fente ombilicale étroite, d'une couleur cornée, à peine transparente, presque lisse ; — spire assez aiguë, ovoïde, conique ; sommet petit ; — 6 tours assez convexes, croissant rapidement et séparés par une suture bien marquée ; dernier tour grand, convexe, égalant à peu près en hauteur les 2/3 de l'ensemble de la coquille, descendant lentement et régulièrement vers l'ouverture, et présentant un bord libre droit et à peu près vertical. — Ouverture à peine oblique, arrondie, très-faiblement anguleuse vers le haut ; péristome très-légèrement épaissi et faiblement évasé ; bord columellaire un peu réfléchi, bord externe régulièrement arqué.

Opercule corné, marron-rougeâtre, un peu concave vers son *nucleus* qui est fort rapproché du bord columellaire ; il est orné de stries d'accroissement spirescentes très-fines et très-élégantes, et assez profondément enfoncé dans l'ouverture de la coquille.

Haut., 6^{mm} ; — diam., 4^{mm}.

Cette nouvelle Amnicole, que nous nous faisons un plaisir de dédier à notre honorable correspondant et ami, M. J. A. Macé, de Cannes (Alpes Maritimes), a été récoltée à Antunez, près de Barcelone (Espagne).

Figure 75. Comparison between original descriptions of *M. emiliana*, *M. balearica*, *M. maceana* and *M. lanceolata* taken from Paladilhe (1869). Red lines remark the colour of the shells and operculum according to the author´s criterion.

Neither did our survey of the vicinities of Burgau, Lagos and Praia da Figuera, Portugal. Holyoak et al. (2017) previously proposed the synonymy of *M. edmundi* with *M. tachoesnsis* on the basis of conchological and anatomical characters, however, we were unable to support their synonymy with molecular data.

According to our data, the population previously reported as *M. bayonnensis* from a stream in Bidart by Boeters and Falkner (2017) actually comprises specimens of *M. tachoensis*. *Mercuria bayonnensis* was originally described from Lake Moriscot, in La Negresse, France (Locard 1894) and later, Boeters and Falkner

(2017) expanded the range of the species to the localities of Bidart, Biarritz, La Nive, Le Teich and La Tresne, in France and the city of Bilbao, in Spain. In both the original description and the aforementioned article, the authors described the species as having a conical shell and a short, pigmented penis. However, *M. tachoensis* is also described as having a short, pigmented penis and conical shell (Boeters 1988) and all of the male individuals that we dissected from Bidart and Mouguerre presented short, pigmented penises. Given that the species was not found in the type locality, it was not included in our molecular analyses. Therefore, until material from the type locality can be integrated into our study, it is not possible to know with certainty if *M. bayonnensis* is *M. tachoensis* or another species.

We also found *M. tachoensis* in some localities previously reported for *M. anatina*. The type locality of the latter species is the "*l'embouchure de la Somme*" and "*Les environs de Paris*", France (Poiret, 1801). Despite many surveys of areas surrounding Paris, the species has not been found again in the area of the type locality (Boeters and Falkner 2017). Boeters and Falkner (2017) assigned the species to three *Mercuria* populations located in the Netherlands. We sampled a population from one of these localities, the distributary of Oude Maas in Hoogvliet, Netherlands. Our molecular and morphological studies of these specimens indicate that they belong to a population of *M. tachoensis* and not *M. anatina*, as previously thought. As in the case of *M. bayonnensis*, additional field surveys in France will be needed to clarify the taxonomic status of *M. anatina* and its validity as a species.

The systematics of *Mercuria* populations that occur in the British Islands has always been confusing as many authors have attributed these populations, seemingly indiscriminately, to species distributed over the Mediterranean (i.e., *M. similis*) or in Atlantic coastal streams (i.e., *M. anatina*). Kerney (1999) and Anderson and Rowson (2020) cited all of the collected populations as *M. confusa*. Further assignations corrected the name, at least for the cases involving *M. similis*, though the authors cautiously named the species as *M.* cf. *similis* (Anderson 2005, Byrne et al. 2009). Kadolsky (2011) attributed populations occurring in the Arun River to *M. anatina*. Our molecular survey, which included a population from this river and another from Barking, London, indicate that these snails are *M. tachoensis*.

Our integrative study has revealed that *M. tachoensis* is distributed along a large section of the Atlantic coastal area of Europe and in the British Islands. A similar geographic distribution pattern has also been documented in other freshwater taxa,

including fishes (Reyjol et al. 2007), the genus *Unio* (Unionidae: Bivalvia) (Araujo et al. 2017) and the genus *Theodoxus* (Neritidae: Gastropoda) (Sands et al. 2019). In this pattern, the Atlantic slope presents lower species richness compared with the Mediterranean region, consistent with our observations of *Mercuria* and its higher diversity in the latter region.

b) Identified Mercuria *species after using an integrative approach*

Our assignment of the collected populations to species using an integrative approach changes the number of species that should be currently recognised and the extent of their distributions. Although Boeters and Falkner (2017) found a total of six species (*M. bayonnensis*, *M. baudoniana*, *M. sarahae*, *M. anatina*, *M. similis* and *M. meridionalis*) in the continental area of France, in our study, which included populations of four of these species (*M. bayonnensis*, *M. anatina*, *M. similis* and *M. meridionalis*), we could only identify two species (*M. tachoensis* and *M. similis*). For the Iberian Peninsula and the Balearic Islands, Glöer et al. (2015b), Boeters (1988) and Boeters and Falkner (2017) mentioned a total of six species (*M. tachoensis*, *M. edmundi*, *M. similis*, *M. balearica*, *M. bayonnensis* and *M. emiliana*). In our study, we found evidence for only three of these species (i.e., *M. tachoensis*, *M. similis* and *M. balearica*), which appear to have a wider distribution than previously thought. The inclusion of new populations from the Iberian Peninsula has revealed the existence of three new species (*M. egarensis* sp. nov., *M. carrillorum* sp. nov. and *M. felixi* sp. nov.). Likewise, new populations from the Italian Peninsula have uncovered two new species, although we only showed data for one (*M. lupiaensis* sp. nov.). Our molecular studies also confirm the North African species found by Boulaassafer et al. (2018) and with the inclusion of new populations, have revealed a new species in the central region of Tunisia (*M. veronicae* sp. nov.).

4.2.3. Comparison of phylogenetic patterns among hydrobiid genera

Geographic factors such as topography, elevation and habitat type can influence gene flow between populations of freshwater species and, consequently, the evolutionary patterns of species (Hughes 2007, Hughes et al. 2009). Also, climatic events such as glaciations can lead to the extinction of faunal groups, thus affecting the topology of phylogenies (Benke et al. 2009, Wilke et al. 2010, Benke et al. 2011). To assess the influence of these factors on the phylogenetic patterns of hydrobiids, we inferred the evolutionary history of four genera that have a high number of species and a relatively wide distribution and that inhabit different areas of the elevational range of hydrobiids.

Among the studied genera, *Islamia* is the oldest, with species first diverging ca. 12 Mya, followed by *Corrosella* ca. 10 Mya (see Figure 72). The estimated age of the latter group coincides with the one obtained by Delicado et al. (2013). Both genera present geographically restricted clades, however, species of *Islamia* dispersed (jumped) more frequently during the early stages of its history, likely promoted by its occurrence at lower elevations compared with *Corrosella* (see section 4.3). Springsnail species, such as those of *Corrosella*, seem to have evolved in allopatry, with the isolation of the different lineages being maintained by habitat fragmentation due to physical or climatic barriers (Wilke et al. 2010, Delicado et al. 2015, Delicado et al. 2018).

According to our phylogenetic study, the species clades inferred for *Corrosella* and *Islamia* present restricted distributions within the Iberian, Italian and Balkan peninsulas. These results suggest high speciation within these peninsular regions, supporting previous assumptions about Mediterranean peninsulas serving as evolutionary centres for hydrobiids (Manganelli et al. 2001, Arconada and Ramos 2003, Miller et al. 2018). Old speciation events that likely originated through physical isolation contrast with the speciation processes suggested for other hydrobiid species that occur in lakes or coastal hydrographic systems. Stelbrink et al. (2020), for instance, stated that the high diversity of young hydrobiid flocks discovered in Lake Ohrid (which evolved ~2 Mya) may be the result of adaptive radiation facilitated by ecological opportunity rather than of physical isolation. Indeed, hydrobiid genera living in the lower courses of rivers (i.e., *Ecrobia*, *Hydrobia*, *Pseudamnicola* and *Mercuria*) are generally younger in age [e.g., *Pseudamnicola* < ca. 5 Mya (Delicado et al. 2014) or *Ecrobia* < ca. 3 Mya (Vandendorpe et al. 2019)] than those of springsnails. Also, their evolutionary patterns are highly associated with dispersal processes (Wilke et al. 2000b, Delicado et al. 2015, Vandendorpe et al. 2019). As a result, clades comprising genera found in lowlands typically present wide geographic ranges that subsequently overlap, as depicted in our phylogenetic inferences of *Pseudamnicola* and *Mercuria* (see Figure 72). Also, in the case of *Pseudamnicola*, the clades and distributions found by Boulaassafer et al. (2020) and Delicado et al. (2014) were recovered in our study, supporting the evidence showing a double colonisation towards the Balearic Islands from Europe and Africa, followed by isolation.

Unlike more recent splits, basal relationships within the studied genera still remain unclear. In cladistics and phylogenetic approaches, the phenomenon of polytomies (i.e., multifurcating relationships among the branches of a phylogenetic tree) is still an active topic of discussion. A polytomy can be soft or hard. As asserted by

Walsh et al. (1999), a soft polytomy can be resolved by increasing the number of molecular markers or sources of information used, leading to the bifurcation of the branches in question. By contrast, a hard polytomy is one in which the multifurcated relationships remain in spite of the addition of more information. In this context and on the basis of biogeographic hypotheses, fossil data and geological evidence, cases of hard polytomies can often be attributed to cladogenetic, though not necessarily dichotomous, events that lead to simultaneous and multiple speciation events.

Unresolved basal relationships in other hydrobiid genera have been related to incomplete taxon sampling or the lack of molecular data (Delicado et al. 2013b, Delicado et al. 2015). Other studies that included more nuclear and mitochondrial markers, such as H3, 12S rRNA, 18S rRNA and EF1-α, have been able to adequately resolve basal relationships when using a concatenated dataset (Colgan et al. 2007a, Delicado et al. 2019). Nevertheless, we suggest that, based on our data, the polytomies found in Mercuria, as well as in Pseudamnicola, are hard, resulting from fast cladogenetic events that could not be detected by our slow-evolving molecular markers.

Uncorrected pairwise distances for COI in *Mercuria* ranged from 1.3% to 9.3% and averaged 7.5%. This is similar to the situation found in the Mediterranean genus *Pseudamnicola*, which range from 0.5% to 10% and averaged 6.7% (Delicado et al. 2015). By contrast, for *Corrosella*, Delicado et al. (2015) found an average genetic distance close to 8% (5.39%–11.15%), which is similar to, or higher than, the values obtained for other hydrobiid genera, such as the North America genera *Floridobia* Thompson & Hershler, 2002; *Marstonia* Baker, 1926 and *Pyrgulopsis*, which ranged 0.5%–6.1%, 1.0%–8.5% and 2.8%–11.2%, respectively (Hershler et al. 2003). Highly variable genetic distances have also been observed in other hydrobioid groups, such as the spring snail genus *Bythinella*, which occurs at relatively high elevations over glacial zones and ranged 1.8%–4.7% (Feher et al. 2013), or the groundwater dwelling species of the snail genus *Bythiospeum* Bourguignat, 1882, which ranged 3.6–16.4% (Richling et al. 2017). Our study provides the first global molecular analysis of the genus *Islamia*, which showed the genetic divergence of COI ranged from 0% to 18.5%, which is most similar to the ranges estimated for *Bythiospeum* and *Pyrgulopsis*.

4.3 Biogeography of hydrobiid genera: the role of habitat type and elevation

4.3.1 Time of divergence and biogeography of *Corrosella*, *Islamia*, *Pseudamnicola* and *Mercuria*

The timing of speciation events can be estimated from genetic data using phylogenies calibrated through molecular clock approaches. Knowledge of timescales has important implications for reconstructing the biogeographic history of taxonomic groups and inferring their underlying processes. This framework has generated controversy, particularly in terms of how it handles heterogeneity rate and calibration. The development of new methodologies that treat variation in substitution rates has increased the accuracy of (and confidence in) the heterogeneity parameter; however, the issue of calibration is still regarded as a source of uncertainty. Calibration refers to the incorporation of independent, non-molecular time information into a phylogeny, which transform relative into absolute time of divergence. Calibration methods are considered internal when the calibration source is based on ancestral DNA, fossils and/or biogeographic events and external when the source is an external molecular clock rate (Wilke et al. 2009). Although the first approach is considered to be more accurate, external substitution rates are more widely used due to the lack of robust fossil records for many animal groups.

In the case of Hydrobiidae, it is common to find descriptions of imprecisely dated fossil species whose placement within the family is questionable. Given this situation, we used, as a calibration source, the COI substitution rates calculated for the genera *Pyrgulopsis* (Hershler and Liu 2008) and *Salenthydrobia* (Wilke 2003, Wilke et al. 2009). These rates, calculated using biogeographic calibration bounds, have proven effective in the estimation of divergence times and speciation processes in other genera within Hydrobiidae (Föller et al. 2015, Szarowska et al. 2016, Delicado et al. 2019, Boulaassafer et al. 2020). However, caution must be applied when drawing conclusions from results obtained using this approach, particularly when correlating a speciation event with a certain time frame or inferring the evolutionary mechanisms that underlie the event (Wilke et al. 2009).

By applying external calibration rates, *Corrosella* and *Islamia* dated to approximately the same age (ca. 11 Mya) in our dated species tree, while *Mercuria* and *Pseudamnicola* both dated to a notably lower age (ca. 7 Mya). According to the diversification theory, groups that are older should present more species as they had a longer time to diversify (McPeek and Brown 2007). When we contrast the age of these genera with the number of species comprising each, we found a

notable difference between the number of extant species presented by *Corrosella* (17) and *Islamia* (45) and a greater difference between *Mercuria* (26) and *Pseudamnicola* (70). The disparity in the species richness observed among these genera may be related to two main factors: first, differences in environmental preferences such as in habitat type, elevational range or water parameters; and second, differences in dispersal strategies (Rivadeneira et al. 2015, Delicado et al. 2018). We discuss these points in greater detail in section 4.3.2.

Given the estimated ages of the four genera, we placed the origins of their diversification in the Miocene (from 23 to 5.3 Mya), which was a period characterised by intense tectonic activity and numerous climatic changes in the western Mediterranean (Harzhauser et al. 2002, Rosenbaum et al. 2002). The earliest divergences within *Mercuria* and *Pseudamnicola* appear to have taken place during the Messinian Salinity Crisis (~5.96–5.33 Myr), a period in which, due to the uplift of the Betic-Rifean reliefs, seaways to the Atlantic Ocean gradually closed, producing considerable water volume losses and partial desiccation of the basin (Krijgsman et al. 1999, Duggen et al. 2003, Rindi et al. 2019). This event resulted in Europe and North Africa being connected through land bridges between Iberia and Morocco (Strait of Gibraltar) and between Tunisia, Sicily and Italy (Strait of Sicily), leading to faunal interchange between the two continents (Figure 76). The subsequent refilling of the Mediterranean Sea acted as an important barrier to gene flow between populations of freshwater taxa living throughout the Mediterranean, including gastropods and thus promoted speciation (Sherpa et al. 2018). We found evidence of some dispersal events across the Mediterranean Sea that coincided with the presence of these land connections and common bird migration routes. For instance, we found dispersal events in the lineage leading to *M. balearica* and *M. felixi* sp. nov. and to *M. tensiftensis*, closely related species distributed in the southern Iberian Peninsula and Northwest Africa and in the lineage leading to *M. lupiaensis* sp. nov., *M. veronicae* sp. nov. and *M. saharica*, closely related species whose distribution includes the area around Italy, Sicily and Tunisia. The distribution of some species of *Pseudamnicola,* namely *P. conovula*, *Pseudamnicola* sp2, *Pseudamnicola* sp4 and *Pseudamnicola* sp5, can also be explained by the Italy, Sicily and Tunisia land connections.

As the sister group of the genera *Mercuria* and *Islamia* is still unknown, we can only estimate the ancestral area of their initial divergence (or crown split), which was the Iberian Peninsula for *Mercuria* and the Apennine Peninsula for *Islamia*. A sister group relationship between *Pseudamnicola* and *Corrosella* has not been supported by previous phylogenetic studies (Delicado et al., 2015); however,

together with the genus *Diegus* Delicado, Machordom & Ramos, 2016, these three genera form a well-supported clade that likely originated in the Iberian Peninsula, approximately 22 Mya (Delicado et al., 2015). However, according to our BioGeoBEARS analysis (see Figure 72), which included more species from newly surveyed regions, the Apennine Peninsula is the ancestral area of the diversification within *Pseudamnicola*.

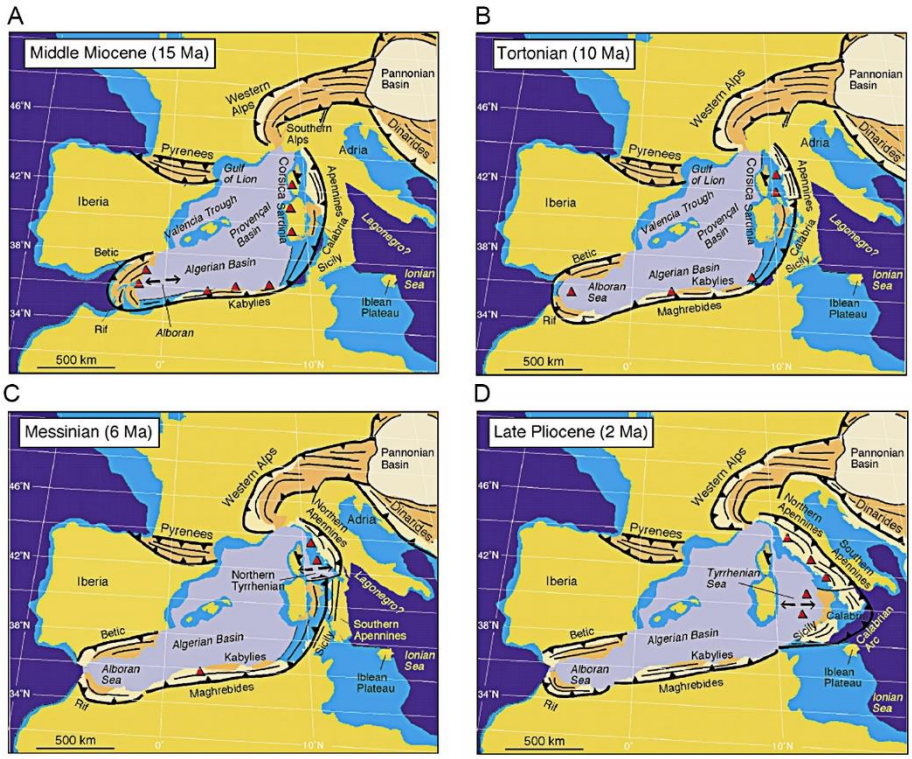


Figure 76. Paleogeographic reconstruction of the western Mediterranean area (modified from Rosenbaum et al. (2002). **A**, Middle Miocene; **B**, Tortonian stage ; **C**, Messinian stage; **D**, Late Pliocene.

The biogeographic history of *Corrosella* inferred here suggests a pattern of isolation by distance produced by vicariance processes, a hypothesis previously proposed by Delicado et al. (2015) and Delicado et al. (2013b). According to Delicado et al. (2013b), these processes were associated with geological events including the uplift of the Pyrenees, which isolated populations in the north of Spain and the south of France and for the southern Iberian clade, the tectonic activity in the Alboran Sea that resulted in the formation of the Betic mountains. Climatic conditions also played an important role in the speciation that took place

in the different regions of the Iberian Peninsula, with potential extinction events occurring in the northern and central areas during glaciation events (Delicado et al. 2013b).

Geological and climatic processes also influenced speciation in *Islamia*, concurrent with that in *Corrosella*, during three main periods, approximately 10 Mya, 5 Mya and 2 Mya. Cladogenesis within *Islamia* involved a combination of founder events from the Apennine Peninsula eastwards and westwards and narrow (within-ecoregion) sympatry. The earliest period of speciation was associated with several geological processes that occurred in the Mediterranean area, including the final formation of the Rif–Betic Cordillera ca. 10 Mya in the southern Iberian Peninsula (Rosenbaum et al. 2002) and the alpine orogenesis between 23 and 11 Mya, which uplifted the Alps and the Pyrenees, the main mountainous areas in western Europe (Frisch et al. 2000). These geological changes made the occurrence of jumps between ecoregions less likely.

The later speciation periods, 5 Mya and 2 Mya, are likely related to isolation by glaciations. The Messinian was an intense climatic period that affected more than southern Europe with the desiccation of the Mediterranean basin. According to Jansen et al. (1990), this period was also characterised by the presence of ice-sheets throughout the northern hemisphere ca. 6 Mya and in the period between 2.8 and 0.7 Mya, by cyclic climatic changes interleaving glacial epochs with warms periods. All of these conditions likely played a fundamental role in preventing gene flow between populations (Hewitt 2000, Joger et al. 2007, Savić 2008).

The division of the Hercynian Belt (ca. 25 Mya in the Oligocene; Rosenbaum et al., 2002) pre-dates the speciation events within the Mediterranean genera *Pseudamnicola* and *Mercuria*. Thus, the occurrence of these groups in the current Hercynian partitions is likely a result of a series of dispersal and colonisation events followed by subsequent isolation, rather than by range fragmentation. Our results are consistent with previous phylogenetic studies in which this type of process is evidenced. One example is the double colonisation of *Pseudamnicola* populations from Europe and Africa to the Balearic Islands (Delicado et al. 2015, Boulaassafer et al. 2020).

Mercuria species appear to exhibit different patterns of dispersal to the Balearic archipelago compared with those of *Pseudamincola*. For instance, on the island of Majorca, we found *M. similis*, a species widely distributed from the southern Iberian Peninsula to the region near Nice, France, whereas on the island of Minorca, we only found *M. balearica*, a species also distributed throughout the

southern Iberian Peninsula. However, in contrast to the pattern of dispersal found in *Pseudamnicola*, we found that, in *Mercuria*, both dispersal events originated in the Iberian Peninsula. Despite the fact that *M. balearica* is related to *M. tensiftensis*, a species from North Africa, our results indicate that the entire clade (*M. balearica* + *M. tensiftensis* + *M. felixi* sp. nov.) originated in the southern Iberian Peninsula, with subsequent diversification occurring towards the Balearic Islands and North Africa (see Figure 72). Colonisation patterns similar to the ones found for *Mercuria* towards the islands have also been reported for other freshwater groups (e.g., Abellan, Millan, & Ribera, 2009; Lazaro et al., 2009).

Our biogeographic reconstruction method has several limitations in estimating ancestral areas. For instance, given the low support at basal branches, we are not able to accurately infer the ancestral areas from which the groups originated during their evolution. An explanatory example is the earliest ancestral area estimations within *Islamia*: the low probabilities obtained in the reconstruction do not support the selected targeted areas as those that can be reasonably related to the first dispersal events in the history of the genus. According to our BioGeoBEARS analysis, this genus originated in the Italian Peninsula, then long jumped to the Anatolian and Iberian peninsulas and not to neighbouring regions such as continental France or the Balkans. This observation may be, in part, due to the fact that our study did not include a number of known species of these genera and their geographic regions. For example, newly described species of *Islamia* from Morocco (Glöer et al. 2020, Mabrouki et al. 2021) and the northern Iberian Peninsula (Ruiz-Cobo et al. 2018) were not included in our study. Neither were all Aegean and Asia Minor species of *Pseudamnicola*, although the genus assignment of many of these species is doubtful (e.g., Delicado et al., 2016), nor were many of the North African species of *Mercuria*, especially those from Algeria and Tunisia. Despite these caveats, multiple more recent speciation events promoted by dispersal or vicariance are well supported across the inferred phylogenies. Therefore, in general, the biogeographic history of the four genera studied here can be inferred with confidence and compared using our calibrated phylogenies.

4.3.2 Dispersal strategies affecting species richness

Dispersal modes have important consequences on the evolution and species richness of organismal groups (Shurin and Allen 2001, Ricklefs 2004). For freshwater organisms, variation in dispersal strategies have been attributed to differences in habitat types, elevational preferences or body sizes (Shurin 2000, Ribera et al. 2003, Shah et al. 2017). Habitat types (e.g., headwater springs, lakes, subterranean waters, brackish waters) linked to factors such as elevation or

latitude differ from each other in the extent of their geographic connectedness, natural environmental conditions and anthropogenic impact (Miller et al. 2018), which affect the survival and dispersal of populations. The observed distribution of threaten hydrobiid species along the elevational gradient (see Figure 73) may reflect an interplay of these factors as the highest extinction risk peaks at 1,500 m.a.s.l (Miller et al. 2018). However, hydrobiids have a limited dispersal capacity and need other vectors to colonise new territories, such as continuous water flows or accessibility of birds or fishes to ecosystems inhabited by hydrobiids. These particularities were revealed by our GLM since connectivity between ecoregions and the peninsula effect were identified as factors affecting the distribution of species richness (see Figure 21).

Our biogeographic analysis also reflects these features: for example, two genera of similar ages, *Corrosella* and *Islamia*, have accumulated species at different rates, likely influenced by abiotic factors such as the habitat preferences and elevational ranges of their species. Species of *Corrosella*, which primarily inhabit the most isolated high mountain ecosystems, dispersed mainly by connections between ecoregions (high *d* parameter and low *j*), while species of the other genera including *Islamia*, which inhabit lower areas, dispersed mainly by jumps between more distant ecoregions (Figure 73). Several factors may have accounted for the inferred long-distance jump dispersals: 1) better access for birds at low elevations, 2) greater connectivity of lower river courses (that evolved into larger rivers) during glacial times (Dias et al. 2014), which could have promoted anagenetic events of dispersal in *M. tachoensis* in the Atlantic and in *P. subproducta* in the Mediterranean and 3) greater ecological plasticity of the species of *Mercuria* and *Pseudamnicola*, which can also account for the high *d* and *j* parameters (Delicado et al., 2018).

Living snails have been found on the feathers, feet and even in the guts of water birds (Wilke 2003, Van Leeuwen et al. 2012, Wada et al. 2012), supporting the idea of aerial dispersal. Although there is no direct evidence of hydrobiids being transported by birds, this mechanism has been proposed to explain the unusual distribution patterns of some hydrobiid genera including *Ecrobia* (Haase et al. 2010, Vandendorpe et al. 2019), *Pyrgulopsis* (Liu et al. 2003, Hershler et al. 2015) and *Pseudamnicola*, at least within the Mediterranean islands (Delicado et al. 2014, Szarowska et al. 2016, Boulaassafer et al. 2020).

One may argue that our explanation cannot be applied to the difference in species richness observed between *Mercuria* and *Pseudamnicola*, considering the higher species richness of *Pseudamnicola* despite the fact that both genera co-inhabit the

same elevations. However, this disparity could be due to two main factors: 1) the species richness of the two genera is not fully known (e.g., species attributed to *Pseudamnicola* that occur in Asia Minor probably do not belong to this genus) and species of *Mercuria* are likely yet to be discovered, as evidenced by the number of newly described species in our study; and 2) *Mercuria* could have fewer species because of its greater ecological plasticity and euryhaline character, allowing it to inhabit systems with low salinities to almost brackish ones, which have been shown to promote higher levels of dispersion (and gene flow) for some hydrobiid species (e.g., species of *Ecrobia* and *Hydrobia*; (Haase et al. 2010, Vandendorpe et al. 2019).

Our biogeographic analyses have revealed (jump) dispersal events among the studied groups (e.g., *Pseudamnicola* and *Mercuria* to the islands of Majorca or Minorca). Our data, unlike for other organisms (Fenchel and Finlay 2004, Shurin et al. 2009), did not evidence a direct relationship between dispersal and body size (i.e., smaller organisms are less constrained by dispersal limitations than bigger ones; (Shurin 2000, Shurin et al. 2009) in these events. For instance, despite its smaller shell dimensions, *Islamia* presented a lower dispersion index than *Mercuria*. However, the robustness of this relationship should be further evaluated for hydrobiids.

Constraints on dispersion affect populations at the local level; however, if these persist over time, global patterns of species richness can be affected (Cai et al. 2018). Dispersal limitations in freshwater gastropods can be geographic or ecological (Strong et al. 2008). Factors that affect dispersal include the following: ecological and physiological tolerance of individuals, tolerance to salinity or low temperatures, reproductive and developmental strategies, ability to survive desiccation and vagility (Lassen and Kristensen 1978, Hylleberg and Siegismund 1987, Siegismund and Hylleberg 1987, Komendantov and Smurov 2009). Hydrobiids in general have low vagility and low dispersal capacities as they depend exclusively on the aquatic environment. Also, their life history traits such as having direct development [except for *Peringia ulvae* (Pennant, 1777) which produces larvae; Wilke and Davis (2000)], separate sexes and small body sizes make them more dependent on the breadth of ecological niches, the connectivity between habitats and the presence of other vectors for colonisation.

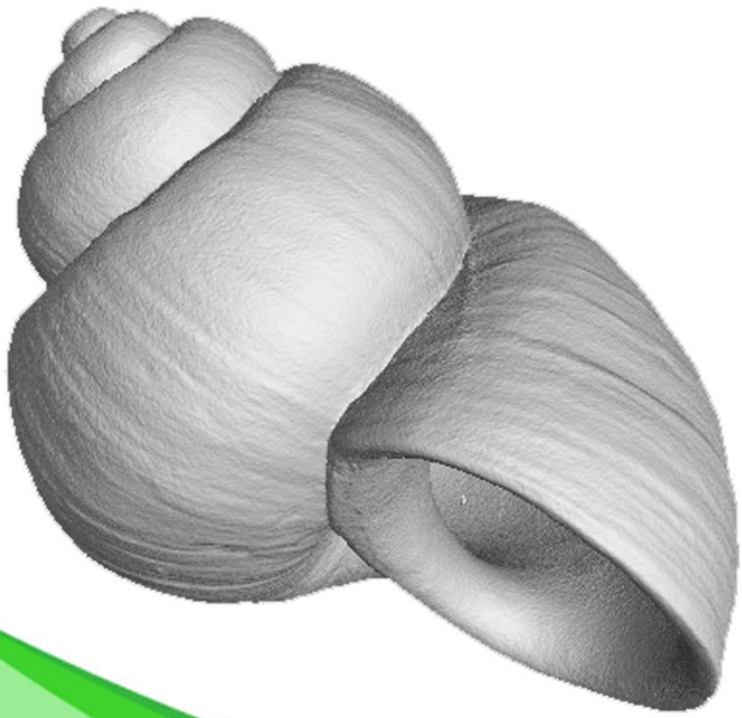
Dispersal into new disjunct biogeographic zones may promote founder event speciation (Lam et al. 2020, Tsang et al. 2020), given the low probability of continued gene flow. In our study, the genera with the highest species richness (*Pseudamnicola* and *Islamia*) are those that have dispersed mainly by jump

dispersal. This process is highly linked to speciation and involves a jump of the ancestor to another area with little to no contact between populations. On the contrary, anagenetic events of dispersal into adjacent zones involve the range expansion of a species but not an increase in the number of species within a clade (Ree and Smith 2008). The genera with the lowest number of species (*Corrosella* and *Mercuria*) showed a high dispersion *d* parameter that is not related to speciation but rather to range expansion along branches.

Overall, our study has shown that dispersal strategy and evolutionary history have influenced the species richness of the studied genera of hydrobiids, as shown for other molluscs (Cai et al. 2018, Czaja et al. 2020). A variable that can positively influence the dispersal parameter *j*, more than *d*, is elevation: a lower elevation may correlate with a greater number of scattering vectors and greater connectivity dynamics between water bodies. Another element to consider is the higher extinction rate of highly specialised groups inhabiting more isolated ecosystems, such as *Corrosella*, which may have had a negative influence on the current distribution of species richness.

In conclusion, inferring the evolutionary history of the studied genera is important in order to have a more complete knowledge of the family Hydrobiidae. By understanding the factors that influence how and the extent to which, species dispersed, we can design future conservation strategies. Considering that freshwater ecosystems are one of the most endangered (Dudgeon et al. 2006, Collen et al. 2014), new policies and future strategies will be necessary to guarantee the protection and continuity of the threatened species belonging to this family.

CONCLUSIONS



From the literature review and the biogeographic and systematic results of this doctoral thesis, the following general conclusions can be drawn about the family Hydrobiidae:

1. Following the *sensu stricto* definition of the family, we have compiled geographic data for approximately 900 extant nominal species of hydrobiids and identified hotspots of richness, endemism and threat, mainly in freshwater ecoregions of the Mediterranean basin. Geographic and climatic factors such as latitude, watershed connectivity, precipitation, seasonality and annual temperature range best explain the observed distributions of these species, whereas elevation and geological heterogeneity do not (probably because the spatial aggregation of these two factors concealed their true variability). These findings suggest that more stable climatic conditions, in terms of temperature and precipitation and habitat connectivity contributed to increase hydrobiid species richness.

2. The extinction risk analysis showed that the conservation status of only 42.9% of the Hydrobiidae *s. str.* species have been assessed by the IUCN thus far. Of these, two-thirds have been classified within a threat category, mainly because of their narrow distribution range. As many of the unassessed taxa are endemic to a single ecoregion, the number of threatened species will remain high for this family, particularly in the face of rising global climate and land use change.

3. Our phylogenetic inferences were useful tools for delimiting species in *Mercuria* and inferring evolutionary and biogeographic patterns in different genera. A total of 14 species of *Mercuria* was delimited from our dataset. Of these, nine are known species and five are new to science. Of the applied species delimitation methods, distance-based automatic barcode gap discovery (ABGD) was the one most consistent with the morphological data. By contrast, the methods based on the generation of partitions (GMYC and PTP) greatly overestimated the number of putative species.

4. According to our classic and geometric morphometric results, the shell of *Mercuria* presents wide intra- and interspecific variability, evidence that this character should not be used to propose species hypotheses. Similar results were found for the colour of the operculum. A high level of variability was also found in the reproductive system in characters associated with the penis, penial appendix and bursa copulatrix, indicating

that these characters should be used with caution to delimit species of the genus. To this end, we propose the use of the integrative taxonomic approach for species delimitation.

5. The age and habitat type of the studied genera do not explain their species richness. *Corrosella*, for instance, is one of the oldest of these genera, yet it has the lowest species richness. Further supporting this conclusion, the genera *Mercuria* and *Pseudamnicola*, despite having a similar age and habitat preferences, present a great disparity in species richness.

6. Overall, our study shows that the dispersal strategy and evolutionary history of the hydrobiid genera studied here likely influenced their species richness. Better access for dispersal vectors and greater habitat connectivity at the lower elevations may have facilitated long-distance dispersal, resulting in founder event speciation. On the contrary, highly specialised and isolated groups, such as the species of *Corrosella* inhabiting mountain-top springs, are less likely to disperse and speciate (and, thus, are probably more prone to extinction) compared with those inhabiting lower elevations.

Consideration of these conclusions will prove useful for future works exploring, to a greater extent, the role of external factors such as habitat and elevation in speciation events and, therefore, in the species richness of animal groups with little dispersal power, such as hydrobiids.

De la revisión de la literatura y de los resultados biogeográficos y sistemáticos de esta tesis doctoral, se pueden extraer las siguientes conclusiones generales para futuros estudios de la familia Hydrobiidae:

1. Siguiendo la definición *sensu-stricto*, se han recopilado datos geográficos para aproximadamente 900 especies nominales existentes de hidróbidos identificando *hotspots* de riqueza, endemismo y amenaza principalmente en ecorregiones de agua dulce de la cuenca mediterránea. Los factores geográficos y climáticos como la latitud, la conectividad de la cuenca, la precipitación, la estacionalidad y el rango de temperatura anual explican mejor esta distribución, mientras que la elevación y la heterogeneidad geológica fueron descartadas como variables explicativas probablemente porque su agregación espacial ocultaba su verdadera variabilidad. Estos hallazgos sugieren que condiciones más estables, en términos de temperatura y precipitación, y la conectividad del hábitat han contribuido a aumentar la riqueza de especies de hidróbidos.

2. El análisis del riesgo de extinción mostró que el estado de conservación de solo el 42,9% de los Hydrobiidae *s. str*. especie ha sido evaluada por la UICN hasta el momento. De estos, dos tercios se clasificaron dentro de una categoría de amenaza, principalmente debido a sus estrechos rangos de distribución. Dado que los taxones no evaluados a menudo son endémicos de una sola ecorregión, esto sugiere que la proporción de especies amenazadas seguirá siendo alta para esta familia que enfrenta el cambio climático global y el uso de la tierra.

3. En cuanto a otros hidróbidos, nuestras inferencias filogenéticas fueron herramientas útiles para delimitar especies en *Mercuria* e inferir patrones evolutivos y biogeográficos en diferentes géneros. Revelaron 14 especies de *Mercuria* de nuestro conjunto de datos, nueve ya conocidas y cinco nuevas para la ciencia. Entre los métodos de delimitación de especies aplicados, el método de «Automatic Barcode Gap Discovery» (ABGD) fue el más consistente con la morfología, mientras que los métodos basados en la generación de particiones (GMYC y PTP) sobrestimaron en gran medida el número de especies putativas.

4. Nuestros resultados morfológicos indican que la concha de *Mercuria* presenta una amplia variabilidad intra e interespecífica tanto por morfometría clásica como geométrica, demostrando ser un carácter que no debe utilizarse para proponer hipótesis de especies. Se encontraron resultados similares para

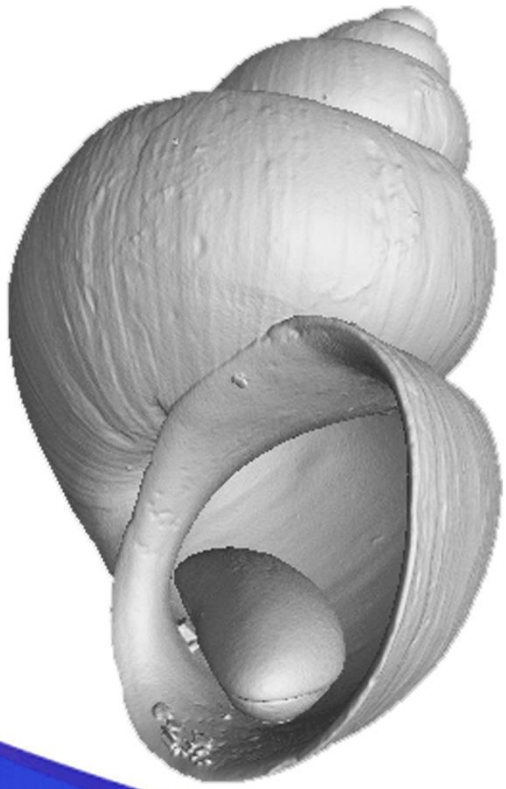
el color del opérculo. En el sistema reproductivo, también se encontró una alta variabilidad en los caracteres del pene, el apéndice del pene y la bursa copulatrix; por lo tanto, deben usarse con precaución para delimitar especies del género. Para ello, proponemos el uso de taxonomía integradora.

5. La edad y el tipo de hábitat de los géneros estudiados no explican la riqueza de especies ya que, por ejemplo, *Corrosella*, al ser uno de los géneros más antiguos, muestra la riqueza de especies más baja. Los géneros *Mercuria* y *Pseudamnicola* también confirman esta conclusión ya que, a pesar de tener una edad y hábitat similares, presentan una gran disparidad en la riqueza de especies.

6. En general, nuestro estudio revela que la estrategia de dispersión y la historia evolutiva de los géneros de hidróbidos estudiados aquí pueden haber influido en su riqueza de especies. Un mejor acceso para los vectores de dispersión y una mayor conectividad del hábitat a menor elevación pueden facilitar la dispersión a larga distancia, lo que resulta en la especiación del evento fundador. Por el contrario, los grupos altamente especializados y aislados, como las especies de *Corrosella* que habitan en los manantiales de alta montañas, tienen menos probabilidades de dispersarse y especiarse (pero probablemente más de extinguirse) que los grupos de baja elevación.

Los trabajos futuros deberían explorar en mayor medida el papel de factores externos como el hábitat y la elevación en los eventos de especiación, y por tanto en la riqueza de especies, de grupos de animales con poco poder de dispersión como los hidróbidos.

APPENDICES



Appendix 1. Elevation of the type locality and the thread category of all extant Hydrobiidae species according to Miller et al. (2018).

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
1	*Adriohydrobia gagatinella*	18.769540	42.484480	LC	179
2	*Agrafia wiktori*	21.631389	39.368611	DD	852
3	*Alzoniella asturica*	-6.002759	43.353831	EN	178
4	*Alzoniella braccoensis*	9.538946	44.287978	NT	536
5	*Alzoniella camocaensis*	-5.472296	43.460359	DD	69
6	*Alzoniella cantabrica*	-4.397045	43.381304	LC	3
7	*Alzoniella cornucopia*	11.402650	43.395060	VU	251
8	*Alzoniella delmastroi*	7.407562	45.232391	EN	662
9	*Alzoniella edmundi*	2.481660	39.652661	EN	158
10	*Alzoniella elliptica*	-1.621819	43.349506	VU	7
11	*Alzoniella fabrianensis*	11.931979	46.568735	VU	1545
12	*Alzoniella feneriensis*	8.315015	45.707552	VU	791
13	*Alzoniella finalina*	7.583640	43.833230	EN	54
14	*Alzoniella galaica*	-7.194932	42.588771	CR	594
15	*Alzoniella haicabia*	-1.734092	43.376380	VU	24
16	*Alzoniella hartwigschuetti*	15.735496	47.973686	NT	615
17	*Alzoniella iberopyrenaica*	-1.376670	42.915723	CR	688
18	*Alzoniella junqua*	-0.417612	43.143610	VU	459
19	*Alzoniella lucensis*	-7.297793	42.439690	LC	639
20	*Alzoniella lunensis*	9.923379	44.185583	VU	42
21	*Alzoniella macrostoma*	9.923379	44.185583	NT	42
22	*Alzoniella manganellii*	11.500000	44.000000	NT	874
23	*Alzoniella marianae*	-6.286799	43.320663	CR	471
24	*Alzoniella microstoma*	9.934680	44.108964	NT	8
25	*Alzoniella montana*	-4.107187	43.167404	NT	625
26	*Alzoniella murita*	-3.060050	42.931412	DD	636
27	*Alzoniella navarrensis*	-1.283039	43.109297	VU	262
28	*Alzoniella onatensis*	-2.410930	43.018162	CR	396
29	*Alzoniella ovetensis*	-4.107187	43.167404	LC	625
30	*Alzoniella pellitica*	-2.631987	42.992071	LC	608
31	*Alzoniella perrisii*	-0.511057	43.865989	VU	100
32	*Alzoniella pyrenaica*	-0.912199	43.063664	DD	646
33	*Alzoniella rolani*	-8.905505	42.221602	NT	10

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
34	*Alzoniella sigestra*	8.855130	44.435180	NT	38
35	*Alzoniella slovenica*	18.659790	48.912382	LC	404
36	*Alzoniella somiedoensis*	-6.255946	43.092695	VU	699
37	*Anadoludamnicola gloeri*	38.008036	38.518900	DD	833
38	*Anagastina gluhodolica*	19.091285	42.243480	EN	5
39	*Anagastina hadouphylax*	18.392414	42.854548	CR	384
40	*Anagastina matjasici*	19.007554	42.631901	CR	256
41	*Anagastina scutarica*	19.323876	42.163201	EN	1
42	*Anagastina vidrovani*	18.933944	42.846681	LC	648
43	*Anagastina zetaevallis*	19.258900	42.467250	DD	49
44	*Andrusovia andrusovi*	52.583333	39.083333	DD	0
45	*Andrusovia brusinai*	52.541667	42.708333	DD	0
46	*Andrusovia dybowskii*	49.599892	37.517630	DD	0
47	*Andrusovia marina*	50.901083	43.541667	DD	0
48	*Antibaria notata*	18.973820	42.197084	LC	18
49	*Arganiella pescei*	1.465278	42.503611	LC	2109
50	*Arganiella tabanensis*	19.219083	42.527550	DD	107
51	*Arganiella wolfi*	-6.625235	37.935942	VU	617
52	*Attebania bernasconii*	-9.723233	29.647996	CR	292
53	*Avenionia berenguieri*	4.807099	43.947766	NT	29
54	*Avenionia bourguignati*	4.442595	48.020849	DD	177
55	*Avenionia brevis*	5.125236	46.250439	LC	222
56	*Avenionia ligustica*	8.836660	44.433180	LC	88
57	*Avenionia parvula*	9.726519	44.225505	NT	97
58	*Avenionia roberti*	4.897844	50.377425	NT	243
59	*Balkanica yankovi*	25.314528	42.956411	DD	353
60	*Balkanospeum schniebsae*	27.014306	45.544111	DD	299
61	*Belgrandia alcoaensis*	-8.938880	39.534849	CR	100
62	*Belgrandia bigorrensis*	1.422639	43.153072	DD	267
63	*Belgrandia bonelliana*	11.868047	42.990714	CR	556
64	*Belgrandia boscae*	-0.179475	38.963204	NT	25
65	*Belgrandia cazioti*	2.100364	42.571009	DD	1524
66	*Belgrandia conoidea*	1.336016	44.018844	EN	79
67	*Belgrandia coutagnei*	4.812741	43.961865	DD	22
68	*Belgrandia dunalina*	3.876716	43.610769	DD	58
69	*Belgrandia gfrast*	7.538333	47.615000	VU	238

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
70	*Belgrandia gibba*	5.353836	44.757882	NT	413
71	*Belgrandia gibberula*	3.574798	43.687292	VU	49
72	*Belgrandia heussi*	-8.946782	39.881834	NT	10
73	*Belgrandia ionica*	19.928657	39.477088	DD	2
74	*Belgrandia latina*	11.939335	42.613014	VU	304
75	*Belgrandia lusitanica*	-8.431838	40.205614	EN	22
76	*Belgrandia marginata*	6.459086	43.522418	DD	188
77	*Belgrandia mariatheresiae*	12.816683	43.332012	LC	474
78	*Belgrandia minuscula*	14.111537	42.053460	DD	2449
79	*Belgrandia moitessieri*	3.877597	43.611463	CR	56
80	*Belgrandia semiplicata*	-0.449876	47.352839	CR	45
81	*Belgrandia silviae*	-8.463443	40.108535	VU	119
82	*Belgrandia sorgica*	5.126380	43.921792	DD	87
83	*Belgrandia stochi*	13.584902	45.783797	VU	15
84	*Belgrandia targoniana*	11.254945	43.770804	DD	66
85	*Belgrandia thermalis*	10.400704	43.716145	LC	10
86	*Belgrandia torifera*	17.370609	43.205176	VU	229
87	*Belgrandia varica*	7.187473	43.705162	CR	25
88	*Belgrandia zilchi*	13.732269	41.463233	DD	86
89	*Belgrandia caprai*	11.218963	43.243970	DD	209
90	*Belgrandiella abchasica*	41.275047	43.034377	DD	500
91	*Belgrandiella adsharica*	41.718810	41.540477	EN	47
92	*Belgrandiella aulaei*	14.231886	47.905674	EN	411
93	*Belgrandiella austriana*	15.433382	47.133312	CR	527
94	*Belgrandiella bachkovoensis*	25.600760	42.419624	CR	288
95	*Belgrandiella boetersi*	13.152248	47.954462	CR	612
96	*Belgrandiella bozidarcurcici*	17.397867	44.552600	DD	394
97	*Belgrandiella bulgarica*	24.195082	42.948544	VU	352
98	*Belgrandiella bumasta*	20.202422	42.664783	DD	748
99	*Belgrandiella bureschi*	23.146968	42.707403	VU	643
100	*Belgrandiella caucasica*	39.362972	44.007658	DD	148
101	*Belgrandiella cavernica*	31.432759	41.276952	CR	48
102	*Belgrandiella croatica*	15.223171	45.265196	VU	316
103	*Belgrandiella crucis*	14.411677	45.729382	VU	574
104	*Belgrandiella dabriana*	16.638056	44.710556	NT	218
105	*Belgrandiella delevae*	26.512800	42.933600	DD	742

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
106	*Belgrandiella edessana*	39.231273	37.752605	VU	708
107	*Belgrandiella elburensis*	52.250483	36.455996	DD	66
108	*Belgrandiella elbursensis*	52.159329	35.555457	DD	1739
109	*Belgrandiella fontinalis*	14.642480	46.088438	LC	270
110	*Belgrandiella fuchsi*	15.716133	48.043568	VU	387
111	*Belgrandiella ganslmayri*	14.660074	47.852559	CR	409
112	*Belgrandiella globulosa*	14.454428	45.763295	VU	629
113	*Belgrandiella haesitans*	21.558754	38.563971	LC	11
114	*Belgrandiella hershleri*	15.019156	46.095537	VU	505
115	*Belgrandiella hessei*	23.409069	43.053154	VU	780
116	*Belgrandiella koprivnensis*	15.980798	44.993129	DD	398
117	*Belgrandiella kreisslorum*	15.620810	47.908445	CR	480
118	*Belgrandiella krupensis*	17.747695	43.052282	DD	2
119	*Belgrandiella kuesteri*	14.466665	46.133336	DD	381
120	*Belgrandiella kusceri*	14.317056	45.811531	DD	569
121	*Belgrandiella libanica*	36.200412	34.008701	NT	1128
122	*Belgrandiella lomica*	26.110794	43.465414	DD	210
123	*Belgrandiella maarensis*	25.045944	43.244917	DD	233
124	*Belgrandiella mimula*	16.344387	48.156403	CR	198
125	*Belgrandiella multiformis*	15.836859	47.653237	CR	698
126	*Belgrandiella nana*	7.430717	36.456603	DD	297
127	*Belgrandiella novoselensis*	17.558752	43.101004	DD	91
128	*Belgrandiella ocalis*	3.943293	44.110868	DD	186
129	*Belgrandiella pageti*	15.326142	45.252539	DD	244
130	*Belgrandiella pandurskii*	24.885456	43.233491	DD	125
131	*Belgrandiella parreyssii*	16.211300	47.965278	CR	272
132	*Belgrandiella pelerei*	16.346818	47.893584	CR	224
133	*Belgrandiella pusilla*	23.408596	43.053066	VU	780
134	*Belgrandiella robusta*	15.353929	45.587457	DD	163
135	*Belgrandiella saxatilis*	2.799745	43.504991	LC	371
136	*Belgrandiella schleschi*	14.466671	45.724934	VU	588
137	*Belgrandiella seminium*	6.641556	36.348893	DD	586
138	*Belgrandiella serbica*	22.774807	43.172182	DD	684
139	*Belgrandiella stanimirae*	25.476417	42.876389	DD	522
140	*Belgrandiella styriaca*	15.367274	47.328998	CR	502
141	*Belgrandiella substricta*	14.318332	45.969384	VU	290

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
142	*Belgrandiella superior*	16.050000	47.983330	VU	362
143	*Belgrandiella wawrai*	10.397441	47.263148	EN	1067
144	*Belgrandiella zermanica*	16.067370	44.150769	VU	266
145	*Belgrandiella hohenackeri*	23.047410	38.859212	DD	43
146	*Birgella subglobosa*	86.850000	34.560000	DD	4962
147	*Boetersiella davisi*	-3.566551	36.988253	VU	787
148	*Boetersiella sturmi*	-3.511390	37.316110	EN	1579
149	*Bracenica spiridoni*	19.088773	42.306994	EN	1
150	*Bracenica vitojaensis*	19.362800	42.325400	DD	14
151	*Bucharamnicola bucharica*	68.550173	38.528956	DD	807
152	*Bullaregia tunisiana*	8.746670	36.558300	DD	185
153	*Caspia baerii*	51.374409	36.798778	DD	0
154	*Caspia brotzkajae*	47.808630	42.622803	DD	0
155	*Caspia gaillardi*	48.881966	38.439838	DD	0
156	*Caspia knipowitchi*	40.306150	47.253980	LC	0
157	*Caspia logvinenkoi*	39.434542	47.154399	DD	0
158	*Caspia makarovi*	50.650800	41.936848	LC	0
159	*Caspia milae*	25.466667	43.616667	DD	26
160	*Caspia obventicia*	29.257175	45.433347	DD	0
161	*Caspia stanislavi*	50.650800	41.936848	DD	0
162	*Caspiohydrobia behningi*	59.722258	45.456943	DD	29
163	*Caspiohydrobia bergi*	59.271345	45.167352	DD	53
164	*Caspiohydrobia borealis*	64.769217	52.634457	DD	100
165	*Caspiohydrobia elongata*	69.214234	38.031181	DD	714
166	*Caspiohydrobia gemmata*	59.582840	45.214220	DD	29
167	*Caspiohydrobia grimmi*	62.025278	55.211111	DD	177
168	*Caspiohydrobia husainovae*	59.971106	45.663406	DD	94
169	*Caspiohydrobia johanseni*	76.931882	52.281028	DD	98
170	*Caspiohydrobia pavlovskii*	69.214234	38.031181	DD	714
171	*Caspiohydrobia sogdiana*	69.214234	38.031181	DD	714
172	*Caspiohydrobia subconvexa*	52.208106	36.699049	DD	0
173	*Caspiohydrobia tadzhikistanica*	69.214234	38.031181	DD	714
174	*Caspiohydrobia turrita*	52.208106	36.699049	DD	0
175	*Caspiohydrobia aralensis*	57.924528	45.184417	DD	143
176	*Caspiohydrobia chrysopsis*	51.862272	36.651044	DD	0

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
177	*Caspiohydrobia conica*	51.005835	41.635160	DD	0
178	*Caspiohydrobia coniformis*	69.213291	38.031629	DD	714
179	*Caspiohydrobia convexa*	49.095172	39.187851	DD	0
180	*Caspiohydrobia curta*	51.005835	41.635160	DD	0
181	*Caspiohydrobia cylindrica*	51.005835	41.635160	DD	0
182	*Caspiohydrobia dubia*	51.005835	41.635160	DD	0
183	*Caspiohydrobia eichwaldiana*	30.719231	46.631506	DD	0
184	*Caspiohydrobia kazakhstanica*	60.133570	45.036013	DD	29
185	*Caspiohydrobia ljaurica*	69.214234	38.031181	DD	714
186	*Caspiohydrobia nikitinskii*	60.133570	45.036013	DD	29
187	*Caspiohydrobia nikolskii*	60.133570	45.036013	DD	29
188	*Caspiohydrobia obrutchevi*	60.133570	45.036013	DD	29
189	*Caspiohydrobia oviformis*	52.208106	36.699049	DD	0
190	*Caspiohydrobia parva*	52.208106	36.699049	DD	0
191	*Caspiohydrobia sidorovi*	60.856240	46.153011	DD	39
192	*Cavernisa zaschevi*	23.250696	42.988528	VU	570
193	*Chilopyrgula sturanyi*	20.704384	41.045442	NT	689
194	*Chirgisia alaarchaensis*	74.482650	42.603383	DD	1890
195	*Chondrobasis levantina*	-0.602530	39.926170	NT	604
196	*Cilgia dalmatica*	17.799132	43.017578	DD	0
197	*Cincinnatia integra*	-91.364238	37.978159	DD	230
198	*Cincinnatia winkleyi*	-70.381667	43.581380	DD	2
199	*Corbellaria celtiberica*	-1.967672	41.619017	DD	1034
200	*Corrosella andalusica*	-3.468120	37.791890	DD	822
201	*Corrosella astierii*	5.900000	43.500000	DD	280
202	*Corrosella bareai*	-2.518519	37.963143	DD	1265
203	*Corrosella falkneri*	-2.506220	37.725070	NT	902
204	*Corrosella hauffei*	-0.572333	39.930000	DD	551
205	*Corrosella hinzi*	-1.300750	40.918877	DD	887
206	*Corrosella hydrobiopsis*	-4.137473	37.157949	VU	589
207	*Corrosella iruritai*	-4.132573	37.176690	DD	497
208	*Corrosella luisi*	-3.280075	37.276924	DD	1008
209	*Corrosella manueli*	-2.894018	37.896438	DD	1427
210	*Corrosella marisolae*	-3.570735	37.000049	DD	748
211	*Corrosella navasiana*	-1.385786	41.818280	DD	353
212	*Costellina turrita*	16.499046	43.540215	CR	13

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
213	*Dabriana bosniaca*	16.639278	44.709642	NT	248
214	*Dalmatella sketi*	15.974791	43.903954	CR	42
215	*Dalmatinella fluviatilis*	15.784410	44.192128	EN	22
216	*Daphniola exigua*	22.530870	39.859490	EN	26
217	*Daphniola louisi*	23.818380	37.996310	CR	268
218	*Daphniola magdalenae*	22.049444	39.877222	DD	311
219	*Daphniola eptalophos*	22.503750	38.593194	DD	965
220	*Daphniola hadei*	22.533820	36.730330	DD	5
221	*Daudebardiella asiana*	35.335003	36.986933	DD	22
222	*Devetakia krushunica*	25.035889	43.242556	DD	241
223	*Devetakia mandrica*	24.969111	43.242278	DD	183
224	*Devetakia pandurskii*	24.886833	43.234250	DD	125
225	*Devetakiola devetakium*	24.936667	43.237222	DD	261
226	*Dianella schlickumi*	21.183333	38.750000	CR	14
227	*Dianella thiesseana*	21.620170	38.563010	CR	11
228	*Diegus gasulli*	1.529130	38.978870	DD	5
229	*Dolapia ornata*	20.735553	41.063761	EN	689
230	*Ecrobia cissana*	14.925730	44.539630	DD	0
231	*Ecrobia grimmi*	47.518000	43.301000	DD	0
232	*Ecrobia maritima*	28.542680	40.554350	DD	5
233	*Ecrobia spalatina*	16.701880	43.457030	DD	11
234	*Ecrobia truncata*	-76.238915	38.654854	DD	0
235	*Ecrobia ventrosa*	11.031380	36.817880	DD	14
236	*Euxinipyrgula azovica*	38.941348	47.203179	DD	35
237	*Euxinipyrgula borysthenica*	32.449583	46.530542	DD	0
238	*Euxinipyrgula grigorievi*	32.449583	46.530542	DD	0
239	*Euxinipyrgula limanica*	31.927335	46.939829	DD	0
240	*Euxinipyrgula lincta*	28.995439	45.431817	DD	0
241	*Euxinipyrgula milachevitchi*	39.290644	47.147275	DD	0
242	*Euxinipyrgula ostroumovi*	28.995439	45.431817	DD	0
243	*Euxinipyrgula ukrainica*	32.449583	46.530542	DD	0
244	*Falniowskia neglectissima*	19.822446	50.204096	DD	367
245	*Falsibelgrandiella bunarica*	36.378947	38.717497	DD	1498
246	*Falsipyrgula bakhtarana*	47.077769	34.327692	DD	1342
247	*Falsipyrgula barroisi*	35.588036	32.824728	EN	0
248	*Falsipyrgula beysehirana*	31.521483	37.772079	CR	1122

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
249	*Falsipyrgula pfeiferi*	30.860170	37.881270	EN	916
250	*Falsipyrgula schuetti*	31.521211	37.772399	DD	1122
251	*Fissuria boui*	5.126880	43.922440	NT	87
252	*Fissuria planospira*	10.681304	43.466555	NT	118
253	*Fissuria raehlei*	20.771282	38.153965	NT	12
254	*Floridobia helicogyra*	-82.597903	28.897603	VU	1
255	*Floridobia mica*	-82.775039	29.950875	VU	14
256	*Floridobia monroensis*	-81.271053	28.837950	VU	0
257	*Floridobia parva*	-81.341063	28.947718	VU	17
258	*Floridobia ponderosa*	-81.381637	28.683738	VU	22
259	*Floridobia vanhyningi*	-81.527875	28.846347	VU	29
260	*Floridobia wekiwae*	-81.425232	28.698422	VU	21
261	*Floridobia floridana*	-81.712881	29.574722	DD	17
262	*Floridobia petrifons*	-81.501460	28.756380	DD	31
263	*Floridobia winkleyi*	-70.375053	43.564833	DD	1
264	*Ginaia munda*	20.794240	41.087140	VU	689
265	*Giustia bodoni*	-8.159218	31.331937	EN	694
266	*Giustia costata*	-5.499825	34.183460	CR	125
267	*Giustia gofasi*	-7.981085	31.629472	EN	465
268	*Giustia janai*	-7.981085	31.629472	EN	465
269	*Giustia mellalensis*	-5.499825	34.183460	CR	125
270	*Giustia midarensis*	-5.008562	34.021626	EN	427
271	*Giustia saidai*	-5.499825	34.183460	CR	125
272	*Globuliana gaillardotii*	36.226230	32.629747	LC	592
273	*Gloeria bulgarica*	23.550028	41.416167	DD	670
274	*Gocea ohridana*	20.780020	40.936250	CR	707
275	*Graecoanatolica anatolica*	30.758807	36.914964	DD	54
276	*Graecoanatolica brevis*	29.972889	37.886329	CR	837
277	*Graecoanatolica conica*	29.673388	37.827899	CR	882
278	*Graecoanatolica dinarica*	30.289440	38.505177	EN	1219
279	*Graecoanatolica kocapinarica*	30.846255	37.699572	VU	962
280	*Graecoanatolica lacustristurca*	30.872870	38.023770	EN	916
281	*Graecoanatolica nageli*	28.299833	37.049083	DD	11
282	*Graecoanatolica pamphylica*	27.677350	41.237817	EN	103
283	*Graecoanatolica tenuis*	29.854815	37.819197	EN	837

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
284	*Graecoanatolica vegorriticola*	21.775670	40.783080	CR	512
285	*Graecoanatolica yildirimi*	34.929919	42.011836	DD	5
286	*Graecoarganiella parnassiana*	22.503889	38.592778	DD	965
287	*Graecorientalia vrissiana*	23.725200	37.987831	CR	76
288	*Graziana adlitzensis*	15.837088	47.653463	CR	698
289	*Graziana alpestris*	8.261432	44.214837	LC	338
290	*Graziana cezairensis*	6.925157	43.660101	EN	309
291	*Graziana klagenfurtensis*	14.289080	46.641729	EN	451
292	*Graziana lacheineri*	14.933730	46.412620	LC	792
293	*Graziana papukensis*	17.661558	45.447518	NT	240
294	*Graziana provincialis*	7.107080	43.723504	EN	328
295	*Graziana pupula*	15.783161	45.870118	LC	139
296	*Graziana quadrifoglio*	8.949826	45.914049	VU	1055
297	*Graziana slavonica*	17.602916	45.522230	VU	926
298	*Graziana trinitatis*	7.309988	43.741646	EN	73
299	*Graziana vrbasensis*	16.449644	44.758642	DD	398
300	*Grossuana angeltsekovi*	24.855022	41.951040	LC	458
301	*Grossuana aytosensis*	27.269139	42.714528	DD	139
302	*Grossuana codreanui*	28.546111	43.993611	DD	9
303	*Grossuana delphica*	22.505279	38.483060	DD	526
304	*Grossuana derventica*	26.538306	42.048000	DD	158
305	*Grossuana falniowskii*	25.640000	42.440250	DD	219
306	*Grossuana hohenackeri*	22.638056	39.973889	DD	7
307	*Grossuana marginata*	23.815833	38.587778	DD	430
308	*Grossuana radostinae*	27.100000	43.266667	DD	133
309	*Grossuana serbica*	20.370833	43.115833	DD	726
310	*Grossuana slavyanica*	23.588617	41.431333	DD	816
311	*Grossuana thracica*	25.017574	42.245148	CR	169
312	*Grossuana vurliana*	20.841667	39.432222	DD	272
313	*Guadiella andalucesis*	-3.034204	38.073765	VU	438
314	*Guadiella arconadae*	-3.709655	42.510473	VU	974
315	*Guadiella ramosae*	-2.564827	38.397413	VU	833
316	*Hadziella anti*	13.395134	46.097743	LC	122
317	*Hadziella deminuta*	14.892540	46.088874	VU	242
318	*Hadziella ephippiostoma*	15.052118	46.319242	LC	441
319	*Hadziella krkae*	14.961393	45.812878	VU	192

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
320	*Hadziella leonorae*	2.818330	39.802469	DD	598
321	*Hadziella rudnicae*	15.535850	46.148012	CR	548
322	*Hadziella sketi*	15.680792	44.196756	VU	92
323	*Hadziella thermalis*	15.519979	45.867230	DD	164
324	*Hauffenia danubialis*	16.535600	48.168273	VU	150
325	*Hauffenia edlaueri*	17.448628	43.026652	DD	0
326	*Hauffenia edlingeri*	22.221715	37.771540	CR	514
327	*Hauffenia erythropomatia*	14.384268	46.134462	DD	542
328	*Hauffenia kerschneri*	15.401494	47.995757	EN	437
329	*Hauffenia kissdalmae*	18.991210	47.919466	DD	483
330	*Hauffenia lucidula*	28.335176	43.433056	DD	129
331	*Hauffenia media*	15.390356	45.636763	VU	154
332	*Hauffenia michleri*	14.292714	45.955198	DD	314
333	*Hauffenia nesemanni*	16.439314	48.005565	EN	184
334	*Hauffenia plana*	15.267466	46.239682	VU	239
335	*Hauffenia subcarinata*	13.603554	46.056031	NT	149
336	*Hauffenia subpiscinalis*	13.543930	46.084350	LC	223
337	*Hauffenia tellinii*	13.854960	45.602720	LC	57
338	*Hauffenia tovunica*	15.325231	45.248910	CR	233
339	*Hauffenia wagneri*	15.299937	46.014301	VU	187
340	*Hauffenia wienerwaldensis*	16.097929	48.096644	EN	394
341	*Heideella andreae*	-9.833630	28.989182	DD	342
342	*Heideella dolichia*	1.016156	33.682200	DD	1316
343	*Heideella knidirii*	-4.001120	34.210220	EN	541
344	*Heideella makhfamanensis*	-8.159629	31.271535	DD	800
345	*Heraultiella exilis*	0.692605	42.911119	VU	505
346	*Horatia klecakiana*	16.822610	43.441120	LC	151
347	*Horatia lucidulus*	27.821628	43.539465	CR	260
348	*Horatia macedonica*	21.427478	41.993353	VU	251
349	*Horatia novoselensis*	20.678819	41.178381	VU	696
350	*Horatia parvula*	36.550769	40.328739	DD	604
351	*Hydrobia accrensis*	-0.219433	5.533661	NT	5
352	*Hydrobia acuta*	3.078920	39.902770	LC	0
353	*Hydrobia anatolica*	30.776058	36.874300	CR	52
354	*Hydrobia balfouri*	53.927189	12.528416	DD	773
355	*Hydrobia brondeli*	3.048297	36.311227	NT	875

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
356	*Hydrobia cattaroensis*	19.346341	42.514208	DD	76
357	*Hydrobia declinata*	19.314474	42.501307	DD	70
358	*Hydrobia djerbaensis*	10.870771	33.802022	VU	24
359	*Hydrobia elachista*	6.599313	36.371868	DD	373
360	*Hydrobia faminensis*	-70.932390	-53.609633	DD	15
361	*Hydrobia gabonensis*	12.726218	-0.808407	DD	225
362	*Hydrobia glaucovirens*	54.433943	18.389224	DD	276
363	*Hydrobia guyenoti*	-4.567148	5.309767	EN	10
364	*Hydrobia knysnaensis*	25.200000	-33.960000	DD	112
365	*Hydrobia lactea*	49.639206	25.399026	NT	148
366	*Hydrobia lineata*	1.477237	6.232824	DD	18
367	*Hydrobia longiscata*	35.576916	32.763362	LC	0
368	*Hydrobia luvilana*	13.045146	-4.526882	VU	345
369	*Hydrobia maroccana*	-5.376437	34.270847	EN	53
370	*Hydrobia montenegrina*	17.890218	43.691734	DD	263
371	*Hydrobia musaensis*	31.208116	30.770660	LC	8
372	*Hydrobia newtoni*	49.095172	39.187851	DD	0
373	*Hydrobia plena*	13.491143	-5.818585	EN	125
374	*Hydrobia pontieuxini*	28.574674	43.805126	DD	8
375	*Hydrobia recta*	-6.585744	34.265724	LC	6
376	*Hydrobia rheophila*	13.444342	-5.826874	CR	12
377	*Hydrobia schoutedeni*	13.491143	-5.818585	EN	125
378	*Hydrobia soosi*	29.532992	40.449765	DD	83
379	*Hydrobia glyca*	-3.089000	48.872000	DD	1
380	*Iberhoratia aurorae*	-5.879406	40.251051	DD	891
381	*Iberhoratia gatoa*	-5.238309	36.727229	VU	501
382	*Iberhoratia morenoi*	-6.267470	36.492410	VU	7
383	*Insignia macrostoma*	24.190314	42.947317	VU	449
384	*Intermaria kermanshahensis*	47.689467	34.476521	DD	1347
385	*Intermaria zagroensis*	47.916667	34.500000	DD	1530
386	*Isimerope semele*	22.522310	37.770110	DD	1227
387	*Islamia anatolica*	27.683039	41.239439	CR	97
388	*Islamia archeducis*	3.004972	39.623867	DD	112
389	*Islamia azarum*	-5.959546	43.314549	VU	357
390	*Islamia bendidis*	26.304986	37.622845	CR	21
391	*Islamia bomangiana*	25.596690	40.499225	VU	122

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
392	*Islamia bosniaca*	17.930792	44.137937	VU	373
393	*Islamia bourguignati*	-0.210500	44.649665	DD	110
394	*Islamia burnarbasa*	27.683485	41.239665	CR	90
395	*Islamia cianensis*	15.234614	37.042009	VU	5
396	*Islamia consolationis*	6.603500	47.173472	DD	724
397	*Islamia dmitroviciana*	17.221983	44.692500	DD	295
398	*Islamia emanuelei*	0.548912	47.346485	DD	63
399	*Islamia epirana*	20.883317	39.666660	VU	464
400	*Islamia gaiteri*	10.394602	42.822075	NT	305
401	*Islamia germaini*	4.631941	44.956717	DD	651
402	*Islamia globulus*	0.620010	41.617590	NT	184
403	*Islamia henrici*	-5.306263	37.816848	EN	123
404	*Islamia lagari*	1.736508	41.252039	VU	98
405	*Islamia laiae*	2.712118	39.757131	DD	89
406	*Islamia lanzai*	11.143706	43.920709	DD	513
407	*Islamia latina*	15.442888	44.186870	DD	133
408	*Islamia minuta*	6.038639	46.775187	LC	783
409	*Islamia montenegrina*	19.362800	42.325400	DD	14
410	*Islamia moquiniana*	3.500493	44.521865	LC	721
411	*Islamia pallida*	-3.471102	40.867002	EN	767
412	*Islamia pezzoliana*	12.913756	43.378845	DD	464
413	*Islamia piristoma*	9.924068	44.069435	LC	143
414	*Islamia pseudorientalica*	30.714730	37.165020	CR	329
415	*Islamia pusilla*	1.346389	42.663056	LC	2213
416	*Islamia ruffoi*	11.640355	45.302979	DD	39
417	*Islamia senensis*	11.108423	43.306405	VU	231
418	*Islamia spirata*	6.343072	47.323129	VU	335
419	*Islamia trichoniana*	21.611030	38.586494	CR	43
420	*Islamia ucetiansis*	4.419190	44.010991	DD	136
421	*Islamia valvataeformis*	18.267110	43.819107	DD	511
422	*Islamia zermanica*	16.067370	44.150769	CR	266
423	*Islamia mienisi*	35.569329	33.132940	DD	71
424	*Islamia burduricus*	30.239809	37.414792	DD	1312
425	*Islamia sulfurea*	12.499434	42.497627	DD	133
426	*Istriana falkneri*	4.875022	44.757893	DD	123
427	*Istriana mirnae*	13.722389	45.379273	NT	258

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428	*Iverakia hausdorfi*	19.221647	42.504867	DD	40
429	*Josefus aitanica*	-0.419880	38.607980	LC	785
430	*Karucia sublacustrina*	19.106250	42.358683	DD	33
431	*Kaskakia khorrasanensis*	59.166667	35.416667	DD	1727
432	*Kerkia brezicensis*	15.609145	45.882237	VU	226
433	*Kerkia jadertina*	16.521850	43.542920	EN	58
434	*Kerkia kareli*	15.112231	44.342039	DD	7
435	*Kerkia kusceri*	14.780176	45.882777	CR	272
436	*Kirelia carinata*	31.512455	37.740256	CR	1122
437	*Kirelia murtici*	31.759561	36.891650	CR	554
438	*Kolevia bulgarica*	23.347481	43.265086	DD	267
439	*Lanzaia bosnica*	16.639331	44.709678	DD	248
440	*Lanzaia edlaueri*	17.624128	43.072238	DD	0
441	*Lanzaia elephantotus*	16.732451	43.448198	DD	225
442	*Lanzaia kotlusae*	16.392876	43.911537	VU	531
443	*Lanzaia pescei*	19.362800	42.325400	DD	14
444	*Lanzaia rudnicae*	15.316808	45.249131	NT	294
445	*Lanzaia skradinensis*	15.824470	43.788457	CR	23
446	*Lanzaia vjetrenicae*	17.984584	42.844878	VU	367
447	*Lanzaiopsis savinica*	14.745794	46.355778	VU	516
448	*Lyhnidia gjorgjevici*	20.745220	40.910160	EN	713
449	*Lyhnidia hadzii*	20.719630	41.159785	CR	689
450	*Lyhnidia karamani*	20.775602	40.929166	CR	689
451	*Lyhnidia stankovici*	20.777830	40.959240	CR	704
452	*Lyhnidia sublitoralis*	20.690689	40.986040	DD	689
453	*Macedopyrgula pavlovici*	20.719230	41.161760	VU	689
454	*Macedopyrgula wagneri*	20.673810	41.150780	VU	689
455	*Malaprespia albanica*	20.940790	40.862520	CR	858
456	*Maroccopsis agadirensis*	-9.637132	30.030135	EN	39
457	*Marstonia agarhecta*	-83.434120	32.142020	EN	72
458	*Marstonia castor*	-83.804870	31.887570	CR	91
459	*Marstonia halcyon*	-81.215059	31.914866	LC	0
460	*Marstonia ogmoraphe*	-85.602459	35.151552	LC	213
461	*Marstonia ozarkensis*	-92.285884	36.207330	CR	125
462	*Marstonia pachyta*	-85.538050	35.058810	DD	194
463	*Marstonia comalensis*	-98.131100	29.705850	DD	192

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
464	*Marstonia hershleri*	-85.850040	33.381500	DD	350
465	*Marstonia lustrica*	-73.319503	42.333650	DD	281
466	*Martensamnicola brevicula*	67.015904	39.669525	DD	705
467	*Martensamnicola hissarica*	68.399290	42.752882	DD	198
468	*Martensamnicola kazakhstanica*	70.342805	42.530884	DD	763
469	*Mercuria anatina*	0.565303	45.757310	LC	314
470	*Mercuria balearica*	4.256740	39.897491	DD	1
471	*Mercuria baudoniana*	-1.051943	44.657701	DD	0
472	*Mercuria bayonnensis*	-1.662854	43.388049	VU	10
473	*Mercuria bourguignati*	2.748119	35.872998	DD	609
474	*Mercuria edmundi*	-8.775897	37.143569	DD	64
475	*Mercuria emiliana*	3.158569	44.529008	DD	1109
476	*Mercuria gauthieri*	-1.856206	35.096228	DD	14
477	*Mercuria globulina*	10.108715	36.369112	DD	761
478	*Mercuria maceana*	2.131138	41.402100	DD	127
479	*Mercuria melitensis*	14.375416	35.937496	DD	48
480	*Mercuria meridionalis*	7.261953	43.710173	EN	28
481	*Mercuria perforata*	-0.633738	35.697654	DD	115
482	*Mercuria punica*	8.567226	33.456301	CR	21
483	*Mercuria pycnocheilia*	6.072152	33.097887	DD	71
484	*Mercuria saharica*	6.087606	33.285572	DD	57
485	*Mercuria sarahae*	-1.540222	47.212329	CR	7
486	*Mercuria similis*	12.675464	45.828458	LC	6
487	*Mercuria tachoensis*	-9.200345	38.714420	DD	142
488	*Mercuria tchernovi*	35.473189	31.559029	EN	0
489	*Mercuria vindilica*	-3.181025	47.336420	EN	50
490	*Mercuria zopissa*	9.315944	39.397369	NT	683
491	*Mercuria bakeri*	-5.460306	35.815194	DD	431
492	*Mercuria kobelti*	14.337808	36.011841	DD	33
493	*Mercuria targouasensis*	-10.339747	29.092192	DD	75
494	*Mercuria tingitana*	-5.727389	35.816806	DD	85
495	*Micropyrgula stankovici*	20.782360	41.101560	VU	689
496	*Microstygia deltchevi*	22.677139	42.848139	DD	667
497	*Milesiana schuelei*	-2.511250	37.729790	NT	872
498	*Montenegrospeum bogici*	19.219083	42.527550	DD	107

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
499	*Motsametia borutzkii*	42.703835	42.271670	DD	154
500	*Myrtoessa hyas*	22.895367	37.110004	DD	91
501	*Narentiana albida*	17.425245	43.085116	NT	1
502	*Narentiana vjetrenicae*	17.983712	42.845808	EN	255
503	*Navarriella elliptica*	-1.621625	43.342586	DD	32
504	*Neofossarulus stankovici*	20.740710	40.920400	VU	689
505	*Nicolaia schniebsae*	44.378417	40.322000	DD	1165
506	*Notogillia wetherbyi*	-82.573231	28.517839	DD	12
507	*Ochridopyrgula macedonica*	20.700356	41.133381	NT	689
508	*Ohridohauffenia depressa*	20.655330	40.915790	EN	689
509	*Ohridohauffenia minuta*	20.819831	41.109179	CR	730
510	*Ohridohauffenia rotonda*	20.646530	41.127590	EN	689
511	*Ohridohauffenia sanctinaumi*	20.747480	40.910610	EN	698
512	*Ohridohauffenia sublitoralis*	20.687600	41.121857	DD	689
513	*Ohridohoratia carinata*	20.785090	40.965290	EN	690
514	*Ohridohoratia pygmaea*	20.799910	41.073040	NT	691
515	*Ohrigocea karevi*	20.687600	41.121857	EN	689
516	*Ohrigocea miladinovorum*	20.712087	41.092583	EN	689
517	*Ohrigocea samuili*	20.633610	41.092390	EN	692
518	*Ohrigocea stankovici*	20.745000	40.911350	EN	708
519	*Palacanthilhiopsis carolinae*	4.688493	44.035823	DD	129
520	*Palacanthilhiopsis kuiperi*	5.202194	43.755092	DD	161
521	*Palacanthilhiopsis margritae*	4.290092	44.436268	VU	165
522	*Palacanthilhiopsis vervierii*	4.443937	44.364979	VU	125
523	*Pauluccinella minima*	12.858589	42.404739	LC	396
524	*Peringia mabilli*	8.733547	41.918802	DD	38
525	*Peringia ulvae*	-3.147000	53.343000	DD	24
526	*Persipyrgula saboori*	49.408333	33.935556	DD	1900
527	*Pezzolia radapalladis*	9.214502	44.376600	EN	126
528	*Plagigeyeria deformata*	5.338428	44.764320	EN	392
529	*Plagigeyeria edlaueri*	17.640146	43.045385	DD	1
530	*Plagigeyeria gladilini*	20.291842	42.658032	VU	513
531	*Plagigeyeria horatieformis*	41.311474	43.080672	DD	535
532	*Plagigeyeria klemmi*	17.371747	43.206862	DD	219
533	*Plagigeyeria minuta*	19.433106	43.934270	DD	1026
534	*Plagigeyeria montenigrina*	19.046649	42.347824	CR	17

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
535	*Plagigeyeria mostarensis*	17.811769	43.338167	DD	61
536	*Plagigeyeria nitida*	18.136683	42.675765	DD	31
537	*Plagigeyeria pageti*	18.698670	42.512740	DD	28
538	*Plagigeyeria piroti*	22.585892	43.152425	DD	371
539	*Plagigeyeria plagiostoma*	18.267110	43.819107	DD	511
540	*Plagigeyeria robusta*	18.383655	42.865822	DD	464
541	*Plagigeyeria tribunicae*	18.426576	42.872281	CR	459
542	*Plagigeyeria valvataeformis*	39.362972	44.007658	DD	148
543	*Plagigeyeria zetaprotogona*	19.067931	42.582356	EN	46
544	*Plesiella guipuzcoa*	-2.168215	43.099437	DD	333
545	*Plesiella navarrensis*	-1.894626	42.975296	DD	759
546	*Polinskiola polinskii*	20.687600	41.121857	VU	689
547	*Polinskiola sturanyi*	20.687600	41.121857	NT	689
548	*Pontobelgrandiella angelovi*	25.332898	42.712315	VU	567
549	*Pontobelgrandiella dobrostanica*	24.932528	41.850111	VU	919
550	*Pontobelgrandiella nitida*	24.195331	42.948708	VU	352
551	*Pontobelgrandiella tanevi*	25.432889	43.200583	DD	84
552	*Pontobelgrandiella zagoraensis*	25.562398	42.463458	VU	633
553	*Pontohoratia birsteini*	41.269144	43.031194	DD	514
554	*Pontohoratia smyri*	40.809003	43.091156	DD	74
555	*Prespolitorea malaprespensis*	20.915997	40.783940	CR	841
556	*Prespolitorea valvataeformis*	20.989890	40.866550	CR	870
557	*Prespopyrgula prespaensis*	21.044290	40.989160	EN	842
558	*Probythinella emarginata*	-96.824709	38.227711	DD	389
559	*Pseudamnicola bacescui*	28.610091	44.052849	VU	0
560	*Pseudamnicola beckmanni*	2.649000	39.746000	DD	173
561	*Pseudamnicola bilgini*	40.633118	37.291080	LC	667
562	*Pseudamnicola boucheti*	6.173402	35.549222	DD	1040
563	*Pseudamnicola brachia*	24.803688	35.219599	DD	2121
564	*Pseudamnicola brusiniana*	51.399272	40.146696	LC	0
565	*Pseudamnicola chia*	26.052101	38.389501	VU	757
566	*Pseudamnicola confinis*	17.798467	44.098710	DD	681
567	*Pseudamnicola constantinae*	6.615518	36.350659	DD	542
568	*Pseudamnicola depressispira*	39.015000	49.333611	DD	110
569	*Pseudamnicola dobrogica*	29.561453	45.087585	DD	1

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
570	*Pseudamnicola dupotetiana*	5.067608	36.731204	DD	4
571	*Pseudamnicola exilis*	22.925874	36.687965	DD	92
572	*Pseudamnicola fineti*	7.856433	36.425917	DD	438
573	*Pseudamnicola geldiayana*	33.970506	39.449791	EN	1132
574	*Pseudamnicola georgievi*	50.016667	34.566667	DD	2276
575	*Pseudamnicola gerhardfalkneri*	2.324424	36.231312	DD	310
576	*Pseudamnicola ghamizii*	7.784987	36.965040	DD	68
577	*Pseudamnicola goksunensis*	36.572033	38.032294	DD	1322
578	*Pseudamnicola granjaensis*	2.559000	39.671000	DD	307
579	*Pseudamnicola ianthe*	28.060169	36.340558	DD	142
580	*Pseudamnicola ilione*	27.906708	36.273606	DD	349
581	*Pseudamnicola intranodosa*	38.969369	37.346777	VU	642
582	*Pseudamnicola kotschyi*	52.656398	27.654672	DD	478
583	*Pseudamnicola krumensis*	20.416700	42.192000	DD	475
584	*Pseudamnicola leontina*	28.954326	44.884191	DD	0
585	*Pseudamnicola leprevieri*	-8.478385	31.006317	CR	1711
586	*Pseudamnicola letourneuxiana*	7.748370	36.899451	DD	2
587	*Pseudamnicola linae*	7.795133	36.430100	DD	250
588	*Pseudamnicola lucensis*	10.536991	42.964873	EN	18
589	*Pseudamnicola luteola*	1.973331	35.848900	DD	1435
590	*Pseudamnicola macrostoma*	23.858905	38.043466	DD	343
591	*Pseudamnicola magdalenae*	22.949444	36.347222	DD	134
592	*Pseudamnicola malickyi*	32.532035	35.095992	VU	176
593	*Pseudamnicola marashi*	36.756283	37.450586	DD	766
594	*Pseudamnicola meloussensis*	3.963430	39.938810	DD	35
595	*Pseudamnicola meluzzii*	8.373366	34.236439	VU	107
596	*Pseudamnicola merali*	36.471544	38.094281	DD	1397
597	*Pseudamnicola moussonii*	11.158840	42.414280	LC	301
598	*Pseudamnicola negropontina*	24.327500	38.039300	DD	47
599	*Pseudamnicola numidica*	7.748370	36.899451	DD	2
600	*Pseudamnicola orsinii*	13.582801	42.856274	DD	130
601	*Pseudamnicola pallaryi*	-8.478385	31.006317	CR	1711
602	*Pseudamnicola penchinati*	27.324953	44.184246	DD	10
603	*Pseudamnicola pieperi*	27.171160	35.549593	VU	270
604	*Pseudamnicola pisolinus*	5.531475	43.567154	VU	465

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
605	*Pseudamnicola raddei*	52.630611	36.077470	DD	2744
606	*Pseudamnicola razelmiana*	28.954326	44.884191	DD	0
607	*Pseudamnicola rouagi*	8.073367	36.372883	DD	1152
608	*Pseudamnicola sciaccaensis*	13.089312	37.511311	DD	79
609	*Pseudamnicola troglobia*	18.136938	42.675557	LC	31
610	*Pseudamnicola vinarskii*	34.034642	36.319041	DD	2
611	*Pseudamnicola virescens*	17.021785	43.294525	DD	21
612	*Pseudamnicola algeriensis*	-1.470878	34.692575	DD	657
613	*Pseudamnicola artanensis*	3.350930	39.694620	DD	136
614	*Pseudamnicola calamensis*	7.794800	36.431770	DD	226
615	*Pseudamnicola chabii*	6.173402	35.549222	DD	1040
616	*Pseudamnicola conovula*	14.991680	44.467220	DD	238
617	*Pseudamnicola pyrenaicus*	-0.426874	43.101892	DD	409
618	*Pseudamnicola subproducta*	2.765150	42.118180	DD	177
619	*Pseudavenionia pedemontana*	7.807720	44.285180	LC	897
620	*Pseudohoratia brusinae*	20.782360	41.101560	VU	689
621	*Pseudohoratia lacustris*	20.718360	41.165690	VU	689
622	*Pseudohoratia ochridana*	20.649520	40.968300	VU	689
623	*Pseudoislamia balcanica*	21.531941	38.602454	CR	14
624	*Pseudopaludinella cygnea*	33.658823	45.908170	DD	0
625	*Pseudorientalia natolica*	29.058771	40.178876	DD	304
626	*Pseudorientalia tzekovi*	24.142770	41.150113	DD	109
627	*Pyrgohydrobia grochmalickii*	20.773850	40.948330	VU	692
628	*Pyrgohydrobia jablanicensis*	20.634230	41.183791	CR	699
629	*Pyrgohydrobia prespaensis*	21.037142	40.884306	EN	841
630	*Pyrgohydrobia sanctinaumi*	20.745123	40.913931	VU	702
631	*Pyrgorientalia zilchi*	30.589198	37.043308	DD	302
632	*Pyrgula abichi*	48.950161	39.105679	DD	0
633	*Pyrgula aenigma*	49.962958	40.592975	DD	0
634	*Pyrgula annulata*	17.410560	43.079730	LC	19
635	*Pyrgula bakuana*	51.802421	36.659526	DD	0
636	*Pyrgula basalis*	51.802421	36.659526	DD	0
637	*Pyrgula behningi*	49.395098	39.319081	DD	0
638	*Pyrgula cincta*	48.950161	39.105679	DD	0
639	*Pyrgula columna*	51.802421	36.659526	DD	0
640	*Pyrgula concinna*	51.802421	36.659526	DD	0

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
641	*Pyrgula curta*	49.943093	40.455681	DD	32
642	*Pyrgula derzhavini*	51.802421	36.659526	DD	0
643	*Pyrgula dimidiata*	49.943093	40.455681	DD	32
644	*Pyrgula dubia*	51.802421	36.659526	DD	0
645	*Pyrgula ebersini*	48.950161	39.105679	DD	0
646	*Pyrgula eulimellula*	51.380028	40.487463	DD	0
647	*Pyrgula fedorovi*	51.802421	36.659526	DD	0
648	*Pyrgula grimmi*	48.950161	39.105679	DD	0
649	*Pyrgula isseli*	48.950161	39.105679	DD	0
650	*Pyrgula kolesnikoviana*	48.950161	39.105679	DD	0
651	*Pyrgula kowalewskii*	48.950161	39.105679	DD	0
652	*Pyrgula lencoranica*	48.950161	39.105679	DD	0
653	*Pyrgula lirata*	48.950161	39.105679	DD	0
654	*Pyrgula marginata*	51.802421	36.659526	DD	0
655	*Pyrgula nana*	48.950161	39.105679	DD	0
656	*Pyrgula nossovi*	51.802421	36.659526	DD	0
657	*Pyrgula pallasii*	48.950161	39.105679	DD	0
658	*Pyrgula pseudobacuana*	48.950161	39.105679	DD	0
659	*Pyrgula pseudodimidiata*	48.950161	39.105679	DD	0
660	*Pyrgula pseudospica*	48.950161	39.105679	DD	0
661	*Pyrgula pulla*	48.950161	39.105679	DD	0
662	*Pyrgula rudis*	48.950161	39.105679	DD	0
663	*Pyrgula schorygini*	50.958333	40.125000	DD	0
664	*Pyrgula similis*	48.950161	39.105679	DD	0
665	*Pyrgula simplex*	48.950161	39.105679	DD	0
666	*Pyrgula sowinskyi*	48.950161	39.105679	DD	0
667	*Pyrgula turkmenica*	48.950161	39.105679	DD	0
668	*Pyrgula ulskii*	48.950161	39.105679	DD	0
669	*Pyrgula uralensis*	48.950161	39.105679	DD	0
670	*Pyrgulopsis acarinatus*	-102.063680	26.986570	DD	740
671	*Pyrgulopsis aloba*	-115.722188	38.931474	EN	1672
672	*Pyrgulopsis amargosae*	-116.423630	35.681630	VU	60
673	*Pyrgulopsis castaicensis*	-118.615104	34.429270	DD	303
674	*Pyrgulopsis wongi*	-118.631320	37.409520	LC	1759
675	*Pyrgulopsis aardahli*	-118.494013	37.846649	DD	1653
676	*Pyrgulopsis anatina*	-115.708060	38.897830	DD	1652

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
677	*Pyrgulopsis anguina*	-114.131930	38.698840	DD	1705
678	*Pyrgulopsis archimedis*	-121.829800	42.347810	DD	1259
679	*Pyrgulopsis arizonae*	-110.154696	33.176939	DD	781
680	*Pyrgulopsis augustae*	-117.506496	39.981893	DD	1691
681	*Pyrgulopsis aurata*	-119.192680	40.763230	DD	1192
682	*Pyrgulopsis avernalis*	-114.473329	36.579292	VU	411
683	*Pyrgulopsis bacchus*	-113.870679	34.857981	VU	1886
684	*Pyrgulopsis basiglans*	-116.907266	40.264439	DD	1843
685	*Pyrgulopsis bedfordensis*	-111.564100	46.353700	DD	1214
686	*Pyrgulopsis bernardina*	-109.263566	31.317685	EN	1126
687	*Pyrgulopsis bifurcata*	-116.909220	40.127740	DD	1557
688	*Pyrgulopsis blainica*	-111.749811	45.208588	DD	1582
689	*Pyrgulopsis breviloba*	-115.023280	38.422620	DD	1613
690	*Pyrgulopsis bruesi*	-119.335920	40.862600	DD	1225
691	*Pyrgulopsis bruneauensis*	-115.718130	42.776280	CR	798
692	*Pyrgulopsis bryantwalkery*	-116.103970	40.713820	DD	1496
693	*Pyrgulopsis californiensis*	-116.513008	32.724040	DD	1066
694	*Pyrgulopsis carinifera*	-114.473329	36.579292	DD	411
695	*Pyrgulopsis cedrocensis*	-115.217154	28.203436	DD	196
696	*Pyrgulopsis chamberlini*	-111.990200	38.763300	DD	1611
697	*Pyrgulopsis chihuahua*	-106.580531	30.405821	DD	1225
698	*Pyrgulopsis chupaderae*	-107.043247	33.783452	DD	1588
699	*Pyrgulopsis cinerana*	-120.818570	41.142510	DD	1523
700	*Pyrgulopsis coloradensis*	-114.432756	36.389144	DD	471
701	*Pyrgulopsis conica*	-113.641050	35.066120	VU	956
702	*Pyrgulopsis cruciglans*	-114.480570	40.067710	EN	1994
703	*Pyrgulopsis crystalis*	-116.322600	36.420770	DD	675
704	*Pyrgulopsis cybele*	-115.744976	40.657325	DD	1597
705	*Pyrgulopsis davisi*	-103.836520	30.633130	DD	1485
706	*Pyrgulopsis deaconi*	-115.418380	36.147150	DD	1101
707	*Pyrgulopsis deserta*	-113.477620	37.322480	DD	2947
708	*Pyrgulopsis diablensis*	-121.380630	37.424120	VU	347
709	*Pyrgulopsis dixensis*	-119.110180	40.770730	DD	1198
710	*Pyrgulopsis eremica*	-120.462630	40.471680	DD	1391
711	*Pyrgulopsis fairbanksensis*	-116.341570	36.490370	DD	692
712	*Pyrgulopsis falciglans*	-120.629370	41.456520	DD	1365

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
713	*Pyrgulopsis fausta*	-115.358070	36.439130	DD	895
714	*Pyrgulopsis fresti*	-117.182918	42.529040	DD	1252
715	*Pyrgulopsis fusca*	-111.990977	38.201351	DD	1938
716	*Pyrgulopsis gibba*	-120.022270	41.834570	DD	1600
717	*Pyrgulopsis gilae*	-118.428700	35.307470	VU	840
718	*Pyrgulopsis giuliani*	-118.067870	35.589230	VU	1341
719	*Pyrgulopsis glandulosa*	-111.830150	34.320030	VU	1624
720	*Pyrgulopsis gracilis*	-116.041380	38.580720	DD	1790
721	*Pyrgulopsis greggi*	-118.924710	34.916300	VU	647
722	*Pyrgulopsis hamlinensis*	-114.053448	38.225624	DD	2247
723	*Pyrgulopsis hendersoni*	-119.054100	43.586270	DD	1266
724	*Pyrgulopsis hovinghi*	-114.739328	41.336897	DD	1798
725	*Pyrgulopsis hualapaiensis*	-113.431400	35.578600	DD	1299
726	*Pyrgulopsis hubbsi*	-115.216130	37.598570	DD	1188
727	*Pyrgulopsis humboldtensis*	-115.525448	41.453093	DD	1877
728	*Pyrgulopsis idahoensis*	-116.933770	43.617670	DD	681
729	*Pyrgulopsis ignota*	-101.801835	30.467286	DD	612
730	*Pyrgulopsis imperialis*	-118.122580	41.697220	DD	1334
731	*Pyrgulopsis inopinata*	-111.990200	38.763300	DD	1611
732	*Pyrgulopsis intermedia*	-117.144580	43.727620	DD	802
733	*Pyrgulopsis isolata*	-116.295870	36.359400	DD	672
734	*Pyrgulopsis kolobensis*	-113.279950	37.266650	DD	1082
735	*Pyrgulopsis landyei*	-114.898000	39.497810	DD	1832
736	*Pyrgulopsis lasseni*	-120.828570	41.014890	DD	1594
737	*Pyrgulopsis lata*	-115.011680	38.439670	DD	1625
738	*Pyrgulopsis lentiglans*	-114.168350	41.541870	DD	1601
739	*Pyrgulopsis leporina*	-115.283770	41.222020	DD	1677
740	*Pyrgulopsis licina*	-116.378560	36.407190	DD	659
741	*Pyrgulopsis limaria*	-119.183830	41.328570	DD	1319
742	*Pyrgulopsis lockensis*	-115.775040	38.554930	DD	1469
743	*Pyrgulopsis longiglans*	-119.409610	38.736580	DD	1517
744	*Pyrgulopsis longinqua*	-115.827428	33.314828	DD	0
745	*Pyrgulopsis manantiali*	-102.014720	26.912020	DD	703
746	*Pyrgulopsis marcida*	-115.075020	38.636060	DD	1638
747	*Pyrgulopsis marilynae*	-108.268100	33.290900	DD	1855
748	*Pyrgulopsis merriami*	-115.108870	37.247130	DD	983

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
749	*Pyrgulopsis metcalfi*	-104.648513	30.038214	EN	952
750	*Pyrgulopsis micrococcus*	-116.759230	36.916620	LC	1031
751	*Pyrgulopsis militaris*	-119.167520	41.397400	DD	1421
752	*Pyrgulopsis millenaria*	-114.325300	41.515200	DD	1563
753	*Pyrgulopsis minkleyi*	-102.070436	26.990236	DD	747
754	*Pyrgulopsis montana*	-114.247970	38.241130	DD	1971
755	*Pyrgulopsis montezumensis*	-111.752610	34.648995	VU	1094
756	*Pyrgulopsis morrisoni*	-111.894070	34.765950	DD	1069
757	*Pyrgulopsis nanus*	-116.295870	36.359400	DD	672
758	*Pyrgulopsis neomexicana*	-106.891417	34.058400	CR	1402
759	*Pyrgulopsis neritella*	-114.912520	39.503830	DD	1858
760	*Pyrgulopsis nonaria*	-111.709300	39.174240	DD	1645
761	*Pyrgulopsis notidicola*	-119.183830	41.328570	DD	1319
762	*Pyrgulopsis orbiculata*	-114.891390	39.538342	DD	1827
763	*Pyrgulopsis owensensis*	-118.322480	37.510600	DD	1606
764	*Pyrgulopsis owyheensis*	-117.447604	42.761524	DD	1357
765	*Pyrgulopsis palomasensis*	-97.613610	30.562823	DD	214
766	*Pyrgulopsis papillata*	-115.717782	38.929949	DD	1668
767	*Pyrgulopsis patzcuarensis*	-101.637968	19.629135	DD	2037
768	*Pyrgulopsis pecosensis*	-104.298000	32.180400	DD	1019
769	*Pyrgulopsis peculiaris*	-112.089660	39.015240	DD	1998
770	*Pyrgulopsis pellita*	-116.384790	39.305490	DD	2080
771	*Pyrgulopsis perforata*	-117.323330	37.032330	DD	1012
772	*Pyrgulopsis perturbata*	-118.414220	37.507700	DD	1281
773	*Pyrgulopsis pictilis*	-117.506500	39.982690	DD	1691
774	*Pyrgulopsis pisteri*	-116.315030	36.427470	DD	688
775	*Pyrgulopsis plicata*	-111.977970	38.067480	DD	1986
776	*Pyrgulopsis robusta*	-110.674920	43.892830	LC	2063
777	*Pyrgulopsis roswellensis*	-104.488772	33.425216	VU	1088
778	*Pyrgulopsis rupinicola*	-121.512651	40.984499	DD	869
779	*Pyrgulopsis sadai*	-117.021230	40.208715	DD	1758
780	*Pyrgulopsis sanchezi*	-116.316170	36.472000	DD	714
781	*Pyrgulopsis sathos*	-115.022420	38.850461	DD	1681
782	*Pyrgulopsis saxatilis*	-114.038330	39.459390	DD	1613
783	*Pyrgulopsis serrata*	-114.779470	40.114650	DD	1966
784	*Pyrgulopsis similis*	-108.109700	33.340500	DD	1938

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
785	*Pyrgulopsis simplex*	-111.493480	34.407820	DD	1798
786	*Pyrgulopsis sola*	-112.015430	34.383630	DD	1199
787	*Pyrgulopsis stearnsiana*	-122.271120	37.804370	LC	18
788	*Pyrgulopsis sterilis*	-116.803120	38.372290	DD	2411
789	*Pyrgulopsis sublata*	-114.375820	37.936910	DD	1661
790	*Pyrgulopsis sulcata*	-114.912550	39.505590	DD	1852
791	*Pyrgulopsis taylori*	-120.622520	35.314070	DD	199
792	*Pyrgulopsis thermalis*	-108.595300	32.955100	CR	1371
793	*Pyrgulopsis thompsoni*	-110.398415	31.457320	NT	1910
794	*Pyrgulopsis transversa*	-112.758020	39.914670	DD	1790
795	*Pyrgulopsis trivialis*	-109.937830	33.571120	CR	1637
796	*Pyrgulopsis turbatrix*	-115.999750	36.443010	DD	1545
797	*Pyrgulopsis umbilicata*	-119.194360	41.376010	DD	1355
798	*Pyrgulopsis variegata*	-113.965690	41.078500	DD	1298
799	*Pyrgulopsis varneri*	-119.167520	41.337570	DD	1324
800	*Pyrgulopsis ventricosa*	-122.680730	38.876050	CR	641
801	*Pyrgulopsis villacampae*	-115.697820	38.936880	DD	1710
802	*Pyrgulopsis vinyardi*	-115.135540	41.568550	DD	2077
803	*Pyrgulopsis wabashensis*	-88.020031	37.925879	DD	111
804	*Radomaniola albanica*	20.778381	40.615544	LC	870
805	*Radomaniola bosniaca*	16.449644	44.758642	DD	398
806	*Radomaniola bulgarica*	25.474306	42.453000	DD	404
807	*Radomaniola callosa*	14.008531	42.159414	VU	622
808	*Radomaniola caputlacus*	37.558199	37.752494	NT	873
809	*Radomaniola curta*	19.258130	42.468070	LC	72
810	*Radomaniola elongata*	19.135682	42.274080	CR	126
811	*Radomaniola feheri*	22.347972	37.093694	DD	458
812	*Radomaniola gaillardoti*	36.581544	35.269221	DD	260
813	*Radomaniola lacustris*	19.221967	42.163285	CR	8
814	*Radomaniola montana*	18.842500	42.288070	LC	10
815	*Radomaniola rhodopensis*	24.704280	41.856063	VU	824
816	*Radomaniola seminula*	22.540118	37.622500	DD	1025
817	*Radomaniola tritonum*	22.717500	37.551944	DD	5
818	*Radomaniola strandzhica*	27.357583	42.151028	DD	262
819	*Rhapinema dacryon*	-85.231793	30.815010	DD	41
820	*Sadleriana affinis*	35.365738	38.668458	DD	1233

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
821	*Sadleriana bulgarica*	24.740896	42.133549	DD	165
822	*Sadleriana byzanthina*	29.060778	40.188509	DD	201
823	*Sadleriana cavernosa*	15.326299	45.251084	CR	241
824	*Sadleriana fluminensis*	13.466900	45.792600	LC	0
825	*Sadleriana minuta*	31.423511	41.280415	DD	17
826	*Sadleriana robici*	14.768361	45.886611	DD	305
827	*Sadleriana sadleriana*	14.353257	45.928516	LC	309
828	*Sadleriana schmidtii*	14.821390	45.890697	LC	319
829	*Sadleriana supercarinata*	15.353970	44.819562	VU	469
830	*Salenthydrobia ferrerii*	18.194170	40.460000	EN	0
831	*Sarajana apfelbecki*	18.269287	43.819130	DD	497
832	*Sardohoratia islamioides*	9.494127	40.289439	EN	135
833	*Sardohoratia sulcata*	9.494127	40.289439	CR	135
834	*Sarkhia sarabensis*	47.054395	34.285450	DD	1457
835	*Saxurinator brandti*	17.650893	43.054170	VU	3
836	*Saxurinator hadzii*	19.218540	42.858683	DD	1890
837	*Saxurinator labiatus*	15.657454	44.780753	CR	738
838	*Saxurinator microbeliscus*	17.344939	43.199926	DD	164
839	*Saxurinator montenegrinus*	18.422196	42.865140	EN	412
840	*Saxurinator orthodoxus*	19.067905	42.580902	CR	48
841	*Saxurinator schlickumi*	20.202561	42.663273	DD	933
842	*Saxurinator sketi*	18.089801	42.650338	EN	22
843	*Shadinia bjniensis*	44.674056	40.463250	DD	1523
844	*Shadinia terpoghassiani*	44.171170	40.142880	DD	854
845	*Sheitanok amidicus*	40.246287	37.891318	NT	578
846	*Sivasi bodoni*	38.304167	38.656111	DD	829
847	*Sogdamnicola pallida*	45.566700	37.333300	DD	1267
848	*Sogdamnicola shadini*	67.248243	39.409578	DD	987
849	*Spathogyna fezi*	-1.722570	39.763150	EN	874
850	*Spilochlamys gravis*	-81.578185	29.080047	DD	22
851	*Stankovicia baicaliiformis*	20.698283	40.951931	CR	689
852	*Stiobia nana*	-85.834967	33.614271	DD	206
853	*Stoyanovia stoyanovi*	24.388611	43.228889	DD	195
854	*Strandzhia bythinellopenia*	27.363694	42.151278	DD	236
855	*Strugia ohridana*	20.631970	41.184140	VU	706
856	*Sumia macedonica*	20.632461	41.183131	DD	707

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
857	*Tadzhikamnicola likharevi*	70.028322	38.104757	DD	1231
858	*Tadzhikamnicola pavlovskii*	70.023301	38.096854	DD	1219
859	*Tanousia zrmanjae*	15.739636	44.208368	CR	14
860	*Tarraconia gasulli*	-0.512940	39.850790	CR	395
861	*Tarraconia rolani*	0.563592	40.745848	EN	2
862	*Tefennia tefennica*	29.772814	37.309455	VU	1165
863	*Terranigra kosovica*	21.077900	42.369718	NT	632
864	*Torosia proschwitzi*	29.846333	37.124556	DD	1804
865	*Trachyochridia filocincta*	20.769866	41.062348	CR	689
866	*Trichonia kephalovrissonia*	20.563824	40.011279	DD	641
867	*Trichonia trichonica*	21.556182	38.565941	CR	11
868	*Turkmenamnicola lindholmi*	62.324895	35.275355	DD	641
869	*Turkmenamnicola smaragdovae*	57.740574	38.012598	DD	773
870	*Turricaspia andrussowi*	51.608055	39.929312	DD	0
871	*Turricaspia astrachanica*	48.008542	46.343736	DD	0
872	*Turricaspia bogatscheviana*	51.608055	39.929312	DD	0
873	*Turricaspia bogensis*	32.004323	46.916669	DD	0
874	*Turricaspia boltovskoji*	38.937566	47.200307	DD	27
875	*Turricaspia borceana*	34.528333	44.785333	DD	121
876	*Turricaspia caspia*	47.464588	42.993426	LC	28
877	*Turricaspia chersonica*	32.614584	46.620563	DD	0
878	*Turricaspia conus*	47.464588	42.993426	DD	28
879	*Turricaspia crimeana*	35.083750	44.826090	DD	36
880	*Turricaspia dagestanica*	48.950161	39.105679	DD	0
881	*Turricaspia derbentina*	51.608055	39.929312	DD	0
882	*Turricaspia eburnea*	51.608055	39.929312	DD	0
883	*Turricaspia elegantula*	51.608055	39.929312	DD	0
884	*Turricaspia iljinae*	34.518611	44.786667	DD	220
885	*Turricaspia ismailensis*	29.235443	45.177689	VU	2
886	*Turricaspia lincta*	29.000944	45.432000	LC	0
887	*Turricaspia martensii*	51.608055	39.929312	DD	0
888	*Turricaspia meneghiniana*	49.952382	40.390515	DD	63
889	*Turricaspia nevesskae*	34.518611	44.786667	DD	220
890	*Turricaspia ovum*	51.608055	39.929312	DD	0
891	*Turricaspia pullula*	50.679918	41.499393	DD	0

ID	SPECIES	LONGITUDE	LATITUDE	RED LIST STATUS	ELEVATION
892	*Turricaspia sajenkovae*	48.994975	45.899028	DD	0
893	*Turricaspia spasskii*	48.519033	41.897396	DD	0
894	*Turricaspia spica*	51.608055	39.929312	DD	0
895	*Turricaspia triton*	47.464588	42.993426	LC	28
896	*Turricaspia trivialis*	52.408512	36.696761	DD	0
897	*Turricaspia turricula*	51.608055	39.929312	DD	0
898	*Turricaspia variabilis*	48.523052	46.160649	LC	0
899	*Turricaspia vinogradovi*	51.608055	39.929312	DD	0
900	*Valvatamnicola archangelskii*	71.804192	39.983914	DD	1441
901	*Valvatamnicola schahimardanica*	71.801674	39.984481	DD	1432
902	*Vinodolia fiumana*	14.758913	45.163130	EN	141
903	*Vinodolia lacustris*	20.987720	40.871030	CR	850
904	*Xestopyrgula dybowskii*	20.736000	40.914400	VU	690
905	*Zaumia kusceri*	20.744676	40.912129	CR	708
906	*Zaumia sanctizaumi*	20.775042	40.937137	CR	689

Appendix 2. Summary statistic for the hydrobiid species richness of 115 ecoregions and the potential predictors of this diversity. Each variable is shown according to its unit or in the unit we calculated it.

	Mean	**Standard deviation**	**Median**	**Min**	**Max**	**Range**	**Skew**	**Kurtosis**	**Standard Error**	**Variance**
Latitude (decimal degrees)	35.08	16.51	38.06	-45.42	67.37	112.80	-1.92	6.25	1.54	272.90
Area (Km^2)	40.92	73.22	18.87	0.31	540.59	540.27	4.21	20.97	6.83	5361.96
Mean elevation (m)	673.93	546.83	533.76	-23.50	2314.35	2337.86	1.10	0.40	50.99	299030.00
Elevational range (m)	2712.83	1513.34	2845	117.00	7159.00	7042.00	0.19	-0.58	141.12	2290184.00
BIO12 Annual Precipitation (mm)	666.68	417.42	584.76	34.80	1729.33	1694.52	0.58	-0.69	38.93	174244.00
BIO15 Precipitation Seasonality	48.85	26.95	42.51	12.43	153.31	140.88	0.93	0.76	2.51	726.30
BIO1 Annual Mean Temperature (ºC*10)	132.34	68.20	127.85	-26.52	275.91	302.43	0.12	-0.59	6.36	4652.25
BIO7 Temperature Annual Range(ºC*10)	320.48	86.61	323.54	104.38	496.89	392.51	-0.47	-0.12	8.08	7502.73
Temperature of the Last Glacial Maximum(ºC*10)	49.10	110.8	59.17	-262.22	241.82	504.03	-0.67	0.30	10.34	12288.30
Geological heterogeneity	1.98	1.25	1.79	0.10	6.00	5.90	0.79	0.16	0.12	1.58
Connectivity (number of boundaries in common)	3.84	2.15	4	0.00	11.00	11.00	0.62	0.43	0.20	4.62
Peninsula	Factor of two levels: 0 – absence, 1 - presence									
Biogeographic realm	Factor of four levels: Palearctic, Nearctic, Neotropic, Afrotropical									

Appendix 3. Comprised dataset of all the ecoregions and all the predictors used to perform the General Lineal Model (GLM).

ECOREGION	LONGITUDE	LATITUDE	SPRICH	TREATENED	ENDEMICS	AREA	ALTMEAN	ALTRANGE	PRECMEAN	PRECANNUALRANGE	TEMPMEAN	TEMPANNUALRANGE	TEMPLASTGLACIALMAX	PENINSULA	HGEO	ECOZONE	CONNECT
Dniester - Lower Danube	23.483194	45.865987	12 8	37	78	75.94	376.52	2883	683.72	29.81	92.27	310.85	12.37	0	94	PAL	8
Southeast Adriatic Drainages	20.122341	41.390123	90	58	67	4.74	771.89	2708	1127.53	34.52	107.26	276.28	49.08	1	15	PAL	4
Dalmatia	16.439078	43.940309	68	27	32	4.41	611.21	2188	1162.03	26.32	107.82	263.15	40.07	0	10	PAL	3
Caspian Marine	51.047619	40.470373	65	0	51	32.10	-14.75	203	366.44	40.38	144.69	333.65	101.13	0	20	PAL	4
Cantabric Coast - Languedoc	1.036893	44.003417	59	26	42	24.80	499.68	3904	894.70	20.63	111.88	244.62	59.92	1	60	PAL	5
Italian Peninsula & Islands	13.075832	40.920544	49	17	27	19.51	429.71	3296	710.90	40.62	139.21	241.27	83.21	1	45	PAL	2
Eastern Iberia	-0.539802	41.126222	37	7	24	16.01	723.59	3306	608.27	27.78	121.71	262.04	76.07	1	45	PAL	3
Gulf of Venice Drainages	10.541621	45.250560	35	8	8	15.53	724.92	4720	974.82	22.70	95.92	272.53	27.80	1	29	PAL	6
Mediterranean Northwest Africa	3.918004	34.804721	35	4	28	49.12	659.72	3350	332.61	44.90	166.42	311.71	116.46	0	71	PAL NE	2
Lahontan	-117.457422	39.628314	31	2	29	21.79	1738.70	3296	261.53	25.80	85.20	395.53	-6.54	0	60	A	7
Upper Danube	15.218524	47.864995	31	20	21	29.96	596.24	3816	851.31	29.86	75.89	289.52	-6.96	0	54	PAL	3
Thrace	26.135422	41.157419	27	10	18	14.27	479.39	2879	638.27	34.80	116.96	293.39	59.17	0	53	PAL	4
Central & Western Europe	10.643414	51.054765	22	5	14	182.93	216.57	4577	722.19	22.91	84.72	258.71	-30.06	0	22 7	PAL	6
Southern Iberia	-4.510574	38.068463	22	9	18	17.43	537.02	3498	526.87	51.25	154.92	294.93	108.54	1	47	PAL	2
Atlantic Northwest Africa	-8.288225	30.188383	21	13	18	42.19	684.49	4153	247.46	71.04	186.03	302.85	144.62	0	76	PAL NE	2
Bonneville	-112.920827	40.041101	21	1	20	16.45	1814.21	2627	337.10	20.79	74.95	413.10	-28.77	0	31	A	4
Ionian Drainages	21.564456	38.275625	20	7	16	3.52	546.95	2389	902.33	63.05	141.20	270.00	87.46	1	13	PAL NE	3
Vegas - Virgin	-114.497792	37.311785	19	1	14	3.51	1443.19	3115	259.16	26.56	124.48	393.04	37.08	0	16	A	4
Southern Anatolia	33.935003	37.090183	18	8	17	10.20	1025.86	3661	650.70	67.27	126.58	315.82	75.29	0	37	PAL	5
Aral Sea Drainages	59.995146	44.274668	17	0	5	48.23	99.71	721	130.30	43.84	103.59	463.28	39.91	0	27	PAL	5
Western Iberia	-6.027838	40.793087	17	5	9	24.46	674.74	2503	688.68	41.52	127.85	265.90	84.09	1	51	PAL NE	3
Death Valley	-117.118333	35.974782	15	2	9	8.07	1161.22	4409	182.48	51.14	151.53	373.12	72.42	0	27	A	5
Dnieper - South Bug	31.273173	50.865280	15	0	3	76.66	160.36	717	584.76	29.64	71.60	339.05	-50.76	0	56	PAL	5

ECOREGION	LONGITUDE	LATITUDE	SPRICH	TREATENED	ENDEMICS	AREA	ALTMEAN	ALTRANGE	PRECMEAN	PRECANNUALRANGE	TEMPMEAN	TEMPANNUALRANGE	TEMPLASTGLACIALMAX	PENINSULA	HGEO	ECOZONE	CONNECT
Aegean Drainages	23.228604	38.288941	14	2	9	2.80	425.88	2441	617.39	59.21	153.30	271.70	100.58	1	15	PALNE	2
Gila	-111.101158	33.130850	13	5	10	15.45	1178.71	3419	374.54	57.31	162.08	361.23	95.50	0	40	ANE	3
Florida Peninsula	-82.072706	28.881094	12	7	10	14.44	28.76	169	1307.84	43.41	211.63	254.83	154.40	1	17	ANE	2
Sacramento - San Joaquin	-120.757410	38.148124	12	5	7	18.88	811.96	4409	642.30	77.50	126.94	323.55	59.50	0	39	A	5
Central Anatolia	32.617183	38.034474	11	9	10	7.31	1233.39	2863	448.47	50.58	103.56	331.48	49.94	0	33	PAL	3
Don	40.580507	49.462805	11	0	4	63.18	130.62	967	501.16	22.33	76.17	378.55	-23.13	0	48	PAL	5
Kura - South Caspian Drainages	46.274894	39.888515	11	0	7	24.48	1192.41	5120	530.82	42.52	95.88	342.73	53.52	0	72	PAL	6
Vardar	21.922598	40.806075	11	5	6	5.28	656.43	2845	609.48	26.08	114.41	315.08	56.04	1	22	PAL	5
Western Anatolia	28.184957	38.204987	11	4	7	8.23	588.85	2504	704.05	70.63	142.09	298.97	89.94	0	27	PAL	4
Upper Tigris & Euphrates	45.559210	34.596102	10	1	6	49.69	1264.11	4387	456.84	82.84	150.80	379.56	96.55	0	12 8	PAL	11
Crimea Peninsula	34.332797	45.284008	9	0	4	2.98	132.33	1530	480.43	17.62	109.97	307.07	35.10	1	4	PAL	2
Ob	74.369958	59.109468	9	0	5	540.59	177.09	4559	447.48	42.45	-18.18	473.07	-134.56	0	39 0	PAL	4
Volga Delta - Northern Caspian Drainages	50.053812	45.555752	9	0	6	10.80	-23.51	117	175.52	18.33	106.03	405.00	39.74	0	4	PALNE	4
Colorado	-110.625720	37.384639	7	4	4	51.68	1771.84	4383	304.56	33.79	98.74	398.02	9.86	0	84	A	10
Northern Anatolia	33.880500	40.157181	7	1	5	22.10	1056.98	3838	521.43	38.37	99.27	312.96	47.97	0	69	PAL	6
Volga - Ural	49.000372	54.061369	7	0	6	281.02	160.59	1692	488.84	27.75	42.73	424.40	-69.66	0	17 0	PAL	9
Western Transcaucasia	40.846327	41.768682	7	1	7	9.15	1260.02	4755	934.73	27.58	80.61	304.06	32.07	0	34	PAL	5
Jordan River	35.633038	31.532620	6	2	4	3.90	533.77	3165	241.02	100.56	186.03	280.79	137.63	0	11	PAL	2
Upper Amu Darya	66.640834	36.435257	6	0	5	46.60	2104.34	7159	435.29	78.96	78.37	385.82	30.00	0	86	PAL	5
Western Caspian Drainages	45.424466	44.255410	6	0	4	23.38	538.54	5633	465.14	36.39	88.19	359.33	34.13	0	17	PAL	7
Lower Tigris & Euphrates	43.594071	33.750717	5	1	1	33.13	201.98	2725	204.24	87.27	215.10	385.04	162.13	0	22	PALNE	2
Mobile Bay	-86.972345	33.152385	4	0	2	11.07	157.87	1258	1441.67	19.57	165.99	327.00	81.76	0	12	A	4

ECOREGION	LONGITUDE	LATITUDE	SPRICH	TREATENED	ENDEMICS	AREA	ALTMEAN	ALTRANGE	PRECMEAN	PRECANNUALRANGE	TEMPMEAN	TEMPANNUALRANGE	TEMPLASTGLACIALMAX	PENINSULA	HGEO	ECOZONE	CONNECT
Northeast US & Southeast Canada Atlantic Drainages	-70.080136	44.633403	5	0	2	40.48	245.02	1954	1108.12	12.43	56.69	391.46	-180.18	0	53	NEA	3
Columbia Unglaciated	-117.887040	44.799254	4	1	3	36.63	1262.84	3726	671.45	38.53	62.34	358.19	-48.59	0	61	NEA	6
Lower Congo Rapids	14.179507	-4.889032	4	4	2	1.42	408.67	824	1236.23	78.68	242.69	137.20	207.79	0	3	AFR	3
Northern British Isles	-5.685864	54.887288	4	0	0	26.75	178.30	1342	1152.30	21.71	81.40	175.88	-39.19	0	54	PAL	1
Pecos	-103.948578	32.597694	4	1	3	11.03	1268.94	3547	352.47	63.78	153.03	368.96	70.54	0	19	NEA	4
Southern California Coastal - Baja California	-114.648690	29.432743	4	0	4	16.09	439.66	3496	248.84	81.86	184.29	265.94	139.08	1	51	NEA	3
Apalachicola	-84.705744	32.097716	3	2	1	5.14	152.14	1310	1344.73	21.63	178.34	307.83	107.33	0	16	NEA	5
Appalachian Piedmont	-80.159471	34.864166	3	1	2	30.64	143.55	1853	1207.37	17.82	157.97	327.39	65.88	0	26	NEA	5
East Texas Gulf	-99.491077	31.638118	3	0	2	25.49	535.38	1441	686.11	41.25	178.88	349.33	79.99	0	36	A	5
Lower & Middle Syr Darya	68.541617	45.224097	3	0	3	59.48	357.42	4130	210.13	42.58	86.36	462.23	27.26	0	10 1	PAL	7
Namak	50.554366	34.935741	3	0	3	10.41	1596.21	3589	214.88	77.47	136.16	408.48	87.46	0	34	PAL	2
Ogooue - Nyanga - Kouilou - Niari	12.123932	-2.152700	3	2	1	23.16	333.81	1028	1729.33	69.28	245.81	130.95	212.25	0	12	AFR	2
Ozark Highlands	-92.238221	36.186895	2	1	1	8.58	257.55	792	1174.01	19.09	142.28	376.00	8.39	0	9	NEA	4
Rio Salado	-101.326823	27.383335	3	0	3	5.65	803.13	2913	394.19	64.83	206.72	301.41	138.01	0	12 19	A	1
Sahara	8.386470	27.685694	3	1	0	325.94	402.99	3456	34.80	62.14	227.67	352.08	177.30	0	9	PAL	3
Teays - Old Ohio	-83.616377	39.160697	3	0	1	38.98	330.47	1698	1096.88	16.91	109.65	365.97	-60.69	0	27	NEA	6
Upper Missouri	-106.209269	46.080737	3	0	2	79.13	1134.17	3767	395.09	57.31	56.81	440.16	-148.63	0	70	A	6

ECOREGION	LONGITUDE	LATITUDE	SPRICH	TREATENED	ENDEMICS	AREA	ALTMEAN	ALTRANGE	PRECMEAN	PRECANNUALRANGE	TEMPMEAN	TEMPANNUALRANGE	TEMPLASTGLACIALMAX	PENINSULA	HGEO	ECOZONE	CONNECT
Upper Rio Grande - Bravo	-106.350576	34.209391	3	2	3	15.98	1918.71	3488	339.83	61.67	107.71	384.12	28.31	0	45	NEA	6
Bight Drainages	3.031498	7.680422	2	0	0	14.92	219.45	1022	1278.32	69.32	265.67	144.93	229.83	0	10	AFR	1
Central Prairie	-94.143639	37.620843	2	0	1	16.19	286.36	717	1031.15	31.91	133.67	392.64	-26.54	0	29	NEA	5
Chesapeake Bay	-77.197458	40.023012	2	0	0	15.10	303.63	1495	1026.89	14.36	102.10	359.17	-70.46	0	18	NEA	4
Coastal Levant	35.325133	32.994549	2	0	0	2.59	466.63	3080	654.55	100.49	178.87	247.54	137.46	0	8	PAL	3
Columbia Glaciated	-117.529102	48.778095	2	0	1	31.43	1230.12	3466	637.17	31.33	43.01	348.59	-138.58	0	64	NEA	2
Eburneo	-5.299983	7.633066	2	1	1	20.80	251.23	1243	1270.73	66.35	263.83	150.95	229.14	0	2	AFR	2
Guzman - Samalayuca	-107.385706	30.453308	2	1	2	11.81	1602.55	2240	357.77	90.48	153.82	360.60	94.67	0	28	NEA	2
Kavir & Lut Deserts	56.097555	33.199897	2	0	0	48.54	1290.14	4160	127.80	82.86	172.19	393.66	121.75	0	98	PAL	5
Laurentian Great Lakes	-84.313900	45.170912	2	0	0	87.65	258.86	1081	848.39	22.74	56.06	398.20	-199.87	0	10 7	NEA	6
Lower Nile	32.489590	19.199791	2	0	0	96.59	433.26	2275	151.18	119.09	263.38	285.71	218.96	0	77	PAL	1
Malebo Pool	15.534466	-4.154022	2	2	0	0.32	491.10	484	1495.15	65.84	243.67	141.91	209.06	0	1	AFR	2
Middle Amu Darya	63.161956	39.357105	2	0	2	46.51	336.98	4322	189.05	75.68	146.55	410.92	98.68	0	30	PAL	4
Nile Delta	31.010286	30.491802	2	0	0	5.30	77.66	925	54.59	91.08	205.81	265.40	158.87	0	6	PAL	2
Northern Baltic Drainages	19.769334	63.005863	2	0	0	156.95	302.31	2494	630.34	30.48	19.40	323.79	-220.01	1	16 8	PAL	2
Norwegian Sea Drainages	11.281744	63.856576	2	0	0	29.99	535.02	2301	1347.67	26.13	22.07	225.90	-164.91	1	48	PAL	2
Oregon & Northern California Coastal	-123.006039	42.050286	2	0	1	11.60	860.89	3756	1202.64	68.09	91.06	285.31	33.77	0	28	NEA	3
Oregon Lakes	-119.642666	42.673203	2	0	2	5.83	1560.37	1758	316.74	29.91	68.32	368.25	-20.21	0	17	NEA	4
Orontes	36.646076	35.458754	2	0	1	2.53	669.80	3027	548.82	84.08	159.20	311.95	113.48	0	10	PAL	4
Sangha	15.297075	1.570269	2	2	0	22.89	528.65	969	1633.55	47.52	243.01	130.52	208.28	0	3	AFR	3
Tennessee	-85.181490	35.592437	2	0	1	10.53	436.49	2019	1390.65	14.86	134.37	341.22	33.61	0	19	NEA	5

ECOREGION	LONGITUDE	LATITUDE	SPRICH	TREATENED	ENDEMICS	AREA	ALTMEAN	ALTRANGE	PRECMEAN	PRECANNUALRANGE	TEMPMEAN	TEMPANNUALRANGE	TEMPLASTGLACIALMAX	PENINSULA	HGEO	ECOZONE	CONNECT
Turan Plain	55.293823	40.631647	2	0	0	31.66	235.81	3967	158.85	49.68	141.71	398.98	90.88	0	25	PALNE	8
Upper Mississippi	-91.744453	42.961817	2	0	0	52.83	297.72	553	837.10	41.30	79.28	432.29	-132.62	0	67	ANE	7
Upper Snake	-112.761827	43.238055	2	1	0	10.29	1841.69	3350	380.99	25.08	45.07	411.12	-69.20	0	37	ANE	5
US Southern Plains	-100.738640	36.408447	2	0	0	41.99	958.23	4229	578.49	53.29	131.08	403.70	3.34	0	4813	A	8
Arabian Interior	45.233350	24.628353	1	0	0	207.74	621.59	2921	93.19	96.09	239.05	317.01	187.73	1	5	PAL	6
Ashanti	-1.918635	6.043854	1	0	0	6.02	162.71	839	1508.88	55.22	260.87	118.34	224.36	0	322	AFR	2
Barents Sea Drainages	44.546421	67.374181	1	0	0	312.90	185.82	1742	502.61	30.74	-26.52	376.88	-226.36	0	4	PAL	4
Cape Fold	21.349351	-33.344345	1	0	1	12.83	541.01	2288	414.42	35.93	162.22	243.43	125.51	0	8	AFR	0
Cape Verde	-23.980330	15.946127	1	0	0	0.33	346.11	2740	241.82	153.31	218.78	104.37	194.10	0	2	AFR	0
English - Winnipeg Lakes	-98.224796	50.610178	1	0	0	81.97	384.89	629	519.99	51.89	15.64	493.32	-262.21	0	75	ANE	4
Iceland - Jan Mayen	-18.537232	65.028997	1	0	0	19.59	509.52	2200	1177.42	18.70	11.76	164.28	-131.97	0	5	PAL	0
Irgyz -Turgai	63.020239	49.512059	1	0	0	29.06	204.79	1049	220.22	21.81	52.19	496.89	-24.99	0	47	PAL	4
Kuban	40.314053	44.494019	1	0	0	6.26	654.63	4926	787.09	26.88	92.56	308.75	36.43	0	10	PAL	3
Lake Issyk Kul - Upper Chu	76.193410	42.485589	1	0	1	5.21	2314.35	4577	348.30	56.06	16.20	417.13	-32.78	0	23	PALNE	2
Lerma - Chapala	-100.549757	20.107452	1	0	1	6.67	2175.83	4778	781.17	93.96	164.35	237.14	125.35	0	36	ANE	1
Lower Mississippi	-90.268789	32.942626	1	0	0	23.76	71.79	278	1427.21	18.67	173.46	323.61	74.92	0	11	ANE	7
Lower Rio Grande - Bravo	-100.827865	27.964762	1	0	0	13.91	586.64	3711	470.69	60.40	207.94	310.10	136.28	0	22	ANE	6
Middle Missouri	-99.874475	41.511952	1	0	0	64.11	906.29	4132	578.17	54.94	86.74	429.86	-94.96	0	70	A	6
Northern Central Asian Highlands	72.438108	41.583045	1	0	1	22.56	1889.35	5546	423.05	52.76	51.26	411.58	2.81	0	58	PAL	3
Northern Hormuz Drainages	55.457430	27.735397	1	0	0	8.19	851.83	3797	160.13	110.02	219.92	299.66	180.00	0	27	PAL	2
Orumiyeh	46.013415	37.362750	1	0	1	5.27	1737.50	2464	404.36	65.11	96.23	382.39	37.05	0	23	PAL	2

ECOREGION	LONGITUDE	LATITUDE	SPRICH	TREATENED	ENDEMICS	AREA	ALTMEAN	ALTRANGE	PRECMEAN	PRECANNUALRANGE	TEMPMEAN	TEMPANNUALRANGE	TEMPLASTGLACIALMAX	PENINSULA	HGEO	ECOZONE	CONNECT
Ouachita Highlands	-93.909303	33.571711	1	0	0	11.21	134.93	804	1268.71	18.75	168.90	347.41	53.80	0	10	NEA	5
Panuco	-98.779682	21.813614	1	0	0	11.78	886.94	3760	1023.45	75.47	211.63	224.57	163.83	0	39	NEA	2
Patagonia	-69.452435	-45.423647	1	0	1	127.48	561.81	5124	612.99	28.65	87.51	228.80	32.37	0	22 5	NEO	0
Sabine - Galveston	-95.077901	31.413856	1	0	0	13.02	102.08	462	1182.00	20.60	187.93	322.68	86.47	0	13	NEA	4
Socotra	53.793091	12.495364	1	0	1	0.32	297.20	1481	94.13	64.45	255.33	139.81	228.14	0	1	AFR	0
Southwestern Arabian Coast	44.573317	18.517677	1	0	1	45.60	779.45	3646	108.39	80.34	238.46	224.49	203.87	1	91	PAL	1
St. Lawrence	-74.253694	47.248129	1	0	0	46.46	354.41	1743	980.65	21.84	24.91	438.75	-252.53	0	65	NEA	2
Tibetan Plateau Endorheic Drainages	86.059965	33.225557	1	0	1	59.17	5026.75	2468	177.99	109.87	-45.31	363.24	-96.46	0	11 7	PAL	0
Volta	-1.478542	10.510952	1	0	0	34.12	254.61	957	981.59	94.78	275.91	192.31	241.82	0	11	AFR	3
West Florida Gulf	-86.443459	31.135137	1	0	0	3.25	77.06	211	1501.32	20.75	186.08	297.48	113.53	0	8	NEA	2
West Texas Gulf	-98.791153	28.374211	1	0	0	6.71	188.43	741	644.35	43.84	212.55	307.36	130.98	0	9	NEA	2

Appendix 4. Information on the sampling locations of the studied populations, including water parameters.

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
41.586217	2.038500	354.70	FW2308	*M. egarensis* sp. nov.	Font de les Canyes, Terrassa	Barcelona	Catalonia	Spain	N/A	N/A	Jordi Corbella	09/10/2016
41.586217	2.038500	354.70	FW2439	*M. egarensis* sp. nov.	Font de les Canyes, Terrassa	Barcelona	Catalonia	Spain	6.96	1413	Miguel Carrillo, Jonathan Miller	02/07/2017
36.587509	-5.574243	379.72	FW2415	*M. carrillorum* sp. nov.	Stream Canuto Gallina, Montes de Propio, Jeréz	Cádiz	Andalucía	Spain	N/A	N/A	Félix Ríos	01/06/2017
36.570760	-5.593999	462.67	FW2543	*M. carrillorum* sp. nov.	Stream in Canuto de Las Palas, Montes de Propio de Jerez	Cádiz	Andalucía	Spain	N/A	1048	Félix Ríos	09/06/2018
36.587509	-5.574243	379.72	FW2547	*M. carrillorum* sp. nov.	Stream in Canuto Gallina, Montes de Propio, Jeréz	Cádiz	Andalucía	Spain	7.34	179	Félix Ríos	15/06/2018
36.570853	-5.593393	450.15	FW2548	*M. carrillorum* sp.nov.	Stream in Canuto de Las Palas, Montes de Propio, Jerez	Cádiz	Andalucía	Spain	7.02	80	Fernando García, Félix Ríos	15/06/2018
44.943579	12.021272	0.00	USGB8015	*M. lupiaensis* sp. nov.	Giammatteo Creek, Frigole	Ferrara	Emilia-Romagna	Italy	N/A	N/A	D. Ferreri	16/03/2003

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
40.292000	17.812000	1.47	USGB16237	*M. lupiaensis* sp. nov.	Risorgenza di Torre Castiglione, Porto Cesareo	Lecce	Puglia	Italy	N/A	N/A	Marco Bodon	28/05/2000
40.208000	17.926000	2.66	USGB16236	*M. lupiaensis* sp. nov.	Palude del Capitano Pond, Sant´Isidoro, Ionian coast, Nardó, Apulia	Lecce	Puglia	Italy	N/A	N/A	Marco Bodon	16/04/2000
36.477025	-5.592611	673.97	FW2584	*M. felixi* sp. nov.	Stream in Canuto de la Tala	Cádiz	Andalucía	Spain	N/A	292	José Manuel Amarillo, Félix Ríos	27/07/2018
36.479256	-5.624502	389.93	FW2585	*M. felixi* sp. nov.	Stream in Canuto del Zapato	Cádiz	Andalucía	Spain	N/A	75	José Manuel Amarillo, Félix Ríos	27/07/2018
36.477025	-5.592611	673.97	FW2685	*M. felixi* sp. nov.	Stream in Canuto de la Tala	Cádiz	Andalucía	Spain	N/A	N/A		
36.479256	-5.624502	389.93	FW2686	*M. felixi* sp. nov.	Stream in Canuto del Zapato	Cádiz	Andalucía	Spain	N/A	N/A		
34.381933	7.933450	278.67	USGB17271	*M. veronicae* sp. nov.	El Waha Spring, Oasis Waterfall, Tamerza	N/A	Tozeur	Tunisia	N/A	N/A	Afef Brahmi, Mustapha Bejaoui, Nourddine Khalloufi	11/08/2015
34.381933	7.933450	278.67	USGB17272	*M. veronicae* sp. nov.	El Waha Spring, Oasis Waterfall, Tamerza	N/A	Tozeur	Tunisia	N/A	N/A	Afef Brahmi, Mustapha Bejaoui, Nourddine Khalloufi	11/08/2015

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
34.377053	7.912929	249.58	USGB17274	*M. veronicae* sp. nov.	Lekbir Spring, Big Waterfall, Tamerza	N/A	Tozeur	Tunisia	N/A	N/A	Afef Brahmi, Mustapha Bejaoui, Nourddine Khalloufi	12/08/2015
34.322167	7.939950	152.77	USGB17276	*M. veronicae* sp. nov.	Echbicka Spring, Echbika Waterfall, Echbika	N/A	Tozeur	Tunisia	N/A	N/A	Afef Brahmi, Mustapha Bejaoui, Nourddine Khalloufi	13/08/2015
36.817200	-5.299117	522.01	FW2236	*M. balearica*	Spring in Montercorto	Málaga	Andalucía	Spain	N/A	N/A	Francisco de Erit Vázquez, Javier Ripoll	13/04/2015
36.906854	-4.766804	301.65	FW2249	*M. balearica*	El Chorro Spring, Ardales	Málaga	Andalucía	Spain	N/A	N/A	Francisco de EritVázquez, Javier Ripoll	08/09/2015
36.640146	-4.498938	41.87	FW2309	*M. balearica*	El Canalillo Stream, Churriana	Málaga	Andalucía	Spain	N/A	N/A	Francisco de Erit Vázquez	22/09/2016
36.911883	-4.770083	228.22	FW2322	*M. balearica*	Venta El Pilar Spring	Málaga	Andalucía	Spain	N/A	N/A	Francisco de Erit Vázquez	21/12/2016
36.505680	-5.523525	182.11	FW2335	*M. balearica*	Los Granados Trough	Málaga	Andalucía	Spain	N/A	N/A	Francisco de Erit Vázquez	14/02/2017
36.811683	-5.300633	432.99	FW2350	*M. balearica*	Stream in Montecorto	Málaga	Andalucía	Spain	8	1362	Amanda Aguado Miguel Carrillo, Jonathan Miller	09/04/2017

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
36.817200	-5.299117	522.01	FW2351	*M. balearica*	Montecorto Spring	Málaga	Andalucía	Spain	7.53	1284	Amanda Aguar, Miguel Carrillo, Jonathan Miller	09/04/2017
36.911883	-4.770083	228.22	FW2354	*M. balearica*	Venta El Pilar Spring	Málaga	Andalucía	Spain	7.7	2750	Amanda Aguado, Miguel Carrillo, Jonathan Miller	10/04/2017
36.740500	-4.834083	311.18	FW2357	*M. balearica*	Valentín Spring, Alozaina	Málaga	Andalucía	Spain	N/A	N/A	Amanda Aguado Miguel Carrillo, Jonathan Miller	11/04/2017
37.675750	-2.973300	781.82	FW2386	*M. balearica*	Caño de la Rambla Stream, Fontanar	Jaén	Andalucía	Spain	N/A	N/A	Amanda Aguado Miguel Carrillo, Jonathan Miller	12/05/2017
37.140033	-1.848983	124.03	FW2395	*M. balearica*	Arabic Spring in Mojácar	Almería	Andalucía	Spain	7.79	878	Amanda Aguado, Miguel Carrillo, Jonathan Miller	14/05/2017
37.585534	-1.121782	486.77	FW2503	*M. balearica*	Sierra de La Muela, Cartagena	Murcia	Murcia	Spain	N/A	N/A	Luis Murillo	27/09/2017
37.910767	-3.124290	539.44	FW2568	*M. balearica*	Fuente de los Cinco Caños Spring, Peal del Becerro	Jaén	Andalucía	Spain	6.83	1395	Miguel Carrillo, Fernando García	12/07/2018

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
39.897517	4.250167	6.45	FW2603	*M. balearica*	Colarsega, Mahón, Minorca	Baleares	Balearic Islands	Spain	N/A	N/A	Miguel Carrillo, Jonathan Miller	14/08/2018
40.007883	4.130183	43.56	FW2604	*M. balearica*	Spring near Sant Joan de Carbonell, Minorca	Baleares	Balearic Islands	Spain	N/A	N/A	Miguel Carrillo, Jonathan Miller	14/08/2018
39.872983	4.131233	21.18	FW2605	*M. balearica*	Cala en Porter, Minorca	Baleares	Balearic Islands	Spain	N/A	N/A	Miguel Carrillo, Jonathan Miller	14/08/2018
39.912300	4.066283	5.52	FW2609	*M. balearica*	Barranc des Bec, Son Bou, Minorca	Baleares	Balearic Islands	Spain	N/A	N/A	Miguel Carrillo, Jonathan Miller	15/08/2018
35.894600	14.338770	147.48	UGSB 19506	*M. melitensis*	Wied-tal Bahrija spring, Il-Bahrija	Malta	Malta	Malta	N/A	N/A	Diana Delicado, Torsten Hauffe	20/11/2016
36.035590	14.233430	71.91	UGSB 19507	*M. melitensis*	Spring in Xlendi Valley, Fontana, Gozo	Malta	Malta	Malta	N/A	N/A	Diana Delicado, Torsten Hauffe	21/11/2016
34.910920	-3.565550	424.65	UGSB 19508	*M. midarensis*	Two parallel irrigation channels, Midar	N/A	Eastern	Morocco	N/A	N/A	Torsten Hauffe, Diana Delicado	03/06/2015
35.076861	-2.924750	51.94	UGSB 19933	*M. midarensis*	Selouane River, Nador	N/A	Eastern	Morocco	N/A	N/A	Younes Mabrouki	30/04/2016
35.163889	-3.110000	79.84	UGSB 19934	*M. midarensis*	Izerouane river	N/A	Eastern	Morocco	N/A	N/A	Younes Mabrouki	12/05/2015
35.105194	-2.345833	1.97	UGSB 19935	*M. midarensis*	Marres Cherraba	N/A	Eastern	Morocco	N/A	N/A	Younes Mabrouki	28/04/2016

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
35.063500	-2.906389	68.91	UGSB 19939	*M. midarensis*	Ouzej River	N/A	Eastern	Morocco	N/A	N/A	Younes Mabrouki	30/04/2016
35.306000	-2.977472	95.94	UGSB 19940	*M. midarensis*	Mariouari River	N/A	Eastern	Morocco	N/A	N/A	Younes Mabrouki	12/05/2016
35.287472	-2.943806	14.39	UGSB 19937	*M. midarensis*	Rio de Oro River	N/A	Melilla	Spain	N/A	N/A	Younes Mabrouki	18/05/2015
32.638533	-16.938772	140.92	UGSB 19970	*M. rolani*	Spring in Arco da Calheta	Madeira	Madeira	Portugal	N/A	N/A	U. Jueg	27/02/2017
33.881750	10.083500	11.48	UGSB 17283	*M. saharica*	Gabés Stream	N/A	Gabés	Tunisia	N/A	N/A	Afef Brahmi, Mustapha Bejaoui, Nourddine khalloufi	12/08/2015
33.758733	10.206417	21.52	UGSB 17287	*M. saharica*	Ketana Oasis	N/A	Gabés	Tunisia	N/A	N/A	Afef Brahmi, Mustapha Bejaoui, Nourddine Khalloufi	12/08/2015
33.933335	10.078586	8.96	UGSB 17292	*M. saharica*	Ghanouch Stream, Ghanouch	N/A	Gabés	Tunisia	N/A	N/A	Afef Brahmi, Mustapha Bejaoui, Nourddine Khalloufi	12/08/2015
34.108450	9.982117	17.14	UGSB 17295	*M. saharica*	Al Akarit Stream, El Akarit	N/A	Gabés	Tunisia	N/A	N/A	Afef Brahmi, Mustapha Bejaoui, Nourddine Khalloufi	12/08/2015
42.978767	3.011600	8.99	FW2436	*M. similis*	Buddle in La Palme	Aude	Languedoc-Roussillon	France	7.21	10750	Miguel Carrillo, Jonathan Miller	01/07/2017

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
42.850650	2.935617	7.74	FW2434	*M. similis*	Fonte Dame, Salses-le-Château	Pyrénées-Orientales	Languedoc-Roussillon	France	7.38	3300	Miguel Carrillo, Jonathan Miller	01/07/2017
42.859033	2.958550	25.11	FW2435	*M. similis*	Font d´Estramar, Salses-le-Château	Pyrénées-Orientales	Languedoc-Roussillon	France	7.33	8570	Miguel Carrillo, Jonathan Miller	01/07/2017
43.531600	5.108100	17.75	FW2473	*M. similis*	Stream near La Suriane, Etang de Berre	Bouches-du-Rhône	Provence-Alpes-Cote d'Azur	France	7.23	948	Jonathan Miller	01/08/2017
43.513900	6.479250	160.49	FW2475	*M. similis*	La Foux-de-Draguignan Stream	Var	Provence-Alpes-Cote d'Azur	France	7.44	5150	Jonathan Miller	03/08/2017
42.110033	0.349550	519.75	FW2412	*M. similis*	Saltwater stream near Aguinaliu	Huesca	Aragon	Spain	7.89	23200	Miguel Carrillo, Jonathan Miller	04/06/2017
41.991983	0.386550	526.84	FW2413	*M. similis*	Stream in Peralta de la Sal	Huesca	Aragon	Spain	7.64	2760	Miguel Carrillo, Jonathan Miller	04/06/2017
41.987400	0.386050	514.70	FW2414	*M. similis*	Sosa River in Peralta de la Sal	Huesca	Aragon	Spain	7.03	9810	Miguel Carrillo, Jonathan Miller	04/06/2017
42.129579	0.378749	518.06	FW2512	*M. similis*	Fuente del Molino Spring, Barranco Sarrón, Torres del Obispo, Graus	Huesca	Aragon	Spain	N/A	N/A	Cristobal Rubio	17/11/2017
41.432050	-0.466317	154.32	FW2411	*M. similis*	Stream in Barranco del Agua Salada, Gelsa	Zaragoza	Aragon	Spain	7.57	12290	Miguel Carrillo, Jonathan Miller	03/06/2017

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
41.297850	2.113417	2.39	FW2437	*M. similis*	La Ricarda Pond	Barcelona	Catalonia	Spain	7.68	1493	Miguel Carrillo, Jonathan Miller	02/07/2017
40.673117	0.593583	0.17	FW2440	*M. similis*	Ullals de Baltasar	Tarragona	Catalonia	Spain	7.3	2650	Miguel Carrillo Jonathan Miller	03/07/2017
38.522900	-1.601317	497.64	FW2398	*M. similis*	Cordovilla Saltings	Albacete	Castilla-La Mancha	Spain	7.99	6200	Amanda Aguado Miguel Carrillo, Jonathan Miller	14/05/2017
39.278017	-1.308367	601.03	FW2404	*M. similis*	La Salaboreja Spring	Albacete	Castilla-La Mancha	Spain	6.87	5230	Amanda Aguado Miguel Carrillo Pacheco, Jonathan Miller	15/05/2017
39.278483	-1.308967	601.88	FW2405	*M. similis*	La Cañada Stream near La Salaboreja Spring	Albacete	Castilla-La Mancha	Spain	7.55	2700	Amanda Aguado Miguel Carrillo Pacheco, Jonathan Miller	15/05/2017
39.253400	-1.557450	641.78	FW2406	*M. similis*	Stream feeding Galayo's Pond	Albacete	Castilla-La Mancha	Spain	7.91	1524	Amanda Aguado, Miguel Carrillo, Jonathan Miller	15/05/2017
41.195950	-2.708300	967.80	FW2366	*M. similis*	Saltings of Riba de Santiuste	Guadalajara	Castilla-La Mancha	Spain	7.3	25230	Gerson Cárdenas García, Miguel Carrillo, Jonathan Miller	15/04/2017

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
41.139767	-2.769467	913.07	FW2368	*M. similis*	Alcolea Stream, tributary of Salado River, road from Imon to Santamera	Guadalajara	Castilla-La Mancha	Spain	7.77	4240	Gerson Cárdenas García, Miguel Carrillo, Jonathan Miller	15/04/2017
41.131950	-2.775133	939.15	FW2369	*M. similis*	Salado River in Santamera	Guadalajara	Castilla-La Mancha	Spain	7.5	5800	Gerson Cárdenas García, Miguel Carrillo, Jonathan Miller	15/04/2017
41.128250	-2.774200	919.11	FW2370	*M. similis*	Salado River in Santamera	Guadalajara	Castilla-La Mancha	Spain	7.7	5770	Gerson Cárdenas García, Miguel Carrillo, Jonathan Miller	15/04/2017
40.904300	-2.325350	1004.38	FW2382	*M. similis*	La Vega Stream in Saelices de la Sal	Guadalajara	Castilla-La Mancha	Spain	7.69	2770	Amanda Aguado, Miguel Carrillo, Jonathan Miller	22/04/2017
38.541467	-0.815733	479.21	FW2464	*M. similis*	Vinalopó River, Sax	Alicante	Valenciana	Spain	7.8	17100	Miguel Carrillo, Jonathan Miller	21/07/2017
38.621883	-0.030900	25.88	FW2466	*M. similis*	Braña's ravine, near L'Olla Beach	Alicante	Valencia	Spain	7.62	3610	Miguel Carrillo, Jonathan Miller	21/07/2017
40.375233	0.400750	0.07	FW2467	*M. similis*	Marsh in Peñíscola	Castellón de la Plana	Valencia	Spain	7.34	3020	Miguel Carrillo, Jonathan Miller	22/07/2017

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
40.019783	0.001083	2.22	FW2468	*M. similis*	Irrigation ditch in Moli de la Font	Castellón de la Plana	Valencia	Spain	7.43	1963	Miguel Carrillo, Jonathan Miller	22/07/2017
40.058183	-0.458033	376.61	FW2469	*M. similis*	Irrigation ditch in Cirat	Castellón de la Plana	Valencia	Spain	8.01	1323	Miguel Carrillo Jonathan Miller	22/07/2017
39.044467	-0.516133	100.22	FW2463	*M. similis*	Fuente Amarga Spring	Valencia	Valencia	Spain	7.8	6380	Miguel Carrillo, Jonathan Miller	21/07/2017
38.246639	-1.002462	299.70	FW2459	*M. similis*	Chícamo stream near La Umbría	Murcia	Murcia	Spain	N/A	N/A	Antonio José García	31/03/2017
38.036018	-1.431197	264.21	FW2460	*M. similis*	Mula River	Murcia	Murcia	Spain	N/A	N/A	Antonio José García	23/04/2017
37.906457	-1.127844	478.42	FW2504	*M. similis*	Spring near Puerto de la Cadena,	Murcia	Murcia	Spain	N/A	N/A	Luis Murillo	11/10/2017
39.766833	3.086600	0.10	FW2597	*M. similis*	Font de Son Sant Joan Spring, Majorca	Baleares	Balearic Islands	Spain	N/A	N/A	Miguel Carrillo, Jonathan Miller, Marian Ramos	11/08/2018
39.792900	3.074750	0.00	FW2598	*M. similis*	Albufera of Majorca, Siquia de Son Senyor	Baleares	Balearic Islands	Spain	N/A	N/A	Miguel Carrillo, Jonathan Miller, Marian Ramos	11/08/2018
39.784050	3.079900	0.00	FW2599	*M. similis*	Albufera of Majorca, Siquia d´en Moix	Baleares	Balearic Islands	Spain	N/A	N/A	Miguel Carrillo, Jonathan Miller, Marian Ramos	11/08/2018

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
36.891733	-2.010050	70.74	FW2392	*M. similis*	Las Negras ravine	Almería	Andalucía	Spain	8.46	3150	Amanda Aguado, Miguel Carrillo Pacheco, Diego Moreno, Jonathan Miller	13/05/2017
36.792833	-5.558117	389.55	FW2352	*M. similis*	Spring Pilar de los Playeros, Villamartín	Cádiz	Andalucía	Spain	6.65	868	Amanda Aguado, Miguel Carrillo, Jonathan Miller	09/04/2017
36.865650	-5.665133	128.87	FW2353	*M. similis*	Fuente la Zarza Spring, Villamartín, Cádiz	Cádiz	Andalucía	Spain	7.3	1347	Amanda Aguado, Miguel Carrillo, Jonathan Miller	09/04/2017
36.466830	-5.928288	244.86	FW2501	*M. similis*	Fuente Grande Spring, Medina Sidonia	Cádiz	Andalucía	Spain	N/A	N/A	Félix Ríos	24/09/2017
36.678274	-5.840829	50.34	FW2516	*M. similis*	Fuente Platero Spring	Cádiz	Andalucía	Spain	N/A	N/A	Félix Ríos	28/01/2018
37.020614	-5.426859	267.20	FW2521	*M. similis*	Balneario de Pozo Amargo, Puerto Serrano	Cádiz	Andalucía	Spain	N/A	N/A	Félix Ríos	29/03/2018
36.538426	-5.744773	212.28	FW2522	*M. similis*	Alcalá de los Gazules	Cádiz	Andalucía	Spain	N/A	N/A	Félix Ríos	01/04/2018
36.865650	-5.665133	128.87	FW2525	*M. similis*	Fuente la Zarza Spring, Villamartín	Cádiz	Andalucía	Spain	N/A	930	Félix Ríos Jiménez	15/04/2018

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
36.880310	-5.409620	387.91	FW2540	*M. similis*	El Algarrobo Spring, Algodonales	Cádiz	Andalucía	Spain	N/A	1068	Félix Ríos	03/06/2018
36.460313	-5.928664	261.55	FW2545	*M. similis*	La Salá Spring, Medina Sidionia	Cádiz	Andalucía	Spain	7.3	1500	Fernando García, Félix Ríos	15/06/2018
36.460313	-5.928664	261.55	FW2546	*M. similis*	La Salá Spring (upper part), Medina Sidionia	Cádiz	Andalucía	Spain	7.3	1500	Fernando García, Félix Ríos	15/06/2018
36.538371	-5.744319	205.69	FW2549	*M. similis*	Las Presillas Spring, Alcalá de los Gazules	Cádiz	Andalucía	Spain	6.55	890	Fernando García, Félix Ríos	15/06/2018
36.460230	-5.614306	399.71	FW2583	*M. similis*	El Berrueco Spring	Cádiz	Andalucía	Spain	N/A	4034	Félix Ríos	29/07/2018
37.104117	-3.725750	725.33	FW2347	*M. similis*	Spring in La Malahá	Granada	Andalucía	Spain	7.6	3890	Amanda Aguado, Miguel Carrillo, Jonathan Miller	08/04/2017
37.104150	-3.724400	715.22	FW2348	*M. similis*	Fuente la Pucha Spring, La Malahá	Granada	Andalucía	Spain	7.38	3190	Amanda Aguado, Miguel Carrillo, Jonathan Miller	08/04/2017
43.510350	5.119283	0.92	FW2474	*M. similis*	Arc River near Les Cabanes	Bouches-du-Rhône	Provence-Alpes-Côte D'azur	France	7.51	28900	Jonathan Miller	02/08/2017
43.454920	-1.399673	47.64	FW2593	*M. tachoensis*	Stream near Chemin d'Elizaberry, Mouguerre	Pyrénées-Atlantiques	Aquitaine	France	7.33	592	Miguel Carrillo, Fernando García, Jonathan Miller	29/07/2018

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
43.463483	-5.466433	38.37	FW2449	*M. tachoensis*	Fuente Tebia Spring, Asturias	Camoca	Asturias	Spain	7.44	1805	Miguel Carrillo, Jonathan Miller	14/07/2017
40.198933	-8.432283	23.84	FW2477	*M. tachoensis*	Fonte dos Amores Spring, Quinta das Lagrimas	Coimbra	Coimbra	Portugal	7.41	823	Miguel Carrillo, Jonathan Miller	11/08/2017
40.209117	-8.417617	89.44	FW2478	*M. tachoensis*	Fonte da Nogueira Spring	Coimbra	Coimbra	Portugal	7.61	359	Miguel Carrillo Jonathan Miller	11/08/2017
39.628250	-8.958333	38.30	FW2480	*M. tachoensis*	Nascente Sr. Jordão Spring, Alpedriz	Leiria	Leiria	Portugal	7.34	431	Miguel Carrillo, Jonathan Miller	11/08/2017
39.628633	-8.958183	41.22	FW2481	*M. tachoensis*	Nascente da Moura Spring, Alpedriz	Leiria	Leiria	Portugal	7.01	434	Miguel Carrillo, Jonathan Miller	11/08/2017
39.429900	-9.094700	35.81	FW2482	*M. tachoensis*	Spring in Freguesia de Salir de Matos, Caldas da Rainha	Leiria	Leiria	Portugal	7.66	609	Miguel Carrillo, Rui Manuel da Costa Mendes, Jonathan Miller	12/08/2017
39.351450	-9.055533	146.39	FW2483	*M. tachoensis*	Padre Antonio Spring in São Gregorio de Fanadia, Caldas de Rainha	Leiria	Leiria	Portugal	7.29	883	Miguel Carrillo, Rui Manuel da Costa, Jonathan Miller	12/08/2017

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
39.324750	-9.178800	27.78	FW2486	*M. tachoensis*	Spring in São Mamede	Leiria	Leiria	Portugal	7.4	949	Miguel Carrillo, Rui Manuel da Costa, Jonathan Miller	12/08/2017
39.264367	-8.776767	24.10	FW2484	*M. tachoensis*	Spring beside the road N114 at Vila Nova da Babeca	Santarém	Santarém	Portugal	7.61	754	Miguel Carrillo Pacheco, Rui Manuel da Costa, Jonathan Miller	12/08/2017
39.175700	-8.865533	93.66	FW2485	*M. tachoensis*	Spring in Ereira	Santarém	Santarém	Portugal	6.83	663	Miguel Carrillo, Rui Manuel da Costa, Jonathan Miller	12/08/2017
39.091085	-9.211042	98.69	FW2420	*M. tachoensis*	Paserihnos Spring, Matacães	Lisboa	Lisboa	Portugal	7.2	1130	Miguel Carrillo, Rui Manuel da Costa, Jonathan Miller	03/06/2017
39.193400	-9.062200	265.50	FW2487	*M. tachoensis*	Buddle in Pragança	Lisboa	Lisboa	Portugal	7.27	441	Miguel Carrillo, Rui Manuel da Costa, Jonathan Miller	12/08/2017
38.938233	-9.387900	48.56	FW2489	*M. tachoensis*	Fonte dos Tritões Spring, Mafra	Lisboa	Lisboa	Portugal	8.14	638	Miguel Carrillo Pacheco, Rui Manuel da Costa, Jonathan Miller	13/08/2017
38.933633	-9.406533	79.14	FW2490	*M. tachoensis*	Spring in Valbom, Carvoeira	Lisboa	Lisboa	Portugal	7.53	750	Miguel Carrillo, Rui Manuel da Costa, Jonathan Miller	13/08/2017

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
38.925800	-9.339083	127.99	FW2491	*M. tachoensis*	Fonte da Ribeira Spring, Ribeira de Maciel Forro, Mafra	Lisboa	Lisboa	Portugal	7.35	1089	Miguel Carrillo, Rui Manuel da Costa Jonathan Miller	13/08/2017
38.824950	-9.273300	271.17	FW2492	*M. tachoensis*	Spring in Vale de Lobos	Lisboa	Lisboa	Portugal	7.52	466	Miguel Carrillo Pacheco, Rui Manuel da Costa Mendes, Jonathan Miller	13/08/2017
38.511200	-9.026200	105.86	FW2493	*M. tachoensis*	Fonte de Oleiros Spring	Setúbal	Setúbal	Portugal	7.69	675	Miguel Carrillo, Jonathan Miller	13/08/2017
37.555250	-7.533450	12.49	FW2502	*M. tachoensis*	Guadiana river banks, Baixo Alentejo	Beja	Beja	Portugal	N/A	N/A	Geraldine A. Holyoak	18/04/2014
51.867026	4.336805	1.44	UGSB23178	*M. tachoensis*	Wet reed land, small stream, Hoogvliet	Zuid-Holland	Zuid-Holland	Netherlands	N/A	N/A	N/A	N/A
51.855717	4.347396	1.60	UGSB23179	*M. tachoensis*	Junction of the running surface in the tidal area, Hoogvliet	Zuid-Holland	Zuid-Holland	Netherlands	N/A	N/A	N/A	N/A
N/A	N/A	N/A	UGSB23177	*M. tachoensis*	Ruigeplaatbosch Park, wet reed land, Oude Maas River	Zuid-Holland	Zuid-Holland	Netherlands	N/A	N/A	N/A	N/A

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
38.961783	-9.415383	36.03	FW2488	*M. tachoensis*	Fonte do Cabo Spring, Ericeira	Lisboa	Lisboa	Portugal	7.53	1118	Miguel Carrillo, Rui Manuel da Costa, Jonathan Miller	12/08/2017
43.444333	-1.585850	54.10	FW2596	*M. tachoensis*	Stream in Bidart	Pyrénées-Atlantiques	Aquitaine	France	7.67	660	Miguel Carrillo, Fernando García, Jonathan Miller	29/07/2018
50.860060	-0.547830	0.63	UGSB 21169	*M. tachoensis*	Stream outflowing from Swanbourne Lake, Arundel	West Sussex	England	United Kingdom	N/A	N/A	Dietrich Kadolsky	04/09/2013
51.529330	0.074560	5.81	UGSB 21193	*M. tachoensis*	Reed belt of Hand Trough Creek (tributary of River Roding), high intertidal	Barking and Dagenham	England	United Kingdom	N/A	N/A	Dietrich Kadolsky	31/08/2013
N/A	N/A	N/A	FW2669	*M. tachoensis*	Arun river banks, Burpharm	West Sussex	England	United Kingdom	N/A	N/A	Martin Willing	21/11/2018
33.053450	-5.414690	1248.09	UGSB 17912	*M. targouasensis*	Oum Rbii Spring, Khenifra,	N/A	Centre-South	Morocco	N/A	N/A	Torsten Hauffe, Diana Delicado	02/06/2015
33.052730	-5.415000	1258.33	UGSB 16943	*M. targouasensis*	Spring flowing into Oued Oum Rbii	N/A	Centre-South	Morocco	N/A	N/A	Torsten Hauffe, Diana Delicado	02/06/2015
29.271944	-10.019444	346.15	FW2258	*M. targouasensis*	Arbáa Mesti	N/A	South	Morocco	N/A	N/A	Alberto Sánchez	13/12/2015

Latitude	Longitude	Elevation	Code	Species	Locality	Province	Region	Country	pH	Conductivity (mS/cm^2)	Collector	Date
28.133597	-10.398539	493.67	FW2283	*M. targouasensis*	Oued Assaka spring, Oued Noun	N/A	South	Morocco	N/A	N/A	Alberto Sánchez	13/08/2016
29.583529	-10.030107	41.37	UGSB 17955	*M. targouasensis*	Irrigation channel North Mirheleft	N/A	South	Morocco	N/A	N/A	Khadija Boulaassafer, Mohamed Ghamizi	02/02/2015
31.632500	-9.584722	124.82	UGSB 17918	*M. tensiftensis*	Pond near Lahjar Spring, Essaouira	N/A	Tensift	Morocco	N/A	N/A	Khadija Boulaassafer, Mohamed Ghamizi	28/11/2015
31.493556	-8.785194	409.45	UGSB 17910	*M. tensiftensis*	Ditch in Sidi Bouzid, near Chichaoua, S of Marrakech	N/A	Tensift	Morocco	N/A	N/A	Khadija Boulaassafer, Mohamed Ghamizi	28/11/2015
31.384861	-8.127358	598.89	UGSB 19944	*M. tensiftensis*	Ditch in Agadir N'tachraft	N/A	Tensift	Morocco	N/A	N/A	Khadija Boulaassafer, Mohamed Ghamizi	20/02/2017
31.375819	-8.127308	608.83	UGSB 19945	*M. tensiftensis*	Spring near Lalla Takerkoust Dam, Marrakech	N/A	Tensift	Morocco	N/A	N/A	Khadija Boulaassafer, Mohamed Ghamizi	20/02/2017
31.676292	-7.267164	770.89	UGSB 19946	*M. tensiftensis*	Ditch in Talkount, NE of Marrakech	N/A	Centre	Morocco	N/A	N/A	Khadija Boulaassafer	21/02/2017
31.632500	-9.584972	122.00	UGSB 17918	*M. tensiftensis*	Ditch in Haddada Bouzerktoun, Essaouira	N/A	Tensift	Morocco	N/A	N/A	Khadija Boulaassafer	28/11/2015

Appendix 5. Summary of the Genetic uncorrected pairwise distances

COI

		1	2	3	4	5	6	7	8	9	10	11	12	13
M. balearica	**1**													
M. egarensis sp. nov.	**2**	8.1												
M. carrillorum sp. nov.	**3**	7.9	1.3											
M. similis	**4**	7.5	3.7	3.8										
M. tachoensis	**5**	7.5	7.7	7.9	8									
M. felixi sp. nov.	**6**	5.3	8.2	8	7.3	7.4								
M. lupiaensis sp. nov.	**7**	7.6	7.4	7.9	7.3	8.1	8							
M. targouasensis	**8**	7.2	8.2	8.3	7.9	7.6	6.8	8.4						
M. midarensis	**9**	8.6	8.3	8.2	8.8	8.5	8	9.3	4					
M. tensiftensis	**10**	5.2	9	8.7	7.9	8.1	5.1	8.6	6.2	7.1				
M. melitensis	**11**	8.6	9	8.8	8.5	8.9	7.9	7.8	7.3	8.5	8.2			
M. veronicae sp. nov.	**12**	7.7	7.4	7.7	7.4	7.5	7.3	3.9	7.4	8.3	8.4	6.2		
M. saharica	**13**	8.3	8.8	8.9	8.3	9.1	8.6	7.3	8.1	9.1	8.6	7.4	7.2	

16S

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
M. tachoensis	**1**														
M. balearica	**2**	2.5													
M. egarensis sp. nov.	**3**	2.2	2.8												
M. carrillorum sp. nov.	**4**	2.4	3	0.2											
M. similis	**5**	3.1	3.6	1.3	1.6										
M. felixi sp. nov.	**6**	2.4	1.8	2.7	2.9	3.7									
M. lupiaensis sp. nov.	**7**	3.7	4.6	3.5	3.7	4	4.2								
M. targouasensis	**8**	3.2	4.1	3	3.2	3.6	3.6	4.5							
M. midarensis	**9**	3.1	4.2	2.8	3	3.4	3.6	4.1	2.1						
M. tensiftensis	**10**	2.9	2.4	2.7	2.9	3.3	1.8	4.1	3.8	3.7					
M. melitensis	**11**	3.2	3.7	2.1	2.2	2.7	3.6	3	3.1	3.2	3.2				
M. rolani	**12**	3.5	3.7	2	2.1	2.6	4	2.9	3.6	3.8	3.3	0.7			
M. veronicae sp. nov.	**13**	3.7	4.8	3.6	3.8	4.1	4.3	1.4	4.4	4.3	3.8	3.2	3.2		
M. saharica	**14**	3.5	3.7	3.3	3.5	4.3	4	4.3	4.2	4.1	4	3.5	3.5	4.4	

28S

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
M. balearica	**1**														
M. egarensis sp. nov.	**2**	0.6													
M. carrillorum sp. nov.	**3**	0.5	0.3												
M. similis	**4**	0.6	0.5	0.5											
M. tachoensis	**5**	0.7	0.5	0.5	0.6										
M. felixi sp. nov.	**6**	0.6	0.2	0.2	0.5	0.5									
M. lupiaensis sp. nov.	**7**	0.9	0.6	0.7	0.7	0.5	0.6								
M. targouasensis	**8**	1.2	1.2	1.2	1.2	1.3	1.2	1.3							
M. midarensis	**9**	1.6	1.3	1.3	1.3	1.2	1.3	1.3	1.4						
M. tensiftensis	**10**	0.5	0.6	0.5	0.6	0.7	0.6	0.9	1.3	1.6					
M. melitensis	**11**	0.9	0.5	0.6	0.8	0.7	0.5	0.6	1.4	1.3	0.9				
M. rolani	**12**	0.7	0.8	0.8	0.8	0.9	0.8	1.1	1.6	1.8	0.4	1.1			
M. veronicae sp. nov.	**13**	0.9	0.7	0.7	0.8	0.5	0.7	0.4	1.4	1.3	0.9	0.7	1.1		
M. saharica	**14**	0.9	0.7	0.7	0.8	0.6	0.7	0.4	1.1	1.2	0.9	0.7	1.1	0.5	

Appendix 6. Descriptive statistics such as mean, standard deviation and minimum and maximum values were used to summarise intra- and interspecific variation of the shell.

Species	Code	N	SL	SW	AL	AW	AH	LBW	WBW	WAW	WPW
M. similis	FW2347	n=14	1.9 ± 0.19; 0.1 (2.27-1.51)	1.5 ± 0.12; 0.08 (1.7-1.28)	1.43 ± 0.13; 0.09 (1.66-1.18)	1.21 ± 0.1; 0.08 (1.35-1.02)	0.97 ± 0.07; 0.07 (1.05-0.83)	0.95 ± 0.07; 0.07 (1.05-0.82)	0.71 ± 0.06; 0.08 (0.82-0.59)	0.67 ± 0.06; 0.09 (0.8-0.54)	0.36 ± 0.05; 0.14 (0.46-0.28)
M. similis	FW2348	n=14	2.28 ± 0.12; 0.05 (2.51-1.97)	1.77 ± 0.1; 0.06 (1.96-1.57)	1.73 ± 0.11; 0.06 (1.89-1.46)	1.4 ± 0.1; 0.07 (1.61-1.18)	1.16 ± 0.08; 0.07 (1.26-0.98)	1.12 ± 0.08; 0.07 (1.21-0.94)	0.85 ± 0.07; 0.08 (0.97-0.73)	0.78 ± 0.06; 0.08 (0.93-0.68)	0.42 ± 0.03; 0.07 (0.48-0.37)
M. similis	FW2352	n=17	2.32 ± 0.2; 0.09 (2.65-1.98)	1.8 ± 0.15; 0.08 (2.06-1.45)	1.72 ± 0.14; 0.08 (1.96-1.42)	1.43 ± 0.11; 0.08 (1.63-1.25)	1.15 ± 0.09; 0.08 (1.31-1)	1.13 ± 0.08; 0.07 (1.29-1)	0.86 ± 0.07; 0.08 (0.95-0.74)	0.81 ± 0.07; 0.09 (0.94-0.66)	0.45 ± 0.06; 0.13 (0.56-0.37)
M. similis	FW2404	n=38	3.71 ± 0.3; 0.08 (4.32 - 3.26)	2.87 ± 0.17; 0.06 (3.18 - 2.49)	1.85 ± 0.12; 0.06 (2.09 - 1.62)	1.31 ± 0.09; 0.07 (1.46 - 1.18)	1.84 ± 0.12; 0.07 (2.07 - 1.55)	2.75 ± 0.18; 0.07 (3.03 - 2.41)	2.22 ± 0.14; 0.06 (2.5 - 1.97)	1.29 ± 0.13; 0.1 (1.62 - 1.07)	0.69 ± 0.09; 0.13 (0.84 - 0.47)
M. similis	FW2405	n=24	3.8 ± 0.28; 0.07 (4.37-3.2)	3.11 ± 0.23; 0.07 (3.57-2.6)	2.01 ± 0.12; 0.06 (2.29-1.72)	1.4 ± 0.12; 0.09 (1.66-1.13)	2.03 ± 0.11; 0.05 (2.3-1.72)	2.93 ± 0.2; 0.07 (3.44-2.51)	2.4 ± 0.14; 0.06 (2.64-2.1)	1.31 ± 0.1; 0.08 (1.48-1.06)	0.66 ± 0.07; 0.11 (0.8-0.47)
M. similis	FW2406	n=34	3.57 ± 0.68; 0.19 (4.61-2.33)	2.68 ± 0.76; 0.28 (4.6-1.77)	2.08 ± 0.41; 0.2 (4.12-1.74)	2.11 ± 0.39; 0.18 (3.33-1.67)	2.77 ± 0.52; 0.19 (3.53-1.34)	2.86 ± 0.65; 0.23 (3.65-1.2)	1.7 ± 0.63; 0.37 (3.31-1.23)	0.8 ± 0.14; 0.18 (1.36-0.55)	1.42 ± 0.17; 0.12 (1.65-0.71)
M. similis	FW2411	n=35	4.05 ± 0.35; 0.09 (4.78-3.41)	3.08 ± 0.22; 0.07 (3.42-2.55)	2 ± 0.12; 0.06 (2.25-1.76)	1.49 ± 0.1; 0.07 (1.68-1.24)	2 ± 0.12; 0.06 (2.2-1.68)	2.99 ± 0.21; 0.07 (3.4-2.52)	2.37 ± 0.17; 0.07 (2.67-2.03)	1.4 ± 0.15; 0.11 (1.7-1.18)	0.79 ± 0.1; 0.13 (1.01-0.57)
M. similis	FW2412	n=30	4.01 ± 0.25; 0.06 (4.57-3.6)	3.23 ± 0.2; 0.06 (3.57-2.85)	2.07 ± 0.11; 0.05 (2.33-1.78)	1.56 ± 0.1; 0.06 (1.7-1.32)	2.08 ± 0.11; 0.05 (2.3-1.8)	3.04 ± 0.18; 0.06 (3.41-2.63)	2.46 ± 0.15; 0.06 (2.86-2.22)	1.35 ± 0.13; 0.1 (1.67-1.07)	0.72 ± 0.09; 0.13 (0.99-0.56)
M. similis	FW2413	n=38	3.85 ± 0.33; 0.09 (4.84-3.35)	3.09 ± 0.25; 0.08 (3.75-2.74)	1.95 ± 0.13; 0.07 (2.21-1.67)	1.97 ± 0.16; 0.08 (2.26-1.4)	1.48 ± 0.19; 0.13 (2.25-1.28)	2.91 ± 0.24; 0.08 (3.5-2.49)	2.33 ± 0.16; 0.07 (2.79-1.95)	1.36 ± 0.14; 0.1 (1.7-1.14)	0.71 ± 0.11; 0.15 (1.05-0.55)
M. similis	FW2434	n=37	3.55 ± 0.27; 0.08 (4.18-3.08)	2.84 ± 0.2; 0.07 (3.33-2.45)	2.65 ± 0.2; 0.08 (3.14-2.3)	2.16 ± 0.16; 0.07 (2.51-1.88)	1.83 ± 0.13; 0.07 (2.15-1.53)	1.79 ± 0.13; 0.07 (2.14-1.48)	1.35 ± 0.09; 0.07 (1.62-1.14)	1.23 ± 0.11; 0.09 (1.47-1.04)	0.67 ± 0.06; 0.09 (0.82-0.53)
M. similis	FW2435	n=22	3.71 ± 0.26; 0.07 (4.26-3.26)	3.09 ± 0.18; 0.06 (3.41-2.71)	2.88 ± 0.16; 0.06 (3.19-2.59)	2.28 ± 0.13; 0.06 (2.52-1.96)	1.95 ± 0.11; 0.06 (2.14-1.7)	1.9 ± 0.11; 0.06 (2.11-1.66)	1.48 ± 0.09; 0.06 (1.66-1.36)	1.28 ± 0.1; 0.08 (1.54-1.13)	0.65 ± 0.09; 0.14 (0.85-0.49)
M. similis	FW2436	n=35	3.74 ± 0.37; 0.1 (4.74-3.1)	3.01 ± 0.23; 0.08 (3.66-2.63)	1.88 ± 0.17; 0.09 (2.23-1.59)	1.93 ± 0.17; 0.09 (2.3-1.62)	1.4 ± 0.12; 0.09 (1.69-1.18)	2.79 ± 0.23; 0.08 (3.31-2.42)	2.27 ± 0.17; 0.07 (2.75-2.02)	1.34 ± 0.13; 0.1 (1.67-1.16)	0.71 ± 0.08; 0.11 (0.86-0.56)
M. similis	FW2437	n=8	3.79 ± 0.38; 0.1 (4.33-3.15)	3.06 ± 0.32; 0.1 (3.6-2.52)	2.86 ± 0.29; 0.1 (3.28-2.34)	2.32 ± 0.19; 0.08 (2.59-1.95)	1.91 ± 0.18; 0.09 (2.12-1.57)	1.82 ± 0.19; 0.1 (2.07-1.49)	1.38 ± 0.16; 0.12 (1.56-1.13)	1.26 ± 0.12; 0.1 (1.41-1.02)	0.66 ± 0.07; 0.11 (0.74-0.55)
M. similis	FW2440	n=16	3.64 ± 0.38; 0.1 (4.35-2.98)	2.93 ± 0.26; 0.09 (3.46-2.53)	1.67 ± 0.1; 0.06 (1.87-1.52)	1.71 ± 0.09; 0.05 (1.9-1.57)	1.31 ± 0.09; 0.07 (1.48-1.14)	2.65 ± 0.22; 0.08 (3.05-2.3)	2.21 ± 0.2; 0.09 (2.53-1.85)	1.31 ± 0.15; 0.11 (1.52-1.04)	0.73 ± 0.13; 0.18 (0.91-0.46)

Species	Code	N	SL	SW	AL	AW	AH	LBW	WBW	WAW	WPW
M. similis	FW2474	n=20	4.05 ± 0.33; 0.08 (4.7-3.48)	3.27 ± 0.26; 0.08 (3.79-2.73)	2 ± 0.18; 0.09 (2.39-1.7)	2.07 ± 0.19; 0.09 (2.44-1.77)	1.48 ± 0.15; 0.1 (1.72-1.15)	3.04 ± 0.25; 0.08 (3.62-2.56)	2.49 ± 0.17; 0.07 (2.86-2.17)	1.43 ± 0.11; 0.08 (1.64-1.22)	0.76 ± 0.08; 0.11 (0.91-0.6)
M. similis	FW2673	n=8	3.76 ± 0.21; 0.06 (3.99-3.39)	3.04 ± 0.15; 0.05 (3.23-2.81)	1.91 ± 0.08; 0.04 (2.06-1.81)	1.93 ± 0.08; 0.04 (2.04-1.8)	1.43 ± 0.12; 0.08 (1.62-1.26)	2.8 ± 0.17; 0.06 (3.1-2.6)	2.26 ± 0.12; 0.05 (2.47-2.09)	1.32 ± 0.1; 0.08 (1.46-1.18)	0.69 ± 0.08; 0.12 (0.81-0.6)
M. tachoensis	FW2420	n=30	3.15 ± 0.16; 0.05 (3.43-2.82)	2.29 ± 0.09; 0.04 (2.5-2.12)	2.24 ± 0.09; 0.04 (2.44-2.09)	1.77 ± 0.09; 0.05 (1.98-1.57)	1.54 ± 0.07; 0.05 (1.67-1.37)	1.51 ± 0.07; 0.05 (1.64-1.36)	1.14 ± 0.05; 0.04 (1.26-1.01)	1.08 ± 0.05; 0.05 (1.19-0.97)	0.61 ± 0.05; 0.09 (0.75-0.51)
M. tachoensis	FW2477	n=30	2.9 ± 0.19; 0.07 (3.35-2.48)	2.08 ± 0.12; 0.06 (2.38-1.86)	2.04 ± 0.13; 0.06 (2.37-1.71)	1.61 ± 0.09; 0.06 (1.82-1.47)	1.36 ± 0.09; 0.06 (1.55-1.11)	1.34 ± 0.08; 0.06 (1.53-1.1)	1.06 ± 0.06; 0.06 (1.19-0.93)	0.98 ± 0.07; 0.07 (1.11-0.87)	0.61 ± 0.06; 0.1 (0.73-0.51)
M. tachoensis	FW2478	n=18	2.88 ± 0.26; 0.09 (3.3-2.3)	2.1 ± 0.16; 0.08 (2.36-1.79)	2.06 ± 0.15; 0.07 (2.31-1.77)	1.62 ± 0.11; 0.07 (1.78-1.37)	1.36 ± 0.11; 0.08 (1.55-1.12)	1.35 ± 0.12; 0.09 (1.53-1.06)	1.05 ± 0.08; 0.07 (1.15-0.87)	0.97 ± 0.08; 0.08 (1.1-0.79)	0.58 ± 0.08; 0.13 (0.71-0.42)
M. tachoensis	FW2481	n=31	3.19 ± 0.2; 0.06 (3.58-2.8)	2.25 ± 0.13; 0.06 (2.51-1.95)	2.21 ± 0.13; 0.06 (2.48-1.9)	1.71 ± 0.1; 0.06 (1.91-1.53)	1.48 ± 0.08; 0.06 (1.68-1.34)	1.45 ± 0.09; 0.06 (1.63-1.29)	1.11 ± 0.06; 0.05 (1.22-0.98)	1.06 ± 0.07; 0.06 (1.17-0.93)	0.63 ± 0.05; 0.08 (0.73-0.53)
M. tachoensis	FW2482	n=7	2.86 ± 0.23; 0.08 (3.21-2.55)	2.13 ± 0.14; 0.07 (2.36-1.94)	2.1 ± 0.14; 0.07 (2.35-1.94)	1.71 ± 0.12; 0.07 (1.89-1.53)	1.44 ± 0.08; 0.06 (1.56-1.34)	1.42 ± 0.09; 0.06 (1.56-1.31)	1.06 ± 0.08; 0.07 (1.18-0.97)	1.02 ± 0.08; 0.08 (1.14-0.93)	0.55 ± 0.08; 0.14 (0.65-0.39)
M. tachoensis	FW2483	n=24	3.18 ± 0.26; 0.08 (3.69-2.84)	2.46 ± 0.17; 0.07 (2.78-2.22)	2.37 ± 0.18; 0.08 (2.71-2.09)	1.92 ± 0.13; 0.07 (2.15-1.64)	1.64 ± 0.11; 0.07 (1.83-1.41)	1.62 ± 0.12; 0.07 (1.82-1.4)	1.18 ± 0.09; 0.08 (1.36-1.04)	1.13 ± 0.09; 0.08 (1.33-1.01)	0.6 ± 0.06; 0.1 (0.75-0.52)
M. tachoensis	FW2484	n=33	3 ± 0.18; 0.06 (3.28-2.61)	2.18 ± 0.11; 0.05 (2.33-1.9)	2.11 ± 0.1; 0.05 (2.33-1.85)	1.71 ± 0.09; 0.05 (1.85-1.52)	1.42 ± 0.06; 0.04 (1.49-1.26)	1.38 ± 0.06; 0.04 (1.45-1.22)	1.09 ± 0.07; 0.07 (1.26-0.96)	0.99 ± 0.05; 0.05 (1.16-0.89)	0.64 ± 0.06; 0.09 (0.74-0.53)
M. tachoensis	FW2485	n=22	3.32 ± 0.23; 0.07 (3.7-2.87)	2.35 ± 0.15; 0.06 (2.58-2)	2.29 ± 0.15; 0.07 (2.54-2)	1.82 ± 0.12; 0.06 (2.12-1.64)	1.53 ± 0.1; 0.07 (1.71-1.3)	1.5 ± 0.1; 0.07 (1.67-1.27)	1.2 ± 0.09; 0.07 (1.42-1.05)	1.11 ± 0.09; 0.08 (1.29-0.93)	0.68 ± 0.07; 0.11 (0.82-0.54)
M. tachoensis	FW2489	n=23	2.09 ± 0.48; 0.23 (3.44-1.59)	1.56 ± 0.3; 0.19 (2.42-1.28)	0.99 ± 0.19; 0.19 (1.58-0.78)	1.03 ± 0.19; 0.18 (1.63-0.82)	0.72 ± 0.14; 0.2 (1.1-0.54)	1.55 ± 0.34; 0.22 (2.48-1.21)	1.17 ± 0.22; 0.18 (1.78-0.93)	0.74 ± 0.15; 0.21 (1.17-0.53)	0.4 ± 0.09; 0.23 (0.66-0.28)
M. tachoensis	FW2669	n=18	3.45 ± 0.22; 0.06 (3.95-3.18)	2.49 ± 0.13; 0.05 (2.84-2.26)	1.54 ± 0.07; 0.05 (1.68-1.45)	1.59 ± 0.08; 0.05 (1.75-1.49)	1.16 ± 0.07; 0.06 (1.33-0.99)	2.46 ± 0.13; 0.05 (2.82-2.27)	1.92 ± 0.12; 0.06 (2.26-1.77)	1.2 ± 0.11; 0.09 (1.49-1.08)	0.7 ± 0.07; 0.1 (0.85-0.59)
M. balearica	FW2335	n=9	2.45 ± 0.16; 0.07 (2.73-2.22)	1.84 ± 0.1; 0.05 (2.03-1.71)	1.77 ± 0.09; 0.05 (1.93-1.64)	1.43 ± 0.08; 0.06 (1.58-1.3)	1.21 ± 0.08; 0.07 (1.32-1.09)	1.17 ± 0.1; 0.09 (1.32-1.01)	0.9 ± 0.05; 0.06 (1-0.82)	0.83 ± 0.06; 0.07 (0.97-0.76)	0.47 ± 0.05; 0.11 (0.56-0.42)
M. balearica	FW2351	n=5	2.75 ± 0.06; 0.02 (2.89-2.67)	2.1 ± 0.05; 0.02 (2.16-1.98)	2.07 ± 0.04; 0.02 (2.12-1.97)	1.71 ± 0.04; 0.02 (1.79-1.62)	1.28 ± 0.04; 0.03 (1.37-1.22)	1.24 ± 0.04; 0.03 (1.34-1.18)	1.03 ± 0.03; 0.03 (1.09-0.99)	0.94 ± 0.02; 0.02 (0.98-0.91)	0.56 ± 0.04; 0.07 (0.61-0.48)
M. balearica	FW2354	n=26	2.81 ± 0.15; 0.05 (3.29-2.47)	2.17 ± 0.1; 0.05 (2.51-1.92)	2.11 ± 0.1; 0.05 (2.48-1.87)	1.73 ± 0.09; 0.05 (2-1.5)	1.4 ± 0.06; 0.04 (1.64-1.23)	1.35 ± 0.06; 0.04 (1.54-1.2)	1.04 ± 0.06; 0.06 (1.23-0.89)	0.97 ± 0.06; 0.06 (1.16-0.84)	0.57 ± 0.05; 0.09 (0.69-0.43)

Species	Code	N	SL	SW	AL	AW	AH	LBW	WBW	WAW	WPW
M. balearica	FW2357	n=31	2.82 ± 0.15; 0.05 (3.3-2.43)	2.22 ± 0.1; 0.05 (2.59-2)	2.13 ± 0.09; 0.04 (2.52-1.92)	1.74 ± 0.08; 0.05 (2.03-1.55)	1.46 ± 0.05; 0.03 (1.69-1.31)	1.39 ± 0.05; 0.04 (1.6-1.23)	1.03 ± 0.05; 0.05 (1.18-0.92)	0.98 ± 0.05; 0.05 (1.09-0.86)	0.51 ± 0.04; 0.08 (0.62-0.4)
M. balearica	FW2395	n=17	2.84 ± 0.17; 0.06 (3.35-2.44)	2.21 ± 0.12; 0.05 (2.62-1.9)	2.14 ± 0.13; 0.06 (2.54-1.74)	1.77 ± 0.08; 0.05 (2.02-1.57)	1.46 ± 0.06; 0.04 (1.67-1.28)	1.4 ± 0.06; 0.04 (1.57-1.24)	1.08 ± 0.07; 0.06 (1.25-0.88)	0.99 ± 0.07; 0.07 (1.2-0.77)	0.53 ± 0.07; 0.13 (0.76-0.3)
M. balearica	FW2568	n=10	2.73 ± 0.21; 0.08 (3.08-2.42)	2.1 ± 0.13; 0.06 (2.32-1.91)	2.05 ± 0.14; 0.07 (2.27-1.88)	1.6 ± 0.11; 0.07 (1.84-1.46)	1.31 ± 0.08; 0.06 (1.4-1.16)	1.26 ± 0.09; 0.07 (1.36-1.1)	1 ± 0.07; 0.07 (1.1-0.86)	0.95 ± 0.07; 0.07 (1.08-0.86)	0.56 ± 0.06; 0.11 (0.69-0.46)
M. balearica	FW2604	n=13	3.1 ± 0.42; 0.14 (3.62-1.82)	1.64 ± 0.18; 0.11 (1.93-1.45)	1.8 ± 0.46; 0.26 (3.19-1.45)	2.04 ± 0.44; 0.22 (2.65-1.44)	1.82 ± 0.47; 0.26 (2.51-1.39)	1.32 ± 0.46; 0.35 (2.43-1.03)	2.21 ± 0.54; 0.24 (2.72-1.02)	0.6 ± 0.06; 0.1 (0.71-0.48)	1.14 ± 0.05; 0.04 (1.22-1.07)
M. maceana	MZB77-8445	N=27	4.51 ± 0.32; 0.07 (5.22-3.97)	3.43 ± 0.16; 0.05 (3.78-3.06)	2.26 ± 0.22; 0.1 (2.83-1.93)	2.33 ± 0.18; 0.08 (2.95-2.05)	1.63 ± 0.11; 0.07 (1.82-1.36)	3.45 ± 0.22; 0.06 (4.01-2.96)	2.58 ± 0.3; 0.12 (3.84-2.06)	1.53 ± 0.17; 0.11 (1.81-1.18)	0.83 ± 0.12; 0.14 (1.08-0.6)
M. egarensis sp. nov.	FW2439	n=32	3.58 ± 0.28; 0.08 (4.31-2.95)	2.5 ± 0.19; 0.08 (2.8-1.78)	1.71 ± 0.15; 0.09 (1.93-1.28)	1.46 ± 0.39; 0.27 (2.68-0.92)	1.44 ± 0.24; 0.17 (1.85-1.09)	2.75 ± 0.21; 0.08 (3.33-2.4)	2.13 ± 0.16; 0.07 (2.46-1.8)	1.35 ± 0.19; 0.14 (2.19-1.12)	0.69 ± 0.08; 0.12 (0.9-0.54)
M. carrillorum sp. nov.	FW2542	n=18	3.33 ± 0.21; 0.06 (3.82-2.84)	2.42 ± 0.18; 0.07 (2.77-2)	1.5 ± 0.11; 0.07 (1.66-1.23)	1.47 ± 0.12; 0.08 (1.65-1.2)	1.08 ± 0.08; 0.07 (1.25-0.86)	2.33 ± 0.17; 0.07 (2.67-1.94)	1.8 ± 0.11; 0.06 (2.05-1.57)	1.19 ± 0.08; 0.07 (1.36-1.02)	0.66 ± 0.06; 0.09 (0.78-0.57)
M. carrillorum sp. nov.	FW2543	n=33	2.87 ± 0.28; 0.1 (3.54-2.4)	2.18 ± 0.17; 0.08 (2.5-1.84)	1.36 ± 0.1; 0.07 (1.55-1.17)	1.4 ± 0.09; 0.06 (1.59-1.2)	1.05 ± 0.1; 0.1 (1.24-0.89)	2.11 ± 0.19; 0.09 (2.48-1.78)	1.68 ± 0.13; 0.08 (1.94-1.42)	0.98 ± 0.08; 0.08 (1.16-0.85)	0.55 ± 0.07; 0.13 (0.69-0.39)
M. lupiaensis sp. nov.	USGB16236	n=5	3.46 ± 0.15; 0.04 (3.71-3.3)	2.64 ± 0.06; 0.02 (2.7-2.56)	1.6 ± 0.07; 0.04 (1.71-1.54)	1.62 ± 0.09; 0.06 (1.77-1.52)	1.25 ± 0.05; 0.04 (1.32-1.19)	2.48 ± 0.12; 0.05 (2.65-2.35)	2.07 ± 0.09; 0.04 (2.21-1.98)	1.27 ± 0.1; 0.08 (1.39-1.13)	0.71 ± 0.06; 0.08 (0.78-0.61)
M. lupiaensis sp. nov.	USGB8015	n=5	3.24 ± 0.12; 0.04 (3.39-3.11)	2.56 ± 0.1; 0.04 (2.65-2.46)	1.52 ± 0.17; 0.11 (1.79-1.37)	1.6 ± 0.14; 0.09 (1.81-1.43)	1.19 ± 0.1; 0.08 (1.29-1.08)	2.44 ± 0.1; 0.04 (2.56-2.33)	2.04 ± 0.08; 0.04 (2.11-1.93)	1.19 ± 0.04; 0.03 (1.23-1.14)	0.64 ± 0.04; 0.06 (0.7-0.6)
M. felixi sp. nov.	FW2685	n=34	3.21 ± 0.31; 0.1 (3.83-2.57)	2.48 ± 0.2; 0.08 (2.9-2.05)	1.59 ± 0.12; 0.08 (2-1.38)	1.62 ± 0.09; 0.06 (1.85-1.45)	1.15 ± 0.12; 0.1 (1.42-0.94)	2.4 ± 0.25; 0.1 (2.82-1.41)	1.85 ± 0.19; 0.1 (2.11-0.95)	1.1 ± 0.12; 0.11 (1.38-0.83)	0.61 ± 0.08; 0.13 (0.76-0.46)
M. veronicae sp. nov.	USGB17271	n=6	4.62 ± 0.43; 0.09 (5.27-4.19)	3.37 ± 0.43; 0.13 (4.06-2.94)	1.98 ± 0.2; 0.1 (2.3-1.77)	2.03 ± 0.19; 0.09 (2.34-1.87)	1.57 ± 0.16; 0.1 (1.81-1.42)	3.39 ± 0.42; 0.12 (4.13-2.95)	2.72 ± 0.33; 0.12 (3.25-2.38)	1.72 ± 0.14; 0.08 (1.92-1.56)	0.88 ± 0.11; 0.13 (1.02-0.73)

Appendix 7. Global species richness of hydrobiid snails determined by climate and evolutionary history

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Global species richness of hydrobiid snails determined by climate and evolutionary history

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Abstract

1. Efforts to describe spatial patterns of freshwater diversity and to evaluate their underlying factors have traditionally been focused on some animal groups (e.g. amphibians, fish or dragonflies). Despite being a dominant component in continental aquatic ecosystems and crucial for determining priority areas for conservation, broad-scale data on gastropod total species, endemic and threatened species richness are limited. Based on these biodiversity indices, we identify global hotspots, extinction risk along the elevational gradient and the drivers of species richness patterns in the largest group of freshwater gastropods, the family Hydrobiidae.
2. Given the strong dependency to a nonmarine aquatic environment, the observed richness patterns of extant hydrobiid species could be significantly influenced by large-scale geography and dispersal processes as well as climatic conditions affecting continental ecosystems. Therefore, we tested several predictions for species richness derived from ecological and evolutionary hypotheses postulated for other freshwater groups.
3. Based on a comprehensive literature and biodiversity database review, we compiled the number of total, endemic and threatened species per freshwater ecoregion. We classified ecoregions as hotspots if each biodiversity index was in the top 25% of its range and assessed the effect of 13 environmental and evolutionary factors on species richness using generalised linear models.
4. We identified 906 species and 157 genera of Hydrobiidae showing mainly a Nearctic–Palearctic distribution and 19 biodiversity hotspots, most located across the Mediterranean Basin. In our data set, 83% of the species were endemic to a single ecoregion. Of the 43% non-data deficient species, we found almost three times more threatened than non-threatened species, and extinction risk peaked at 1,500 m a.s.l. Species richness was unequally distributed over biogeographic realms, increased with higher connectivity among ecoregions, and was negatively related with annual temperature range. Latitude and precipitation seasonality explained part of the richness variation by a nonlinear relationship.
5. The identified hotspots correspond with those of other freshwater taxa. The hump-shaped relationship of extinction risk with elevation is likely the consequence of decreasing natural and anthropogenic perturbations at higher elevations. Global hotspots of Hydrobiidae richness represent areas of climatic stability with medium precipitation and temperature seasonality that are well

connected with other hydrological basins. Our results illustrate that both evolutionary and environmental factors determine these global patterns and that future changes of the latter factors may affect hydrobiid richness.

KEYWORDS
biodiversity, distribution, endemism, freshwater gastropods, threats

1 | INTRODUCTION

Maps of species richness and threats are considered fundamental tools in the field of biodiversity conservation to identify hotspots of diversity (Myers, 1988) and to explore the potential causes of their spatial variability. However, our knowledge of species richness and threat patterns is still limited to only a few taxonomic groups (Tisseuil et al., 2013), particularly for freshwater taxa compared with terrestrial faunas (Beck et al., 2012). Furthermore, even within freshwater groups, information is still biased in terms of geography and taxonomy (Dudgeon et al., 2006; Strayer & Dudgeon, 2010). Global species richness patterns of highly diverse freshwater groups, such as gastropods, are still poorly understood, although these invertebrates play an important role in ecosystem function (Johnson, Preston, Hoverman, & Richgels, 2013 and references therein) and show rapidly declining levels of diversity (Lydeard et al., 2004). However, describing global patterns of freshwater gastropod diversity presents enormous difficulties. The number of species is certainly underestimated as a considerable number of freshwater gastropod species are often (a) endemic to only a few square metres and can easily remain undetected during field surveys and (b) described based on shell characteristics alone, which can hide greater diversity as most of the species present featureless shells (Strong, Gargominy, Ponder, & Bouchet, 2008). Moreover, regions other than North America, Europe and Australia remain poorly inventoried (Lydeard et al., 2004).

Kabat and Hershler (1993) and Strong et al. (2008) estimated a number of ca. 1,000 species for Hydrobiidae Stimpson, 1865, one of the largest families of continental aquatic gastropods. This estimate suggests that Hydrobiidae hold up to a quarter of the total freshwater gastropod diversity; however, due to the aforementioned limitations in species detection and description, these authors also recognised that this value might depict only ca. 25% of the actual species diversity. However, until the application of molecular tools, brackish and freshwater snails were all typically classified as hydrobioids (= Hydrobiidae sensu *lato* [*s. l.*]; Davis, 1979) due to their small size (between 0.5 and 8 mm, maximally 15 mm) and featureless shells. This likely resulted in an overestimation of the family's actual species richness. During the last two decades, molecular data have been used to support taxonomic assignments and to infer evolutionary relationships among hydrobiid taxa. The modern sensu stricto (*s. str.*) family definition, based on morphological and molecular evidence (Criscione & Ponder, 2013; Wilke et al., 2001, 2013), recognised some former hydrobiid subfamilies, such as Cochliopinae, Lithoglyphinae and Moitessieriinae, as independent families outside Hydrobiidae (see Bouchet & Rocroi, 2005; Bouchet et al., 2017). Therefore, the taxon Hydrobiidae, as previously defined based on morphology alone, was not monophyletic. The family Hydrobiidae *s. str.* (herein referred to as hydrobiids) is currently more narrowly delimited and confined to the western Palearctic, eastern Nearctic, northern Neotropic and South Africa (Wilke et al., 2013).

Within this distribution range, hydrobiids are found in nearly all aquatic continental ecosystems, with the great majority of the species inhabiting spring and cave interstitial systems (Strong et al., 2008). These ecosystems contain a highly endemic hydrobiid fauna of poorly dispersing animals, requiring perennial waters and specific habitat characteristics. As a consequence, their distribution is often restricted to a single or a few sites, thus being vulnerable to habitat loss and fragmentation, which can lead to the extinction of species in extreme cases (e.g. Hershler, Ratcliffe, Liu, Lang, & Hay, 2014; Szarowska, 2006). Furthermore, stochastic natural processes and anthropogenic actions have profoundly and diversely impacted hydrobiid species (Collen et al., 2014; Cowie, Regnier, Fontaine, & Bouchet, 2017; Sisk, Launer, Switky, & Ehrlich, 1994), making the group a priority for conservation studies over the past two decades (e.g. Bouchet & Gargominy, 1998; Hershler, Liu, & Howard, 2014). As a result, 1,107 species of Hydrobiidae *s. l.* are reported in the IUCN Red List of Threatened Species (2017), of which 31 are listed as extinct and 526 (~50%) are considered at high risk of extinction. However, given the new definition of Hydrobiidae *s. str.* based on molecular evidence and the rapid grow of species discoveries with ca. 120 species of Hydrobiidae described during the last two decades (e.g. Arconada, Delicado, & Ramos, 2007; Delicado, Machordom, & Ramos, 2012; Glöer, Boeters, & Walther, 2015; Hershler, Liu, & Bradford, 2013; Radea, Parmakelis, Mourikis, & Triantis, 2013; Ramos, Arconada, & Moreno, 2000), estimates of species richness should be reexamined and adjusted accordingly. This calls for new investigations of global-scale patterns of hydrobiid species richness and endemicity, which will provide a baseline for macroecological studies and conservation actions.

Unlike for other taxonomic groups, global biogeographic studies of the Hydrobiidae family are scarce (e.g. Davis, 1982; Hershler & Liu, 2017; Lassen, 1975). However, regional-scale studies of hydrobiid diversity reported remarkable richness in spring systems on the Apennine, Iberian and Balkan peninsulas (Arconada & Ramos, 2003; Bodon, Manganelli, & Giusti, 2001; Radoman, 1983), the Great Basin in North America (Hershler, 1998), the ancient lakes Ohrid and Prespa on the Balkan Peninsula (Radoman, 1983) and in brackish environments such as the Caspian Sea (Vinarski & Kantor, 2017). These studies also

provide evidence for the restricted distribution ranges of the gastropods and question the taxonomic delimitation of widely distributed species. In fact, although few widespread hydrobiids exist (e.g. Hershler, Thompson, & Liu, 2011), there is genetic evidence that some of these taxa actually consist of a complex of cryptic, geographically limited species (Delicado & Ramos, 2012; Hershler, Liu, et al., 2014; Hershler, Ratcliffe, et al., 2014; Liu, Hershler, & Clift, 2003; Wilke & Pfenninger, 2002), with a few being old, unique phylogenetic clades (Khalloufi, Béjaoui, & Delicado, 2017). Further phylogeographic studies are therefore needed to reappraise the taxonomic status of several hydrobiid species and to identify targets for conservation strategies.

Given the dependency to a nonmarine aquatic environment, these observed richness patterns of extant hydrobiid species could be significantly influenced by large-scale geography and dispersal processes as well as climatic conditions affecting continental ecosystems. Variation in species richness of freshwater organisms is often associated with latitude (Abell et al., 2011; Georgopoulou, Neubauer, Harzhauser, Kroh, & Mandic, 2016; Hof, Brändle, & Brandl, 2008; Iversen, Jacobsen, & Sand-Jensen, 2016; Perez-Losada, Bond-Buckup, Jara, & Crandall, 2009; Pérez-Quintero, 2015), which is, in turn, related to climatic gradients. For instance, the stable temperature and precipitation conditions in the tropics are thought to increase net diversification for a wide range of taxa, resulting in increased species richness (Gaston, 2007; Oberdorff et al., 2011; Pyron & Wiens, 2013). Elevation is another potentially important geographic factor influencing species richness. A high number of freshwater endemic species is reported from higher elevations, presumably as the result of greater habitat specialisation and isolation affecting montane populations (Hughes & Eastwood, 2006; Steinbauer et al., 2016) and more stable climate conditions than in lower elevations (Jetz, Rahbek, & Colwell, 2004; Ohlemüller et al., 2008).

Distribution of species richness may also depend on regional factors, such as the ancestral geographic area of the group, dispersal limitations and habitat heterogeneity (Hortal, Triantis, Meiri, Thébault, & Sfenthourakis, 2009; Iversen et al., 2016). Considering that most hydrobiids are habitat specialists and poor dispersers, we expect to observe a positive relationship between connectivity among ecosystems and species richness, as shown in other organisms (Braaker, Obrist, Ghazoul, & Moretti, 2017; Ribera, Foster, & Vogler, 2003). Therefore, remote areas within the global distribution of hydrobiids or isolated territories, such as peninsulas or islands, might contain fewer species (see the "classic peninsula" effect of Simpson, 1964). As with habitat connectivity, habitat heterogeneity also increases species numbers due to more niche space. Indeed, a positive relationship between heterogeneity and diversity has been widely documented for a variety of groups (Hortal et al., 2009; Kohn & Walsh, 1994; Tews et al., 2004; Weisberg et al., 2014), including freshwater molluscs (Hauffe, Schultheiß, Van Bocxlaer, Prömmel, & Albrecht, 2014).

In this work, we examine diversity patterns and investigate factors that may explain the observed distribution of extinction risk and species richness in one of the most diverse groups of freshwater organisms. To do this, we first evaluated the global distribution of Hydrobiidae *s. str.* species, their endemism and threat status, to identify priority areas for conservation (i.e. biodiversity hotspots sensu Myers, 1988). Second, we determined the extinction risk along the elevational gradient. Third, we analysed the effect of large-scale climatic, geographic and regionally restricted evolutionary variables on global patterns of hydrobiid species richness. Our study provides the first large-scale data set of Hydrobiidae *s. str.* species distribution and suggests a set of determining factors to consider for future conservation assessments of freshwater molluscs.

2 | METHODS

2.1 | Quantifying Hydrobiidae *s. str.* species richness, endemism and threat status

To obtain the biodiversity measures analysed here, we generated a global database of the geographic distribution of extant Hydrobiidae *s. str.* species by conducting a comprehensive literature search for systematics and species distribution in the original descriptions (see Supporting Information Appendix S1). Renowned biodiversity websites including the International Union for Conservation of Nature (IUCN, 2017), the Pan-European Species Directories Infrastructure, PESI (de Jong et al., 2015), Fauna Europaea (Bank, 2017), the Integrated Taxonomic Information System, ITIS (Integrated Taxonomic Information System, 2010), the World Register of Marine Species, WORMS (Horton et al., 2017), MolluscaBase (MolluscaBase, 2016) and AnimalBase (AnimalBase, 2016) were used to avoid taxonomic incongruences and duplicated information caused by synonyms. Owing to the lack of detailed range maps for many species and precision of some of the type localities, the number of hydrobiid species per freshwater ecoregion (Abell et al., 2008) was used to quantify hydrobiid species richness. These ecoregions represent mainly drainage basins and were delineated based on freshwater vertebrate assemblages. Moreover, they were previously used as biogeographic units to calculate species richness and to create conservation plans for numerous freshwater invertebrate groups (Perez-Losada et al., 2009).

A list of species was compiled, registering their type locality coordinates, presence in ecoregions, endemism and conservation status. We defined endemic species as those occupying a single ecoregion. The conservation status of each species was assigned as one of the following Red List categories as defined by the IUCN (IUCN, 2017): Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT), Least Concern (LC) and Data Deficient (DD). Species not yet assessed by the IUCN were assigned as data deficient.

Biodiversity hotspots have been identified based on different operational criteria, for instance, considering the top 2.5% of grid cells or 5% of the total land area (Huang et al., 2016; Orme et al., 2005) of highest species richness. These thresholds may not be suitable for a low number of geographic units comprised of large areas, such as the freshwater ecoregions in this study. Therefore, we defined hotspots as the richest 25% of ecoregions in terms of species richness, endemics and threatened species (i.e. those in the categories CR, EN and VU) following Abell et al. (2011), who used the same geographic units as in this study. Additional scenarios (top 5%

and 10%) were applied to assess the efficiency of the selection. We mapped hydrobiid species richness, endemics, threatened species and hotspots in ArcGIS 10.4 (ESRI, 2011).

We evaluated the robustness of our hotspot identification against species misidentification and erroneous occurrence reports by randomly re-assigning 50% of the species of an ecoregion to other species and repeating the hotspot selection. A complete random species assignment is an unrealistic scenario because typically the regional species pool is considered by taxonomists and ecologists. We therefore sampled species from the same biogeographic realm with a probability inversely proportional to the geographic distance to the focal ecoregion. We run the simulation 1000 times in the R 3.3.2 statistical environment (R Core Team, 2017) with the distance among ecoregions calculated by the geosphere 1.5-5 package (Hijmans, 2016).

2.2 | Geographic and climatic data

We assessed the effect of 13 geographic and climatic predictors (see Supporting Information Appendix S2) on hydrobiid species richness. These variables constitute proxies for different geographic, ecological and evolutionary processes of species richness distribution.

Several geographic and climatic factors may explain variation in hydrobiid species richness at the global scale. Utilising the freshwater ecoregion maps published by Abell et al. (2008), we obtained the latitude and longitude of each region using the coordinates of their centroids by applying the functions Feature to Point and Calculate Geometry in ArcGIS. We used only the absolute values of latitude as a predictor of hydrobiid richness, whereas both measures were needed to evaluate the robustness of our statistical analyses (see below). We calculated ecoregion area by utilising the geosphere package. Latitude and area are not direct drivers of species richness but often are co-correlated with numerous ecological factors, such as primary productivity and habitat diversity, respectively. Based on the digital elevation model GMTED2010 with a spatial resolution of 30 s, we calculated the mean elevation of each ecoregion and its elevational range using 3D Analyst tools in ArcGIS. Bioclimatic variables were extracted following the same method but using the digital raster model from the WorldClim database (Hijmans, Cameron, & Parra, 2006) with a spatial resolution of 30 s for the variables BIO1 = Annual Mean Temperature, BIO7 = Annual Temperature Range, BIO12 = Annual Precipitation and BIO15 = Precipitation Seasonality. The annual mean temperature of the Last Glacial Maximum according to the CCSM4 climatic projection was sourced from the same database with a spatial resolution of 2.5 min.

Proxies for the influence of evolutionary and dispersal processes included the following smaller-scale factors: connectivity among ecoregions, the peninsula effect, the biogeographic affiliation (Holt et al., 2013; Udvardy, 1975) and habitat heterogeneity. As a measure of connectivity, we calculated the number of neighbours for each ecoregion using the rgdal 1.2-4 package for R (Bivand, Keitt, & Rowlingson, 2014). The peninsula effect was coded by zero (for ecoregions not on any peninsula) and one (for ecoregions on a peninsula). As a proxy for habitat heterogeneity, we used the number of geological entities per ecoregion, derived from the lithological map of the world gridded to 0.5° spatial resolution (Hartmann & Moosdorf, 2012). This was carried out using the Join and Statistic functions in ArcGIS. Because the number of geological units increases with area, we standardised this variable by ecoregion size. The matrix used for the analyses with all the data is available in the supplementary material section (see Supporting Information Appendix S3).

2.3 | Statistical analysis

We tested the influence of elevation on extinction risk by performing an ordinal regression model with the IUCN Red List category as the ordered response and linear, quadratic and cubic terms of elevation as predictors. Because the position of the data-deficient category within that ordered sequence of extinction risk is not defined, we excluded those species. Given the narrow range for most hydrobiid species, we used the elevation of each type locality as an indicator of the elevational range (Supporting Information Appendix S4). We fitted the regression model using a vector generalised linear model with the proportional odds family implemented in the VGAM 1.0-4 package for R (Yee, 2017). We selected the model with the lowest Akaike Information Criterion (AIC) (Akaike, 1974). Using the likelihood ratio test, we tested the proportional odds assumption. This evaluates whether individual risk categories differ in their response to elevation, such as whether the proportion of one category increases with elevation as another decrease.

Regression analysis of species richness was performed as described by Zuur, Ieno, and Elphick (2010). To standardise and compare regression coefficients of individual predictors, all variables were centred to zero and scaled to one standard deviation. We detected potential outliers by boxplots of each variable in R. A strong collinearity among predictors may affect the estimate of the regression parameters. We detected that latitude, mean annual temperature and temperature of the Last Glacial Maximum were correlated with a Pearson's correlation coefficient $>|0.7|$ (Dormann et al., 2013). Therefore, we excluded the latter two predictors from our models. We tested the effect of our variables on species richness using a generalised linear model (GLM). Preliminary analysis using a Poisson error distribution, which is typically recommended for count data (Cameron & Trivedi, 2013), indicated a problem with overdispersion. Overdispersion was the result of the variance in hydrobiid richness exceeding its mean and biased regression analyses. We therefore utilised the GLM of the MASS 7.3-45 package for R (Venables & Ripley, 2002) with a negative binomial distribution of model errors. Following Zuur et al. (2010), we assessed potential violations of GLM assumptions by inspecting the QQ plots.

Using a stepwise selection of predictors, we identified the model with the lowest AIC. In addition to excluding correlated predictors prior to model fitting, we utilised the car 2.1-3 package for R (Fox, Weisberg, & Bates, 2010) to calculate the generalised variance inflation factor, which indicates remaining multicollinearity by values greater than 10 (Quinn & Keough, 2002). However, in our case, all values were well below this threshold.

A lack of independence between observations can produce potential biases in estimated regression coefficients (e.g. Dormann et al., 2007; Zuur, Ieno, Walker, Saveliev, & Smith, 2009). Therefore, model residuals were tested for spatial autocorrelation using Moran's *I* statistic (Moran, 1950), ranging from −1 to +1. The correlog function of the pgirmess 1.6.5 package for R (Giraudoux, 2013) indicated low but significant spatial autocorrelation for the first distance class ($I_{780\ km} = 0.2$, $p < 0.001$), after the progressive Bonferroni correction (Hewitt et al., 1997). Only a few methods to account for spatial autocorrelation can handle count data (reviewed in Dormann et al., 2007). In our case, none of these classical methods removed the spatial autocorrelation, which was likely due to the lower observed spatial autocorrelation than in Dormann et al. (2007). We therefore opted to use the residuals autocovariate (RAC) GLM, which, in simulation studies, revealed unbiased regression coefficients (Crase, Liedloff, & Wintle, 2012). It is a regression with all previously identified predictors plus the RAC. In our case, the RAC is, for each ecoregion, the sum of the nonspatial GLM residuals weighted by the inverse squared distance to the focal ecoregion. The residuals of this regression analysis showed no spatial autocorrelation. A second AIC-based model selection identified the predictors of hydrobiid species richness that were previously only selected because of unaccounted spatial autocorrelation. A fully reproducible script of all analyses is available as Supporting Information Appendix S5.

3 | RESULTS

3.1 | Species richness distribution and hotspots

Our data set comprised a total of 906 extant species classified in 157 genera of Hydrobiidae *s. str.* distributed in 115 freshwater ecoregions (Supporting Information Appendix S1). The taxonomic status of a few records of the brackish-water genus *Hydrobia* Hartmann, 1821 reported from the equatorial zone of Africa and South America should be verified. Figure 1a–c shows distribution maps of hydrobiid species richness, endemics and threatened species. We found the highest species richness values in ecoregions from southern Europe, northern Africa, northwestern United States of America and the Caspian Sea (see Figure 1a). The Dniester-Lower Danube region was the richest ecoregion, with over 14% of the global hydrobiid species richness (Table 1). In our data set, 83% of the species were endemic to a single ecoregion, of which 39% are characterised by a type locality at high elevation (>500 metres above sea level [m a.s.l.]).

Table 1 shows ecoregions that contain the top quartile of species richness, endemics and threatened species. Congruence among these diversity measures was found in 19 ecoregions, qualifying them as biodiversity hotspots sensu Myers (1988). These were mainly found across the Mediterranean basin (Figure 1d) and only in three ecoregions from North America. This number decreases when applying lower thresholds than 25%, with identified hotspots located mainly in southern Europe (Figure 1d–f). The analysis of robustness of hotspot selection against species misidentification showed that ecoregions characterised by a biodiversity close to the lower end of the top 25% were not always classified as hotspots, whereas the most biodiverse regions were robustly classified as hotspot (Table 1).

3.2 | Risk distribution

According to our data set, of the 906 species of Hydrobiidae *s. str.*, 284 (31.4%) are classified within the threatened categories (CR, EN, VU) of the IUCN and 104 (11.5%) are of least concern or near threatened species (Figure 2a). The remaining 518 (57.1%) species in our data set are either listed as data deficient by the IUCN or have not yet been assessed. The highest number of threatened species was found in the Southeast Adriatic Drainages ecoregion, with a total of 54 threatened species listed by the IUCN. With the lowest AIC, our model selection identified a combination of quadratic and cubic terms of elevation as the best predictor of IUCN Red List category (Table 2). We could only robustly test the proportional odds assumption for a simple linear and quadratic relationship with elevation. More complex models did not converge because of the high number of coefficients. However, for the two converging models, the simpler proportional odds model was preferred (linear $\chi^2 = 1.9$, $df = 3$, $p = 0.6$; quadratic: $\chi^2 = 0.5$, $df = 3$, $p = 0.9$). Using the coefficients of the best-fit model to predict the proportions of IUCN Red List categories along the elevational gradient showed an increase in threatened categories until 1,500 m a.s.l, after which, threat risk decreases (Figure 2b).

3.3 | Determinants of species richness distribution

With the lowest AIC, the best-fit nonspatial regression model included as predictors of species richness latitude, connectivity, biogeographical realm, precipitation seasonality, annual precipitation and annual temperature range (Table 3). However, this model was potentially biased due to the low but significant spatial autocorrelation (see Methods). In contrast, the spatial regression model did not indicate autocorrelation and revealed that annual precipitation was a false positive selected predictor of hydrobiid species richness. Comparison of the estimated coefficients of the nonspatial and spatial regression models signified no major differences (Table 3), indicating no further bias in estimated regression coefficients.

As shown by the coefficients of the spatial regression model (Table 3), there was a positive relationship between species richness and connectivity, whereas annual temperature range showed a negative influence. Latitude and precipitation seasonality showed a nonlinear relationship with hydrobiid species richness (Figure 3).

With an $R^2 = 0.53$, our nonspatial regression model explained more than 50% of the variance in hydrobiid species richness.

4 | DISCUSSION

In this study, we evaluated global patterns of species richness, endemism and threatened species of the family Hydrobiidae *s. str.* by compiling data for 906 described extant species and their geographic

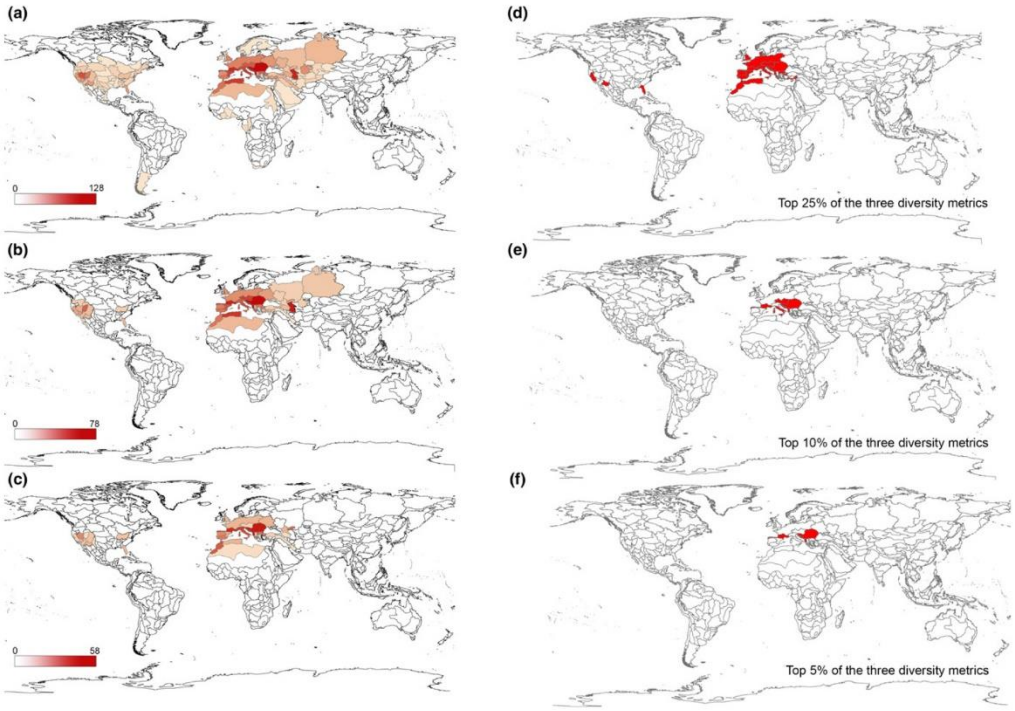

FIGURE 1 Distribution maps for three measures of hydrobiid diversity: (a) species richness, (b) endemic species and (c) threatened species. The inferred hotspots sensu Myers (1988) at the top 25% (d), 10% (e) and 5% (f) of these metrics are shown in red.

distribution based on the freshwater ecoregions proposed by Abell et al. (2008). We identified diversity hotspots within this family, characterised by a high number of species, endemics and threatened species. We also assessed the extinction risk of hydrobiid species according to the IUCN categories by elevation of the type localities and found the number of species catalogued within threatened categories peaking at 1,500 m a.s.l. Our selected richness model identified a positive effect of connectivity among ecoregions and a negative effect of annual temperature range on hydrobiid species richness. The principal findings are interpreted in the context of how large- and regional-scale variables shape hydrobiid diversity among ecoregions. We also discuss potential processes shaping the species richness distribution of hydrobiids and their conservation.

4.1 | Spatial variation of richness, endemism and threat

With an estimated 1,000–1,250 described species (Kabat & Hershler, 1993; Strong et al., 2008), hydrobioids represent one of the most speciose groups of nonmarine aquatic molluscs. Recent molecular and morphological studies have shown that hydrobioids actually comprise several microgastropod families (Wilke et al., 2001, 2013) and defined the sensu stricto character of the family Hydrobiidae. However, an overall approximation of the total species number of this family was unknown, until now. Based on the molecular definition of Hydrobiidae *s. str.* and a comprehensive literature and database review, we found 906 valid hydrobiid species described so far. This estimate nearly equals previous observations of species richness for Hydrobiidae *s. l.*, suggesting that either other hydrobioid families are species poor or we know little about the actual diversity of the remaining hydrobioids. According to Strong et al. (2008), we may know only 25% of these species. Given that, for instance, Australian hydrobioid genera alone comprise ~300 species (Ponder & Colgan, 2002), the global richness of hydrobioid is more likely underestimated than those other hydrobioid families being species poor. Moreover, spring systems of some regions, such as Africa or South America, remain to be extensively surveyed, which may increase these numbers even more.

We found congruence among the distribution of high species richness, endemicity and number of threatened hydrobiid species in 19 freshwater ecoregions mainly from Europe and North Africa, qualifying them as biodiversity hotspots (Figure 1d). Other

TABLE 1 List of ecoregions representing hotspots of hydrobiid diversity, including their species richness, number of threatened and endemic species, and the robustness of hotspot selection against species misidentification

Ecoregion	Species richness	Threatened species	Endemic species	Robustness
Dniester–Lower Danube	128	37	78	1.000
Southeast Adriatic Drainages	90	58	67	1.000
Dalmatia	68	27	32	1.000
Cantabric Coast–Languedoc	59	26	42	1.000
Italian Peninsula and Islands	49	17	27	1.000
Eastern Iberia	37	7	24	0.998
Gulf of Venice Drainages	35	8	8	0.995
Mediterranean Northwest Africa	35	4	28	0.995
Upper Danube	31	20	21	0.990
Thrace	27	10	18	0.997
Southern Iberia	22	9	18	0.992
Central & Western Europe	22	5	14	0.944
Atlantic Northwest Africa	21	13	18	1.000
Ionian Drainages	20	7	16	0.931
Southern Anatolia	18	8	17	0.982
Western Iberia	17	5	9	0.745
Gila	13	5	10	0.698
Florida Peninsula	12	7	10	0.305
Sacramento—San Joaquin	12	5	7	0.287

ecoregions, such as in the Caspian Sea or the majority of western North America, also present high species richness (Hershler, Liu, et al., 2014; Kantor, Vinarski, Schileyko, & Sysoev, 2010; Vinarski & Kantor, 2017) but a low number of assessed species according to the IUCN criteria. This lack of conservation attention invalidates these areas as hydrobiid hotspot (sensu Myers, 1988); however, this may change with future conservation assessments. Our simulation evaluating the robustness of hotspots against species misidentification confirms this because the IUCN status seems to be the crucial factor for our type of simulation. By re-assigning 50% of all species names, species richness remained typically the same, except in cases where a randomly sampled species is already present in the focal ecoregion. Endemism, the second hotspot criteria, was slightly lower in the simulated data than in the observed data given the high share of endemic species. The IUCN status turned out to determine hotspot robustness. A high number of threatened species per biogeographic realm likely results in a replacement of an endangered species by another one; consequently, the limited assessment for the Nearctic species may explain the low hotspot robustness of North American ecoregions (Table 1).

In general, our results are consistent with the findings that the Mediterranean basin is a global hotspot of biodiversity for various taxa (Cuttelod, García, Abdul Malak, Temple, & Katariya, 2009; de Figueroa, Lopez-Rodriguez, Fenoglio, Sanchez-Castillo, & Fochetti, 2013; Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000) and contains the highest proportion of threatened freshwater species in Europe (Skoulikidis et al., 2017). Previous studies also identified some of these ecoregions as important areas of hydrobiid species richness. For instance, Arconada and Ramos (2003) and Strong et al. (2008) identified the mountainous regions in southern France and Spain, southern Alps, the Balkan Peninsula and southwestern USA as biodiversity hotspots. Other authors found a very high number of endemic hydrobiid species in the Balkan region, such as in the Zrmanja River in Croatia and in the ancient lakes Ohrid, Prespa and Skadar (Beran, Osikowski, Hofman, & Falniowski, 2016; Falniowski & Beran, 2015; Hadžišče, 1956; Pešić & Glöer, 2013; Radoman, 1963). In North America, Howard et al. (2015) considered the Sacramento river basin and the Lahontan ecoregion as zones with high species richness and vulnerability for molluscs and crustaceans. Likewise, Brown, Lang, and Perez (2008) highlighted the Great Basin, Rio Grande, Colorado River and Florida drainages as zones with high diversity of the families Pleuroceridae and Hydrobiidae, consistent with our results. Notably, we scored the North American genus *Pyrgulopsis* Call & Pilsbry, 1886 as the richest genus within Hydrobiidae with 134 species (but see Hershler et al. (2013), who estimated 137 species), with its highest diversity occurring in the Great Basin (Hershler & Sada, 2002).

The high number of endemic hydrobiid species listed in this work (i.e. 754 of 906 species) supports the results of previous taxonomic and ecological studies of this family. Overall, hydrobiid species present high endemicity and, in general, a very restricted distribution, sometimes occurring in only one or two localities. Habitat type, which in most cases are isolated springs, and the low dispersal capacity of most of the species may account for this distribution pattern (Arconada & Ramos, 2003; Delicado, Machordom, & Ramos, 2013; Hershler, 1998; Strong et al., 2008). The high endemicity could also be related to the fact that numerous species included in our study were described based only on a single population. Therefore, their actual distribution may not be completely known and thus underestimated.

We applied the monophyly criterion of the family Hydrobiidae (sensu Wilke et al., 2013) to calculate the number of threatened species assessed by the IUCN. Therefore, we found 284 Hydrobiidae *s. str.* species catalogued under a threatened category, representing ~54% of the total number of threatened hydrobioid species listed in the IUCN. The number of threatened species is likely even higher as more than 50% of the Hydrobiidae *s. str.* species are classified as data deficient. These findings indicate that hydrobiids face a high degree of threat, mainly caused by habitat degradation and/or the intrinsic characteristics of the species (Strong et al., 2008). By comparing the threat category assigned by the IUCN with the elevation

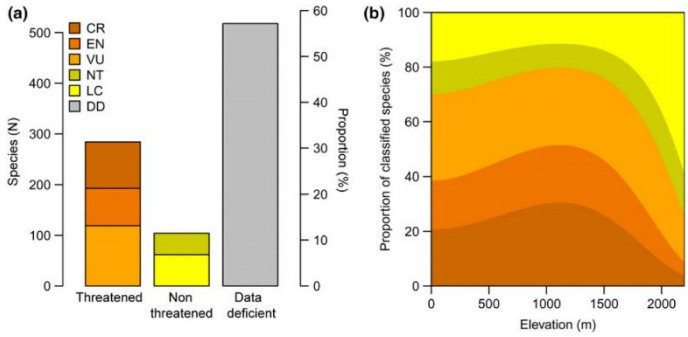

FIGURE 2 Hydrobiid extinction risk. (a) Number and proportion of species according to the IUCN Red List categories. We classified all species not listed by the IUCN as data deficient. (b) Proportion of IUCN Red List categories along the elevation gradient predicted by our nonlinear ordinal regression model

TABLE 2 Risk models for hydrobiid extinction. Different models represent increasing complexity in the nonlinear relationship between the IUCN Red List category of a species and the elevation of its type locality. Log-likelihood indicates absolute model fit and ΔAIC the complexity-penalised difference in model fit in comparison with the best-fit model. ΔAIC < 2 indicates no substantial difference in fit

Model	Log-likelihood	Parameters	ΔAIC
Constant	−602.3	1	2.6
Linear	−602.2	2	4.3
Quadratic	−602.3	2	4.5
Cubic	−601.8	2	3.6
Linear + quadratic	−600.5	3	2.9
Linear + cubic	−599.8	3	1.6
Quadratic + cubic	−599.0	3	0.0
Linear + quadratic + cubic	−598.7	4	1.3

of the type localities, we found a relatively high proportion of threatened species up to 1,000 m a.s.l., with a peak at 1,500 m a.s.l., after which, it decreases with higher elevation. This pattern is unexpected as most of the high-elevation species are narrow range endemics and therefore should be more vulnerable to, for instance, habitat loss compared with lowland species. However, the relatively lower risk at higher elevations could be related to the fact that high-elevation populations are less accessible and therefore more protected from human activities than those living at lower elevations (Angeli, Cantonati, Spitale, & Lange-Bertalot, 2010). Vörösmarty et al. (2010) found that 65% of the global river discharges, and the ecosystems supported by these waters, were under a moderate to high threat with an estimated 10,000–20,000 species considered at risk or extinct. This estimation supports the idea that freshwaters are the most endangered ecosystems in the world (Camargo & Alonso, 2006; Dudgeon et al., 2006; Markovic, Carrizo, Kärcher, Walz, & David, 2017).

4.2 | Predictors of hydrobiid species richness distribution

Climate and geographic variables are often strong descriptors of broad-scale species richness patterns (Hawkins et al., 2003), reflecting the sorting of species along ecological gradients (Gaston, 2000). Evolutionary and dispersal processes also play an important role in richness distribution (Wiens & Donoghue, 2004). Our regression model included, as predictors of hydrobiid richness, variables that reflect all three mechanisms, as observed in other freshwater animal groups (Tisseuil et al., 2013).

Freshwater gastropods present an overall pattern of high species richness in tropical zones that generally decreases with increasing latitude, such that lower richness and endemicity are observed at higher latitudes (Strong et al., 2008). This pattern has also been found for other groups of freshwater organisms, such as fish, amphibians and dragonflies (Heino, 2011; Tisseuil et al., 2013). Nevertheless, incongruences with this general pattern are common in freshwater ecosystems: For instance, mayflies and stoneflies show a reversed latitudinal gradient with higher species richness at high latitudes, whereas caddisflies and salamanders lack latitudinal richness gradients (Heino, 2011). For hydrobiids, latitudinal gradient follows a hump-shaped pattern, peaking at mid-latitudes (30°–40°), with minimums at warm tropical areas and boreal latitudes. Like most latitudinal richness gradients (Mittelbach et al., 2007), this distribution likely results from the interaction of many factors, such as geography, climate, evolutionary history or water availability, to facilitate the coexistence of species. In fact, this mid-latitudinal zone, which harbours the highest number of hydrobiid species, features relatively low temperature seasonality and intermediate values of precipitation seasonality (Figure 3). These findings suggest that hydrobiids prefer areas with more stable climatic conditions, thus supporting the climatic stability theory (Klopfer, 1959; Klopfer & MacArthur, 1960), as found for other freshwater organisms (Oberdoff, Guégan, & Hugueny, 1995; Oberdorff et al., 2011; Whitton, Purvis, Orme, & Olalla-Tárraga, 2012).

TABLE 3 Drivers of hydrobiid richness. Influence ($\beta \pm$ standard error) of ecological and evolutionary predictors on hydrobiid species richness estimated with a nonspatial and a spatial generalised linear model (GLM). All effects are in comparison with the Afrotropical realm (i.e. the intercept level). A superscript of 2 indicates a nonlinear influence of the respective predictor. We evaluated the significance (p) of individual predictors by the Wald test statistic z. Note that we used an information theory approach for predictor selection through maximising model fit by penalising for additional predictors. Therefore, not all predictors were significant at $\alpha = 0.05$

	Nonspatial GLM			Spatial GLM		
	β	z	p	β	z	p
Intercept	2.27 ± 0.54	4.23	<0.001	2.18 ± 0.52	4.19	<0.001
Latitude^2	−0.33 ± 0.08	−4.00	<0.001	−0.37 ± 0.08	−4.55	<0.001
Connectivity	0.31 ± 0.11	2.87	0.004	0.25 ± 0.10	2.43	0.015
Biogeographic realm						
Nearctic	−0.42 ± 0.58	−0.73	0.468	−0.35 ± 0.56	−0.61	0.540
Neotropic	−2.72 ± 1.41	−1.94	0.053	−2.61 ± 1.38	−1.90	0.057
Palearctic	0.52 ± 0.52	1.01	0.314	0.67 ± 0.50	1.33	0.182
Precipitation seasonality	−0.53 ± 0.13	−3.99	<0.001	−0.42 ± 0.13	−3.25	0.001
Precipitation seasonality^2	−0.19 ± 0.10	−2.00	0.046	−0.22 ± 0.10	−2.24	0.025
Annual precipitation	−0.33 ± 0.14	−2.37	0.018	−0.22 ± 0.14	−1.56	0.118
Annual temperature range	−0.78 ± 0.16	−4.76	<0.001	−0.68 ± 0.16	−4.26	<0.001
Residual autocovariate				0.31 ± 0.10	3.13	0.002

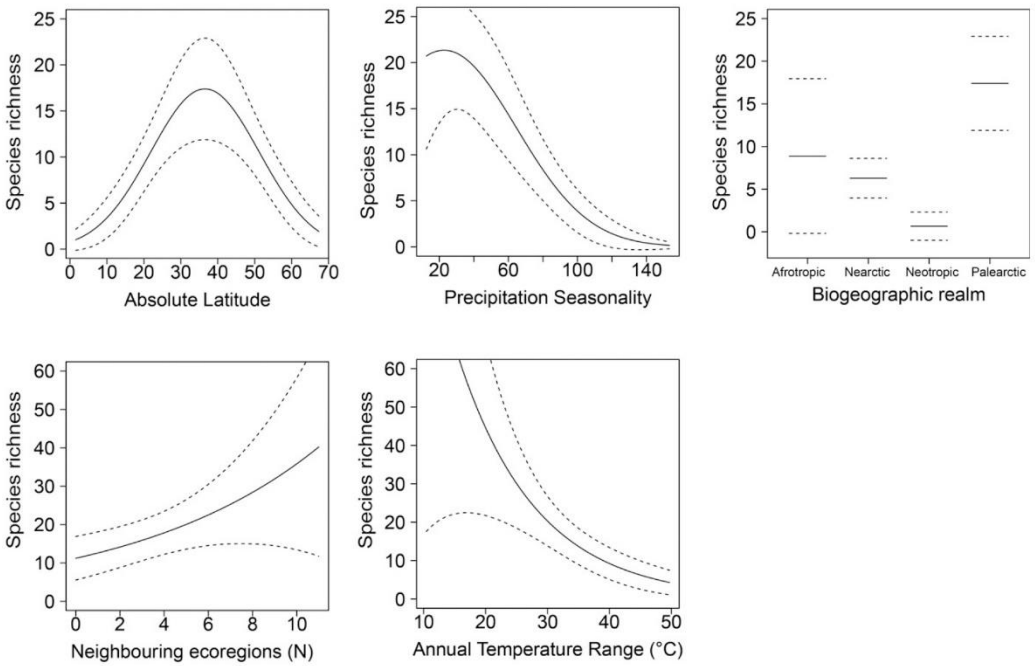

FIGURE 3 Response plots. Predicted hydrobiid species richness (solid line) and the 95% prediction interval (dashed lines) according to the best-fit spatial generalized linear model along ecological and evolutionary predictors. We fixed all continuous predictors, except the focal one, to their mean and the biogeographic realm to the Palearctic, showing the sole response of richness to the predictor of interest. The GLM specification of the negative binomial model errors caused the nonlinear impression of the richness relationship with annual temperature range and connectivity; however, both correlations are monotonic

Climatic stability during certain Quaternary periods in Europe has been shown to promote the geographic expansion and increase in species richness of lacustrine gastropod assemblages (Georgopoulou et al., 2016). Likewise, in our study, past temperatures (i.e. temperature of the Last Glacial Maximum) were strongly correlated with latitude and present-day temperatures and, therefore, may also explain the present-day distribution of hydrobiid species richness. In fact, the regions showing a great share of the richness distributed in the southern Palearctic and Nearctic realms coincide with the permafrost line in these realms during the Last Glacial Maximum (Frenzel, 1992). However, glacial refugia do not always correspond with regions of high gastropod diversity, as demonstrated in the European spring snail genus *Bythinella* Moquin-Tandon, 1856 (Benke, Brändle, Albrecht, & Wilke, 2011), suggesting that climate may differentially affect the evolutionary history of lentic and lotic species (Dehling, Hof, Brändle, & Brandl, 2010). However, testing this hypothesis is beyond the scope of this study and requires more information about the environment and species' ecology.

Areas of high elevation have served as evolutionary centres of endemism driven by complex topographies that generate heterogeneous environments (Allouche, Kalyuzhny, Moreno-Rueda, Pizarro, & Kadmon, 2012). Moreover, these areas feature strong climatic changes along elevational gradients, which may have buffered against extinction events during periods of climate oscillations in the past by facilitating short-distance dispersal (Jetz et al., 2004; Ohlemüller et al., 2008). Contrary to what Pérez-Quintero (2015) showed for European freshwater gastropods, the elevation range of ecoregions did not contribute to explain hydrobiid species richness in this study. Differences in taxonomic and spatial scale may account for this disparity: Pérez-Quintero (2015) used point data, whereas we used the mean value and range of elevation per ecoregion as a proxy of the elevation in which a species lives, due to limitations in determining the exact geographic range of all 906 species. Likewise, we did not detect an influence of geological heterogeneity on hydrobiid species richness, suggesting that either the geographic unit of our study (i.e. ecoregions) is too large to detect such a relationship or habitat heterogeneity may indeed not influence the species richness distribution of the family Hydrobiidae. Although habitat heterogeneity was shown to play an important role in the species richness distribution of freshwater African molluscs (Hauffe et al., 2014), we found no such a significant correlation with our ecoregion binning. In particular, we did not find substantial support for the species–area relationship either (i.e. no effect of ecoregion size on species richness was found), which is thought to be associated with habitat heterogeneity (Ricklefs & Lovette, 1999). This lack of correlation may indicate that hydrobiid species richness is more likely attributable to past and present climatic conditions affecting a particular ecoregion, which contribute to create more potential habitats for hydrobiid species, rather than to their geographic extent.

Factors influencing dispersal, including some analysed here, such as connectivity among ecoregions, peninsula effect and biogeographic affiliation, can influence certain evolutionary processes and shape species richness distributions. Geographic connectivity favours dispersal processes by having common boundaries and creating corridors for movement (Taylor, Fahrig, Henein, & Merriam, 1993). Despite the low dispersal ability of most hydrobiid species, which are, in many cases, restricted to a single ecoregion (but see Wilke, Rolán, and Davis (2000) and Haase, Naser, and Wilke (2010) for a wider species distribution of the brackish genera *Hydrobia* and *Ecrobia* Stimpson, 1865), we have found a positive relationship between species richness and connectivity. This pattern has also been observed in other molluscs and groups such as fish and odonates (Amoros & Bornette, 2002; Ward, Tockner, & Schiemer, 1999; Wepfer, Guénard, & Economo, 2016). However, our findings indicated that peninsular ecoregions, which are supposed to be geographically isolated and species poor, are actually as diverse as nonpeninsular ecoregions. The peninsula effect may have been counteracted by past colonisation of the southern peninsulas during glacial episodes (as mentioned above) and long-distance dispersal by birds, which has also been suggested to play an important role in the geographic distribution of certain hydrobiid species (Delicado, Machordom, & Ramos, 2014; Haase et al., 2010; Szarowska, Osikowski, Hofman, & Falniowski, 2016). The importance of historical factors is also reflected in the variation of species richness found among biogeographic realms (Figure 3). The inclusion of biogeographic realm adds explanatory power to richness models by accounting for common ancestry of species and diversification dynamics in a region (e.g. Griffiths, McGonigle, & Quinn, 2014; Tedesco, Oberdorff, Lasso, Zapata, & Hugueny, 2005). These findings demonstrate that the species richness distribution of Hydrobiidae *s. str.* can be explained by the joint effect of climate and evolutionary history.

We conclude that more stable conditions in terms of temperature and precipitation as well as habitat connectivity may contribute to increase hydrobiid species richness. Moreover, our study revealed a lower proportion of threatened species in highlands than in lowlands, although with the caveat that conservation assessments are lacking for more than half of the known hydrobiid species. Global climate change models predict perturbations of most of the world's ecosystems mainly due to changes in temperature and precipitation seasonality (Carpenter, Fisher, Grimm, & Kitchell, 1992; Walther et al., 2002); therefore, hydrobiid species and their ecosystems may be at elevated risk in the future. Although our study compiles a large portion of the known extant species, a fine-scale geographic study of hydrobiid gastropods potentially could identify additional predictors of diversity hotspot.

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SUPPORTING INFORMATION

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

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