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Living on the mountains: Patterns and causes of diversification in the springsnail subgenus *Pseudamnicola* (*Corrosella*) (Mollusca: Caenogastropoda: Hydrobiidae)

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ABSTRACT

Hydrobiidae is one of the largest families of freshwater gastropods comprised of approximately 400 genera and 1000 species. Despite this high level of diversity, most hydrobiid species inhabit fragile ecosystems in restricted distribution areas. In this work, we analyze modes of speciation and causes of diversification in the hydrobiid springsnail subgenus *Pseudamnicola* (*Corrosella*). Species of this group typically live in nutrient poor springs and streams and are restricted to mountainous regions of the Iberian Peninsula (Spain) and Southern France. Previous morphological and molecular (based only on the cytochrome *c* oxidase subunit I (COI) gene) studies revealed 11 nominal *Corrosella* species. In this study, we enhance published molecular results by generating new data from mitochondrial (16S rRNA and COI) and nuclear ribosomal regions (18S and 28S rRNA) from 50 *Corrosella* populations. As a result of this study we have identified one new species, making a total of twelve recognized species in the subgenus *Corrosella*. Our phylogenetic results also reveal the existence of three lineages within the subgenus, and the estimation of time divergence indicates the occurrence of three main speciation events during the upper Miocene to Pleistocene. We test the influence of several geographical and ecological variables and observe that diversification patterns are related to habitat fragmentation rather than environmental conditions. This result suggests that the high level of diversity observed within the subgenus may have resulted from a non-adaptive radiation. The formation of the Iberian Peninsula mountain ranges (the Pyrenees in the north and the Betic Cordillera in the south) and the configuration of the Iberian current hydrographic system played important roles in *Corrosella* speciation. Additionally, during the Miocene the Iberian Peninsula experienced a gradient of increasing temperature and dryness from north to south, which together with a high level of tectonic activity, may have caused the majority of the diversity found in the southern Iberian Peninsula.

1. Introduction

The Miocene (23.3–5.2 Ma) was a crucial period in the history of the Earth and of life on Earth because of major palaeogeographic, palaeoclimatic and palaeoenvironmental events that occurred during this time. Among them were continental drift and climate change, which induced, especially in temperate areas, a cooling towards the Pleistocene. Western regions of the Mediterranean, including the Iberian Peninsula, were profoundly affected by the dramatic events that occurred at the end of Miocene (Aguirre, 2003). Geological processes in the Iberian Peninsula during the Alpine orogeny have critically affected the diversification and dispersion of many species of plants and animals (Joger et al., 2007; Miguel et al., 2007; Pardo et al., 2008). These processes included: (i) the second and last uprising of the Pyrenees in the north (Barbadillo et al., 1997; Meléndez-Hevia, 2004); (ii) the creation of the Betic Cordillera and the active plate tectonics of the Alboran Sea area in the south (approximately 10 Ma according to Rosenbaum et al. (2002)) and (iii) the Messinian salinity crisis (MSC) (between 5.96–5.33 Ma according to Krijgsman et al. (1999), or 6.5/6.6–5.3 Ma according to Aguirre (2003)). These palaeogeographical events led to allopatric speciation, which increased Iberian species richness and changes in dispersion patterns (the closure of the Strait of Gibraltar allowed faunal interchange between Africa and Europe), both of which led to greater species diversification. The great biodiversity of the Iberian Peninsula was also the result of the southwards Pleistocene migration of species that were unable to withstand strong climatic oscillations caused by alternating glacial and interglacial periods (700,000 years ago to present day). The Iberian Peninsula, as well as the Balkan and Italian Peninsulas,

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served as refuges for many species and are considered "evolutionary centres" (Davis, 1982) for freshwater mollusks.

Mountainous regions in Spain and Southern France are considered hotspots for freshwater gastropod species, particularly hydrobioids families (Strong et al., 2008). The prosobranch mollusk family Hydrobiidae Stimpson, 1865 belongs to this group and represents one of the most speciose groups in freshwater biotopes (although some brackish species exist). According to classical classifications, this group of tiny gastropods (between one and eight mm) contained more than 400 genera and between 1000 and 1250 species worldwide (Kabat and Hershler, 1993; Strong et al., 2008). However, recent studies have pointed to the existence of many more independent radiations at the same rank within the superfamily Rissooidea (Ponder et al., 2008; Zielske et al., 2011). Recent molecular phylogenies delineate the family Hydrobiidae s.s. within Rissooidea with a distribution range constrained to the western Palearctic, eastern Nearctic, northern Neotropic and South Africa (Wilke et al., 2013). Although a parallel study, performed by Criscione and Ponder (2013), has demonstrated that this family does not belong any longer to the superfamily Rissooidea but to Truncatelloidea. Despite being widely distributed, most hydrobiids species are restricted to a small number of localities and/or fragile ecosystems (e.g., springs, streams and ponds), which are largely endangered due to human activities or water shortages. For detailed systematic studies, as well as for conservation efforts, it is necessary to know the species diversity of the group; although in reality, the number of currently described taxa is likely an underestimation of the total number of existing taxa (Strong et al., 2008). However, one difficulty in determining the true number of hydrobiid taxa is due to the recognition of species boundaries as most of them have unsculptured shells and simplification of anatomical structures due to miniaturization (Arconada and Ramos, 2003), which leads to high levels of homoplasy and cryptic species (e.g., Haase, 2008; Delicado and Ramos, 2012; Liu et al., 2003). Cladistic analyses of hydrobiid genera using morphological characters generated incongruent phylogenies (Bodon et al., 2001; Falniowski and Szarowska, 1995; Haase, 2008; Szarowska, 2006). Morphology alone cannot resolve the systematics of the group, thus data from other disciplines, such as genetics, ecology and biogeography, are required.

Therefore, in an attempt to apply a multidisciplinary approach and to reinforce the morphological and anatomical work previously performed (Boeters, 1988; Delicado et al., 2012; Delicado and Ramos, 2012), we study the phylogeny and the evolutionary history of *Pseudamnicola* Paulucci, 1878, one of the most diverse genera within Hydrobiidae. Within the Mediterranean, this freshwater genus consists of 41 European (Delicado et al., 2012; Delicado and Ramos, 2012; Fauna Europaea, 2011), 13 North African (Boeters, 1976; Ghamizi et al., 1997; Glöer et al., 2010; Pallary, 1926) and three Turkish nominal species (Shütt and Bilgin, 1970; Shütt and Sesen, 1993). The classification of *Pseudamnicola* comprises two subgenera: *Pseudamnicola* s. str. and *Corrosella*. In this study, we focus on *Corrosella*. *Corrosella* was first described as a new genus by Boeters in 1970 (Boeters, 1970). However, the same author later recognized that not enough diagnostic characters existed to validate this taxon at this level (Boeters, 1984), and rather established *Corrosella* as a subgenus of *Pseudamnicola*. Currently, 11 species of this subgenus have been described morphologically; ten of them are found in the Iberian Peninsula, while the remaining species, *P.* (*C.*) *astieri* (Dupuy, 1851), is found in Southern France (Alpes Maritimes). Recently, nine out of these species were characterized both morphologically and molecularly using only the cytochrome *c* oxidase subunit I gene (see Delicado and Ramos, 2012; Delicado et al., 2012). With the purpose of enhancing previous works, this study analyzes, for the first time, all 11 species comprising the subgenus, employing a greater number of populations and genes, and thus, provides a more complete data set for testing phylogenetic relationships for this group.

In addition to examining the speciation pattern within *Corrosella*, we investigated the causes of speciation for this group of springsnails. Previous studies demonstrated the importance of habitat fragmentation and genetic drift in the diversification of low-dispersal snails (Gittenberger, 1991; Murphy et al., 2012; Wilke et al., 2010). Two of the most important factors that drive habitat isolation, and consequently, allopatric speciation, are geological barriers and climatic fluctuations (Mayr, 1942). Iberian Hydrobiidae species richness and their high level of endemicity are thought to be mainly due to tectonic events and the limited dispersal capacity of the group (Arconada and Ramos, 2003). However, climatic variables, including air temperature and precipitation, can also strongly influence freshwater mollusk distribution as they directly affect their habitats. The tectonic evolution and geographical features of the Iberian Peninsula resulted in a palaeoclimate that, during the Miocene up to the Pliocene, consisted of an increasing gradient of temperature and dryness from north to south (Jiménez-Moreno et al., 2010), which we hypothesize affected springsnail populations.

Therefore, to study the effects that geographic and climatic changes may have had on the evolution of *Corrosella*, we present the first phylogeny of the subgenus, including the 11 recognized species, plus one new species that was revealed in this study by molecular data. Overall, the objectives of this study are: (1) to reconstruct interspecific relationships based on the analysis of mitochondrial and nuclear DNA; (2) to analyze the tempo and mode of species diversification; and (3) to study the patterns and explore possible causes of diversification of this subgenus.

2. Material and methods

2.1. Material for molecular analyzes

Fifty populations of *Pseudamnicola* (*Corrosella*) from the Iberian Peninsula and the Department of Var, Alpes-Maritimes, Southern France were examined genetically (Fig. 1; and Tables 1 and S1); these regions include the entire known range of the subgenus *Corrosella*. Vouchers and DNA samples are kept in the Tissues and DNA collection at the Museo Nacional de Ciencias Naturales (MNCN), Madrid, Spain (Table 1).

Two mitochondrial regions, 16S rRNA (16S) and cytochrome oxidase subunit I (COI), and one nuclear ribosomal region, 28S rRNA (28S) were analyzed for 102 specimens. Additionally, one specimen per species was sequenced for 18S rRNA (18S) because of its high sequence conservation. A final data set of 98 *Corrosella* specimens from the 50 localities, in which the 11 recognized species are represented, were analyzed as an ingroup. Sequences of two specimens of the sister species *Pseudamnicola* (*Pseudamnicola*) *subproducta* (Paladilhe, 1869) were obtained and included in the analyses. The species *Peringia ulvae* (Pennant, 1777) and *Mercuria emiliana* (Paladilhe, 1869) were used as outgroups (Table 1). All new sequences were deposited in GenBank under accession numbers JX081679–JX081776, JX081805–JX081885, JX081890–JX081985. Additional COI sequences from 23 specimens were obtained from GenBank (Delicado and Ramos, 2012; Delicado et al., 2012). Taxonomic identification was based on previously established morphological and morphometric characters (see Delicado and Ramos, 2012; Delicado et al., 2012).

2.2. DNA isolation, amplification and sequencing

Total genomic DNA was only isolated from the foot tissue of the springsnails; the rest of the animal was preserved as voucher