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# Recent expansion and relic survival: Phylogeography of the land snail genus *Helix* (Mollusca, Gastropoda) from south to north Europe ☆

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## ABSTRACT

Little is known about the evolutionary history of *Helix*, despite the fact that it includes the largest land snails in the western Palaearctic, some of which (e.g. *H. pomatia* Linnaeus, 1758) are valuable human food. We compared two groups of *Helix* with apparently contrasting evolutionary histories: the widespread species *H. pomatia* and the group distributed along the Italian Apennine chain, a relatively unknown set of species with a restricted distribution over a range of altitudes. To reconstruct the evolutionary trajectories of these two groups, we analysed morphological (shell and genitalia) and molecular characters (mitochondrial and nuclear markers) in a total of 59 populations from northern and central Europe (*H. pomatia*) and along the Apennine chain (various species). We also reconstructed the phylogeny and the evolutionary history of the genus by combining our data with that currently available in the literature. We found that spatial changes did not merely imply fragmentation of populations, but also implied environmental changes (woodlands *vs.* grasslands) that may have triggered the observed phenotypic diversification. We also found that Anatolia is the ancestral range of *Helix* and is therefore an important area for the Palaearctic diversity. The results provide insights into the evolutionary history of species richness and more generally into the processes that may have shaped the distribution and diversification of these organisms across Europe and the peri-Mediterranean area.

## 1. Introduction

The amazingly wide spectrum of shell colours and shapes observable in land snails gives scientists the opportunity to understand how evolutionary processes proceed and lead to diversity. When these evolutionary processes are sought in a geographical context (i.e. phylogeography; Avise, 1998, 2000; Kidd and Ritchie, 2006), patterns and causes of diversity can be unveiled on a broad geographical scale and "fortuitous" phenotypic resemblances become precise convergences, often with an underlying adaptive meaning (Gould, 1971).

Several genetic studies have attempted to relate molecular divergence patterns with the major palaeoevents (climatic and geological) that determined the current geography of southern Europe. In particular, the peri-Tyrrhenian area is a known hotspot of biodiversity (Blondel and Aronson, 1999) due to its complex geological history (Steininger and Rögl, 1984; Vai and Martini, 2001; Weiss and Ferrand, 2007). Many studies have revealed the importance of Pleistocene glacial refugia in shaping the genetic structure of a number of south European animal species (e.g. zygaenid moths, Cesaroni et al., 1989; cyprinid fish, Stefani et al., 2004; crayfish, Trontelj et al., 2005; cave crickets, Allegrucci et al., 2005; fire-bellied toads, Canestrelli et al., 2006; salamanders, Canestrelli et al., 2014a; newts, Maura et al., 2014). Some of these studies focused on Tyrrhenian land snails (Ketmaier et al., 2006, 2010).

Little is known about the evolutionary history of the *Helix* species, despite the fact that they belong to a diverse and widespread genus including the largest land snails in the western Palaearctic, some of which (e.g. *H. pomatia* Linnaeus, 1758) are valuable human food. The genus occurs in Europe, Mediterranean North Africa (from Egypt to Algeria) and some sectors of western Asia (Asia Minor, the Caucasus, Turan, Iran and the Middle East) and comprises about 40 species with a diversity hotspot centred on the eastern Mediterranean – Asia Minor (Bank, 2011; Welter-Schultes, 2012; Korábek et al., 2014; Psonis et al., 2014; Neubert, 2014). In its westernmost European range it embraces some widespread species, e.g. *H. pomatia* (the Roman snail), *H. lucorum*

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Linnaeus, 1758, and *H. cincta* Müller, 1774, and some putative range-restricted species possibly belonging to an Apennine-Tyrrhenian radiation, such as *H. ceratina* Shuttleworth, 1843, from Corsica and *H. ligata* Müller, 1774, from the central and southern Apennines (Ketmaier et al., 2007; Fiorentino et al., 2013a). Phylogenetic relationships of the eastern Mediterranean species have been investigated by Korábek et al. (2014, 2015) and Psonis et al. (2014). Korábek et al. (2014) re-examined *H. lucorum* sustaining that it included two distinct taxa; Psonis et al. (2014) analysed some Aegean species finding that *H. cincta* is paraphyletic and in need of taxonomic revision. Korábek et al. (2015) addressed the phylogeny of the genus *Helix* and found three major geographical monophyletic groups (Anatolian, Mediterranean and European), but not the monophyly of several species groups, including that of the type species, *H. pomatia*.

In the present analysis we compared two groups of *Helix* species with apparently very different evolutionary histories: *H. pomatia*, a widespread European species and the Italian Apennine group, a little known group of species with high phenotypic diversity and a restricted distribution.

*H. pomatia* has a European distribution, occurring mainly in central and eastern Europe (Bank, 2011; Welter-Schultes, 2012; Neubert, 2014). In Italy, it is only present in the northern regions from Liguria to Friuli (Cesari, 1978). Two main hypotheses have been formulated in the past to explain its present distribution: (1) spontaneous post-glacial colonisation of central and northern Europe (Taylor, 1910); (2) repeated human-mediated translocations, since in England fossil records are only available from the Roman period (Evans, 1972). The predictions for these two not mutually exclusive hypotheses are: if the distribution is due to translocation by humans, then one should expect shared haplotypes between distant regions; otherwise, the distribution of haplotypes should be geographically structured. We aimed at testing these two hypotheses.

On a smaller scale, the evolutionary history of *Helix* along the Apennines (peninsular Italy south of the Ligurian Alps) is also controversial. This diverse group has many forms and variants (in colour and band pattern and length of genital parts), which have from time to time been considered different species/subspecies but no definitive taxonomy has yet been established (Fig. 1). Distinct species are supposed to be recognised on the basis of shell colouration, shell bands and length of genital parts, but quantitative testing for significant differences has never been done (Manganelli et al., 1995, reported three endemic species from the Apennines: *H. delpretiana* Paulucci, 1878, *H. ligata* and *H. mileti* Kobelt, 1906). Many different questions have been raised about the evolutionary history of Apennine *Helix* snails, such as how many different species there are and the adaptive significance of shell colouration. One key hypothesis on land snail shells is that colouration and band patterns are related to habitats and lighter or darker colours are thought to be correlated with exposure or altitude (Cameron et al., 1996; Chiba, 1999) in response to predation (e.g. camouflage) or to solar radiation (lighter shells minimize heat absorption in habitats where solar radiation is strong) (Cain and Sheppard, 1954; Jones et al., 1977; Ozgo, 2005).

Genetic studies on a species of the same family as *Helix* (Helicidae), *Cepaea nemoralis*, (i.e. Murray, 1975; Jones et al., 1977; Richards et al., 2013) have identified the loci responsible for band and colour variation, some of which are linked and inherited together as a "supergene". Evidence of a strong directional selection component in colour and band shell phenotypes has also been shown (Cain and Sheppard, 1954; Cain et al., 1960).

If shell patterns are related to habitats, a categorical factor based on colour and band types should enable prediction of the central trend in the distribution by altitude. The prediction is that darker shells are an adaptation to sheltered habitats (low altitude) and lighter shells to exposed habitats (higher altitude) (Cameron et al., 1996; Chiba, 1999). Here we tested distributional patterns of shell morphology. Analysed in conjunction with a multigene-based phylogeny, this allowed us to describe patterns, from which we inferred the most likely explanations. Did different shell types repeatedly occur together in genetically homogeneous clusters of populations? Are lighter and darker shells an adaptation to environments at different altitudes as expected from observations of other snails (i.e. adaptation to sheltered sites at low altitude and exposed sites at higher altitude – where altitude summarises factors such as humidity, heat, exposure, woodland habitats vs grassland)? What is the likely evolutionary history behind these patterns?

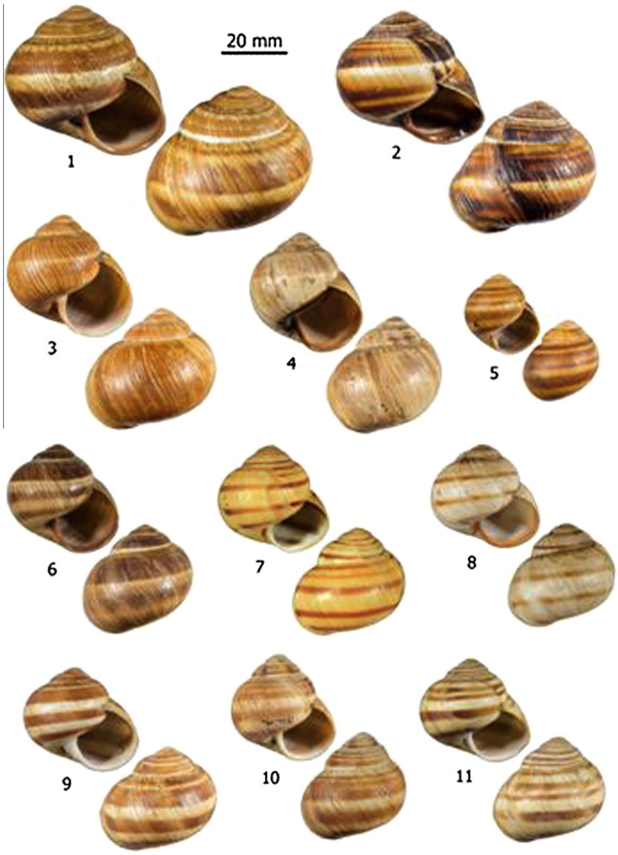

**Fig. 1.** Shell variability in *Helix* species. 1: *Helix straminea* (Italy: Montalbuccio – MON), 2: *Helix lucorum* (Italy: Ponte della Sambiuca – PDS), 3: *Helix pomatia* (Italy: Valle del Rio Freddo – RFR), 4: *Helix melanostoma* (Tunisia: Djebel Zaghouan – ZAG), 5: *Helix ceratina* (Corsica – COR), 6: *Helix cincta* (Italy: Arquà Terme – ARQ), 7: Apennine *Helix* (Italy: Forchia – FOR), 8: Apennine *Helix* (Italy: Filigieno – FIL), 9: Apennine *Helix* (Italy: Monti Reatini, Leonessa – LEO), 10: Apennine *Helix* (Italy: Monti del Matese: Monte Miletto – MIL), 11: Apennine *Helix* (Italy: Monti Reatini, Iaccio Crudele – IAC).

To test our hypotheses on the evolutionary history of *Helix* snails we analysed morphological (shell and genitalia) and molecular data (mitochondrial and nuclear markers; mtDNA and nDNA) on two scales. At the broader scale we reconstructed the phylogeny and evolutionary history of the genus by analysing our data in combination with that already published by Korábek et al. (2014, 2015) and Psonis et al. (2014). We also analysed the population genetic structure and phylogeography of the widespread species *H. pomatia*. We then narrowed the focus to the scale of peninsular Italy, where we investigated evolutionary relationships and diversification among *Helix* populations over time, and the origin of shell diversification. In both cases, we asked whether the distribution and variability patterns of *H. pomatia* in mainland Europe and *Helix* populations