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Molecular systematics and biogeography of land snails of the Mediterranean Basin: the study of *Allognathus*, *Candidula* and *Xerocrassa* (Gastropoda: Helicoidea)

Luis Javier Chueca Simón

Corrigendum

Chueca, L.J. (2016) *Molecular systematics and biogeography of land snails of the Mediterranean Basin: the study of* Allognathus, Candidula *and* Xerocrassa *(Gastropoda: Helicidae)*. University of the Basque Country (UPV/EHU), Spain.

1) In page 15, legend of Figure 1.11 should be as follows:

   A. *Candidula codia* Boliqueime. Faro (Portugal)
   B. *Candidula corbellai* Pla de Busa, Navés. Lleida (Spain)
   C. *Candidula coudensis* Vale da Couda. Leiria (Portugal)
   D. *Candidula gigaxii* La Muela, Algodonales. Cádiz (Spain)
   Scale bar 0.5 cm

2) In page 108, at legend of Figure 4.4, where it says "*X. molinae*" it should read "*X. ebusitana*"

Molecular systematics and biogeography of land snails of the Mediterranean Basin: the study of *Allognathus*, *Candidula* and *Xerocrassa* (Gastropoda: Helicoidea)

A thesis submitted by
Luis Javier Chueca Simón
for the degree of Doctor of Philosophy,
under the supervision of

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University of the Basque Country (UPV/EHU), Vitoria-Gasteiz, 2016

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Contents

Abstract

Human activity is causing a crisis of biodiversity with an elevated extinction rate, so correct species identification and a good knowledge of their habitats and distribution ranges is essential to minimize biodiversity loss through the prioritization of conservation areas and its efficient management. As one of the twenty-five biodiversity hot spots around the world, the Mediterranean Basin is characterized by a great biological richness with a high number of endemic species. Over the last years, molecular systematics has risen as one of the most appropriate disciplines for the discovery of genetic diversity, leading to several phylogenetic and phylogeography works. Moreover, biogeographical studies are plentiful in the region because its geological history is well known, where the main geological events are placed in a well-documented temporal framework. In this way, terrestrial gastropods are considered as a group of great interest in studies of phylogeography and evolution due to their restricted dispersal capacity and intraspecific gene-flow that tend to preserve phylogeographical patterns. The main aim of this thesis is to broaden our knowledge about the systematics and biogeography of three genera of land snails, *Allognathus*, *Candidula* and *Xerocrassa*, with a high number of species endemic to the Mediterranean basin.

Firstly, a nomenclatural revision of the genus *Allognathus* was conducted, with special emphasis on *Allognathus graellsianus*, *A. hispanicus hispanicus* and *A. hispanicus campanyonii*. Furthermore, we include a redescription of the genital anatomy of *A. hispanicus tanitianus* as the original description does not represent accurately the morphology of the genital anatomy. Then, we inferred the evolutionary history of the land snail genus *Allognathus* from a molecular phylogenetic reconstruction. An approximate temporal framework for its colonization of the Balearic Island is provided according to paleaeogeographical events in the western Mediterranean Basin. By the use of molecular species delimitation analyses, three species were delimited within the genus, that should be named *A. graellsianus*, *A. hispanicus* and *A. campanyonii*, the latter having at least five subspecies. The phylogenetic reconstruction showed a high degree of parallelism between the divergence of the main *Allognathus* lineages and the palaeogeography of the Balearic Islands, indicating a colonization of the archipelago from the southeast Iberian Peninsula during the middle Miocene. Sea level fluctuations that took place in the western Mediterranean during the Plio-Pleistocene explained the diversification and secondary contacts of the phylogroups of *Allognathus* within the Gymnesic or within the Pytiusic Islands and islets, as well as their distribution ranges. The presence of *A. campanyonii campanyonii* in the Iberian Peninsula, and *A. campanyonii minoricensis* in Ibiza, are the result of introductions in historic times from Mallorca and Menorca respectively. Finally, several hybrid specimens within the different phylogroups of *A. (I.) campanyonii* have been detected, supporting their consideration as subspecies of the same species under the biological species concept.

As in the genus *Allognathus*, we assessed the evolutionary history of the land snail genus *Xerocrassa* on the Balearic Islands from a molecular phylogenetic reconstruction. We unravelled its present diversity within the archipelago, and investigated its radiation processes according to its current distribution and the palaeogeographical events in the western Mediterranean Basin. Eleven species were delimited within the genus in the Balearic Islands, some of them with several subspecies, four species in the Pytiusic and seven in the Gymensic Islands. Due to the molecular results, a new taxonomic classification has been proposed. The onset of the Mediterranean climate during the transition of the Pliocene to the Pleistocene, and Pleistocene glacial cycles influenced the radiation of the genus within the archipelago.

Finally, we conducted a molecular phylogeny for the genus *Candidula*, including almost all of its 28 species, from DNA sequences of two mitochondrial and nine nuclear loci. New primers were developed for seven of these nuclear loci, obtained from the transcriptomes of three species of *Candidula,* which successfully amplified for several species of Geomitridae and Hygromiidae, too. Our molecular phylogeny recovered at least six *Candidula* lineages. The incorporation of *Candidula* lineages within the Geomitridae phylogeny demonstrated that the genus is polyphyletic. As a consequence, the presence of a single large dart sac is, probably, a character that has appeared independently several times within the family. The six primary groups of *Candidula* show high biogeographical consistence, where western groups were included within the Helicellini whereas central-eastern groups were closely related to the Trochoideini and Cernuellini. Most *Candidula* species analysed were monophyletic. Only *C. camporroblensis* and *C. belemensis* were paraphyletic, being necessary the study of more of their populations to resolve their taxonomy. *C. intersecta* could include more than one species.

KEYWORDS

Biogeography, conservation, diversification, endemism, Mediterranean Basin, Gastropoda, Helicoidea, phylogeography, species, taxonomy.

Resumen

La cuenca mediterránea es una de las regiones más ricas y complejas de la Tierra, y constituye uno de los veinticinco “hotspots” de biodiversidad del planeta. La singularidad y riqueza biológica de esta región se explica por la complejidad de su paleogeografía y paleoclimatología, así como por la presencia de un mosaico de hábitats que han favorecido los procesos de diversificación. Sin embargo, debido principalmente al incremento de la presión humana, la cuenca mediterránea ha experimentado una importante pérdida y fragmentación de hábitat que está provocando un aumento significativo de los procesos de extinción, siendo necesario establecer medidas prioritarias de conservación. De este modo, en términos de conservación, es crucial contar con una adecuada clasificación de los organismos, siendo la taxonomía filogenética una disciplina esencial para alcanzar este objetivo. En los últimos años, la sistemática molecular ha adquirido un papel relevante, proporcionando un conjunto de datos, complementarios de los paleogeográficos, paleontológicos, climáticos, morfológicos, etc., con los que abordar estudios filogenéticos, biogeográficos y evolutivos. Así mismo, el uso de herramientas moleculares ha permitido la identificación de especies crípticas y de unidades evolutivamente significativas dentro de las especies (ESUs), así como sinonimizar taxones que no son más que variedades morfológicas dentro de una misma especie.

El objetivo principal de esta tesis es ampliar el conocimiento existente sobre la taxonomía, la biogeografía y las relaciones filogenéticas de tres géneros de gasterópodos terrestres pertenecientes a la superfamilia Helicoidea (Gastropoda: Pulmonata), mediante la aplicación de diferentes herramientas moleculares. Se trata del primer estudio que aborda el esclarecimiento de las relaciones filogenéticas existentes en los géneros *Allognathus*, Pilsbry 1888 y *Candidula*, Kobelt 1871, así como de las especies del género *Xerocrassa*, Monterosato 1892 presentes en las islas baleares. Estos estudios resultan de gran interés, dado el alto grado de endemicidad que presentan estos géneros en la cuenca mediterránea. Los resultados obtenidos en este trabajo pretenden contribuir de manera significativa al conocimiento de los procesos biogeográficos más relevantes acaecidos en la cuenca mediterránea occidental. Además, esta nueva información permitirá actualizar la clasificación de estos géneros así como delimitar correctamente todos los taxones identificados, tomando en consideración la nueva información molecular. Todo ello redundará en una correcta conservación de su biodiversidad.

Capítulo 1

El primer capítulo expone una introducción general donde se realiza una descripción del área de estudio, la cuenca mediterránea, indicando los principales eventos geológicos que han moldeado la región hasta su disposición actual. Estos eventos geológicos adquieren gran relevancia en el campo de la biogeografía debido a que la distribución actual de numerosos organismos está muy ligada a la historia geológica de la cuenca. También se

incluye una revisión de los principales trabajos realizados sobre gasterópodos terrestres, que demuestran la importancia de la sistemática molecular en los campos de la taxonomía, filogeografía y biogeografía, en la región mediterránea. Por último, se expone una breve descripción de los tres géneros de gasterópodos terrestres seleccionados para este trabajo, así como los aspectos más relevantes por los que resulta interesante abordar su estudio.

Capítulo 2

En el segundo capítulo se definen los objetivos generales y específicos de esta tesis, siendo el principal objetivo *"Incrementar el conocimiento en la sistemática, historia evolutiva y biogeografía de tres géneros de gasterópodos terrestres con un elevado número de especies dentro de la cuenca mediterránea"*.

Capítulo 3

Este capítulo recoge dos de los cuatro artículos incluidos en esta tesis. Ambos trabajos tratan sobre el género *Allognathus*, endémico de las Islas Baleares (Mediterráneo occidental), y tratan de esclarecer la taxonomía y nomenclatura de las especies, así como los aspectos biogeográficos más relevantes en la diversificación del género.

Artículo I: **"Consideraciones nomenclaturales sobre las especies del género *Allognathus* (Gastropoda: Pulmonata: Helicidae) y estudio anatómico de *Allognathus hispanicus tanitianus*"** - "Nomenclatural considerations about *Allognathus* species (Gastropoda: Pulmonata: Helicidae) and anatomical study of *Allognathus hispanicus tanitianus*".

La nomenclatura y taxonomía del género *Allognathus* ha sido muy controvertida desde su descripción, habiendo sido objeto de debate en numerosos trabajos, pero sin que se haya alcanzado un consenso al respecto. En este estudio, se realizó una exhaustiva revisión bibliográfica acerca de la nomenclatura de las especies del género *Allognathus*, consultando las obras originales con las descripciones y figuras aportadas para cada especie nominal, así como las obras más importantes que han tratado el género. Del mismo modo, se realizó una descripción conquiológica de cada taxón basándonos en el estudio de ejemplares procedentes de todo el área de distribución del género y cubriendo todos los taxones descritos de *Allognathus*. Además, se redescribió la anatomía del aparato reproductor de *A. hispanicus tanitianus* debido a que la descripción original incluye algunos datos, que en nuestra opinión, no representan con exactitud su morfología.

Artículo II: "Biogeografía del género de gasterópodos terrestres *Allognathus* (Helicidae): una colonización de las Islas Baleares en el Mioceno medio" - **"Biogeography of the land snail genus *Allognathus* (Helicidae): middle Miocene colonization of the Balearic Islands"**.

En este segundo trabajo sobre *Allognathus* se abordó por primera vez el estudio de las relaciones filogenéticas existentes entre los diferentes taxones descritos hasta el momento para este género. La compleja y controvertida taxonomía del género, aspecto abordado de forma previa a este trabajo, permitió destacar la falta de consenso, tanto en el número de taxones que componen el género, como en la determinación de las relaciones de parentesco existentes entre ellos. Por otro lado, *Allognathus* es el único género de la superfamilia Helicoidea endémico de las Islas Baleares siendo un buen candidato para evaluar las relaciones entre la paleogeografía de las Islas Baleares y la historia evolutiva de los taxones y linajes evolutivos existentes en el género.

Se incluyeron 87 ejemplares en el estudio, representando todas las especies nominales consideradas por los principales especialistas. Se generó una matriz de 2461 pares de bases procedentes del análisis de la región nuclear (5.8S rRNA – ITS2 – 28S rRNA) y dos fragmentos mitocondriales (*COI* y 16S rRNA). Los análisis de máxima verosimilitud e inferencia bayesiana permitieron resolver las relaciones filogéneticas existentes entre los diferentes taxones considerados así como examinar la historia evolutiva del género. Además, se emplearon diferentes herramientas moleculares de delimitación de especies para clarificar la taxonomía del género en el archipiélago. Los resultados obtenidos revelaron la presencia de tres especies dentro del género, una de las cuales contiene al menos cinco subespecies, por lo que se propone una nueva clasificación taxonómica que refleja las relaciones entre taxones de acuerdo a la información filogenética obtenida. Por otro lado, la reconstrucción filogenética mostró un gran paralelismo entre la divergencia de los principales linajes de *Allognathus* y la paleogeografía del Mediterráneo occidental. El género pudo colonizar Mallorca desde el sureste de la Península Ibérica en el Mioceno medio, en paralelo a otros organismos terrestres. Durante el Mioceno inferior y el Plioceno, ocurrieron varios eventos de diversificación dentro del archipiélago que explican su diversidad actual.

Capítulo 4

Artículo III: "Biogeografía y radiación del género de gasterópodos terrestres *Xerocrassa* (Hygromiidae) en las Islas Baleares" - **"Biogeography and radiation of the land snail genus *Xerocrassa* (Hygromiidae) in the Balearic Islands"**.

En este trabajo se evaluó la historia evolutiva del género *Xerocrassa* en las Islas Baleares (Mediterráneo occidental) mediante la inferencia de la primera filogenia molecular para las especies del género endémicas del archipiélago. Debido a las escasas diferencias existentes en el aparato reproductor, la taxonomía de las especies del archipiélago Balear se ha basado tradicionalmente en la morfología de la concha, existiendo clasificaciones muy dispares por parte de los diversos autores que han estudiado el género.

Para elucidar la compleja taxonomía de *Xerocrassa* en las Islas Baleares, así como para investigar su historia evolutiva, se analizaron 170 ejemplares provenientes de 112 poblaciones, generando una matriz de 2540 pares de bases procedentes del análisis de la región nuclear 5.8S rRNA – ITS2 – 28S rRNA y de dos fragmentos mitocondriales, *COI* y 16S rRNA. Los resultados obtenidos sugieren la presencia de al menos 11 especies dentro del archipiélago; cuatro especies en Ibiza, seis en Mallorca y una en Menorca, presentando algunas de ellas varias subespecies. Debido a ciertas incongruencias entre las clasificaciones previas y los resultados obtenidos en la filogenia molecular y los análisis de delimitación de especies, relativas fundamentalmente a la adscripción de subespecies a su especie correspondiente, se proponen varios cambios nomenclaturales que creemos reflejan mejor las relaciones filogenéticas de estos taxones. Del mismo modo, se realizó un estudio biogeográfico para evaluar los procesos de radiación en función de la distribución actual de las especies, así como de los eventos paleogeográficos más importantes que han afectado al Mediterráneo occidental. De acuerdo con los análisis de datación molecular, *Xerocrassa* colonizó el archipiélago en un único evento durante la crisis salina del Messiniense (5.96 – 5.33 Ma) desde el este de la Península Ibérica. Además, estos análisis soportan la hipótesis de diversificación relativamente reciente dentro del género, donde el inicio del clima Mediterráneo durante la transición del Plioceno al Pleistoceno, así como los ciclos glaciares del Pleistoceno, han jugado un papel importante en su radiación dentro del archipiélago.

Capítulo 5

Artículo IV: "La filogenia molecular del género de gasterópodos terrestres *Candidula* (Geomitridae) inferida a partir de genes mitocondriales y nucleares revela la polifilia del genero" - **"Molecular phylogeny of *Candidula* (Geomitridae) land snails inferred from mitochondrial and nuclear markers reveals the polyphyly of the genus"**.

En este trabajo se presenta el estudio filogenético más exhaustivo realizado hasta el momento para el género *Candidula*. Con al menos 28 especies presentes a lo largo de Europa occidental, *Candidula* es uno de los géneros de Helicoideos más diversos dentro de la región mediterránea. Presenta numerosos endemismos en las Islas Canarias, la Península Ibérica, Italia y la Península Balcánica. Aunque el género está bien delimitado en función de la morfología de su aparato reproductor, las relaciones de las especies existentes dentro de él no están todavía resueltas. Debido a su gran número de endemismos y a su distribución en la cuenca del Mediterráneo, *Candidula* es un buen candidato para estudiar las relaciones filogenéticas entre todas las especies y para identificar procesos biogeográficos que han conducido a su distribución actual.

En este trabajo se estudiaron 22 especies pertenecientes al género *Candidula* infiriendo una filogenia molecular basada en dos genes mitocondriales (*COI* y 16S rRNA), una región nuclear ampliamente empleada en filogenias de moluscos (5.8S rRNA – ITS2 – 28S rRNA), y siete regiones de ADN nuclear cuya búsqueda y diseño de primers se realizó expresamente para este estudio (loci 40SS6, 60SL7, 60SL9, 60SL13, 60SL13a, 60SL17 and RPL14) a partir de los transcriptomas de tres especies de *Candidula*.

Como resultado se obtuvo una matriz de datos de 5585 pb, a partir de la cual se realizaron diferentes análisis de máxima verosimilitud e inferencia bayesiana. La filogenia molecular obtenida mostró dos clados principales, con las especies de *Candidula* agrupadas en seis entidades monofiléticas. Un primer clado agrupó a las especies endémicas del sudeste de Francia e Italia junto a *C. unifasciata*, especie ampliamente distribuida por centro Europa. El segundo de los clados principales englobó todas las especies endémicas de la Península

Ibérica junto a *C. intersecta* y *C. gigaxii*, ambas de amplia distribución en Europa, estando también presentes en la Península Ibérica. Por último, dentro de este segundo clado, se obtuvo un linaje diferente formado por *C. ultima*, endémica de las Islas Canarias.

Finalmente, la adición de diferentes géneros pertenecientes a la familia Geomitridae a los análisis filogenéticos, reveló que el género *Candidula* es polifilético, siendo necesario un estudio más completo de la familia para resolver sus relaciones filogenéticas. La polifilia detectada parece indicar que se han producido diversos procesos de homoplasia en las estructuras del aparato reproductor de la familia Geomitridae, y que son la base de la clasificación actual de la familia, lo que cuestiona enormemente el carácter natural de la clasificación genérica en esta familia.

Capítulo 6

Este capítulo recoge una síntesis de las modificaciones taxonómicas propuestas para los géneros estudiados de acuerdo a la nueva información molecular obtenida.

Capítulo 7

En este último capítulo se presentan las principales conclusiones contenidas en los capítulos anteriores y que resumen las contribuciones más relevantes de este trabajo al conocimiento y conservación a las especies de gasterópodos terrestres estudiadas.

Preface

This thesis is divided into seven chapters. The general framework is given by the general introduction (**Chapter 1**), where a reconstruction of the origin of the Mediterranean Basin is made, describing the main palaeogeographical events that have shaped the current topography of the Mediterranean. Then, within the evolutionary biology field, main disciplines and most important concepts applied or discussed in this thesis are briefly described. Finally, a brief description of the target genera of this work was done indicating the main aspects that make interesting these groups for the fields of malacology and biogeography.

The general and specific goals of the thesis are reported in **Chapter 2.**

Chapter 3 is subdivided into two papers (Papers I and II) and comprises a nomenclatural revision, a molecular phylogeny and the historical biogeography of *Allognathus* (Helicidae), a land snail genus endemic to the Balearic Islands.

In **Chapter 4** the molecular phylogeny and radiation processes of the *Xerocrassa* (Geomitridae) species endemic to the Balearic Islands is investigated. Moreover, according to the molecular information obtained a new taxonomic classification is proposed.

Chapter 5 shows the most exhaustive molecular phylogeny for the genus *Candidula* (Geomitridae) based on mitochondrial and nuclear markers.

Chapter 6 summarized the primary taxonomic modifications proposed for the genera studied according to the new molecular information obtained.

Finally, in **Chapter 7** the major findings from this PhD thesis are summarized.

CHAPTER 1

General Introduction

1. Mediterranean Basin

The Mediterranean Basin is one of the richest and most complex regions on Earth from a geological and biological point of view, and constitutes one of the twenty-five biodiversity hot spots considered around the world (Myers *et al.*, 2000), with a high number of endemic species. The uniqueness and richness of this singular region is explained by a complex palaeaogeography and palaeoclimate, as well as by the presence of a mosaic of habitats that have favoured diversification (Blondel & Aronson, 1999). However, as a result of the increment of human population, the Mediterranean Basin has experienced an important habitat loss as well as extinction processes, hence the setting of conservation priorities has become an urgent need (Médail & Quézel, 1999; Cincotta *et al.*, 2000; Brooks *et al.*, 2002; Vogiatzakis *et al.*, 2006). Currently, the main operating criteria for the definition of protected areas are the endemic richness and phylogenetic diversity (Orme *et al.*, 2005; Fonseca *et al.*, 2006; Spathelf & Waite, 2007). Therefore, in terms of conservation, an adequate classification of the organisms living in this region is crucial, being taxonomy an essential discipline to achieve this goal.

The geological history of the Mediterranean Basin, located at the edges of African and Eurasian plates, is relatively well known (Rosenbaum *et al.*, 2002; Meulenkamp & Sissingh, 2003; Rosenbaum & Lister, 2004; Jolivet *et al.*, 2006; Schettino & Turco, 2006), where tectonic events and sea level fluctuations are placed in a well-defined temporal framework. A succession of complicated periods of continental convergence and collisions of tectonic plates since the Mesozoic era (200 Ma), originated the Mediterranean Basin from Tethys Ocean (Blondel & Aronson, 1999; Meulenkamp & Sissingh, 2003). From Middle Jurassic to Eocene, continental plates of Africa, Europe and North America began to drift and led to the formation of the Atlantic Ocean and closing the gap between Europe and Africa

Figure 1.1. Map of the Mediterranean Basin. Dashed line indicates the approximate delimitation of the Mediterranean biogeographical area (redrawn from Quézel & Médail, 2003).

where much of the original Tethys Ocean was eliminated. In this period, the Greco-Italian micro-continent moved until its collision with the Eurasian plate. Then Italy collided with the Balkan block, giving rise to the Apennines. At the same time, a northward movement of Africa initiated the creation of the mountain ranges that encircle the Basin (Hsü, 1971). The Iberian Peninsula was joined to the African plate and shared its westward movement, which prompted the origin of the Pyrenees. Thereafter, at the beginning of the Oligocene (30-25 Ma), as a result of the Alpine orogeny, several continental microplates that formed part of the Hercynian belt (Corsica, Sardinia, Balearic Islands, Calabro-Peloritan massif, the Kabylies and the Beatic-Rift Cordillera) broke up and started drifting from the eastern Iberian Peninsula to their current location. Another short but crucial period was the Messinian salinity Crisis (5.96 – 5.33 Ma) (Hsü *et al.*, 1973; Krijgsman *et al.*, 1999). After the closure of the Isthmus of Suez at the lower Miocene (20 Ma), the Strait of Gibraltar was the unique connection of the Mediterranean Sea to any ocean. The collision between Africa and the Iberian Peninsula isolated the Mediterranean Sea from the Atlantic Ocean and led to the desiccation of the Mediterranean Basin allowing land connections between North Africa, Corsica, Sardinia and Eurasia and between the Balearic Islands and the Iberian Peninsula. The opening of the Strait of Gibraltar about 5.33 Ma allowed the refilling of the Mediterranean Basin and restored the isolation of Island ecosystems. Furthermore, sea level fluctuations took place due to glacial cycles in the Mediterranean during the Plio-Pleistocene (Lisiecki & Raymo, 2005; Sosdian & Rosenthal, 2009; Capraro *et al.*, 2011), modifying the shape and size of emerged lands, allowing different connections periods between islands and mainland. For instance, the last major ice ages (Riss, 200,000 years ago, and Würm, 25,000 years ago) led to the lowering of sea level at 130 and 110 m, respectively in western Mediterranean (Pomar & Cuerda, 1979; Emig & Geistdoerfer, 2004).

Within the biodiversity richness of the Mediterranean Basin, we should highlight Mediterranean islands, which show high endemism rates and constitute unique systems to the study of evolutionary processes addressing speciation (Mayol *et al.*, 2012), where the largest islands (Balearic Islands, Crete, Corsica, Cyprus, Sardinia and Sicily) show endemism rates of around 10 – 12% (Médail & Quézel, 1997).

The major climate changes occurred in the Mediterranean Basin, from the Messinian salinity crisis till the current Mediterranean climate, have contributed to shape the current biodiversity of this hot spot. The transition from a warm and humid climate to a cold and dry climate allowed a progressive increase of herbal plants linked to arid or sub-arid climates in substitution of subtropical flora from previous periods (Fauquette *et al.*, 1998; Suc & Popescu, 2005). This transition is considered as the onset of the Mediterranean climate (Suc, 1984; Thompson, 2005). The current Mediterranean climate is a transitional regime between cold temperate and dry tropical climates and it is characterized by the unique combination of hot dry summers, cool (or cold) humid winters and minimum levels of surface water availability during those months when the sun is at its strongest. Accordingly, the short spring and autumn seasons are critical periods for organism growth.

Finally, passive transport within the Mediterranean Basin has been demonstrated in several organisms (Carranza *et al.*, 2004; Martínez-Solano *et al.*, 2004; Pabijan *et al.*, 2012), including land snails (Uit de Weerd *et al.*, 2005; Welter-Schultes, 2008; Jesse *et al.*, 2011; Grindon & Davison, 2013; Poulakakis *et al.*, 2013; Korábek *et al.*, 2015) being an important factor that has influence the current distribution of many taxa.

2. Molecular systematics and phylogeography

Classification of organisms in the sense of Linneus (1758) have gradually changed into systematics, where classification of organisms considers their evolutionary relatedness in the framework of phylogeny, a hierarchical structure interrelating all biological diversity into the "tree of life" (Avise, 1994; Hillis *et al*., 1996). According to this "tree of life" concept, all organisms share a common ancestor (Woese & Fox, 1977). So, phylogenetic inference is based on a central concept of correspondence of compared characters due to common ancestry. This correspondence is called homology (Fitch, 1970).

Traditional approaches for phylogenetic relationships involved comparisons of phenotypic data from morphology or other characters amenable to observation. Nevertheless, at the end of the twentieth century, molecular systematics was developed with the aim to propose well-corroborated hypothesis for the relationships between genes or species (Sites & Marshall, 2003; Wiens, 2007). The analysis of DNA sequences became a standard for phylogenetic inference, where each nucleotide position in a DNA sequence is considered a character, which can attain only four character states, i.e. nucleotides adenine, cytosine, guanine and thymine. Mitochondrial DNA (mtDNA) markers have several advantageous properties, which make them suitable for phylogenetic inference. They are largely clonal, maternally inherited and generally non-recombining (Macaulay *et al*., 1999). Moreover, nuclear DNA (nDNA) markers show lower mutation rates than mtDNA markers being very useful for suprageneric phylogenies. Multi-locus approaches based on analysis of both, mitochondrial and nuclear markers, are essential for an accurate reconstruction of the evolutionary processes underlying the history of species and their populations. Furthermore, these kinds of approximations allow to infer in what extent, processes like interspecific gene flow or incomplete lineage sorting have shaped the phylogenetic relationships among studied species (Maddison & Knowles, 2006; Knowles & Carstens, 2007; Heled & Drummond, 2010).

Nowadays, the character-based methods [Maximum Parsimony (Edwards & Cavalli-Sforza, 1963; Kluge & Farris, 1969; Farris, 1970; Fitch, 1971), Maximum Likelihood (Cavalli-Sforza & Edwards, 1967; Felsenstein, 1981; see Huelsenbeck & Crandall, 1997 to review the method) and Bayesian analysis (Rannala & Yang, 1996; Huelsenbeck & Ronquist, 2001; Huelsenbeck *et al*., 2001; Lewis & Swofford, 2001)] are the most extended methods to reconstruct phylogenetic trees. Maximum Parsimony method does not require the definition of an evolutionary model, but can incorporate information about the relevance of the characters (assign different weights or differential costs). Maximum Likelihood and

Bayesian analysis require definition of probabilistic models of evolution of the characters. The methods of maximum parsimony (MP) and maximum likelihood (ML) are based on a optimization criteria that assess to evaluate alternative trees and choose the most optimal (in MP the best tree is the one that minimizes the homoplasy, and in ML the more likely tree is selected on the basis of the data and the evolutionary model used). On the other hand, Bayesian inference uses Markov Chain Monte Carlo (MCMC) algorithms (Metropolis *et al*, 1953; Hastings, 1970) to approximate the posterior distribution of parameters of interest, including topology and branches length, and the result is a distribution of optimal trees.

Defining species boundaries is another important goal with significant impacts in several fields such as systematics, evolution and conservation (Sites & Marshall, 2003; Agapow *et al*., 2004; Isaac *et al*., 2004; Padial & Riva, 2006). Advances in DNA sequencing technologies have led to the detection of many cryptic species and to resolve the phylogenetic classification of complex taxonomic groups (Bickford *et al*., 2007). In some cases, morphology alone or any other single data set, may not serve to establish species boundaries and relationships accurately, and hence the usage of different lines of evidence is needed (Padial *et al*., 2010; Derkarabetian & Hedin, 2014; Melville *et al*., 2014). Over the last years, many methods for delimiting species have been developed. One of the most commonly used strategies for species delimitation is to apply single-locus genes discover approaches followed by validation analyses (Carstens *et al*., 2013). Nowadays, several DNA-based exploratory methods are available (Pons *et al*., 2006; Puillandre *et al*., 2012; Ratnasingham & Hebert, 2013; Zhang *et al*., 2013; Kekkonen & Hebert, 2014). This exploratory step is generally efficient to detect highly divergent lineages that most probably correspond to different species (Pante *et al*., 2014). However, single-locus analyses generate many putative independent evolutionary lineages that have to be subsequently validated by using multi-locus, multi-coalescent approaches, and further corroborated by the addition of phenotypic, ecological and geographical information (Camargo *et al*., 2012). The use of information from these different types of data and methodologies was described as "integrative taxonomy" (Dayrat, 2005; Will *et al*., 2005).

Phylogeography is a discipline that serves as a bridge between phylogeny and population genetics in an explicit geographical context, and also includes additional information from other disciplines (paleontology, geology, ethology, etc.), which have been widely used in fields such as evolutionary biology, ecology or conservation (Avise, 2000). Phylogeography allows to establish patterns of distribution of genetic diversity from a geographical and temporal context, determining demographic processes as gene flow and effective population size, and to distinguish between historical processes (fragmentation or expansions) and demographic events (gene flow, migration, genetic drift, etc.) (Avise, 2000; Templeton, 2004). Comparing phylogenies of different co-distributed taxa it is possible to recognize common evolutionary patterns and thus better understand the historical processes involved in the population structure of different taxa (Arbogast & Kenagy, 2008; Robertson *et al*., 2009; Gutiérrez-García & Vázquez-Domínguez, 2011).

The estimation of the timing of evolutionary events from DNA sequence information has been one of the main goals of biogeography. Over the last years, different Bayesian methods have been developed allowing the incorporation of uncorrelated variation of substitution rates along the tree obtained, with the aim to estimate the posterior distribution of the rates and divergence times. These estimates offer the possibility of determining the age of many evolutionary processes and mechanisms providing a time framework to date the origin and expansion of lineages, as well as dispersion and vicariance events allowing the study of the speciation and extinction processes.

Phylogenetic estimates of evolutionary timescales can be obtained from nucleotide sequencing data using a molecular clock. These estimates are important for our understanding of evolutionary processes across all taxonomic levels (Duchêne *et al.*, 2014). All molecular clocks need to be calibrated by adding independent sources of information, to transform genetic distances into absolute divergence times (Bidegaray-Batista & Arnedo, 2011). Fossil records, biogeographical and palaeoecological data are the main sources of information to date molecular phylogenies. Correct knowledge of the information for calibration points is crucial to accurately infer molecular dates. Fossil records are considered the most adequate information to calibrate phylogenies. Nevertheless, fossils show important problems like their correct location into the classification system and their position in the phylogeny as one stem lineage or one crown group (Near & Sanderson, 2004). Moreover, fossil records are not available for many taxa, so the use of geological and palaeoclimate events has been very helpful for dating, but their appropriate use to calibrate phylogenies requires well-documented geochronology and the demonstration that the assumed barriers constitute a true obstacle to dispersal for the focal group (Bidegaray-Batista & Arnedo, 2011). Increasing the number of calibrations closer to the root improves the estimates of divergence times; however, a single calibration can be effective if it is able to capture much of the among-lineage rate variation in the data (Duchêne *et al.*, 2014).

Until now, phylogeographical investigations have been focused on regions such as North America (Brunsfeld *et al.*, 2001; Soltis *et al.*, 2006) and the Alps (Schönswetter *et al.*, 2005), but have reached most regions including the Arctic (Abbott & Comes, 2004), China (Qiu *et al.*, 2011) and the Southern Hemisphere (Beheregaray, 2008). However, a substantial increase in the number of studies has occurred over the last ten years within the Mediterranean Basin (Bidegaray-Batista & Arnedo, 2011; Santos-Gally *et al.*, 2012; *et al.*, 2014; Nieto, 2014; Planas *et al.*, 2014; Poulakakis *et al.*, 2014). Although most of the studies developed in this region were mainly focused on mammals (Randi, 2007), herps (Carranza *et al.*, 2006, 2008; Brown *et al.*, 2008; Pleguezuelos *et al.*, 2008; Joger *et al.*, 2010) or arthropods (Sanmartín, 2003; Montreuil, 2008; Simaikis & Mylonas, 2008; Micó *et al.*, 2009; Ribera *et al.*, 2010), the Mediterranean land snails have attracted great interest and have received more attention in the last years (Fiorentino *et al.*, 2013; Korábek *et al.*, 2015; Psonis *et al.*, 2015).

3. Terrestrial gastropods as case of study

Terrestrial gastropods are considered a group of great interest in studies of phylogeography and evolution due to their restricted dispersal capacity and intraspecific gene-flow that tend to preserve phylogeographical patterns (Lydeard & Lindberg, 2003; Pfenninger *et al.*, 2007; Hayes *et al.*, 2009; Holland & Cowie, 2009; Hugall & Stanisic, 2011; Johnson *et al.*, 2012; Stankowski & Johnson, 2014). The combination of particular habitat requirements in land gastropods, together with low active dispersal ability, results in numerous differentiated populations at various levels of geographical and genetic populations, preserving the historical patterns of genetic variation (Thomaz *et al.*, 1996; Elejalde *et al.*, 2008; Hoekstra & Schilthuizen, 2011; Kokshoorn & Gittenberger, 2012; 2013; Sauer *et al.*, 2013). The geographic range of many land gastropods is usually quite restricted, showing high endemism rate and strong geographic structuring of populations, making them particularly suited to conduct biogeographical studies at a microgeographical scale (Davison & Clarke, 2000; Hausdorf & Hennig, 2004; Schilthuizen *et al.*, 2004, 2006; Ketmaier *et al.*, 2006). Terrestrial snails have also been extensively used to study biogeographical and evolutionary processes in the Mediterranean region (Hausdorf & Hennig, 2006; Ketmaier *et al.*, 2006; Douris *et al.*, 2007; Fiorentino *et al.*, 2010; Sauer & Hausdorf, 2010; Fiorentino *et al.*, 2013; Giokas *et al.*, 2010; Guiller & Madec, 2010; Kotsakiozi *et al.*, 2012; Korábek *et al.*, 2015; Psonis *et al.*, 2015).

Land snails are one of the most diverse groups within the Mediterranean region. Particularly, the Helicoidea superfamily shows a high number of species, out of which around 80% are endemic (excluding North Africa where in-depth taxonomic studies are needed).

4. Target genera: *Allognathus*, *Candidula* and *Xerocrassa*

Three land snail genera, with a high number of species endemic to the Mediterranean Basin, belonging to two families within the Helicoidea superfamily, Rafinesque, 1815, were selected to investigate evolutionary, biogeographical and phylogeographical patterns in the Mediterranean Basin.

Kingdom	Animalia	
Subkingdom	Eumetazoa	
Phylum	Mollusca	
Class	Gastropoda	
Subclass	Orthogastropoda	
Superorder	Heterobranchia	
Order	Pulmonata	
Suborder	Stylommatophora	
Superfamily	Helicoidea	
Family	Helicidae	***Allognathus***
	Geomitridae	***Candidula***
		Xerocrassa

* Classification followed by the CLECOM system (Bank, 2011). For the Helicoidea we have followed the classification proposed by Razkin *et al.*, (2015).

Allognathus Pilsbry, 1888

The genus *Allognathus* is endemic to the Balearic Islands (Western Mediterranean), but was introduced to the east coast of the Iberian Peninsula in historic times (Gasull, 1963). The genus is present in the entire archipelago, including Cabrera and Ses Bledes archipelagos and only one population is still alive in the Iberian Peninsula. The genus is divided in two subgenera: *Allognathus s. str*. and *Iberellus* (Bank, 2011; Chueca *et al*., 2013). The former is monotypic and the latter is divided into as many as seven taxa ranked as species or subspecies by different authors (Gasull, 1963; Puente, 1994; Pons & Palmer, 1996; Beckmann, 2007; Chueca *et al*., 2013) (Table 1.1). Shell morphology and population isolation have been the most important criteria for species description. The most remarkable character of the genus is the presence of a helicid shell with several interrupted dark bands on a light background (Figures 1.5 – 1.7). This colour pattern has given the common name "caragol de serp" (snake snail) due to its similarity to the skin of snakes. The genital anatomy is similar to other Helicidae, formed by a big rounded dart sac and two branched mucus glands inserted next to the base of the dart sac (Puente 1994) (Fig. 1.2). Due to the absence of differences in genital anatomy, the taxonomic status of the taxa within *Allognathus* is still unclear, being necessary a revised phylogeny to propose a natural classification that could be useful for effective conservation plans.

On the other hand, *Allognathus* is the only genus of the Helicoidea endemic to the Balearic Islands, being an exceptional model to study the biogeographical history of the archipelago.

Table 1.1. Main and most recent classifications proposed for *Allognathus*

Schileyko, 2006	Beckmann, 2007	Bank, 2011
Allognathus grateloupi	*Allognathus (A.) graellsianus*	*Allognathus (A.) graellsianus*
Iberellus balearica	*Allognathus (Iberellus) balearicus balearicus*	*Allognathus (Iberellus) hispanicus*
I. pyrenaicus		*Allognathus (I.) pyrenaicus pyrenaicus*
I. horadadae	*Allognathus (I.) b. horadadae*	*Allognathus (I.) p. horadadae*
	Allognathus (I.) b. minoricensis	*Allognathus (I.) p. minoricensis*
		Allognathus (I.) p. palumbariae
	Allognathus (I.) b. tanitianus	*Allognathus (I.) p. tanitianus*
Nesiberus pythiusensis	*Allognathus (I.) b. pythiusensis*	*Allognathus (I.) p. pythiusensis*

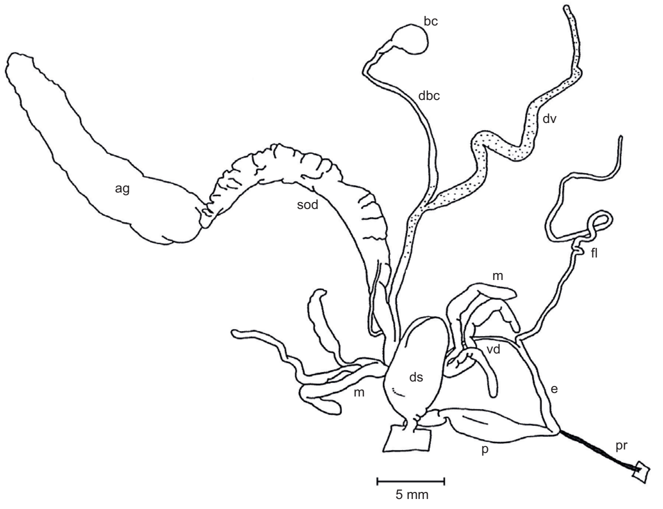

Figure 1.2.
Anatomy of distal genitalia in *Allognathus graellsianus* (from Puente, 1994): **bc** bursa copulatrix, **dbc** duct of bursa copulatrix, **ds** dart sac, **e** epiphallus, **fl** flagellum, **m** mucus gland(s), **p** penis, **pr** penis retractor muscle, **sod** spermoviduct, **v** vagina, **vd** vas deferens.

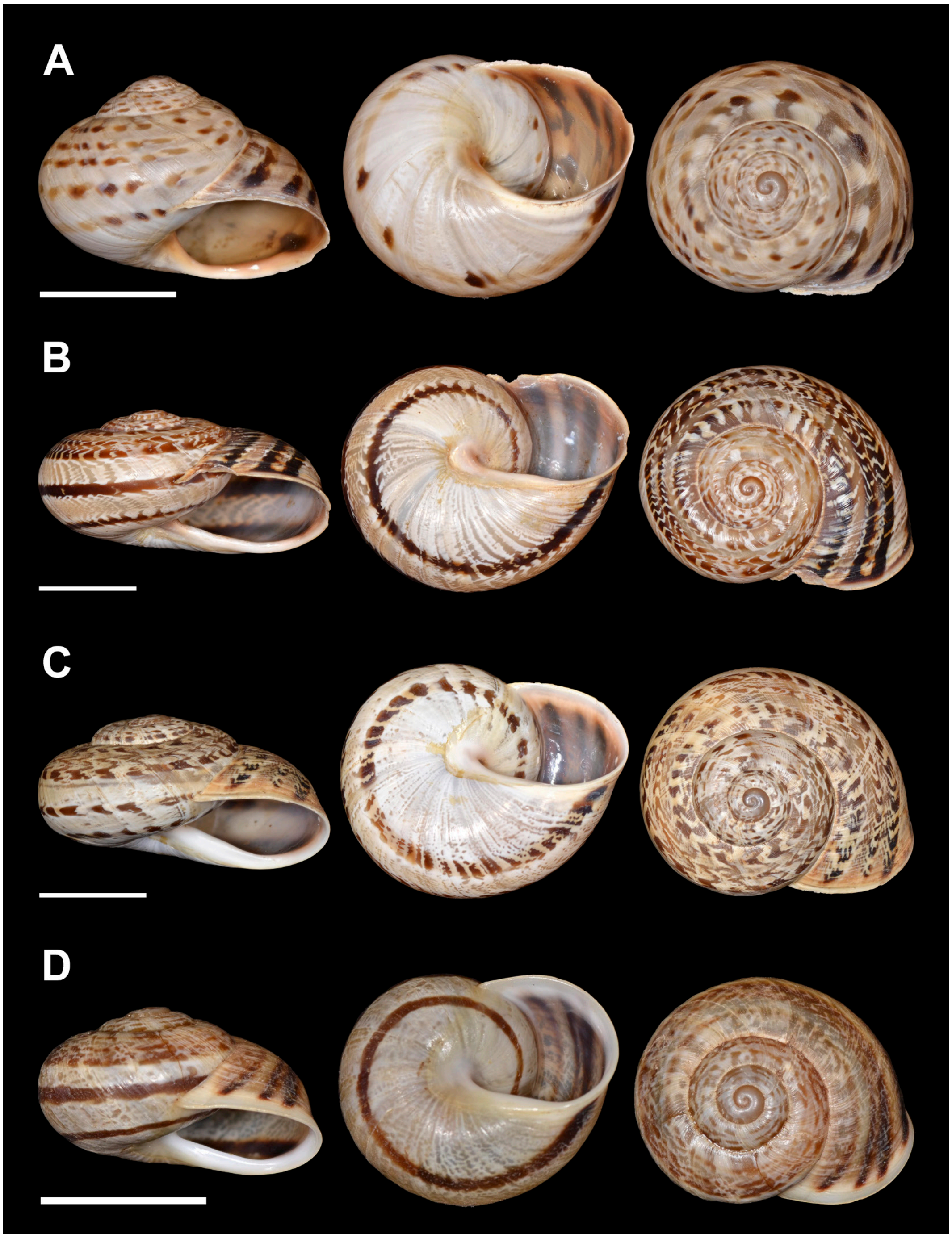

Figure 1.5
A. *Allognathus (Allognathus) graellsianus* Finca Mortixt. Escorca (Mallorca)
B. *Allognathus (Iberellus) hispanicus hispanicus* Puíg de la Verge, Pollença (Mallorca)
C. *Allognathus (Iberellus) hispanicus hispanicus* Sa Guixería, Andratx (Mallorca)
D. *Allognathus (Iberellus) hispanicus campanyonii* Frenchs Monument (Cabrera Archipelago)
Scale bar 1 cm

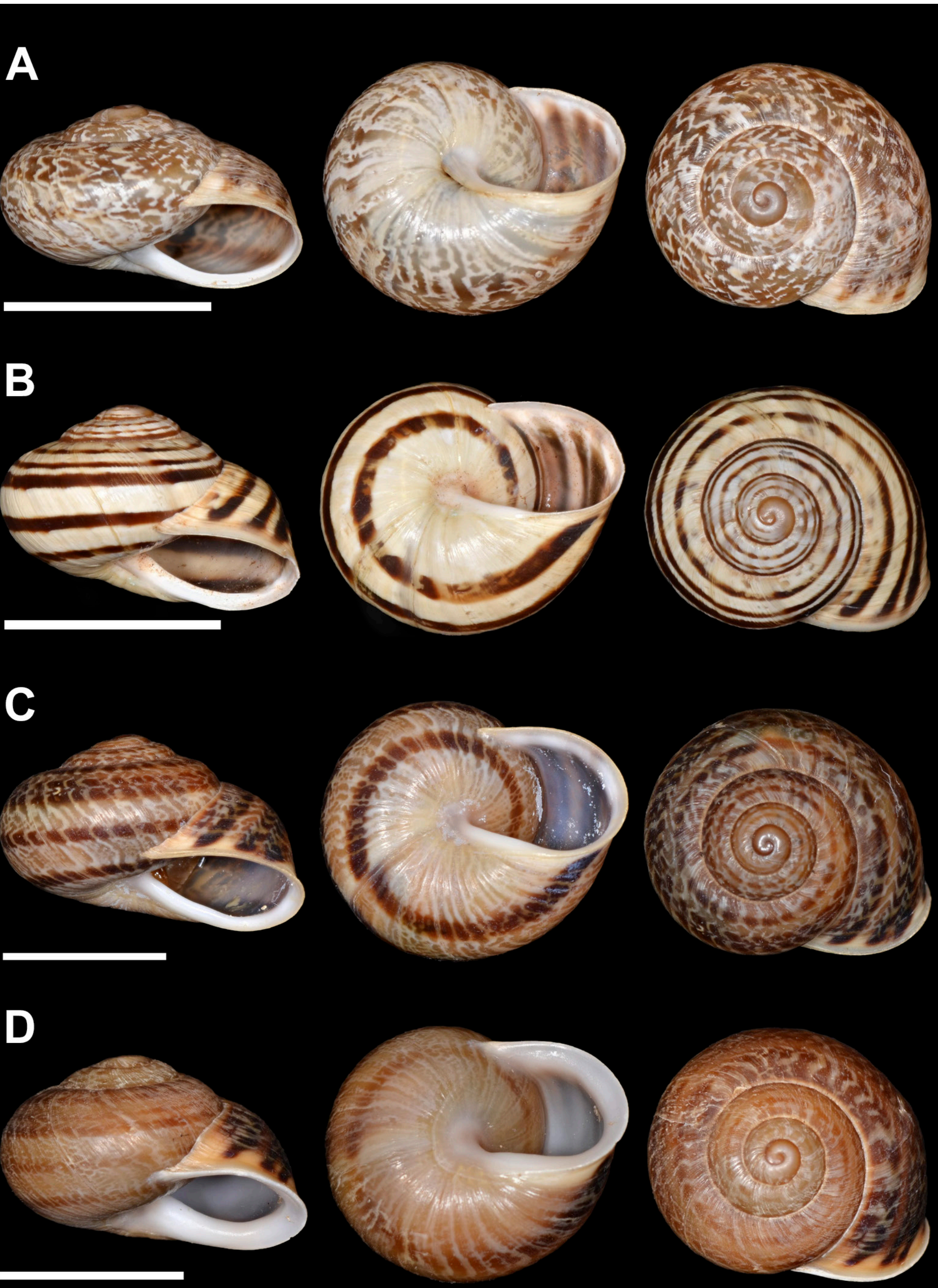

Figure 1.6
A. *Allognathus (Iberellus) hispanicus horadadae* Na Foradada (Cabrera Archipelago)
B. *Allognathus (Iberellus) hispanicus minoricensis* Ses Olles. Es Mercadal (Menorca)
C. *Allognathus (Iberellus) hispanicus minoricensis* Son Saura (Menorca)
D. *Allognathus (Iberellus) hispanicus palumbariae* Illa d'en Colom (Menorca)
Scale bar 1 cm

Figure 1.7
A. *Allognathus (Iberellus) hispanicus pythiusensis* Na Plana (Ses Bledes)
B. *Allognathus (Iberellus) hispanicus tanitianus* Cala Salada (Ibiza)
Scale bar 1 cm

Candidula Kobelt, 1871

The genus *Candidula*, composed by 28 species along western Europe, is disjunctly distributed in the Iberian Peninsula (18 species: *C. arganica, C. arrabidensis, C. belemensis, C. camporroblensis, C. carrapateirensis, C. codia, C. corbellai, C. coudensis, C. gigaxii, C. intersecta, C. najerensis, C. olisippensis, C. ponsulensis, C. rocandioi, C. scabiosula, C. setubalensis, C. strucki* and *C. unifasciata*), Italy (5 species: *C. cavannae, C. fiorii, C. grovesiana, C. spadae* and *C. unifasciata*), Balkan region (4 species: *C. castriota, C. lernaea, C. rhabatoides* and *C. syrensis*), one species in southeastern France (*C. rugosiuscula*) and another species in the Canary Islands (*C. ultima*). Some species are widely distributed throughout Europe (*C. gigaxii, C. intersecta* and *C. unifasciata*), while other species present very narrow distributions (*C. coudensis*, *C. corbellai* or *C. fiorii*, among others). *Candidula* species show high variability in shell characters, from rounded to keeled forms, where several taxa have ribs or hairs (Figures 1.9 – 1.14). Shell size is also very variable, with a diameter size ranging from 4.5 mm in *C. arganica* to 16 mm in *C. spadae* (Welter-Schultes, 2012). Anatomically, the genus is characterized by the presence of a large dart sac arising from the vagina, four mucus glands, simple or bifurcated, and a well-defined penial flagellum (Puente, 1994) (Fig. 1.8). Furthermore, the length of penial flagellum is also an important character to species differentiation (Puente, 1994; Holyoak

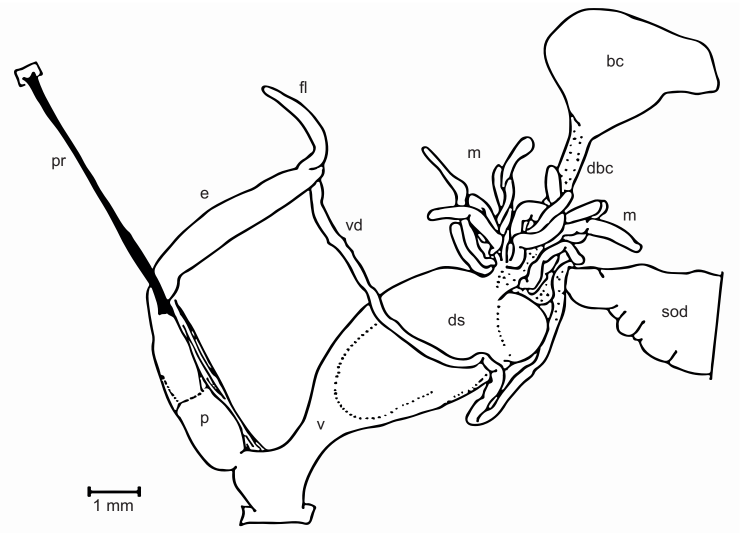

Figure 1.8.
Anatomy of distal genitalia in *Candidula gigaxii* (from Puente, 1994): **bc** bursa copulatrix, **dbc** duct of bursa copulatrix, **ds** dart sac, **e** epiphallus, **fl** flagellum, **m** mucus gland(s), **p** penis, **pr** penis retractor muscle, **sod** spermoviduct, **v** vagina, **vd** vas deferens.

& Holyoak, 2014). Due to its large distribution range, the genus is present in many diverse habitats such as rocky limestone areas, sand dunes and seminatural grasslands into an altitudinal gradient from sea level to high mountains.

Even though *Candidula* is one of the most diverse genera within the Mediterranean Basin, there are only few molecular works restricted to few species, but no global study of the genus has been conducted. The particular distribution of the species belonging to *Candidula* within the Mediterranean Basin makes the genus very interesting in order to study the phylogenetic relationships within the genus and the processes that have led to this biogeographical pattern.

Figure 1.9
A. *Candidula arganica* Berrostegieta. Araba (Spain)
Scale bar 0.5 cm

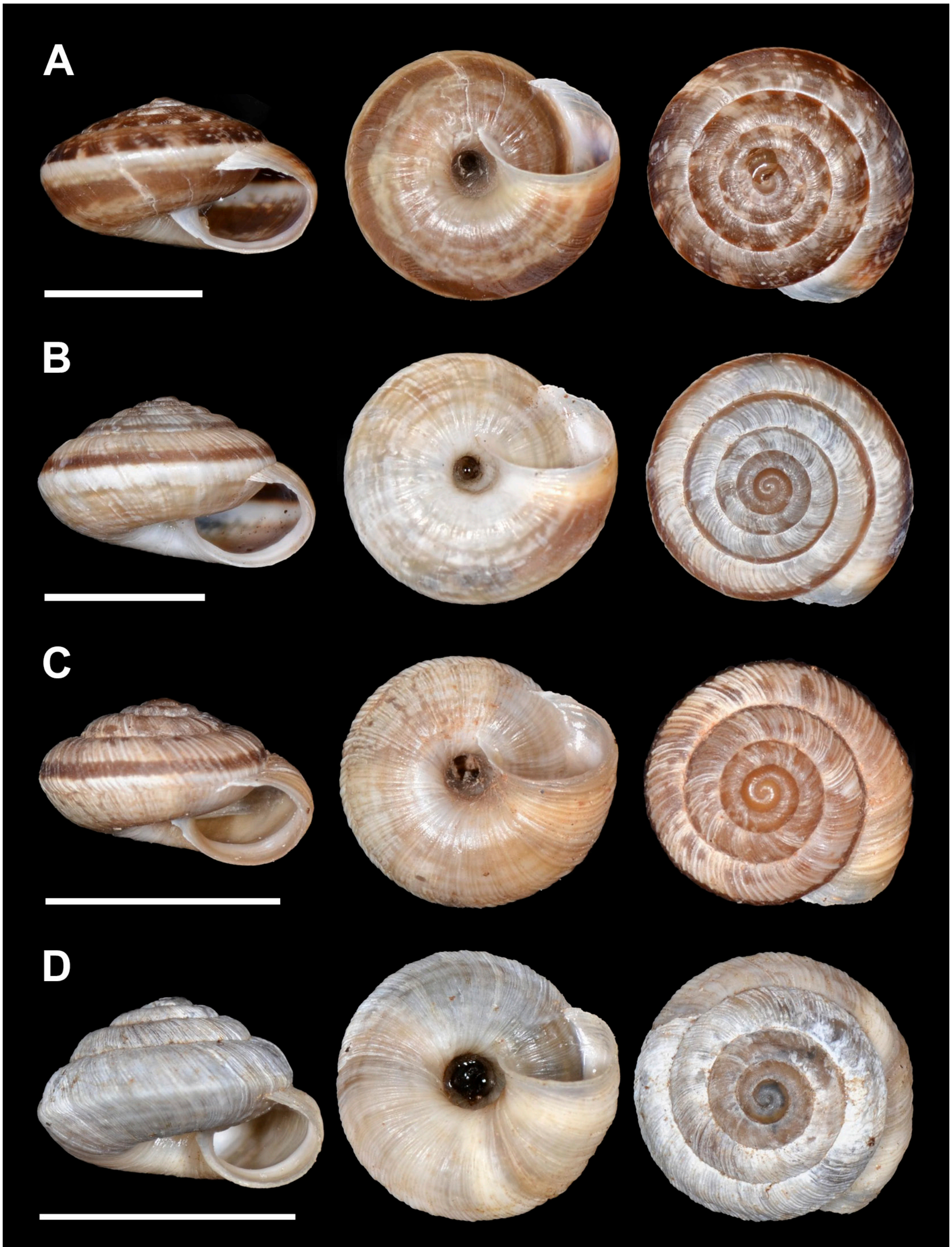

Figure 1.10
A. *Candidula arrabidensis* Serra da Arrabida. Setúbal (Portugal)
B. *Candidula belemensis* Vale da Couda. Leiria (Portugal)
C. *Candidula camporroblensis* Zael. Burgos (Spain)
D. *Candidula cavannae* Campitello Matese. Campobasso (Italy)
Scale bar 0.5 cm

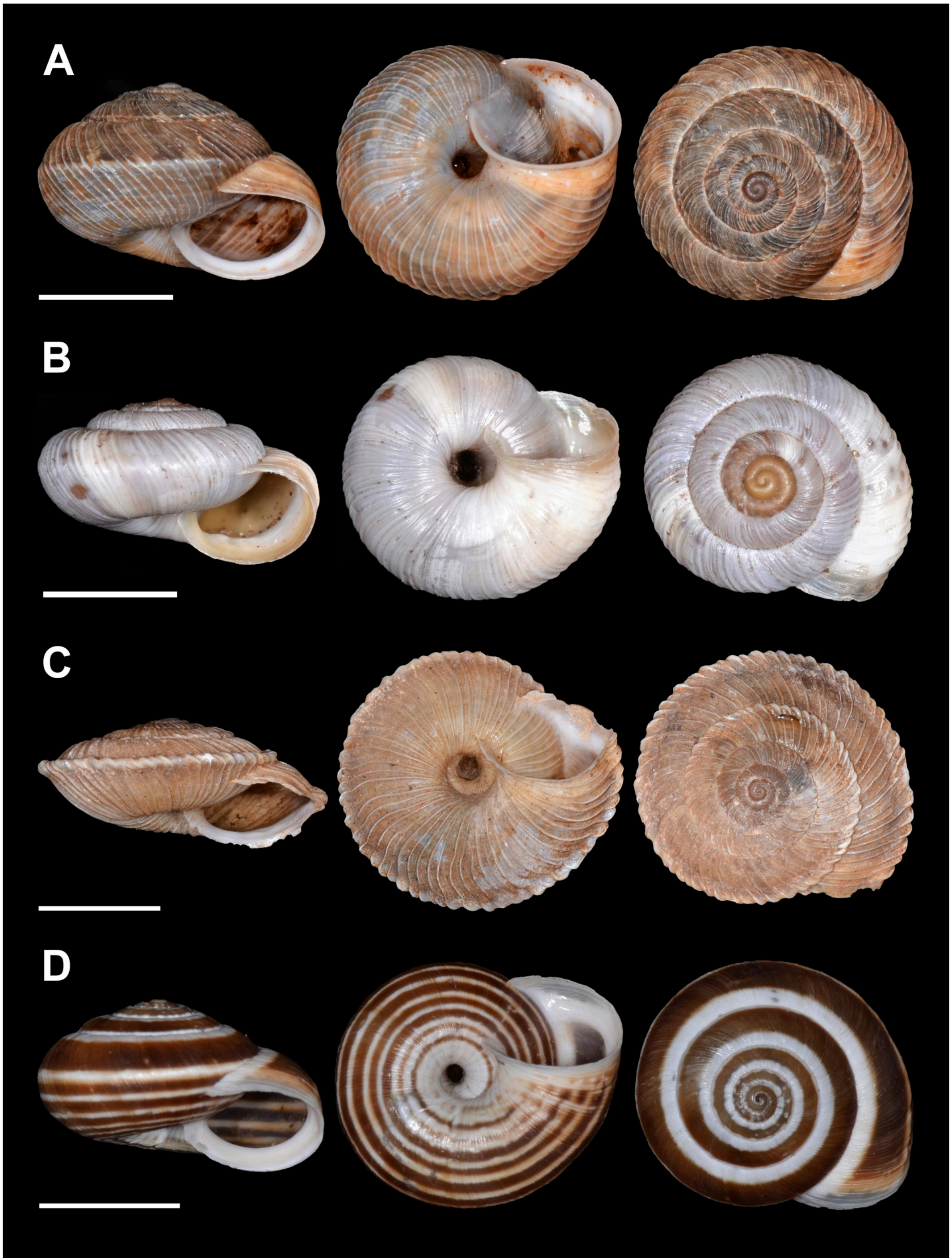

Figure 1.11
D. *Candidula codia* Boliqueime. Faro (Portugal)
A. *Candidula corbellai* Pla de Busa, Navés. Lleida (Spain)
B. *Candidula coudensis* Vale da Couda. Leiria (Portugal)
C. *Candidula gigaxii* La Muela, Algodonales. Cádiz (Spain)
Scale bar 0.5 cm

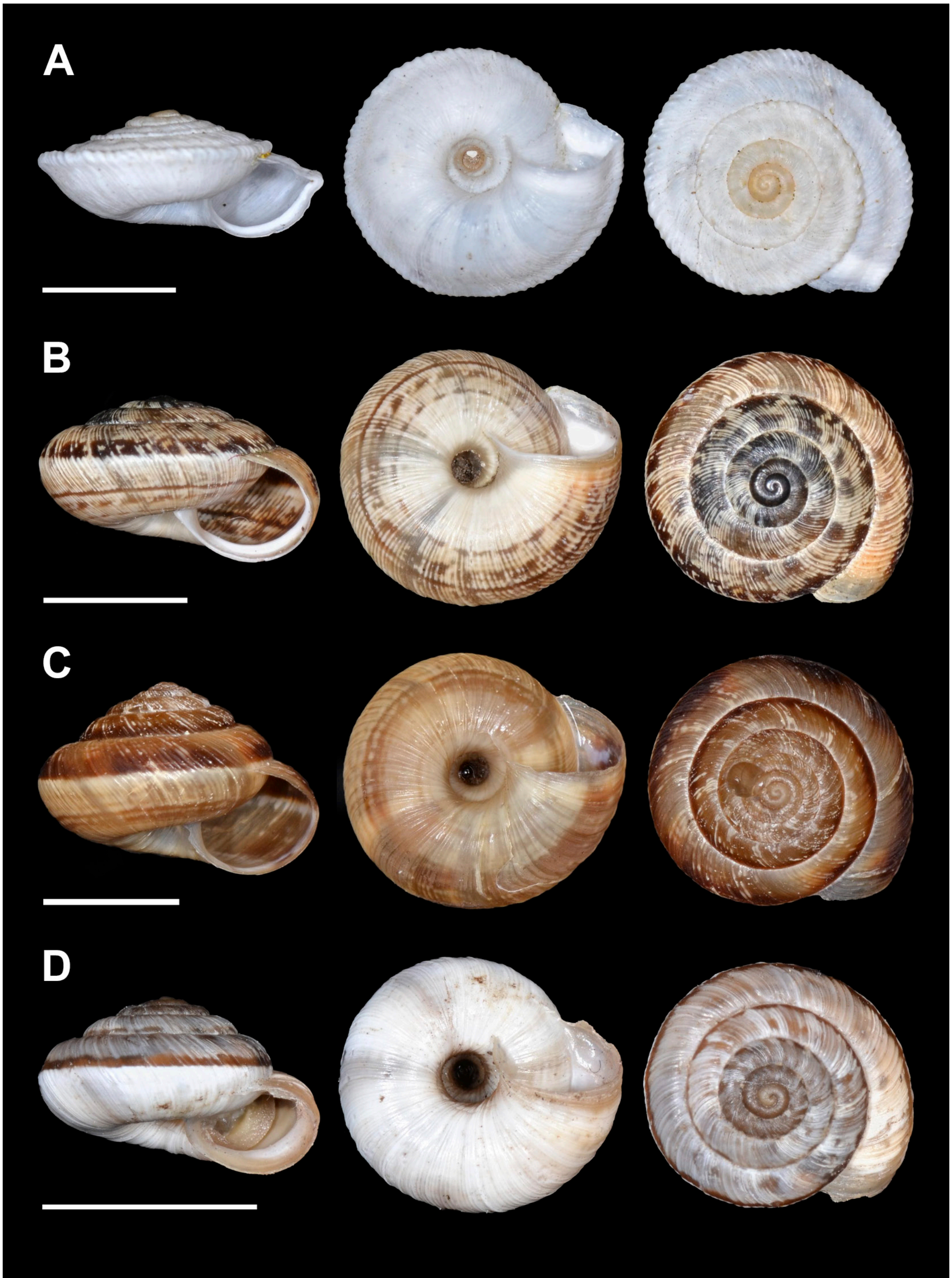

Figure 1.12
A. *Candidula grovesiana* Castrovalva, Gole de Sagittario. L'Aquila (Italy)
B. *Candidula intersecta* Botanic Gardens, Cambridge (England)
C. *Candidula intersecta* Pico Sacro. A Coruña (Spain)
D. *Candidula najerensis* Azagra. Navarra (Spain)
Scale bar 0.5 cm

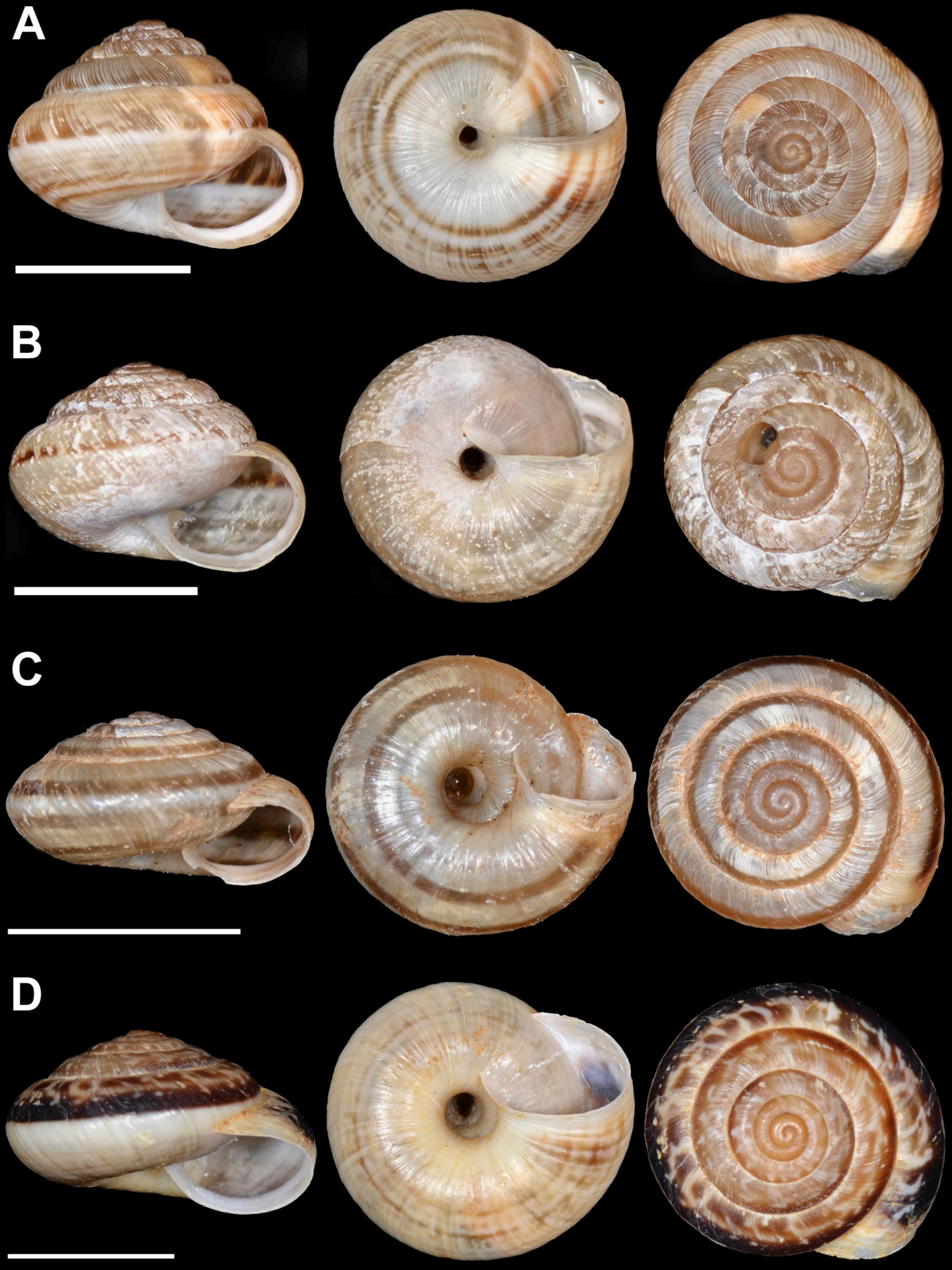

Figure 1.13
A. *Candidula olisippensis* Boliqueime. Faro (Portugal)
B. *Candidula ponsulensis* Pias. Zamora (Spain)
C. *Candidula rocandioi* Besande. León (Spain)
D. *Candidula scabiosula* Moncarapacho. Faro (Portugal)
Scale bar 0.5 cm

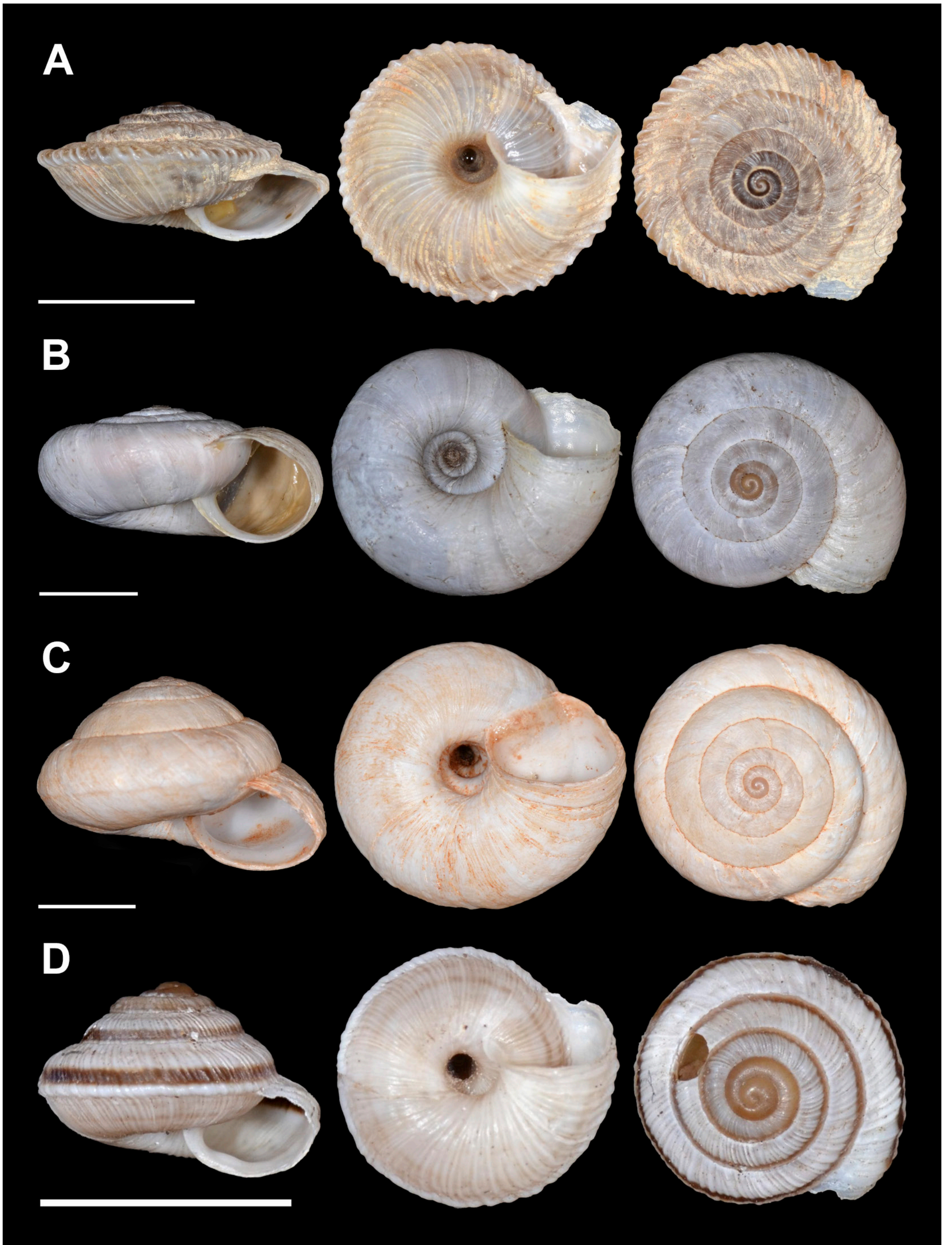

Figure 1.14
A. *Candidula setubalensis* Serra da Arrabida. Setúbal (Portugal)
B. *Candidula spadae* Gran Sasso, Campo Pericoli. L'Aquila (Italy)
C. *Candidula ultima* Lomo del Aceituno, Fuerteventura. Las Palmas (Spain)
D. *Candidula unifasciata* Monte Sagro. Massa-Carrara (Italy)
Scale bar 0.5 cm

Xerocrassa Monterosato, 1892

The genus *Xerocrassa* is distributed along the Mediterranean Basin showing two different geographical centres of diversification, the western (Iberian Peninsula and Balearic Islands) and the eastern Mediterranean (Crete and Middle East). Currently, there are at least 13 recognized species (Beckmann, 2007; Forés, 2015) endemic to the Balearic Islands (10 species in the Gymnesic islands and 3 in the Pityusic islands), with more than 20 subspecies recognized for *X. caroli* and *X. cisternasi* from Ibiza and Formentera islets (Table 1.2). Moreover, another species, *X. molinae*, is present in the Columbretes islands, a small archipelago located between the Iberian Peninsula and the Balearic Islands. *Xerocrassa* species from the Balearic Islands show a high level of variation in shell morphology, and the isolation of many populations in small islets led to consider this high number of subspecies.

Most species show more or less prominent ribs, and while keeled shells characterize Gymnesic taxa, rounded shell forms are the most common in the Pytiusic (Figures 1.18 – 1.26). Moreover, some taxa such as *X. cardonae*, *X. frater pulaensis* and *X. ferrutxensis* (Quintana & Vilella, 2001; Beckmann, 2007; Forés & Altaba, 2014) show hairy shells.

Table 1.2. Main classifications proposed for *Xerocrassa*

Jaeckel, 1952	Gasull, 1964	Beckmann, 2007	Bank, 2011
Xeroplexa frater frater	*Xeroplexa frater frater*	*Xerocrassa frater frater*	*Xerocrassa frater frater*
	X. frater newka	*X. frater newka*	*X. frater newka*
	X. frater muntaneri		
		X. frater pulaensis	
X. pollenzensis		*X. frater pollenzensis*	*X. frater pollenzensis*
X. frater ferreri	*X. frater ferreri*	*X. ferrei ferreri*	*X. ferrei ferreri*
	X. frater pobrensis	*X. ferrei pobrensis*	*X. ferrei pobrensis*
		X. homeyeri	*X. homeyeri*
X. prietoi	*X. prietoi*	*X. prietoi prietoi*	*X. prietoi prietoi*
		X. prietoi muroensis	*X. prietoi muroensis*
		X. moraguesi	*X. moraguesi*
	X. claudinae		
X. nyeli	*X. nyeli*	*X. nyeli*	*X. nyeli nyeli*
		X. ponsi	*X. nyeli ponsi*
		X. cardonae	*X. cardonae*
X. caroli	*X. caroli*	*X. caroli caroli*	*X. caroli caroli*
		X. caroli jaeckeli	*X. caroli jaeckeli*
		X. caroli espartariensis	*X. caroli espartariensis*
		X. caroli alegriae	*X. caroli alegriae*
		X. caroli formenterensis	*X. caroli formenterensis*
X. cisternasi	*X. ortizi cisternasi*	*X. cisternasi cisternasi*	*X. cisternasi cisternasi*
	X. ortizi ortizi	*X. cisternasi ortizi*	*X. cisternasi ortizi*
X. ebusitana vedrae		*X. cisternasi vedrae*	*X. cisternasi vedrae*
X. ebusitana vedranellensis		*X. cisternasi vedranellensis*	*X. cisternasi vedranellensis*
X. ebusitana scopulicola	*X. scopulicola*	*X. cisternasi scopulicola*	*X. cisternasi scopulicola*
X. ebusitana conjungens		*X. cisternasi conjungens*	*X. cisternasi conjungens*
X. ebusitana cala-saladae	*X. calasaladae*	*X. cisternasi calasaladae*	*X. cisternasi calasaladae*
X. ebusitana margaritae	*X. margaritae*	*X. cisternasi margaritae*	*X. cisternasi margaritae*
X. ebusitana muradae		*X. cisternasi muradae*	*X. cisternasi muradae*
	X. ortizi calderensis	*X. cisternasi calderensis*	*X. cisternasi calderensis*
		X. cisternasi mesquidae	*X. cisternasi mesquidae*
		X. cisternasi hortae	*X. cisternasi hortae*
X. ebusitana canae	*X. ortizi canaensis*	*X. cisternasi canae*	*X. cisternasi canae*
X. ebusitana redonae	*X. ortizi redonensis*	*X. cisternasi redonae*	*X. cisternasi redonae*
X. ebusitana ebusitana	*X. ebusitana*	*X. ebusitana ebusitana*	*X. ebusitana ebusitana*

The genital anatomy of *Xerocrassa* is characterized by two small accessory sacs with tubular form, inserted into a long vagina, and 2-4 mucus glands (Puente, 1994) (Fig. 1.15).

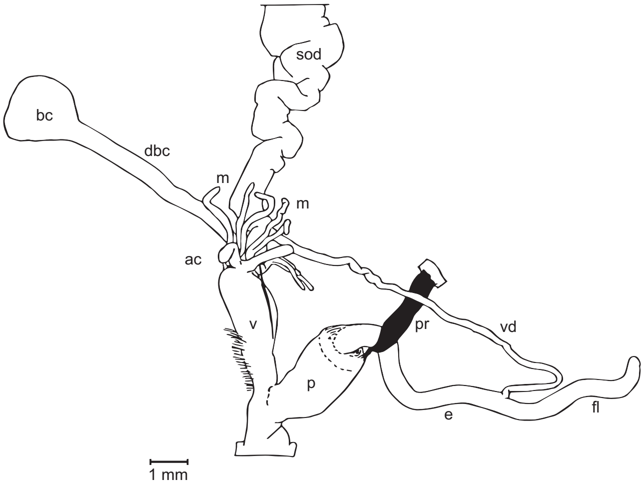

Figure 1.15. Anatomy of distal genitalia in *Xerocrassa ebusitana* (from Puente, 1994): **bc** bursa copulatrix, **dbc** duct of bursa copulatrix, **ac** accessory sacs, **e** epiphallus, **fl** flagellum, **m** mucus gland(s), **p** penis, **pr** penis retractor muscle, **sod** spermoviduct, **v** vagina, **vd** vas deferens.

The taxonomy of the genus has been discussed in several publications (Jaeckel, 1952; Gasull, 1964; Beckmann, 2007; Forés, 2015), but there are different classifications of these species being necessary to perform an exhaustive study to clarify the taxonomy of the genus in the Balearic Islands. Furthermore, due to the high number of *Xerocrassa* taxa and their distribution patterns in the archipelago, this genus is a good candidate for studies of radiation processes on islands.

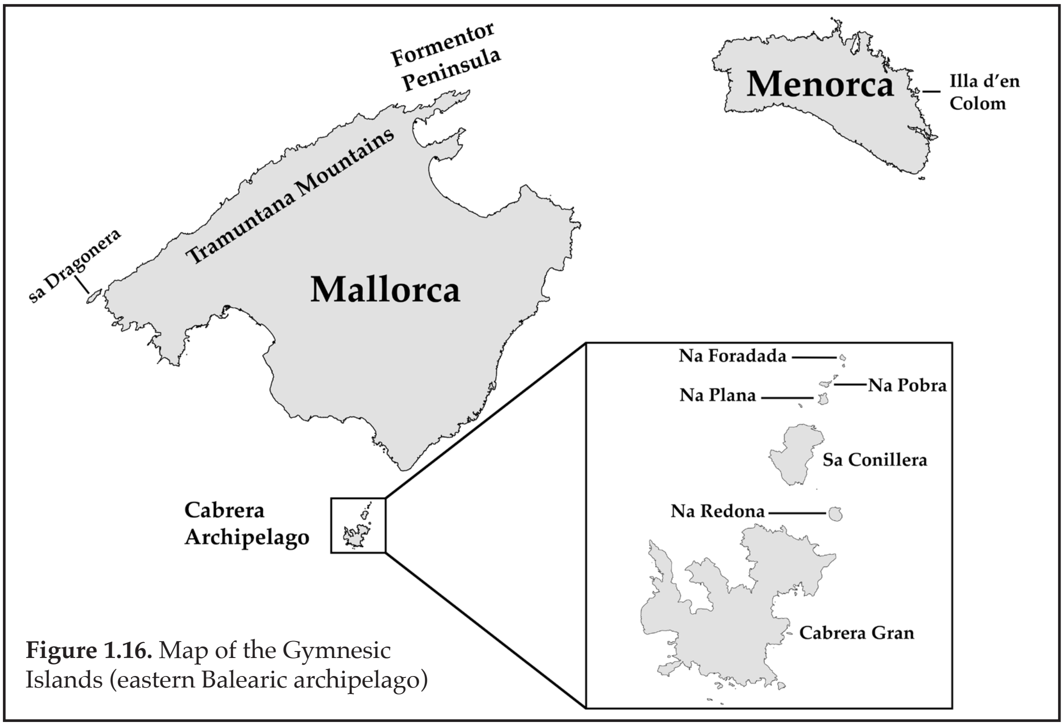

Figure 1.16. Map of the Gymnesic Islands (eastern Balearic archipelago)

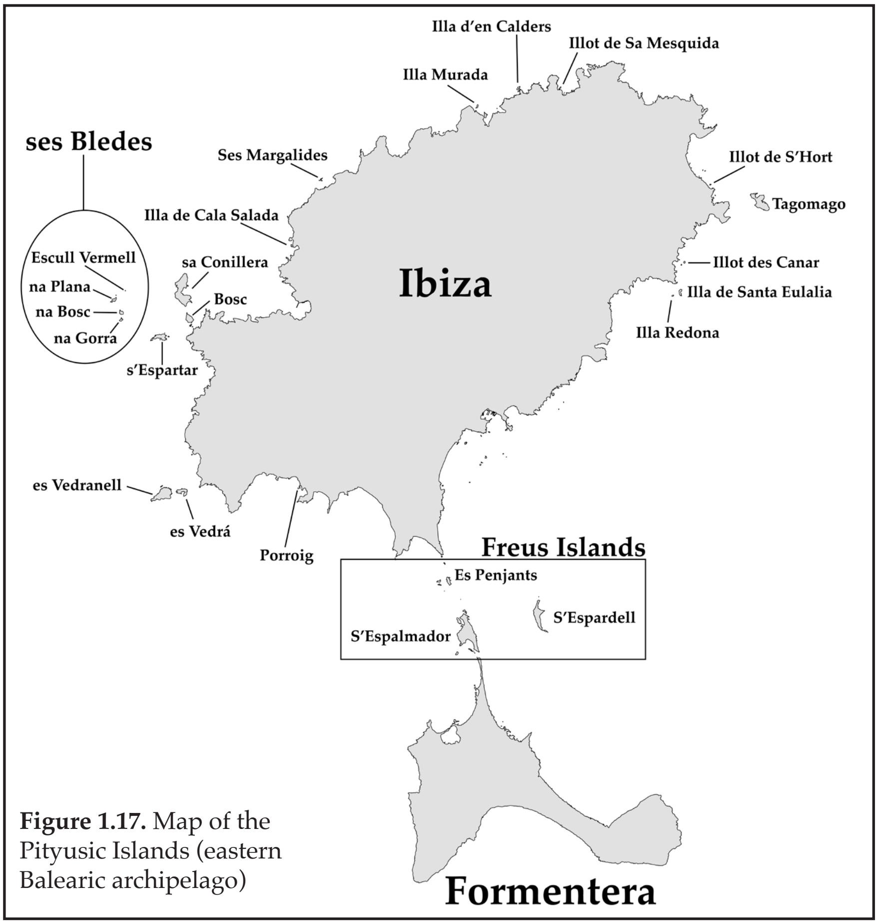

Figure 1.17. Map of the Pityusic Islands (eastern Balearic archipelago)

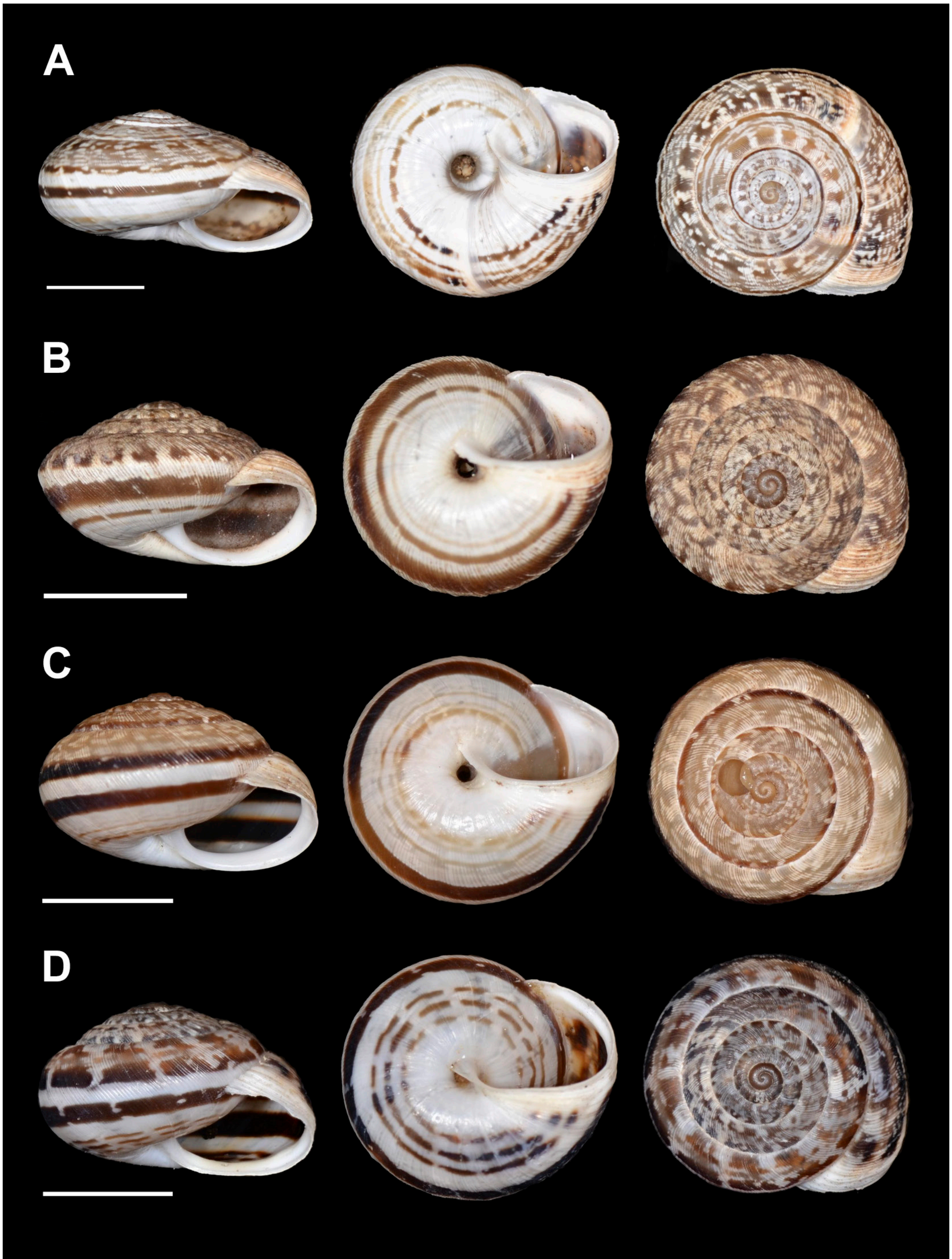

Figure 1.18
A. *Xerocrassa ebusitana* Sant Josep de sa Talaia (Ibiza)
B. *Xerocrassa caroli caroli* Cap des Jueu (Ibiza)
C. *Xerocrassa caroli espartariensis* Illa de s'Espartar (Ibiza)
D. *Xerocrassa caroli formenterensis* Sa Mola (Formentera)
Scale bar 0.5 cm

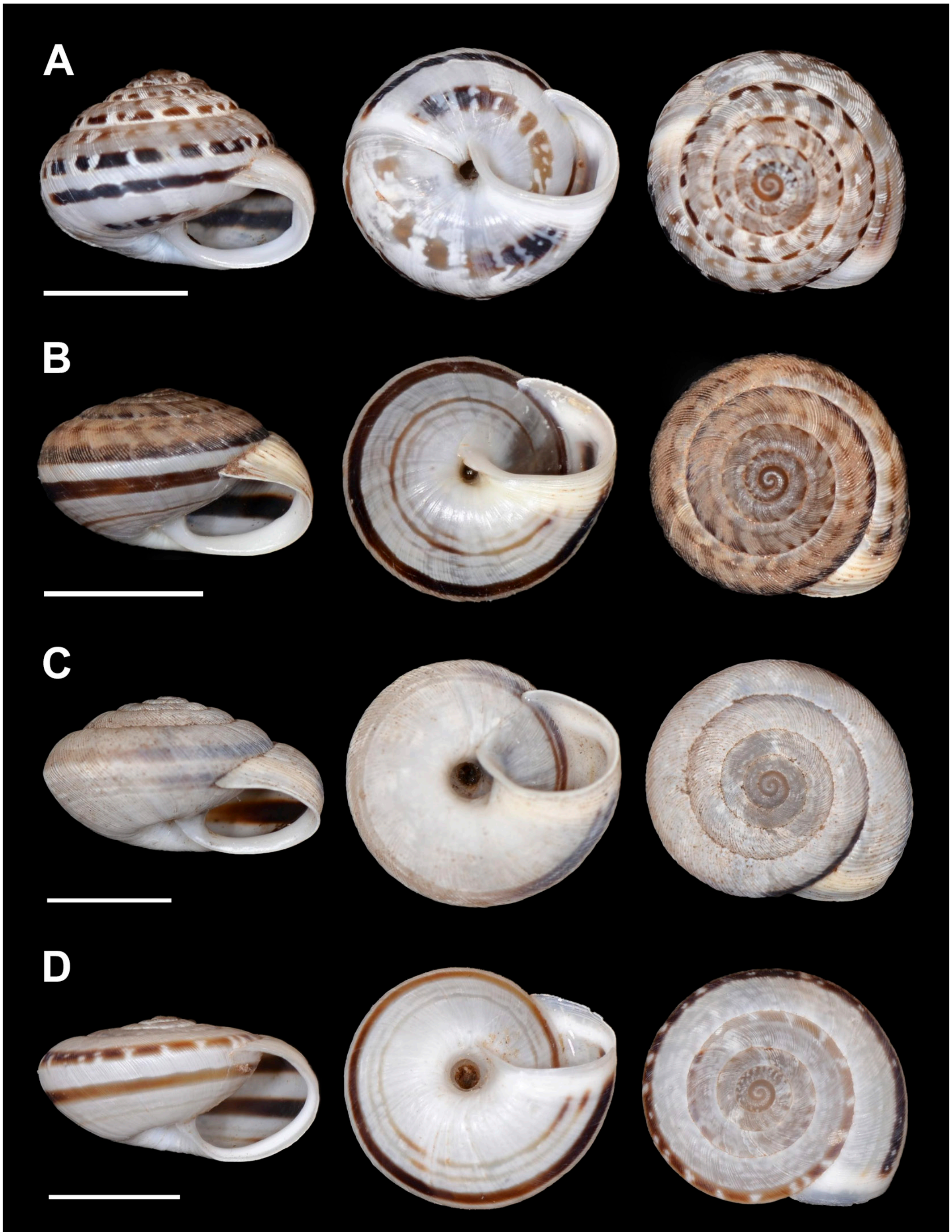

Figure 1.19
A. *Xerocrassa caroli formenterensis* Sa Mola (Formentera)
B. *Xerocrassa caroli jaeckeli* Sa Conillera (Ibiza)
C. *Xerocrassa cisternasi calasaladae* Illa de Cala Salada (Ibiza)
D. *Xerocrassa cisternasi calderensis* Illa d'en Calders (Ibiza)
Scale bar 0.5 cm

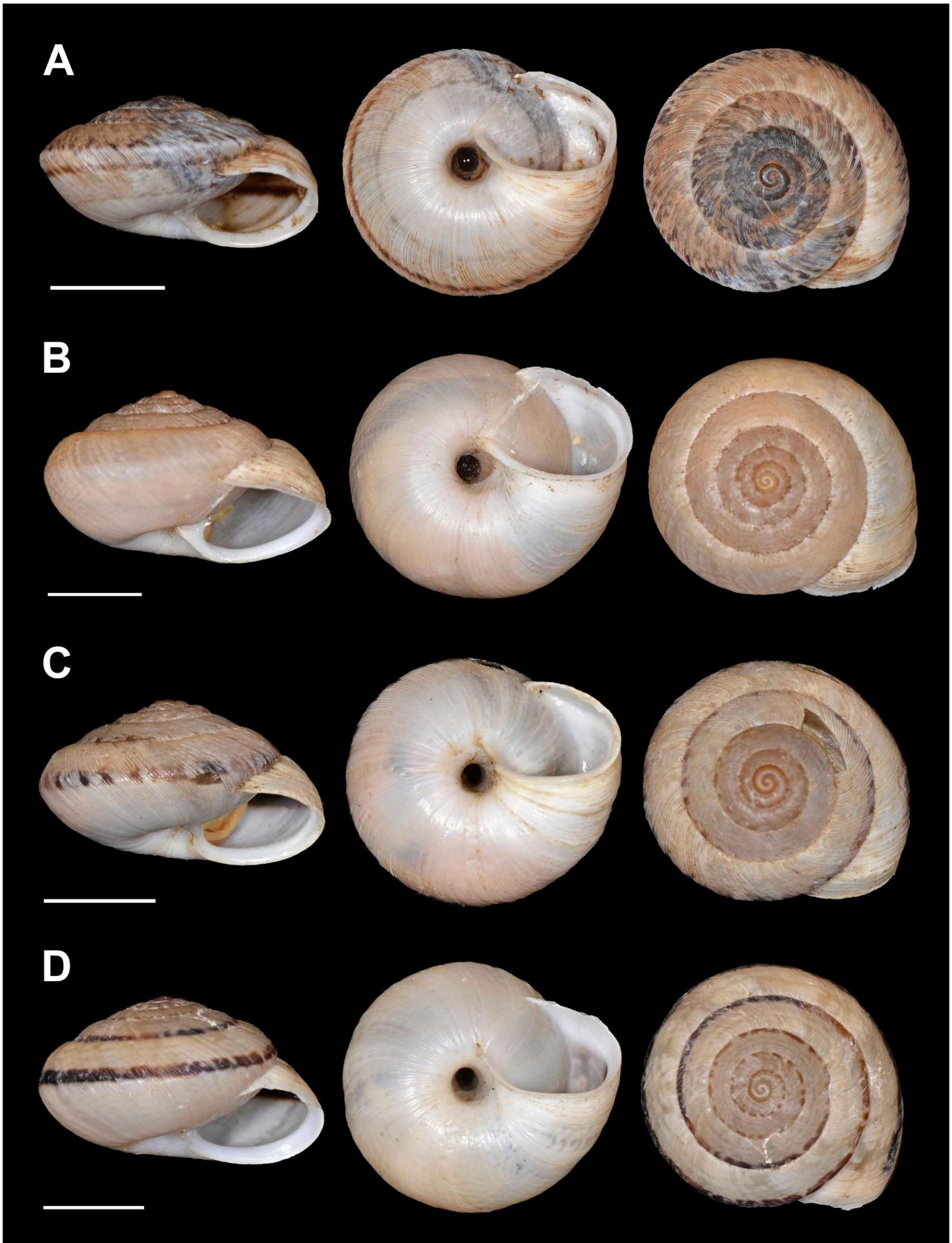

Figure 1.20
A. *Xerocrassa cisternasi canae* Illot des Canar (Ibiza)
B *Xerocrassa cisternasi cisternasi* Illa de Santa Eulalia (Ibiza)
C. *Xerocrassa cisternasi conjungens* Escull Vermell (Ibiza)
D. *Xerocrassa cisternasi margaritae* Ses Margalides (Ibiza)
Scale bar 0.5 cm

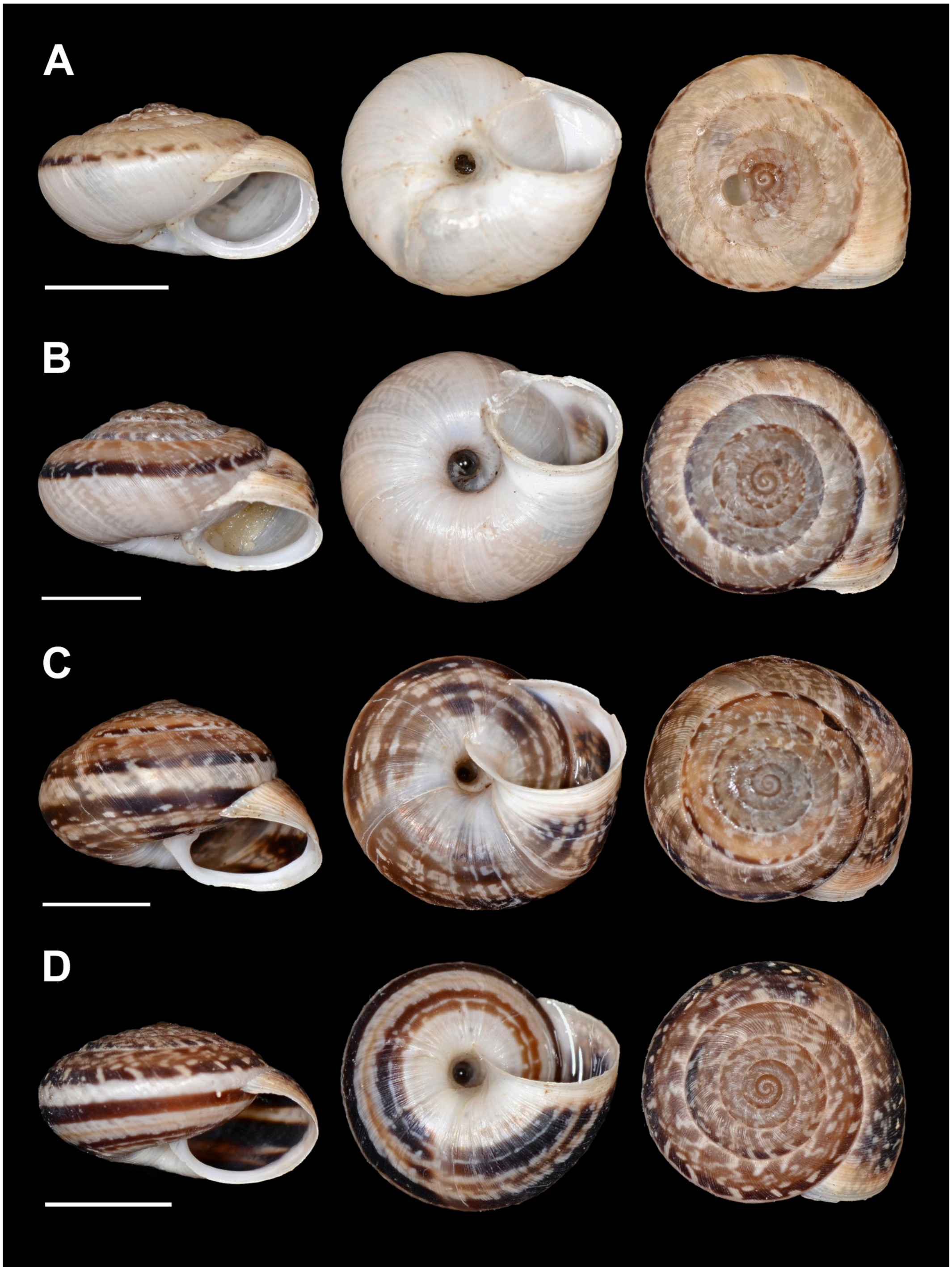

Figure 1.21
A. *Xerocrassa cisternasi mesquidae* Illot de Sa Mesquida (Ibiza)
B. *Xerocrassa cisternasi muradae* Illa Murada (Ibiza)
C. *Xerocrassa cisternasi ortizi* Cala Sant Vicent (Ibiza)
D. *Xerocrassa cisternasi redonae* Illa Redona (Ibiza)
Scale bar 0.5 cm

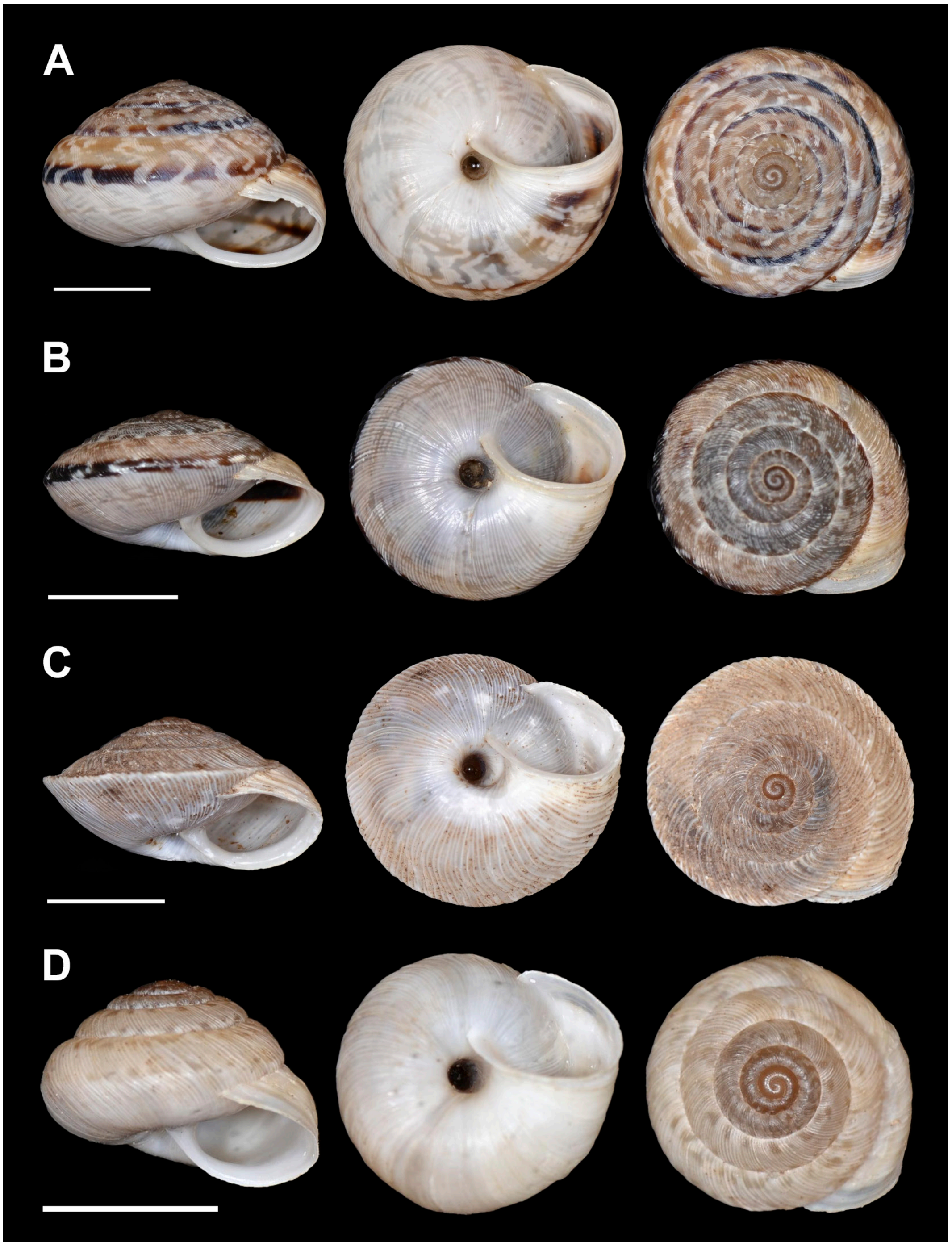

Figure 1.22
A. *Xerocrassa cisternasi scopulicola* Na Plana (Ses Bledes)
B. *Xerocrassa cisternasi vedrae* Es Vedra (Ibiza)
C. *Xerocrassa cisternasi vedranellensis* Es Vedranell (Ibiza)
D. *Xerocrassa ferreri ferreri* Na Foradada (Cabrera Archipelago)
Scale bar 0.5 cm

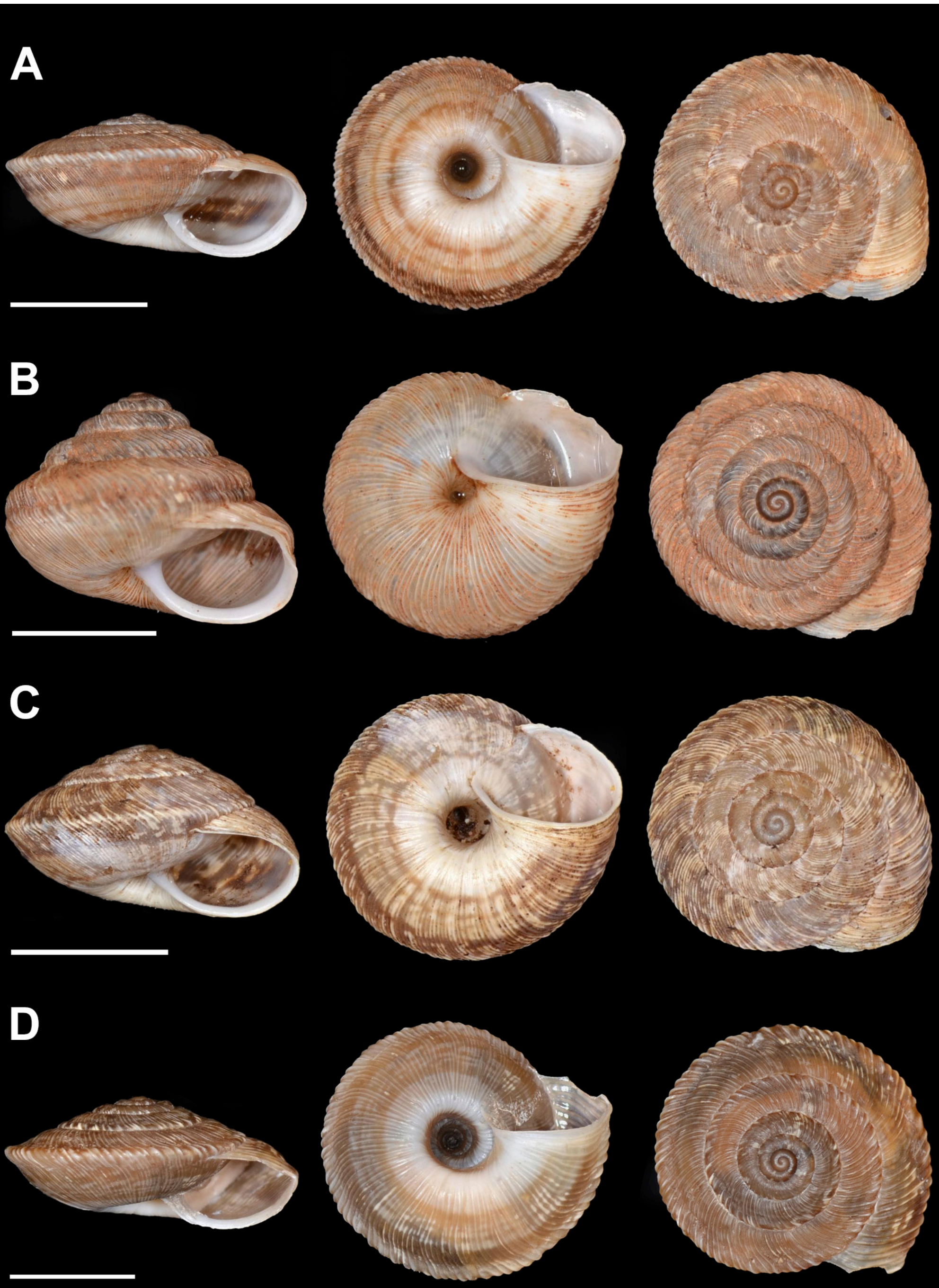

Figure 1.23
A. *Xerocrassa frater frater* Puig de Santa Magadalena. Inca (Mallorca)
B. *Xerocrassa frater newka* Na Burguesa. Genova (Mallorca)
C. *Xerocrassa frater pulaensis* Pula Golf course (Mallorca)
D. *Xerocrassa frater muntaneri* Santuari de Lluc. Escorca (Mallorca)
Scale bar 0.5 cm

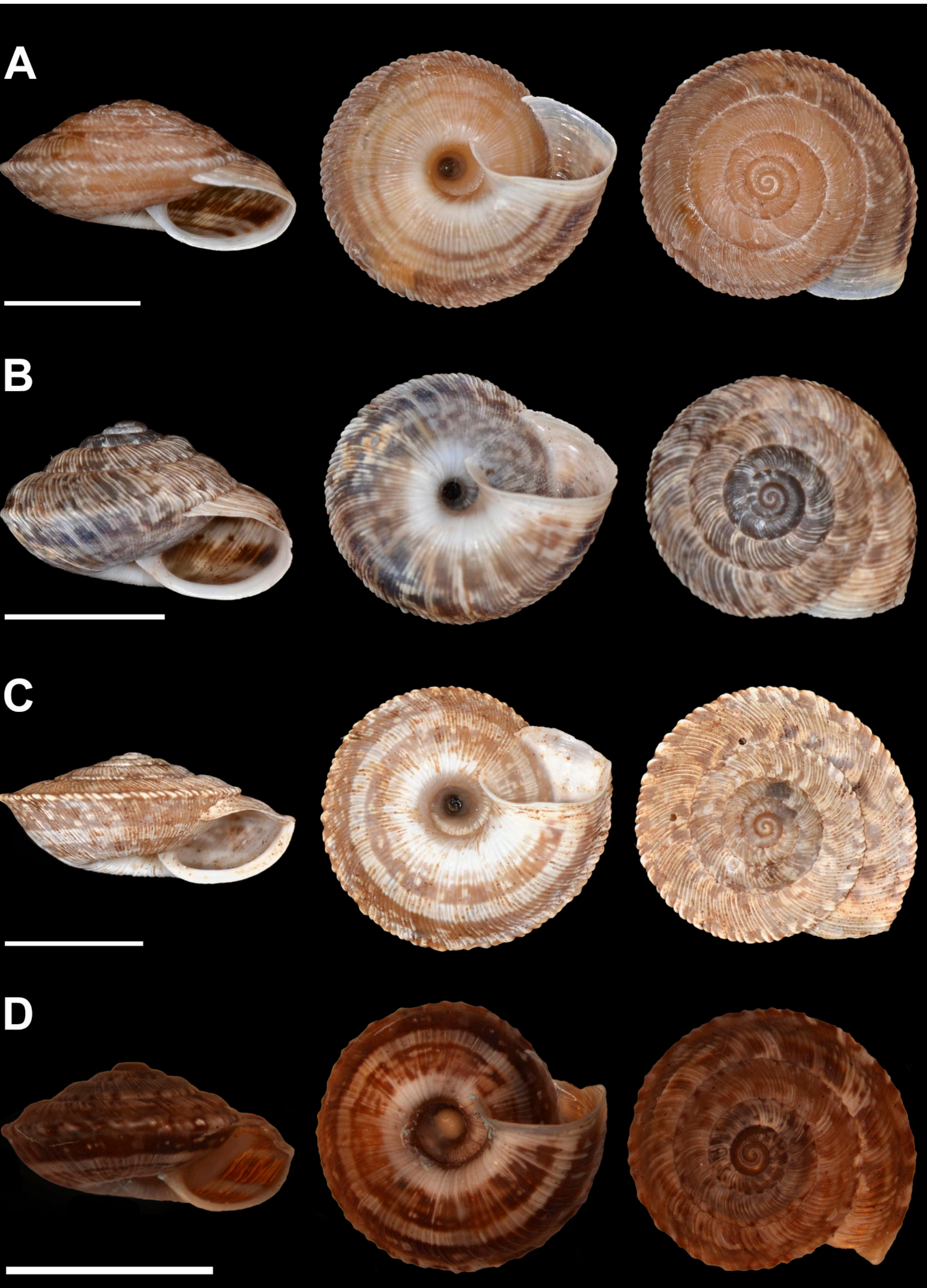

Figure 1.24
A. *Xerocrassa frater pollenzensis* Formentor Peninsula (Mallorca)
B. *Xerocrassa majoricensis* Bunyolí. Establiments (Mallorca)
C. *Xerocrassa prietoi prietoi* Cala Murada. Manacor (Mallorca)
D. *Xerocrassa prietoi muroensis* Platja de Muro (Mallorca)
Scale bar 0.5 cm

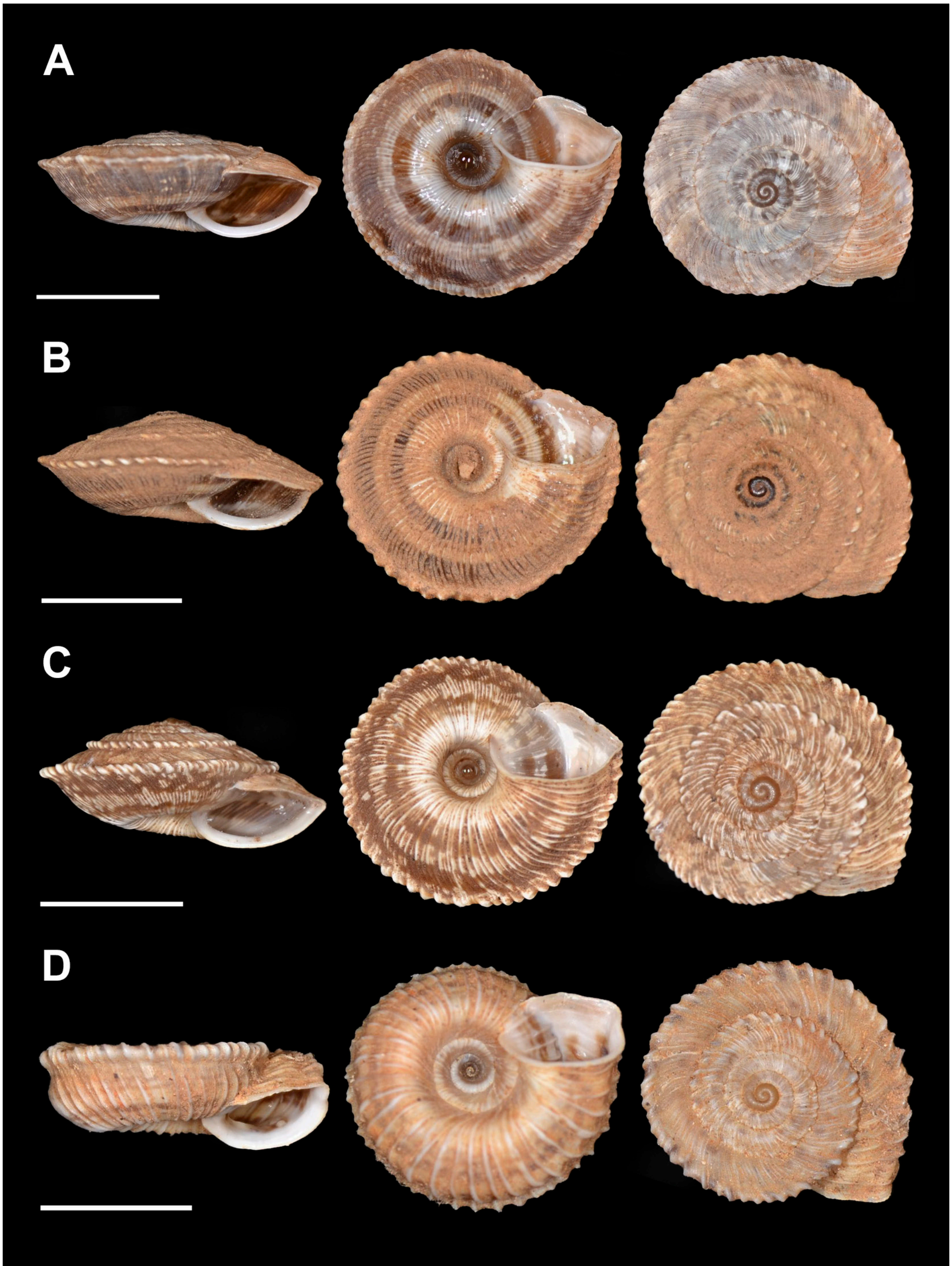

Figure 1.25
A. *Xerocrassa homeyeri* Formentor Cala Romantica. Manacor (Mallorca)
B. *Xerocrassa ponsi* Punta de n'Ensiola (Cabrera Archipelago)
C. *Xerocrassa ponsi* Punta de n'Ensiola (Cabrera Archipelago)
D. *Xerocrassa claudinae* Formentor Peninsula (Mallorca)
Scale bar 0.5 cm

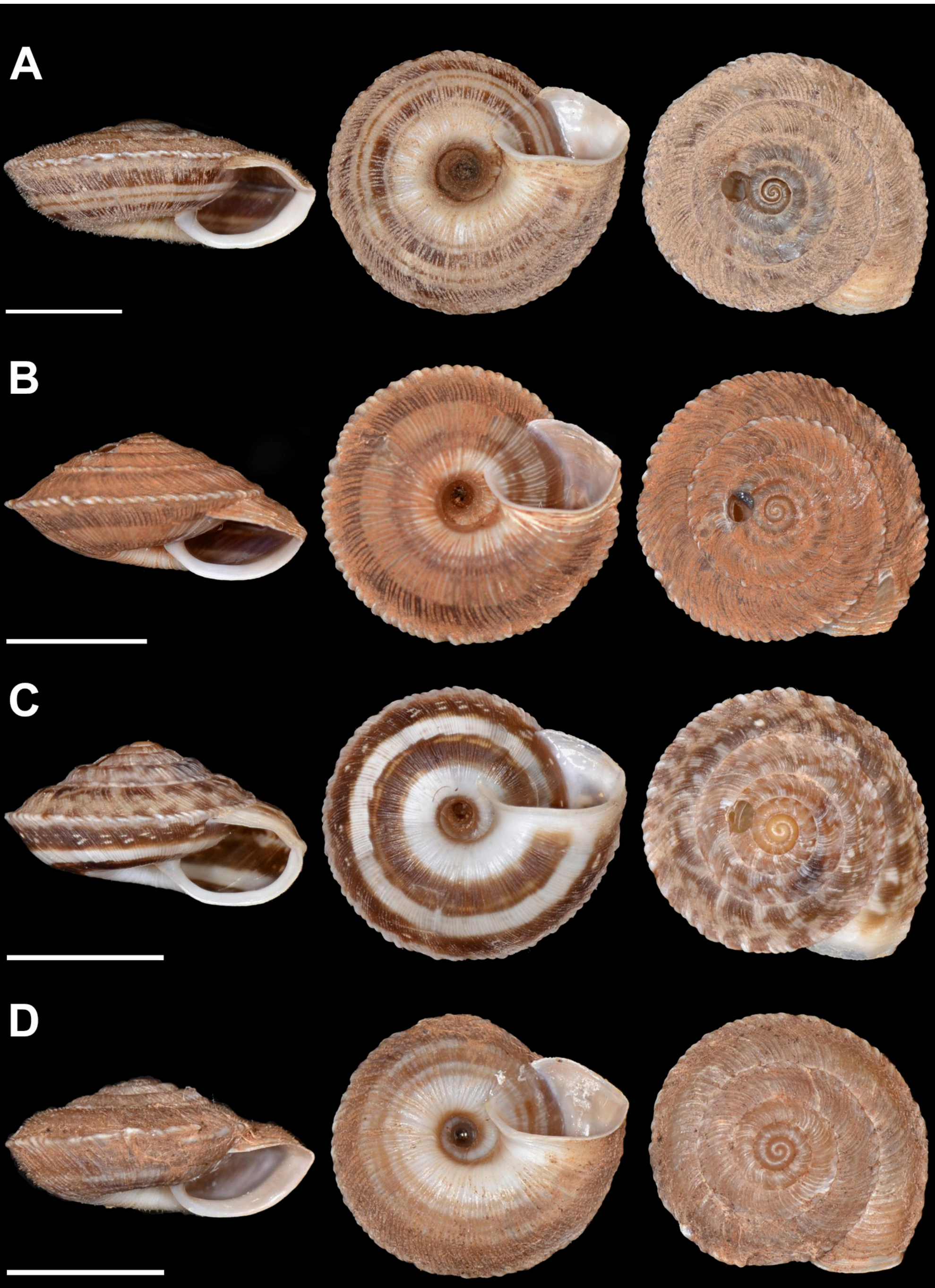

Figure 1.26
A. *Xerocrassa ferrutxensis* Formentor Cala Mesquida. Capdepera (Mallorca)
B. *Xerocrassa nyeli* Alaior (Menorca)
C. *Xerocrassa nyeli* Son Saura. Ciutadella de Menorca (Menorca)
D. *Xerocrassa cardonae* Turdonell de Dalt. Maó (Menorca)
Scale bar 0.5 cm

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CHAPTER 2

Aims of the thesis

Aims of the thesis

The aim of this thesis is to gain insights into the systematics, historical biogeography and evolutionary processes of three genera of land snails belonging to the Helicoidea superfamily (*Allognathus*, *Candidula* and *Xerocrassa*) highly diversified within the Mediterranean Basin, through the application of molecular, morphological and geographical data.

The main goals are to:

1. Infer the first molecular phylogeny for the genus *Allognathus* as well as for the species of *Xerocrassa* endemic to the Balearic Islands, based on both mitochondrial and nuclear rRNA gene sequences.
2. Delimit species within *Allognathus* and *Xerocrassa* complexes using species delimitation methods to assign species or subspecies ranks to the different taxa considered within these two complexes.
3. Propose an updated classification for the genera *Allognathus* and *Xerocrassa* based on the molecular, morphological and geographical data.
4. Evaluate the colonization, dispersion, and radiation processes of *Allognathus* and *Xerocrassa* in the Balearic Islands by applying a relaxed molecular clock analysis in parallel to the palaeogeography and palaeoclimate history of the western Mediterranean Basin.
5. Develop new polymorphic nuclear markers from the transcriptomes of three *Candidula* species.
6. Conduct the first phylogenetic study of western *Candidula* species, using mtDNA and nuclear DNA loci.
7. Investigate the phylogenetic relationships of *Candidula* within the Geomitridae family.

CHAPTER 3

Allognathus

Paper I

Consideraciones nomenclaturales sobre las especies del género *Allognathus* (Gastropoda: Pulmonata: Helicidae) y estudio anatómico de *Allognathus hispanicus tanitianus*

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Nomenclatural considerations about *Allognathus* species (Gastropoda: Pulmonata: Helicidae) and anatomical study of *Allognathus hispanicus tanitianus*

RESUMEN

En el presente trabajo se realiza una revisión nomenclatural para las especies del género *Allognathus* Pilsbry, 1888, endémico del Archipiélago Balear (España), en particular de *Allognathus (Allognathus) grateloupi*, *A. (Iberellus) hispanicus* y *A. (I.) hispanicus campanyonii*. Además, se redescribe la anatomía del aparato reproductor de *A. (I.) hispanicus tanitianus* (Forés & Vilella, 1993) cuya descripción original incluye algunos datos que, en nuestra opinión, no representan con exactitud su morfología.

ABSTRACT

A nomenclatural revision of the species of the genus *Allognathus* Pilsbry, 1888, endemic of the Balearic Islands (Spain) is conducted in the present work. Particular mention is given to *Allognathus (Allognathus) grateloupi*, *A. (Iberellus) hispanicus* and *A. (I.) hispanicus campanyonii*. Furthermore, we include a redescription of the reproductive apparatus of *A. (I.) hispanicus tanitianus* (Forés & Vilella, 1993) as the original description does not accurately represent, in our opinion, the morphology of the reproductive apparatus.

INTRODUCCIÓN

Allognathus Pilsbry, 1888, es un género originalmente endémico del archipiélago Balear, que se encuentra presente en todas las islas principales y en varios de sus islotes periféricos (Gasull, 1963; Beckmann, 2007). Debido a su gran variabilidad conquiológica y al aislamiento geográfico, se han descrito hasta 9 taxones nominales para el género. Uno de estos taxones también ha sido citado en Cataluña y sur de Francia, en las localidades de Banyuls sur Mer (Pirineos Orientales), en las ciudades de Barcelona, Sitges y Tarragona, siendo esta última localidad la única que alberga una población actualmente viva de la especie (Gasull, 1966; Altaba, 2007). Estas poblaciones peninsulares son atribuidas a introducciones antrópicas acaecidas en tiempos históricos (Sacchi, 1957; Gasull, 1663, 1966).

Aún tratándose de un endemismo balear, la primera referencia a una especie del género, proviene de Banyuls sur Mer (Pirineos Orientales, Francia), bajo el nombre de *Helix Companyonii* Aleron in Companyo, 1837, aunque sin ir acompañada de descripción alguna (ver discusión nomenclatural). Rossmässler (1838) aporta la primera descripción para un taxón del género, *Helix hispanica* "Partsch" en cuya sinonimia incluye *H. balearica* de Ziegler. Posteriormente, (Rossmässler, 1839) designa al taxón proveniente de Pirineos Orientales como *Helix hispanica* var. *pyrenaica*, en cuya sinonimia incluye *H. Campanyonii*. Por otra parte, Mittre (1842) describe *Helix minoricensis* de la isla de Menorca. Graells (1846) describe *Helix Grateloupi* procedente de Mallorca, que más adelante Pfeiffer (1848) denominaría como *H. graellsiana*. Pilsbry (1888) crearía el género *Allognathus* para *H. Grateloupi* Graells 1846, mientras que Hesse (1908) erigiría el género *Iberellus* para el grupo de *Helix balearica* y *H. minoricensis*. *Iberellus* es un nombre sustituto para *Balearica* Kobelt, 1904, nombre preocupado por un taxón de aves. La especie tipo de *Allognathus* es *Helix grateloupi*, por designación original; *Iberellus* hereda la especie tipo de *Balearica*, que por lo tanto sería *Helix balearica* Rossmässler, 1838 por tautonomia absoluta.

Tabla 3.1.1. Clasificaciones propuestas para el género *Allognathus s.l.* durante la primera década del s. XXI.

Table 3.1.1. Classifications proposed for the genus Allognathus s.l. *during the first decade of XXI century.*

Bank, 2010	**Beckmann, 2007**	**Schileyko, 2006**
Allognathus (A.) graellsianus	*Allognathus (A.) graellsianus*	*Allognathus grateloupi*
Allognathus (Iberellus) hispanicus	*Allognathus (Iberellus) balearicus balearicus*	*Iberellus balearica*
Allognathus (Iberellus) pyrenaicus		*I. pyrenaicus*
A. (I.) p. horadadae	*A. (I.) b. horadadae*	*I. horadadae*
A. (I.) p. minoricensis	*A. (I.) b. minoricensis*	
A. (I.) p. palumbariae		
A. (I.) p. tanitianus	*A. (I.) b. tanitianus*	
A. (I.) p. pythiusensis	*A. (I.) b. pythiusensis*	*Nesiberus pythiusensis*

Posteriormente, en la primera mitad del siglo XX, se describen varios nuevos taxones de reducida distribución: *Helix pythiusensis* Bofill & Aguilar-Amat, 1924, *Iberellus minoricensis palumbariae* Aguilar-Amat, 1933 e *Iberellus minoricensis horadadae* Jaeckel, 1952. Por último Forés & Vilella (1993), describen *Iberellus tanitianus*, endémico de la isla de Ibiza. Algunas de las principales clasificaciones propuestas para el conjunto de taxones pueden verse en la Tabla 3.1.1.

La validez nomenclatural de *Iberellus campanyonii* ha sido tema de discusión desde su primera cita como *Helix companyonii* Aleron in Companyo 1837, y más recientemente en diversos trabajos (Forés, 2003; Alonso-Zarazaga, 2004; Altaba, 2007; Beckmann, 2007; Alba *et al.*, 2011). Sin embargo creemos que las decisiones adoptadas en estos trabajos no son adecuadas en su totalidad. Del mismo modo, existen una serie de discrepancias en torno al empleo de los nombres *Iberellus hispanicus* e *I. balearicus*, por una parte, y de *Allognathus graellsianus* y *A. grateloupi*, por otra. Por ello hemos considerado oportuno realizar una revisión y proponer una nomenclatura acorde a las disposiciones del Código Internacional de Nomenclatura Zoológica (CINZ).

Por último, las observaciones anatómicas que hemos llevado a cabo sobre ejemplares de *Allognathus (Iberellus) hispanicus tanitianus* (Forés & Vilella, 1993), nos han permitido constatar que la descripción original no representa con exactitud la morfología del aparato reproductor de esta especie, por lo que procedemos a su redescripción.

MATERIAL Y MÉTODOS

Para la discusión nomenclatural se ha procedido a consultar las obras originales con las descripciones y figuras aportadas para cada especie nominal.

La descripción de cada taxón está basada en el estudio del material relacionado en el Apéndice 1. En aquellos casos en que existe una localidad tipo para un taxón, se han incluido topotipos dentro del material analizado. Cuando no existe una localidad tipo concreta, se han analizado ejemplares procedentes del rango de distribución de cada taxón, habiendo confirmado su identificación con las descripciones originales y con obras que han tratado extensamente el género *Allognathus s.l.* (Gasull, 1963, 1966; Beckmann, 2007; Quintana, 2007).

RESULTADOS Y DISCUSIÓN

1. Revisión nomenclatural de las especies del género *Allognathus*

La primera decisión a adoptar es la concerniente al estatus taxonómico de *Allognathus* e *Iberellus*, que son tratados, o bien como géneros diferentes (Alonso-Zarazaga, 2004; Schileyko, 2006; Altaba, 2007; Quintana, 2007; Alba *et al.*, 2011; Welter-Schultes, 2012), o como subgéneros de *Allognathus* (Bank *et al.*, 2001; Beckmann, 2007). Personalmente nos decantamos por clasificar ambos taxones como subgéneros de *Allognathus*. Ello permite reflejar en la clasificación de los helicinos la estrecha relación filogenética existente entre ambos taxones, que consideramos grupos hermanos y, por tanto, más próximos entre sí que con respecto a otros géneros de helicinos (*Otala*, *Pseudotachea*, etc). Por otro lado, esta decisión es concordante con lo propuesto por la clasificación adoptada para los taxones supraespecíficos europeos por Bank *et al.*, (2001). En lo concerniente a la clasificación del taxón de las islas Bledas en un género diferente (*Nesiberus* Haas 1934), no parece justificada ni por la morfología de la concha ni del aparato reproductor, en contra de la clasificación adoptada por Schileyko (2006).

***Helix hispanica* Rossmässler, 1838**

Algunos autores (Schileyko, 2006; Altaba, 2007; Beckmann, 2007; Quintana, 2007) designan a este taxón como *Allognathus (Iberellus) balearicus*. La utilización de *H. balearica* como nombre válido es debida a un supuesto caso de homonimia de *H. hispanica* Rossmässler 1838 con el taxón *Helix lactea* var. *Hispanica* Potiez & Michaud, obra tradicionalmente fechada en 1835, lo que haría que el nombre dado por Rossmässler (1838) no estuviese disponible. Sin embargo Kadolsky (2012) determina fehacientemente que la obra de Potiez y Michaud "Galerie des mollusques, ou catalogue méthodique, descriptif et raisonné des mollusques et coquilles du Muséum de Douai", se desarrolló entre los años 1835 y 1838, pero no hay pruebas de que las partes impresas se encontrasen disponibles por compra o intercambio antes del 27 de Octubre de 1838. Esta es, por tanto, la fecha más antigua referente a la publicación del trabajo mencionado (Kadolsky, 2012). La obra de Rossmässler (1838) data de Junio de 1838, lo que hace que el nombre *H. hispanica* de Rossmässler (1838) tenga prioridad sobre el de Potiez y Michaud (1838), en derivación *Allognathus (Iberellus) hispanicus*, quedando *Helix balearica* Rossmässler (1838) como un sinónimo del mismo, tal y como Rossmässler (1838) propone en su obra original.

***Helix campanyonii* Anton in Rossmässler, 1839**

El problema nomenclatural más controvertido es el referente a la validez del nombre *Allognathus (I.) campanyonii*. Algunos autores como Forés (2003); Forés (2004); Alba *et al.* (2011), consideran que *Helix companyonii* Aleron in Companyo 1837 no sería un *nomen nudum* por lo que lo proponen como el primer nombre disponible válido que debería emplearse. *Helix companyonii*, fue citado por primera vez en el trabajo de Companyo (1837). En este trabajo se describe el contenido de un "cuadro expositor" realizado con conchas representativas de la malacofauna de Pirineos Orientales, que incluye ejemplares de esta especie, si bien no se realiza ninguna descripción, ya que la indicación de que "se parece" a *Helix serpentina* y *H. ondulata* no es un carácter diferencial. Además aquí se puede invocar a la negación de intención (CINZ art. 8.3) debido al uso de las palabras "nous pensons qu'elle n'a pas été décrite". Del mismo modo, la referencia de la especie en tal cuadro, podría entrar, en el caso de etiquetas en colección (CINZ art. 12.3). De acuerdo a estas disposiciones, el nombre *Helix companyonii* sería un *nomen nudum*, por lo que no estaría disponible referido a esta publicación.

Continuando con este problema nomenclatural, Alonso-Zarazaga (2004), propone como nombre válido para este taxón *Helix hispanica* var. *pyrenaica* Rossmässler 1839, en combinación *Allognathus (Iberellus) pyrenaicus*. Sin embargo este nombre es un homónimo primario de *Helix pyrenaica* Draparnaud, 1805 (CINZ art. 57.2) (combinación original de *Norelona pyrenaica*). Ello nos lleva a descartar el uso de *A. (I.) pyrenaicus* (Rossmässler 1839), tal y como ha sido ya mencionado por otros autores (Altaba, 2007; Beckmann, 2007; Alba *et al.*, 2011). En la misma publicación en la que se describe *Helix hispanica var. pyrenaica*, Rossmässler (1839) sinonimiza *Helix Campanyonii* dentro de su nuevo taxón descrito. Este nombre sinonimizado estaría disponible para su utilización (CINZ art. 11.6.1) ya que fue empleado como nombre válido antes de 1961 (Villa y Villa, 1841; Graells, 1846; Martens, 1864).

Algunos autores han considerado que en la obra de Rossmässler (1839) hay una grafía original incorrecta (Altaba, 2007; Beckmann, 2007) por lo que proponen que debería realizarse una corrección del nombre de *campanyonii* a *companyonii* (CINZ art. 32.5.1). No obstante, no hay ninguna evidencia clara de que haya existido un error inadvertido en la publicación original, ya que Rossmässler (1839) no indica en lugar alguno por qué eligió ese nombre. Por otro lado, Welter-Schultes (2012) hace referencia al trabajo de Albers (1850), donde se menciona que Rossmässler (1839) incluye en sinonimia a *H. Campanyonii* debido a una indicación de Hermann Eduard Anton en una carta en la cual dice que ha obtenido este caracol bajo el nombre *campanyonii* de Parreyss y que sospecha que el nombre se refiere a la localidad de "Campana, al pie de los Pirineos, cerca de Perpignan". Al no darse las condiciones para un error inadvertido, consideramos *Allognathus (Iberellus) campanyonii* (Rossmässler 1839) como nombre válido para este taxón, con la descripción y la lámina presentadas en Rossmässler (1839).

Otra cuestión por dilucidar es la concerniente al número de taxones de *Iberellus* presentes en Mallorca. Algunos autores (Beckmann, 2007; Alba *et al.*; 2011) consideran que en Mallorca solo vive una especie de *Iberellus*. En tal caso la especie debería denominarse *Allognathus (Iberellus) hispanicus*, conteniendo varias subespecies. Sin embargo, otros autores (Forés, 2003; Altaba, 2007; Quintana, 2007; Bank, 2010) consideran que existen dos, *A. (I.) hispanicus* en la Sierra de la Tramuntana y *A. (I.) campanyonii* en las zonas bajas de la isla, y que sería esta última la especie introducida en Cataluña y sur de Francia (Sacchi, 1957; Gasull, 1963, 1966). Estos dos taxones se corresponden con los descritos por Rossmässler (1838; 1839), con sus respectivas láminas. Incluso hay autores que consideran que sólo hay una especie de *Iberellus* en todo el archipiélago Balear, sin distinción de subespecies. En la actualidad estamos realizando estudios moleculares basados en la secuenciación de ADN que esperamos permitan resolver la cuestión sobre el número de especies y subespecies presentes en el archipiélago Balear. Los resultados preliminares de estos estudios genéticos (datos no publicados) nos indican que *Iberellus* constituye una única especie según el concepto biológico de especie (Dobzhansky, 1937; Mayr, 1942).

Helix grateloupi Graells, 1846

Este taxón ha sido denominado tradicionalmente *Allognathus graellsianus* (Pfeiffer, 1848), derivado de *Helix graellsiana* (Gasull, 1966; Puente, 1994; Beckmann, 2007; Bank, 2010). Sin embargo, al igual que consideran otros autores (Pilsbry, 1888; Schileyko, 2006; Welter-Schultes, 2012), el primer nombre válido disponible es *Helix grateloupi* Graells (1846), derivado como *Allognathus grateloupi*, nombre que tiene prioridad (CINZ art. 23) sobre *Helix graellsiana* Pfeiffer, 1848. Beckmann (2007) considera *H. grateloupi* Graells, 1846 como *nomen nudum*, lo que es incorrecto, ya que dentro de la obra de Graells (1846) se realiza una correcta descripción del nuevo taxón, acompañada de unas ilustraciones, con una mencionada intencionalidad de que se trata de una nueva especie, y a la que da el nombre de *H. grateloupi* dentro del apartado de "Correcciones y Adiciones" incorporado al final de dicha obra.

2. Estudio de la morfología del aparato reproductor de "*Iberellus tanitianus*" Forés & Vilella, 1993

La descripción de *Iberellus tanitianus* Forés & Vilella (1993) incluye una detallada descripción de concha, aparato reproductor, rádula y mandíbula. Las diferencias morfológicas encontradas, unidas a su aislamiento geográfico, permitieron a estos autores describir esta nueva especie de la isla de Ibiza. En la descripción original Forés y Vilella (1993) indican que los ejemplares de esta especie presentan un "pene de longitud mediana, con el epifalo casi dos veces y media más largo que él, configurado por una doble característica doble asa en forma de ocho". Además, el pene y el epifalo de *I. tanitianus* serían considerablemente más largos que en las otras especies del género (Forés y Vilella, 1993; Quintana, 2007) y, en proporción con el resto de órganos distales, más largos que en el resto de Helicinae. Sin embargo, los individuos de *Iberellus tanitianus* estudiados por nosotros no muestran epifalo y pene tan largos ni con esa forma característica representada en la descripción original.

Según lo observado en 8 ejemplares disecados de tres localidades, incluyendo ejemplares procedentes de las dos estudiadas por Forés y Vilella (1993), el aparato reproductor de *I. tanitianus* se caracterizaría (Figs. 3.1.1A y 3.1.1B) por presentar un pene cilíndrico con un engrosamiento central. Epifalo cilíndrico más corto que la mitad del pene y más delgado que éste. Flagelo filiforme, casi el doble de largo que el conjunto de pene y epifalo. Vagina corta y gruesa sobre la que se inserta un saco del dardo grande y piriforme. Dos glándulas mucosas insertadas en la base del saco del dardo, estando ambas bifurcadas en su parte media (3-5 túbulos en cada glándula en los ejemplares de Cala Sant Vicenç). Bursa copulatrix grande y globosa con un conducto provisto de un divertículo. El divertículo es un 60% más largo que el conducto de la bursa. Oviducto libre corto.

La estructura de asa en forma de ocho representada en el trabajo de Forés y Vilella (1993) puede deberse, o bien al estudio de algún ejemplar teratológico, o a un error de interpretación del lugar de inserción del músculo retractor penial y del conducto deferente,

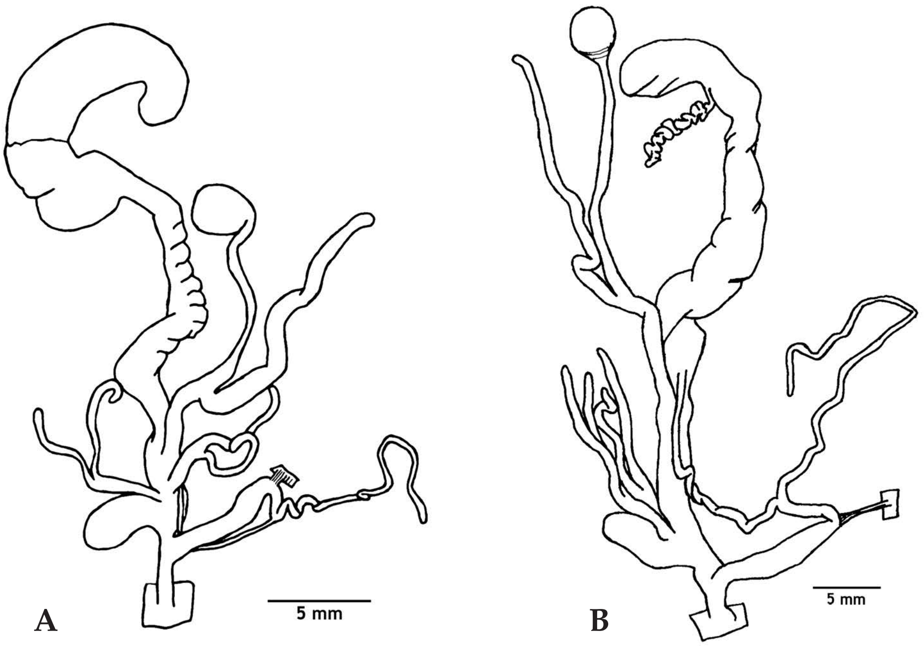

Figura 3.1.1. Aparato reproductor de *Allognathus (Iberellus) hispanicus tanitianus* (Forés y Vilella, 1993). A: Col de sa Creu (Ibiza), B: Cala Salada (Ibiza).

Figure 3.1.1. *Reproductive apparatus of* Allognathus (Iberellus) hispanicus tanitianus *(Forés y Vilella, 1993). A: Col de sa Creu (Ibiza), B: Cala Salada (Ibiza).*

ya que en todos los individuos analizados por nosotros, dichas estructuras se encuentran insertas en una posición similar al resto de especies del género (Puente, 1994; Quintana, 2007).

Taxonomía y descripción conquiológica

De acuerdo con la nueva información morfológica concerniente a la morfología del aparato reproductor de *Iberellus tanitianus* y tomando en consideración los comentarios nomenclaturales expuestos en este trabajo, proponemos la siguiente clasificación para el género *Allognathus*, en espera de que estudios genéticos actualmente en realización, junto a nuevos datos paleontológicos, nos permitan tener un conocimiento más exhaustivo de las relaciones filogenéticas de los taxones aquí considerados y de su historia evolutiva en el archipiélago Balear. Para cada taxón ofrecemos una breve diagnosis basada en la morfología de la concha y su distribución geográfica.

Género ***Allognathus*** **Pilsbry 1888**

Subgénero *Allognathus* Pilsbry 1888

Allognathus (Allognathus) grateloupi **(Graells 1846)***

Helix grateloupi Graells, 1846, *Catalog. Mol. España*: 3, Adiciones, Fig. 7-8 [Localidad tipo: Mallorca].
Helix graellsiana Pfeiffer, 1848, *Monogr. Helic. vivent.*, 1: 437-438 [Localidad tipo: Mallorca].

Concha globosa de paredes finas, frágil, con 4 a 4 ½ vueltas de crecimiento rápido. Última vuelta rápida y bruscamente descendente en la extremidad, bajando desde la tercera a la quinta banda espiral. Presenta 5 bandas espirales bien separadas, normalmente discontinuas. Boca ovalada-redonda con peristoma reflejado, coloreado de color pardo rojizo. Sin ombligo.

Distribución: NE Sierra de la Tramuntana (Mallorca).

Subgénero *Iberellus* Hesse 1908

Allognathus (Iberellus) hispanicus hispanicus **(Rossmässler 1838)**

Helix hispanica Rossmässler, 1838, *Icon.* 2 (1/2): 15, Fig. 460 [Localidad tipo: España].
Helix balearica Rossmässler, 1838, *Icon*. 2 (1/2): 15 (in syn).

Concha globosa-aplanada con 4 ½ vueltas muy planas pero dejando ver la sutura, estriación muy fina, irregular. Última vuelta unas tres veces más ancha que la penúltima, ensanchándose progresivamente hacia la boca. Abertura oblicua, ovalada, descendiendo ligeramente en la extremidad, bajando desde la tercera a la cuarta banda espiral. Peristoma sencillo con engrosamiento interno de color marrón y ligeramente reflejado. Ombligo cerrado. Callosidad columelar bien marcada.

Distribución: Sierra de la Tramuntana (Mallorca).

Allognathus (Iberellus) hispanicus campanyonii **(Rossmässler 1839)**

Helix Companyonii Aléron in Companyo, *Bull. Soc. Philomatic*, 3: 85-104 (*nomen nudum*).
Helix Campanyonii Rossmässler, 1839, *Icon.* 2 (3/4): 11 (in syn).
Helix hispanica var. *pyrenaica* Rossmässler, 1839, *Icon*. 2 (3/4): 11, Fig. 591 [Localidad tipo: Pirineos].
Helix oberndörferi Kobelt, 1882, *Nachr.-Bl. dtsch. malak. Ges.*, 14 (4/5): 69-70 [Localidad tipo: Palma, Mallorca].

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* Revisiones posteriores indican que *Helix grateloupi* Graells, 1846 es un nombre utilizado previamente en *Helix grateloupii* Pfeiffer, 1842, por lo que su correcta designación debe ser *Allognathus (Allognathus) graellsianus* (Pfeiffer, 1848).

Concha globosa de espira cónica poco elevada, 4 ½ a 5 vueltas convexas, marcando bien la sutura, la última doble ancha que la penúltima en las proximidades de la boca. Abertura ovalada, oblicua y descendente. Borde superior de la última vuelta fuerte y bruscamente descendente hacia la extremidad, bajando generalmente más allá de la cuarta banda espiral. Estriación muy fina y regular. Coloración dorsal generalmente marrón oscura, más clara por la parte ventral con las bandas espirales interrumpidas por flamulaciones dispuestas en zigzag, radiales y de color marfil. Peristoma ligeramente engrosado por su interior y algo reflejado, mostrando una callosidad en el borde columelar. Sin ombligo. Localmente (Archipiélago de Cabrera) las conchas son más sólidas, más pequeñas y con engrosamiento interno del peristoma más fuerte.

Distribución: Mallorca, introducida en Tarragona.

Allognathus (Iberellus) hispanicus minoricensis (Mittre 1842)

Helix Minoricensis Mittre, 1842, *Ann. Sci. nat. (Zool.)*, (2) 18: 188 [Localidad tipo: Mahón, Menorca].

Concha con cinco bandas espirales marrones, más o menos continuas, aunque generalmente atravesadas por finas líneas radiales dispuestas en zigzag. La parte inferior de la concha siempre es más clara que la parte superior. Borde superior de la última vuelta fuerte y bruscamente descendente hacia la extremidad, bajando generalmente más allá de la cuarta banda espiral. Sin ombligo.

Dentro de este taxón encontramos una gran variedad en cuanto a la coloración y bandeado de la concha en diferentes poblaciones de la isla de Menorca (ver Quintana, 2007).

Distribución: Menorca, Ibiza y Formentera.

Allognathus (Iberellus) hispanicus palumbariae (Aguilar-Amat 1933)

Iberellus minoricensis palumbariae Aguilar-Amat, 1933, *Butll. Inst. catal. Hist. nat.*, 13 (6/7): 328 [Localidad tipo: Illa d'en Colom, Menorca].

Similar a *A. (I.) h. minoricensis,* del que se diferencia por tener una concha más sólida y por presentar un peristoma blanco, fuertemente engrosado y reflejado. Además las cinco bandas espirales se hacen mucho más oscuras en las proximidades de la abertura. Borde superior de la última vuelta fuerte y bruscamente descendente hacia la extremidad.

Distribución: Illa d'en Colom (Este de Menorca).

Allognathus (Iberellus) hispanicus horadadae (Jaeckel 1952)

Iberellus minoricensis horadadae Jaeckel, 1952, *Mitt. zool. Mus. Berlin,* 28: 84, 103, Abb. 6, Taf. 3 Fig. 1 [Localidad tipo: Isla Horadada].

Concha con 4 ½ vueltas. Color castaño, con las bandas espirales menos contrastadas respecto al color de fondo que en otras especies y difuminadas por las flamulaciones en zigzag. Ombligo imperforado.

Distribución: Illa Na Foradada (Archipiélago de Cabrera).

Allognathus (Iberellus) hispanicus tanitianus **(Forés & Vilella 1993)**

Iberellus tanitianus Forés & Vilella, 1993, *Bol. Soc. Hist. nat. Baleares,* 36: 17-29, Fig. 1, Fig, 2. [Localidad tipo: Cala Sant Vicenç, Coll de sa Creu].

Concha globosa con la espira desde ligeramente convexa a cónica, de 3 ¼ a 3 ¾ vueltas. Sutura marcada. Peristoma bien reflejado, callosidad del borde columelar muy visible. Borde del peristoma blanco puro. Presenta, en general, pliegues junto a la sutura que desde el ápice descienden hacia la concha, claramente visibles a 30X. Boca oblicua y oval transversa. Estriación suave fina y bastante regular, con cinco bandas espirales en general continuas, finas y de color castaño claro. Flamulaciones radiales solo bien visibles entre la sutura y la primera banda espiral, más difuminadas en el resto de la concha. El color de fondo es córneo pálido. Ombligo imperforado. Borde superior de la última vuelta fuerte y bruscamente descendente hacia la extremidad.

Distribución: Ibiza.

Allognathus (Iberellus) hispanicus pythiusensis **(Bofill & Aguilar-Amat 1924)**

Helix (Archelix?) pythiusensis Bofill & Aguilar-Amat, 1924, *Trab. Mus. Cienc. nat.*, 10 (3): 27-28, Lam. 1 Fig. 10-12 [Localidad tipo: Illa Plana, Islas Bledas].

Concha globosa poco calcificada, de estriación muy fina y comprimida, color castaño claro, algo traslúcida, con bandas espirales en general poco marcadas, diluidas en la pigmentación de fondo. Abertura oblicua, ovalada con el borde superior muy descendente en su extremidad, llegando generalmente hasta la quinta banda espiral. Peristoma blanco, algo reflejado. Sin ombligo.

Distribución: Illa Plana y Na Gorra (Islas Bledas).

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APÉNDICE I. MATERIAL ESTUDIADO

Listado de ejemplares estudiados, ordenados por taxones. Abreviaturas: MLL: Mallorca, ME: Menorca, IB: Ibiza. *Los ejemplares de *A. (I.) h. tanitianus* de Cala Xarraca (Ibiza) provienen de depósitos cuaternarios.

ESPECIE	**LOCALIDAD**	**EJEMPLARES**	**COORDENADAS U.T.M.**		
			HUSO	**X**	**Y**
A. (A.) grateloupi	Finca Mortitx (MLL)	3	31 S	493582	4413048
A. (A.) grateloupi	Sa Calobra (MLL)	3	31 S	483684	4411182
A. (A.) grateloupi	Ctra. Ma 10 pk 26 (MLL)	3	31 S	486092	4408729
A. (I.) h. hispanicus	Mirador de Ses Barques (MLL)	3	31 S	478015	4404977
A. (I.) h. hispanicus	Puigpunyent (MLL)	1	31 S	459623	4387551
A. (I.) h. hispanicus	Puig de la Verge (MLL)	3	31 S	499946	4412992
A. (I.) h. hispanicus	Escorça (MLL)	10	31 S	490373	4407310
A. (I.) h. hispanicus	Santuari de Lluc (MLL)	10	31 S	490701	4408136
A. (I.) h. hispanicus	Font des Padro, Biniamar (MLL)	1	31 S	510895	4410466
A. (I.) h. hispanicus	Calviá (MLL)	1	31 S	457202	4376647
A. (I.) h. hispanicus	Finca Mortitx (MLL)	4	31 S	493588	4413288
A. (I.) h. hispanicus	Ctra. Ma 10 pk 106 (MLL)	3	31 S	449046	4384111
A. (I.) h. hispanicus	Ctra. Ma 10 pk 98 (MLL)	1	31 S	452971	4388564
A. (I.) h. hispanicus	Ctra. Ma 10 pk 80 (MLL)	3	31 S	461454	4392524
A. (I.) h. hispanicus	Ctra. Ma 1110 pk 14 (MLL)	2	31 S	468636	4393742
A. (I.) h. hispanicus	Golf "Camp de Mar" (MLL)	1	31 S	449980	4377903
A. (I.) h. hispanicus	La Granja (Esporles) (MLL)	6	31 S	462198	4391224
A. (I.) h. hispanicus	Ctra. Ma 10 pk 45 (MLL)	3	31 S	476556	4404068
A. (I.) h. hispanicus	Ctra. Ma 10 pk 26 (MLL)	4	31 S	486092	4408729
A. (I.) h. hispanicus	Sa Dragonera (MLL)	1	31 S	442261	4382366
A. (I.) h. hispanicus	Ctra. Ma 10 pk 12 (MLL)	1	31 S	493068	4412721
A. (I.) h. hispanicus	Castillo de Alaró (MLL)	1	31 S	482167	4398217
A. (I.) h. hispanicus	Cala Figuera (Paguera) (MLL)	1	31 S	451073	4375703
A. (I.) h. campanyonii	Cala Blava – Cala Pi (MLL)	1	31 S	478300	4367768
A. (I.) h. campanyonii	Palma de Mallorca (MLL)	2	31 S	468760	4380346

ESPECIE	**LOCALIDAD**	**EJEMPLARES**	**COORDENADAS U.T.M.**		
			HUSO	**X**	**Y**
A. (I.) h. campanyonii	Ctra. Ma 10 pk 80 (MLL)	3	31 S	461454	4392524
A. (I.) h. campanyonii	Puig Na Morisca (MLL)	2	31 S	455350	4373179
A. (I.) h. campanyonii	Font de Sa Cala (MLL)	7	31 S	538823	4390660
A. (I.) h. campanyonii	Isla de Cabrera	8	31 S	494500	4333434
A. (I.) h. campanyonii	Isla de Cabrera. Punta de N'Ansiola	3	31 S	493459	4331247
A. (I.) h. campanyonii	Illa dels Conils (Cabrera)	10	31 S	496618	4337582
A. (I.) h. campanyonii	Tarragona murallas	11	31T	353953	4553570
A. (I.) h. horadadae	Na Foradada (Cabrera)	30	31 S	498173	4339780
A. (I.) h. palumbariae	Illa d'en Colom (ME)	4	31 S	609075	4423829
A. (I.) h. minoricensis	Ciutadella de Menorca (ME)	16	31 T	571248	4428299
A. (I.) h. minoricensis	Es Tancants (Algairens) (ME)	8	31 T	578247	4433364
A. (I.) h. minoricensis	Ses Mongetes (ME)	5	31 S	572244	4421521
A. (I.) h. minoricensis	Ses Olles (ME)	9	31 T	599695	4432000
A. (I.) h. minoricensis	Algendar (ME)	7	31 S	583499	4425806
A. (I.) h. minoricensis	Son Febrer (ME)	10	31 S	579926	4426000
A. (I.) h. minoricensis	Canteras Bona Nova (ME)	5	31 T	574426	4428678
A. (I.) h. minoricensis	Son Saura – Es Talaier (ME)	3	31 S	576396	4420157
A. (I.) h. minoricensis	Cala Es Pous (ME)	4	31 T	570505	4433511
A. (I.) h. minoricensis	Cala En Bastó (ME)	4	31 S	570908	4423146
A. (I.) h. minoricensis	Cap de Cavalleria (ME)	2	31 T	593166	4438153
A. (I.) h. minoricensis	Cala de S'Enclusa (ME)	3	31 T	597376	4433575
A. (I.) h. minoricensis	Maó (ME)	3	31 S	602652	4414086
A. (I.) h. minoricensis	Ibiza (IB)	4	31 S	363780	4307778
A. (I.) h. minoricensis	Ses Balandres (IB)	4	31 S	353999	4322934
A. (I.) h. tanitianus	Cala Salada (IB)	8	31 S	352715	4319319
A. (I.) h. tanitianus	Col de Sa Creu (IB)	16	31 S	349341	4309041
A. (I.) h. tanitianus	Cala Sant Vicenç (IB)	11	31 S	378097	4325938
A. (I.) h. tanitianus	Cala Xarraca (IB)*	5	31 S	370759	4329052
A. (I.) h. pythiusensis	Na Plana (Islas Bledas) (IB)	11	31 S	340605	4316155
A. (I.) h. pythiusensis	Na Gorra (Islas Bledas) (IB)	3	31 S	341061	4314868

Paper II

Biogeography of the land snail genus *Allognathus* (Helicidae): middle Miocene colonization of the Balearic Islands

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ABSTRACT

Aim We infer the evolutionary history of the land snail genus *Allognathus* from a molecular phylogeny. An approximate temporal framework for its colonization of the Balearic Islands and diversification within the archipelago is provided according to palaeogeographical events in the western Mediterranean Basin.

Location The Balearic Islands, Western Mediterranean.

Methods A 2461-bp DNA sequence dataset was generated from one nuclear and two mitochondrial gene fragments in 87 specimens, covering all nominal taxa of the genus *Allognathus*. Through maximum-likelihood and Bayesian phylogenetic methods along with a Bayesian molecular clock, we examined the evolutionary history of the group. Ancestral distribution ranges were estimated for divergence events across the tree using a Bayesian approach. We also used genetic species-delimitation models to determine the taxonomy of *Allognathus*.

Results We provided the first molecular phylogeny of *Allognathus*, a genus endemic to the Balearic Islands. The origin of the genus in the Balearic Islands was dated to the middle Miocene based on palaeogeographical events in the Western Mediterranean. During the late Miocene and Pliocene, several diversification events occurred within the archipelago. The ancestral range of *Allognathus* was reconstructed as the north-eastern Tramuntana Mountains of Mallorca.

Main conclusions Three species were delimited within the genus, one of which has at least five subspecies. The phylogenetic reconstruction showed a high degree of parallelism between the divergence of the main *Allognathus* lineages and the palaeogeography of the Balearic Islands. The genus appears to have colonized Mallorca from the south-east of the Iberian Peninsula during the middle Miocene. Sea level fluctuations that took place in the Western Mediterranean from the Messinian to the present are consistent with the diversification and secondary contacts of the phylogroups of *Allognathus*, as well as their distribution ranges. The middle Miocene could have been a period for the colonization of the Balearic Islands by other terrestrial organisms.

Keywords

***Allognathus*, Balearic Islands, biogeography, colonization, Gastropoda, island endemism, Messinian, middle Miocene, phylogeny, Western Mediterranean.**

INTRODUCTION

The Mediterranean Basin has been described as a biodiversity hotspot and is home to many endemic species (Myers, 1990; de Jong, 1998; Blondel & Aronson, 1999; Médail & Diadema, 2009). The Mediterranean region is particularly appropriate for the study of biogeographical and evolutionary processes because of its high species diversity and a complex palaeogeographical history, which has shaped the current distribution patterns of many taxa (Bidegaray-Batista & Arnedo, 2011; Santos-Gally *et al.*, 2012). The high rates of endemicity of the more isolated regions such as the Mediterranean islands makes them especially useful for addressing questions related to speciation (Mayol *et al.*, 2012). The Balearic Islands are the most isolated archipelago in the Mediterranean Sea. The archipelago is divided into two island units. Ibiza and Formentera, including the Ses Bledes archipelago, form the western unit, known as the Pityusic Islands; the eastern unit, or Gymnesic Islands, includes Menorca, Mallorca and the Cabrera archipelago. The geological history of the Balearic Islands is relatively well known (Fig. 3.2.1) and includes tectonic events and sea-level falls assigned to a well-defined temporal framework (Rosenbaum *et al.*, 2002; Rosenbaum & Lister, 2004; Jolivet *et al.*, 2006).

The origin and isolation of the Balearic archipelago, together with other microplates that formed the Hercynian belt, started during the Oligocene (30–25 Ma). The presence of a variety of taxonomically unrelated taxa that currently show similar disjunct distribution patterns in regions arising from the Hercynian microplates, has been explained according to this geological process, including the oak *Quercus suber* (Magri *et al.*, 2007) and several

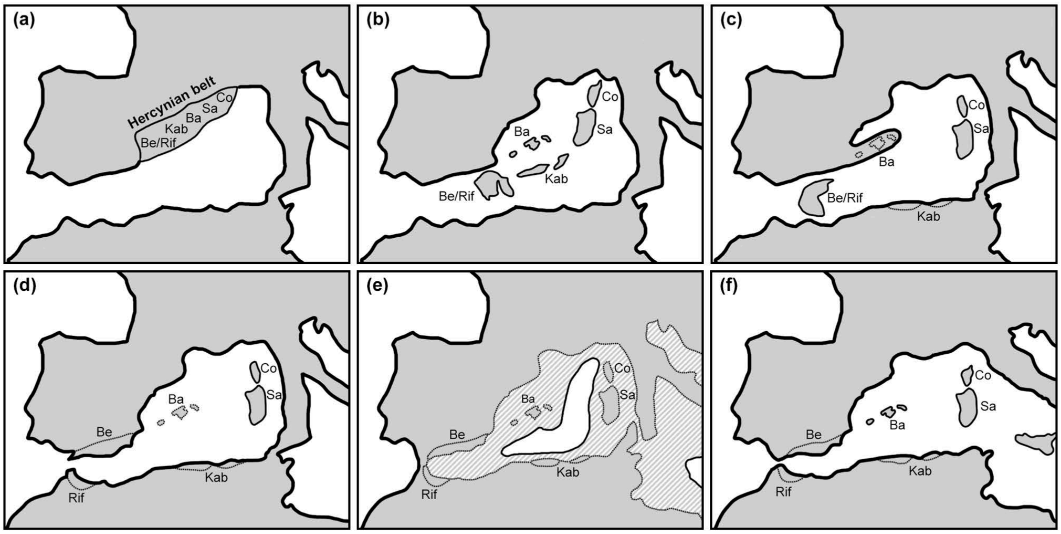

Figure 3.2.1 Reconstruction of the geological history of the western Mediterranean (modified from Rosenbaum *et al.*, 2002). (a) Oligocene (30 Ma); (b) Early Burdigalian (21 Ma); (c) Langhian (15 Ma); (d) Tortonian (10 Ma); (e) Messinian salinity crisis (5.96 - 5.33 Ma) and (f) present. Microplates names of the hercynian belt are indicated as follow: Be/Rif, Betic-Rif system; Kab, Kabylies; Ba, Balearic Islands; Sa, Sardina; Co, Corsica.

invertebrates: *Tyrrhenoleuctra* (Fochetti *et al.*, 2009), *Tudorella* (Pfenninger *et al.*, 2010), *Parachtes* (Bidegaray-Batista & Arnedo, 2011), *Schmidtea mediterranea* (Lázaro *et al.*, 2011) and *Postandrilus* (Pérez-Losada *et al.*, 2011). Other studies have, however, shown that the origins of some biota endemic to the archipelago greatly post-date the separation of the Balearic microplate from the continent. Two different geological episodes are thought to have allowed land connection of the Balearic Islands with the mainland during the Miocene. In the Langhian–Serravallian (16–11.6 Ma), a land-bridge connection is postulated between the Iberian Peninsula and the Balearic Islands as a continuation of the Baetic massif (Riba, 1981; Fontboté *et al.*, 1990; Roca, 1996). The presence of this Baetic–Balearic corridor is well documented by the immigration and settlement of several endemic vertebrates in the Balearic Islands (Adrover *et al.*, 1985; Quintana & Agustí, 2007; Bover *et al.*, 2008). This is the earliest insular vertebrate fauna of the Balearic Islands, detected in several middle Miocene deposits of Mallorca and Menorca, and is closely related to the middle Miocene fauna of the south-eastern Iberian Peninsula (Martín-Suárez *et al.*, 1993; Quintana & Agustí, 2007). Altaba (1997) indicated that the Sub-Baetic Massif (including the Balearic Promontory) that formed 14 Ma was a possible route used by the anuran genus *Alytes* to colonize the Balearic Islands (but see Martínez-Solano *et al.*, 2004). The second Miocene land connection is considered by many authors to be one of the main events driving local diversification in the Mediterranean, and this event has been dated to the Messinian salinity crisis (MSC; 5.96–5.33 Ma), when closure of the Strait of Gibraltar led to desiccation of the Mediterranean Basin (Hsü *et al.*, 1973; Krijgsman *et al.*, 1999). During this dry period, a colonization event from the mainland to the Balearic Islands took place (Fromhage *et al.*, 2004; Lalueza-Fox *et al.*, 2005; Delicado *et al.*, 2014). The refilling of the Mediterranean Basin following the MSC restored the isolation of the Balearic Islands. Further sea-level fluctuations of up to 100 m later took place due to the glacial cycles in the Western Mediterranean during the Plio-Pleistocene (Sosdian & Rosenthal, 2009; Capraro *et al.*, 2011), modifying the shape and size of the emerged islands, and allowing connections between Mallorca (including the Cabrera archipelago) and Menorca (channel around 70 m deep) and between Ibiza, Formentera and Ses Bledes archipelago (channels less than 100 m deep). These connections enabled species migrations and intraspecific gene flow between islands within each of the Gymnesic and Pityusic units of the Balearic Islands (Bover *et al.*, 2008; Brown *et al.*, 2008; Rodríguez *et al.*, 2013; Quintana & Moncunill-Solé, 2014). These post-Messinian sea-level oscillations were not, however, sufficient to reconnect the western and eastern Balearic Islands (separated by a depth of 640 m) or to connect the Balearic Islands to the Iberian Peninsula (depths greater than 800 m). Nonetheless, passive post-Messinian transport across the Mediterranean has been postulated for several organisms (Martínez-Solano *et al.*, 2004; Uit de Weerd *et al.*, 2005; Jesse *et al.*, 2011).

Here, we examine *Allognathus* Pilsbry, 1888, a land snail genus endemic to the Balearic Islands, but introduced to the east coast of the Iberian Peninsula in historical times (Gasull, 1963). The genus inhabits all of the major islands as well as the Cabrera and Ses Bledes archipelagos. According to Bank (2011) and Chueca *et al.* (2013), it has two subgenera:

Allognathus s.str. and *Iberellus*. The former is monotypic and the latter is divided into as many as seven taxa ranked as species or subspecies by different authors (Gasull, 1963; Puente, 1994; Pons & Palmer, 1996; Beckmann, 2007; Chueca *et al.*, 2013). In this report, we follow the taxonomy proposed by Chueca *et al.* (2013), initially considering two species, which are here designated *Allognathus (A.) graellsianus* (Pfeiffer, 1848) and *Allognathus (Iberellus) hispanicus* (Rossmässler, 1838).

Allognathus is a good candidate with which to address the issue of whether relationships among taxa reflect the palaeogeography of the Balearic Islands, and this is the first attempt to explore this genus using molecular methods. The objectives of our study were: (1) to identify phylogroups within the genus and reconstruct the phylogenetic relationships between them; (2) to comprehensively clarify the taxonomy of the genus using species-delimitation models; and (3) to provide a temporal framework for the diversification of the different lineages and the colonization of the islands through the reconstruction of a time-calibrated multilocus species tree using relaxed clock models in combination with independent biogeographical calibration points, and then to determine ancestral areas at divergence events across the tree.

MATERIALS AND METHODS

Taxon sampling

We examined 87 specimens obtained from 51 sampling sites in the Balearic Islands covering all described *Allognathus* taxa, and the single population on the Iberian Peninsula (see Appendix S1 in Supporting Information). Specimens were preserved and stored in 96% ethanol until DNA isolation. Additional specimens were preserved in 70% ethanol to study their anatomy. According to Razkin *et al.* (2015), *Allognathus*, *Hemicycla*, *Iberus* and *Pseudotachea* form a monophyletic group within the Allognathini. Thus, *Hemicycla bidentalis*, *Pseudotachea splendida* and *Iberus gualtieranus* were included in our analyses to determine the phylogenetic relationships of *Allognathus* and date its isolation from its sister taxa. *Helix pomatia* and *Cepaea nemoralis* were also included as outgroups.

Genetic data

Total DNA was extracted from foot muscle tissue using DNeasy Tissue Kit (Qiagen, Hilden, Germany). Two mitochondrial gene fragments and one nuclear gene fragment were selected for multilocus analyses: cytochrome *c* oxidase subunit I (*COI*); the small subunit of ribosomal RNA (16S rRNA); and the cluster formed by the 3′ end of the 5.8S rRNA gene (*c.* 50 bp), the complete ITS2 region (*c.* 600 bp) and the 5′ end of the large subunit (*LSU*; 28S rRNA) gene (*c.* 840 bp). All new sequences obtained were deposited in GenBank (see Appendix S1 for PCR conditions, primers and GenBank accession numbers).

Molecular phylogeny

Sequence alignments for individual gene regions were performed using MAFFT 7 online (Katoh *et al.*, 2002) under its default settings. The Q-INS-i algorithm was applied for 16S and 5.8S–ITS2–28S data, and the 'auto' strategy for *COI* data. The evolutionary model for each partition was estimated prior to analysis with jModelTest 3.7 (Darriba *et al.*, 2012) according to the Akaike information criterion (AIC).

Given that some putative hybrid specimens were identified (see Appendix S2), a reduced dataset of 82 specimens was employed in the phylogenetic analyses to avoid incongruence among lineages. Bayesian-inference (BI) analyses were conducted with MrBayes 3.2.2 (Ronquist *et al.*, 2012), using a partition scheme by genes: *COI* (where the three codon positions were treated independently), 16S rRNA, 5.8S rRNA + ITS2 fragment (treated as a single partition) and 28S rRNA. Two parallel runs were conducted for 50 million generations, sampling every 1000 generations. The evolutionary models were GTR+Γ+I for the mitochondrial genes and HKY for the nuclear marker. The performance of the runs was visualized using Tracer 1.6 (Rambaut *et al.*, 2014). The first 25% of trees were discarded as burn-in and a majority-rule consensus tree was calculated from the remaining trees. Maximum-likelihood analyses were conducted using RAxML 8.0.24 (Stamatakis, 2014) under the GTRGAMMA model, with 1000 nonparametric bootstrap replicates to assess node support.

Molecular species delimitation

Three different methods were used to delimit species using both single-locus and multilocus data.

For primary species delimitation, we used the automatic barcode gap discovery method in abgd (Puillandre *et al.*, 2012). This procedure determines *COI* distances at which a barcode gap occurs and sorts the sequences into putative species based on these distances. The *COI* alignment was uploaded at http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html and abgd was run under default settings.

The second method was the generalized mixed Yule coalescent approach (GMYC; Pons *et al.*, 2006) which delimits mitochondrial clusters on the *COI*+16S rRNA tree and identifies putative independently evolving entities. The GMYC analysis was conducted using R package splits (Ezard *et al.*, 2009) under both the single-threshold and multiple-threshold options, using an ultrametric tree inferred using beast 1.8.0 (Drummond *et al.*, 2012).

Finally, the Bayesian Markov chain Monte Carlo (MCMC) algorithms implemented in bpp (Yang & Rannala, 2010) were used to analyse the phylogeographical data using all the DNA fragments. A guide tree was constructed using *BEAST (Heled & Drummond, 2010) in beast 1.8.0. Running the reversible-jump MCMC analyses for 100,000 generations (sampling interval of five) with a burn-in period of 50,000 produced consistent results across two separate analyses initiated with different starting seeds and using algorithm 0 ($\varepsilon = 15.0$). Each species-delimitation model was assigned equal prior probability. Given that the prior distributions of the ancestral population size (θ) and root age (τ_0) can affect models' posterior probabilities, three different combinations of priors were tested: (1) large ancestral populations sizes and deep divergences: $\theta = G\ (1, 10)$ and $\tau_0 = G\ (1, 10)$; (2)

relatively small ancestral populations sizes and shallow divergences among species: θ = *G* (2, 2000) and τ_0 = *G* (2, 2000); and (3) large ancestral population sizes, θ = *G* (1, 10), and relatively shallow divergences among species, τ_0 = *G* (2, 2000).

Estimation of divergence times

Divergence times within *Allognathus* were estimated using a Bayesian relaxed-clock approach implemented in BEAST 1.8.0, using 7.5×10^7 generations, sampled every 10,000 generations. Models of sequence evolution for each nucleotide sequence partition were determined using the corrected Akaike information criterion in JMODELTEST. The Yule model was chosen as the speciation prior for all three data sets and an uncorrelated lognormal relaxed molecular clock was employed. No fossils of *Allognathus* are known from before the Quaternary. We therefore performed two analyses, one with estimates based on geographical calibration and one based on substitution rates. For the first analysis, we used a palaeogeographical event in the Mediterranean Basin as a calibration point for estimating absolute divergence times. For the main inferred clades, we assumed that the most recent common ancestor of the lineages grouped in clades C3–C8 (Fig. 3.2.2) occurred during the MSC, so 5.33 ± 0.1 Ma was assigned as the prior distribution (normal distribution) for the age of the C3–C8 root node. The second approach to divergence estimation applied locus-specific rates. We performed an analysis using divergence rates from Razkin *et al.* (2015) (2% and 0.24% per million years for mitochondrial DNA and nuclear DNA, respectively) taking 9.1 Ma as the divergence time of the split between *Hemicycla* and *Allognathus*. Burn-in was determined with TRACER 1.6 (Rambaut *et al.*, 2014). The two independent runs were combined and the maximum-clade-credibility tree was identified using LOGCOMBINER 1.8.0 and TREEANNOTATOR 1.8.0 (Drummond *et al.*, 2012).

Ancestral-area reconstruction

The ancestral range at each divergence event was reconstructed using Bayesian binary MCMC analysis (BBM) as implemented in RASP 3.0 (Yu *et al.*, 2014). This program determines the probability of a given ancestral range at a node by averaging over a posterior set of trees, thereby accounting for phylogenetic uncertainty. A consensus tree obtained with BEAST was loaded into RASP. Each sample from the phylogeny was assigned to one of the seven regions: north-eastern Tramuntana Mountains (Mallorca); south-western Tramuntana Mountains; the Cabrera archipelago; Ibiza; the Ses Bledes archipelago; Menorca; and the Iberian Peninsula. Probabilities were estimated for nodes in the phylogeny with a posterior probability > 0.90. We used the F81+G model, and analyses were conducted for 500,000 generations using 10 chains, sampling every 100 generations. The first 20% of generations were discarded as burn-in.

RESULTS

Phylogenetic relationships

The topologies of the combined-dataset trees (*COI* + 16S rRNA + nuclear rRNA gene cluster) obtained by Bayesian and maximum-likelihood analyses provided strong support for basal nodes (Fig. 3.2.2). *Iberus* and *Pseudotachea* grouped together as the sister group to *Allognathus* and *Hemicycla*. The eight main genetic phylogroups identified within *Allognathus* were designated G1, H2, C3, C4, C5, C6, C7 and C8. Clade G1 joined all the *Allognathus graellsianus* specimens together, with full support (BP = 1.0; BS = 100%), and was recovered as the sister group (BP = 1.0; BS = 100%) to the remaining phylogroups, which form the subgenus *Iberellus*. Clade H2 grouped with full support (BP = 1.0; BS = 100%) all the specimens of the morphotype *A. hispanicus* from the north-eastern Tramuntana Mountains. H2 emerged as the sister group to the other clades of subgenus *Iberellus* (C3–C8). Clade C3 grouped with strong support (BP = 1.0; BS = 99%) the specimens of the *A. hispanicus* and *A. campanyonii* morphotypes collected in the south-western Tramuntana Mountains. C4 and C5 grouped together (BP = 1.0; BS = 93%); C4 was composed of the specimens of *A. pythiusensis* morphotype (BP = 1.0; BS= 93%) collected from the Ses Bledes archipelago and northern Ibiza, whereas C5 (BP = 1.0; BS = 96%) comprised *A. tanitianus*-morphotype specimens from Ibiza. All specimens of the *A. minoricensis* morphotype (including one specimen from Ibiza) grouped into two clades (C6 and C7), closely related to phylogroup C8, which combined specimens of the *A. campanyonii* morphotype from Mallorca, the Cabrera archipelago and the city walls of Tarragona. The phylogenetic relationships of clades C6–C8 were not resolved, but these were recovered as the closest relatives of phylogroups C4+C5 (BP = 0.99; BS = 84%). The specimens of *A. horadadae* grouped within clade C8, nested within the specimens of *A. campanyonii*. *Allognathus palumbariae* was nested within clade C6.

The genetic data obtained are provided in Appendix S3. Other gene trees (mitochondrial DNA and nuclear *rRNA*) are presented in the Supporting Information (Figs S1 & S2 in Appendix S2).

Species delimitation

Species limits were tested by different approaches with varying results (Fig. 3.2.3). In ABGD method, the initial partition reached stability at the distance of 0.013, value in which both initial and recursive partitions matched. Considering this distance the method revealed eight groups (*graellsianus*; *hispanicus*; ssp.; *tanitianus*; *pythiusensis*; *minoricensis* 1; *minoricensis* 2 and *campanyonii*). The results of the GMYC analyses based on a *COI*+16S phylogenetic tree were significant, both with single and multiple thresholds. The single-threshold method revealed three putative species clusters, whereas the multiple method revealed eight. The choice of prior distributions in BPP analyses for θ and τ_0 affected the results and corresponding support values. For the analyses assuming the combinations of priors (1) and (3), eight putative species were strongly supported by speciation probabilities, whereas for the combination of priors (2), three putative species were recognized: the phylogroups denoted *graellsianus* and *hispanicus*, and a third formed by the remaining phylogroups.

Temporal framework of *Allognathus* diversification

The different analyses each resulted in similar node-age estimates (Table 3.2.1, Fig. 3.2.4, and Fig. S3 in Appendix S2). The median age for the split between the group formed by

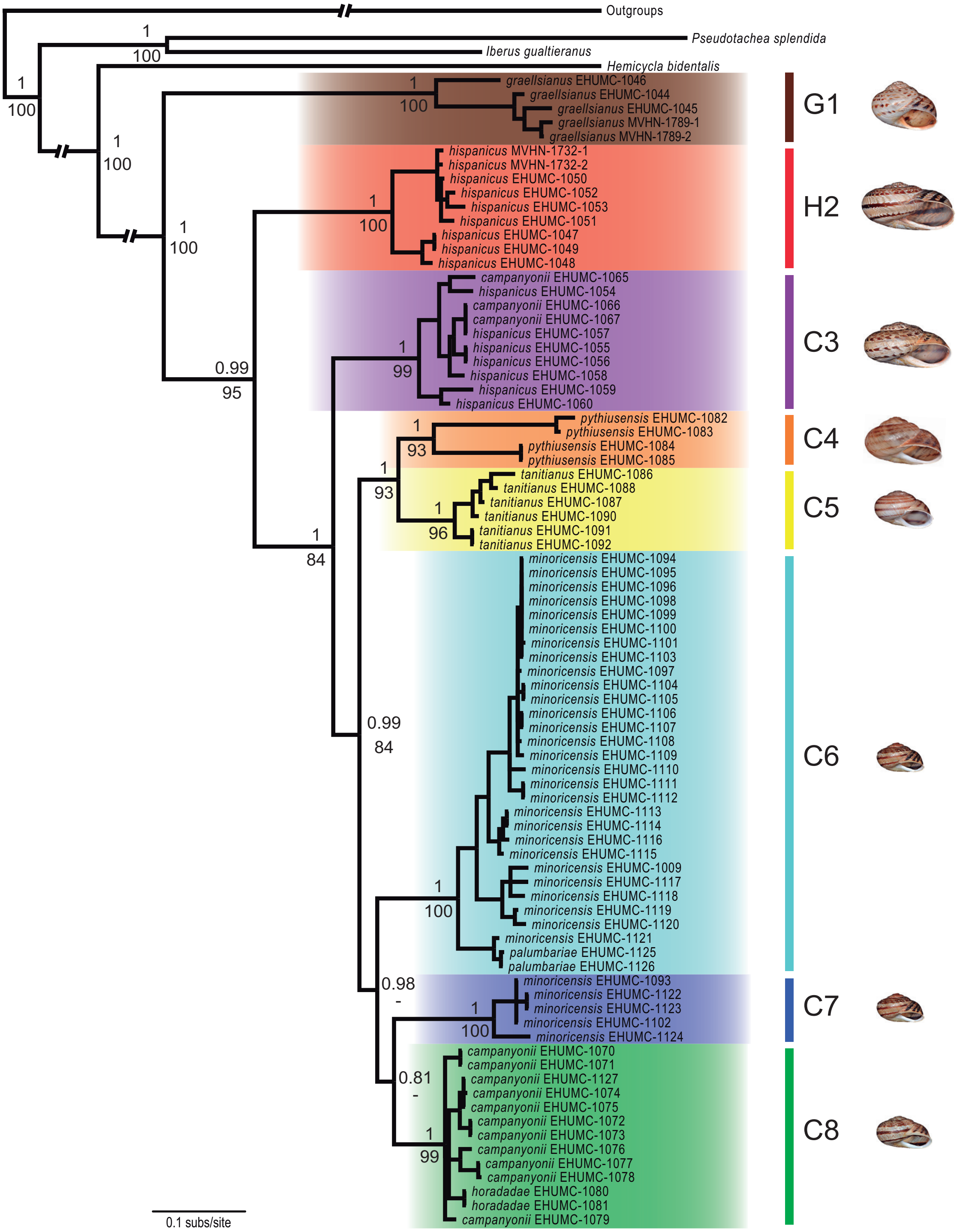

Figure 3.2.2 Bayesian Inference (BI) tree inferred based on a concatenated dataset (*COI*, cytochrome *c* oxidase subunit I; 16S rRNA, partial sequence; 5.8S rRNA, partial sequence; ITS2, internal transcribed spacer 2; 28S rRNA, partial sequence) of *Allognathus* species from the Balearic Islands and Tarragona (Iberian Peninnsula). Numbers on nodes correspond to posterior probabilities (BP) determined in the BI analysis and to bootstrap support (BS) in the maximum-likelihood (ML) analysis. A shell representative of the group's morphology is provided beside each main clade.

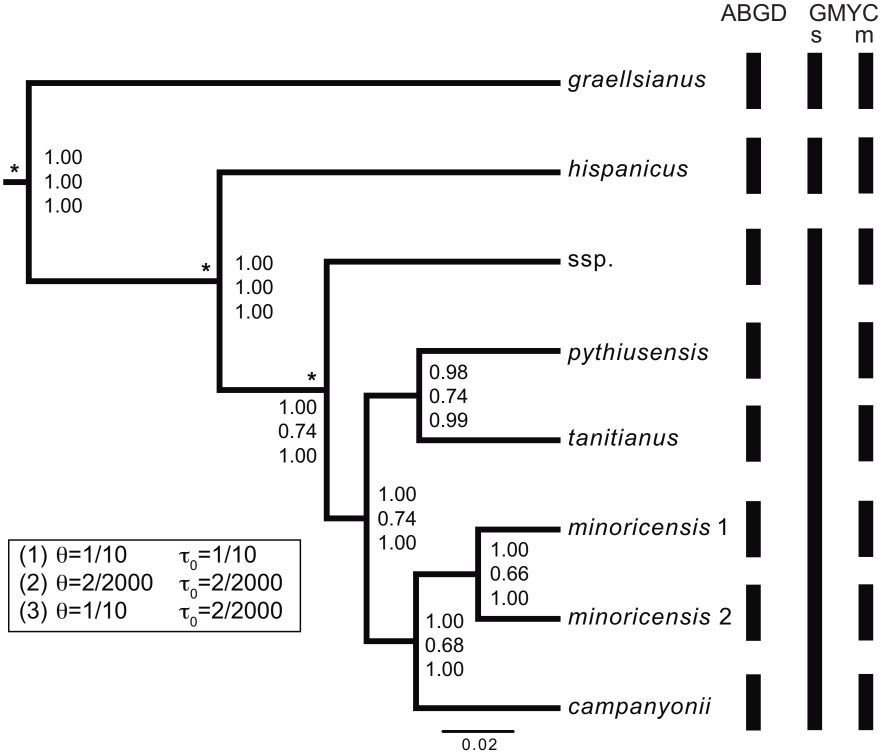

Figure 3.2.3 Summary results of the speceis-delimitation analysis for *Alloganthus*. Maximum-clade-credibility generated using all genetic data in *BEAST. Nodes with an asterisk indicate posterior probabilities derived from BPP analyses using the different sets of priors displayed in the box (bottom left). Black squares next to the tree represent the putative species clusters recovered in the ABGD and GMYC (s, single threshold; m, multiple threshold) analyses

Allognathus and *Hemicycla* from the phylogenetically closest genera *Iberus* and *Pseudotachea* was estimated at around 16.0 Ma. The separation between *Allognathus* and its sister group *Hemicycla* was estimated in both analyses at approximately 12.5 Ma, slightly higher that the value estimated in Razkin *et al.* (2015) where less information about this group was considered. According to the estimates based on geographical calibration (Fig. 3.2.4), the time of the split between *A. graellsianus* (clade G1) and the other *Allognathus* taxa was estimated at 8.19 Ma (95% highest posterior density (HPD), 10.65–6.25 Ma). Clade H2 split 6.76 Ma (95% HPD, 8.37–5.13 Ma). The diversification of clades C3–C8 started 5.32 Ma (95% HPD, 5.52–5.13 Ma), giving rise to at least six divergent lineages during the Pliocene in the different islands of the archipelago.

Ancestral area reconstruction

RASP analysis (Fig. 3.2.4) supported an ancestral range for the *Allognathus* complex (node II) in the north-eastern Tramuntana Mountains (Mallorca). The marginal probability for this basal node reconstruction (P = 96.5%) was much higher than for the alternative area (Iberian Peninsula + NE Tramuntana Mountains, P = 3.5%). The origin of the subgenus *Iberellus* (node III) was also located in the north-eastern Tramuntana Mountains (P = 99.4%). The origin of clades C3–C8 (node IV) in the south-western Tramuntana Mountains

Table 3.2.1 Main node-ages estimates (Ma) from calibrated BEAST analyses of Balearic-endemic *Allognathus* and related genera. Values are means, with highest posterior density intervales (95% HPD) in parentheses. Nodes are indicated in Fig. 3.2.4.

	Geographical calibration	**Substitution rates**
Iberus+Pseudotachea / *Hemicycla+Allognathus* split	**15.89** (21.91-11.07)	**16.24** (22.55-11.02)
Node I	**12.38** (17.08-8.71)	**12.77** (18.03-.8.50)
Node II	**8.19** (10.65-6.25)	**8.89** (12.42-6.04)
Node III	**6.76** (8.37-5.13)	**7 .25** (9.99-5.01)
Node IV	**5.32** (5.52-5.13)	**5.69** (7.75-3.90)

(Mallorca) showed higher support ($P = 58\%$) than any of the alternative areas (north-eastern Tramuntana Mountains, $P = 26\%$; every other area, $P < 6\%$). Finally, our RASP analysis provided strong support for a Menorcan origin of clade C8 ($P = 92.8\%$), which presently inhabits Mallorca, the Cabrera archipelago and the Iberian Peninsula (node V), and much lower probabilities for the alternative origin (Menorca + Cabrera, $P = 6.8\%$).

DISCUSSION

Phylogeny and species delimitation

The results of our study strongly support the monophyly of the genus *Allognathus*. The topologies we obtained showed differences between the mitochondrial and nuclear partitions, although the simultaneous analyses of all genes yielded fully resolved trees with strong support for the main clades. Stochastic sorting of ancestral polymorphisms (Pamilo & Nei, 1988; Wu, 1991) or hybridization (Moore, 1995) may account for the incongruence between mitochondrial and nuclear markers. All phylogenetic analyses supported the basal split of *A. graellsianus* from the remaining phylogroups of the subgenus *Iberellus sensu* Beckmann (2007) and Chueca *et al.* (2013). Within the subgenus *Iberellus*, seven different phylogroups were identified in the mitochondrial and combined trees, but only clades H2 and C3 were well supported in the nuclear-only analysis.

The current taxonomy of *Iberellus* is based on differences in shell morphology (Gasull, 1963; Beckmann, 2007) and some of the phylogenetic lineages obtained within this subgenus were consistent with morphologically defined taxa (Fig. 3.2.5). This occurred for the taxa living in the Pityusic Islands, which formed a monophyletic group: *A. hispanicus pythiusensis* and *A. h. tanitianus*. The former was considered to be endemic to the Ses Bledes archipelago (Gasull, 1963; Pons & Palmer, 1996; Schileyko, 2006), but some specimens of the morphotype *A. h. pythiusensis* collected from northern Ibiza belonged to the *pythiusensis* phylogroup, indicating that it also lives on this island. Fossil shells of this genus found in Ibiza (at Cala Xarraca) also belong to the morphotype *A. h. pythiusensis*, suggesting the long-standing presence of this taxon in Ibiza. Nevertheless, a more extensive sampling

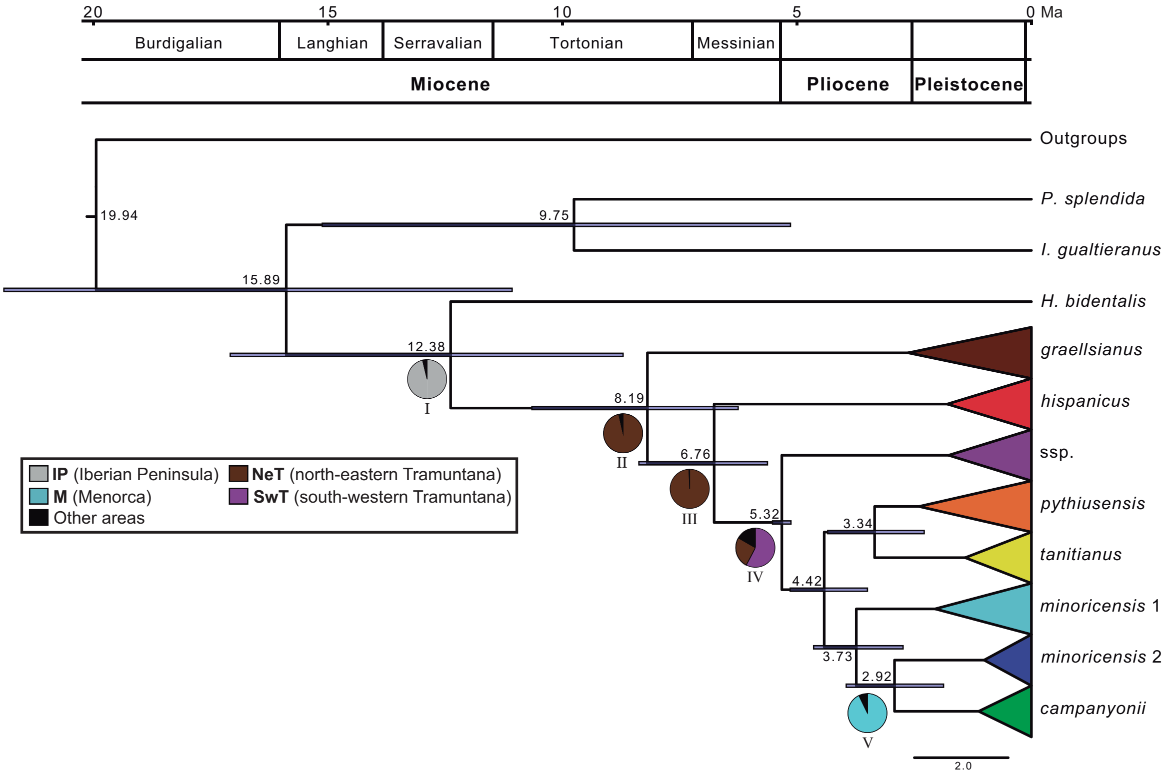

Figure 3.2.4 Time-calibrated multilocus tree for *Allognathus* obtained using BEAST. Bars indicate 95% highest posterior desities of divergence dates, with means estimated in million years ago (Ma) given at nodes. Main clades are colour-coded to match to the phylogeny of Fig. 3.2.2. Pie charts indicate the probability of ancestral areas for nodes with > 0.50 posterior probability support. Colours of ancestral areaas displayed on pie charts are defined in the box (bottom left)

effort is required to improve our understanding of the relationships between these two taxa living in Ibiza, which formed two well-defined phylogroups for mitochondrial but not for nuclear DNA. In the same way, full agreement was not observed between the five phylogenetic lineages obtained for the subgenus *Iberellus* in the Gymnesic Islands and the morphologically defined taxa. This was the case for *A. h. hispanicus* and *A. h. campanyonii*, two morphotypes that did not constitute monophyletic lineages. Snails with large, flat conical shells have been classified as *A. h. hispanicus*, whereas specimens with narrower, more conical shells have been ascribed to *A. h. campanyonii* (Chueca *et al.*, 2013). The presence of haplotypes of both shell morphs mixing with each other is here interpreted as the result of the hybridization of specimens of the *A. h. hispanicus* morphotype (haplogroup C3) with specimens of the *A. h. campanyonii* morphotype (clade C8) in the contact zones. Moreover, shells intermediate between the two morphotypes have been found in these contact areas. Neither of the haplogroups identified in Menorca (C6 and C7) corresponded to a particular shell type or had a localized distribution range on the island. Moreover, specimens collected from the same locality (Ses Mongetes, Ciutadella) and with the same shell morphology belonged to different clades, suggesting interbreeding between the

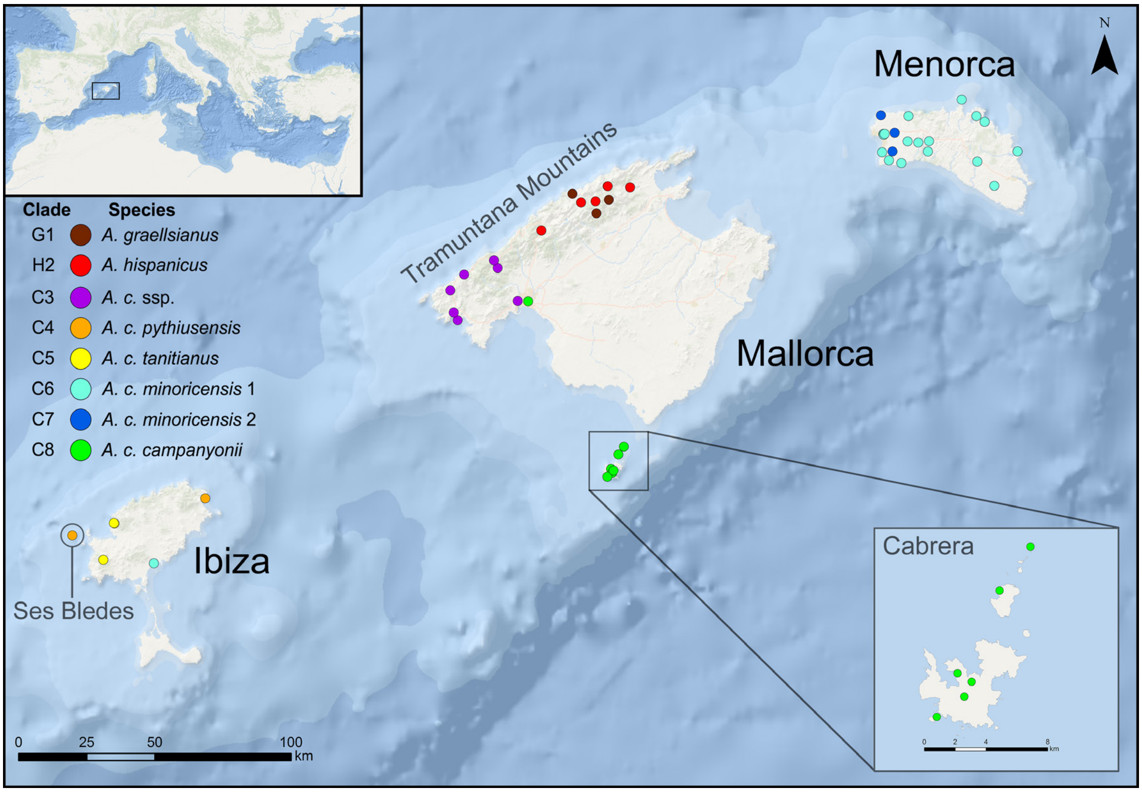

Figure 3.2.5 Map of the Balearic Island (Western Mediterranean) plotting collection localities of *Allognathus* species (samples from Tarragona city, Iberian Peninsula, not shown). Colours corresponds to clades obtained in the phylogeny (Fig. 3.2.2).

two haplogroups. The two remaining morphotypes, *A. h. horadadae* and *A. h. palumbariae* were described based mainly on their isolated distribution on small islets (Chueca *et al.*, 2013). Our results indicate, however, that they are neither valid taxa nor singular evolving lineages or ESUs and should be included in the synonymy of *A. h. campanyonii* and *A. h. minoricensis*, respectively. As well as the putative hybrids found between lineages C3 and C8, we identified hybridization between *A. h. tanitianus* and *A. h. minoricensis* in Cala Salada (Ibiza), where the latter was probably introduced. These results suggest that there is no reproductive isolation between lineages C3–C8. The level of morphological differentiation of the main lineages remains to be investigated and additional studies with other molecular markers would be useful to further investigate the interactions between different clades.

Our species-delimitation analyses recovered between three and eight groups. Although some tests recovered eight putative species, the different hybridization events detected between lineages C3–C8 (*campanyonii* × ssp.; *minoricensis* × *tanitianus*) did not support their consideration as different species under the biological species concept (Mayr, 1942). Based on this information and the results of the species-delimitation analyses, we conclude that three species may be defined within *Allognathus*: *A. (A.) graellsianus*, *A. (I.) hispanicus* and *A. (I.) campanyonii*. The last of these species contains at most five subspecies, mainly defined by biogeographical criteria: (1) *A. c. campanyonii* from the Cabrera archipelago and low areas of Mallorca, also present in Tarragona; (2) *A. c.* ssp., living in the south-western Tramuntana Mountains, which is cryptic to *A. hispanicus* and hybridizes with *A. c. campanyonii* in contact zones; (3) *A. c. minoricensis*, with two mitochondrial haplogroups in Menorca. (4) *A. c. tanitianus*, endemic to Ibiza; and (5) *A. c. pythiusensis*, with populations present in the Ses Bledes archipelago and in northern Ibiza.

Miocene colonization

The presence of many endemic species in the Balearic Islands has been explained by the Tyrrhenian vicariance hypothesis (Fochetti *et al.*, 2009; Pfenninger *et al.*, 2010; Bidegaray-Batista & Arnedo, 2011; Lázaro *et al.*, 2011) or through colonization during the MSC (Fromhage *et al.*, 2004; Lalueza-Fox *et al.*, 2005; Delicado *et al.*, 2014). In the absence of fossil records of *Allognathus* before the Quaternary, we used the MSC as a calibration point for the split between the clades from Ibiza and Menorca from the *A. c.* ssp. clade from Mallorca. A MSC split between congeneric phylogroups has also been described in other organisms that inhabit the Balearic Islands (Lalueza-Fox *et al.*, 2005; Brown *et al.*, 2008). Although passive transport events cannot be entirely ruled out, we have only identified recent passive transport events attributable to human activity at two sites – the ports of Tarragona and Ibiza. Furthermore, the *Allognathus* split occurred between the clades from Ibiza (C4-C5) and Gymnesic Islands (C6-C8) is highly unlikely by passive transport. The estimates based on substitution rates reinforce this hypothesis. According to these assumptions, divergence between *Allognathus* and its sister group (*Hemicycla*) was estimated to have occurred prior to 12.38 Ma, whereas the divergence between these two genera and their closest relatives *Pseudotachea* and *Iberus* was estimated to have taken place 15.89 Ma. These two divergence dates correspond to the middle Miocene. Thus, they largely post-date the Alpine orogeny of the late Oligocene (30–25 Ma), when the Hercynian microplates split from the continent, giving rise to the Balearic Islands (Rosenbaum *et al.*, 2002). For organisms in which diversification by the Tyrrhenian vicariance hypothesis has been shown to correspond to the Oligocene breakup of the Hercynian belt, closely related taxa commonly exist distributed in various regions that originated from the Hercynian microplates (Fochetti *et al.*, 2009; Pfenninger *et al.*, 2010; Bidegaray-Batista & Arnedo, 2011; Lázaro *et al.*, 2011). This is not the case for *Allognathus*, which is endemic to the Balearic microplate. In effect, there are no closely related taxa living in regions that arose from the Hercynian microplates. These results do not support the origin of *Allognathus* in the Balearic Islands through drifting with the Balearic microplate, suggesting that its presence on this archipelago is the result of a subsequent colonization process.

According to our findings, *Allognathus* colonized the Balearic Islands during the middle Miocene. This colonization could have occurred via the Baetic–Balearic corridor dated to the middle Miocene (Riba, 1981; Fontboté *et al.*, 1990; Roca, 1996). *Allognathus* is the only invertebrate taxon whose colonization of the Balearic Islands is assigned to the middle Miocene. This dating supports the hypothesis of a land connection between the archipelago and the Iberian Peninsula during the middle Miocene allowing the entry of terrestrial fauna into the Balearic Islands as suggested for some vertebrates (Adrover *et al.*, 1985; Quintana & Agustí, 2007; Bover *et al.*, 2008).

The position of *Hemicycla* as the sister group of *Allognathus* makes it difficult to pinpoint the colonization age of the Balearic Islands by *Allognathus*. *Hemicycla* is endemic to the Canary Islands, and there are no fossil records of *Hemicycla* in the Iberian Peninsula or the Balearic Islands. Some fossil assemblages from south-eastern France were originally attributed to *Hemicycla*, but these have been recently ascribed to the fossil genus *Megalotachea* (Nordsieck, 2014). These *Megalotachea* are dated from the middle Miocene to the early Pliocene in different places in the eastern Iberian Peninsula. Similarities between the shells of some species of *Megalotachea* and those of the genera *Iberus*, *Pseudotachea*, *Hemicycla* and *Allognathus* suggest that *Megalotachea* could belong to the subfamily Helicinae, closely related to the genera that originated in the western Mediterranean region.

Given the absence of *Hemicycla* fossils, two different scenarios could explain the colonization of the Canary Islands by this genus or its ancestors: (1) from the Iberian Peninsula, after the colonization of the Balearic Islands by ancestors of *Allognathus*, or (2) from the Balearic Islands, once they had been colonized by proto-*Allognathus*+*Hemicycla*. The former scenario would imply ancestors of *Allognathus* colonizing the Balearic Islands during the Serravallian (13.82–11.60 Ma). In contrast, if *Hemicycla* arrived in the Atlantic archipelago from the Balearic Islands, the ancestors of *Hemicycla*+*Allognathus* would have colonized the Balearic Islands in the Langhian (15.97–13.82 Ma). In both cases, these dates fall within the period of connection between the Iberian Peninsula and the Balearic Islands via the Baetic–Balearic corridor that existed during the Langhian–Serravallian (Riba, 1981; Fontboté *et al.*, 1990; Roca, 1996).

Remarkably, *Iberus* and *Pseudotachea* are the closest relatives to *Hemicycla* and *Allognathus*, (Razkin *et al.*, 2015), having their centres of dispersal in the south-east of the Iberian Peninsula (Puente, 1994; Elejalde *et al.*, 2008). This origin supports the hypothesis of colonization of the archipelago via the Baetic–Balearic domain. Similarly, the mammal fauna that colonized the Balearic Islands during the middle Miocene was closely related to the mammal fauna found in the south-eastern Iberian Peninsula (Murchas, Granada) dated to the middle Miocene (Martín-Suárez *et al.*, 1993; Bover *et al.*, 2008).

Later on, during the Tortonian (11.60–7.25 Ma), Mallorca was invaded by an epicontinental sea that reduced it to a cluster of small islands roughly corresponding to its current uplands (Pomar, 1991; Bauzà-Ribot *et al.*, 2011). This could explain the diversification of different *Allognathus* lineages within Mallorca, giving rise to *Allognathus graellsianus*, *A. hispanicus* and *A. campanyonii.* ssp. through vicariant processes. Our dates obtained for the separation of *A. graellsianus* (8.19 Ma) and the divergence of *A. hispanicus* from *A. campanyonii.* ssp. (6.76 Ma) are fairly consistent with this idea. Biogeographical data also support this rationale, because all three taxa live exclusively in the highest mountain system in the Balearic Islands, the Tramuntana Mountains (Gasull, 1963; Pons & Palmer, 1996). *Allognathus graellsianus* and *A. hispanicus* live in the north-east, whereas *A. campanyonii.* ssp. inhabits the south-west of the mountain range.

During the MSC (5.96–5.33 Ma), due to the desiccation of the Mediterranean basin, the entire archipelago was connected (Hsü *et al.*, 1973; Krijgsman *et al.*, 1999) and this allowed dispersal from Mallorca to Menorca and Ibiza. Dispersal from one island to another during the MSC in the Balearic Islands has been confirmed for different taxa (Brown *et al.*, 2008; Bauzà-Ribot *et al.*, 2011). In other cases, the MSC has been suggested as the period for colonization of the Balearic archipelago from the continent (Fromhage *et al.*, 2004; Lalueza-Fox *et al.*, 2005; Delicado *et al.*, 2014). Subsequent filling of the Mediterranean Basin led to the isolation of populations living in the three main emerged islands and triggered a burst of allopatric diversification. The phylogenetic lineages observed here point to different speciation processes within Menorca. According to our RASP analysis, one of the phylogenetic lineages arising in Menorca (*minoricensis* 2) dispersed to Mallorca approximately 3 Ma, ascribing the ancestral range of *A. c. campanyonii* to Menorca (P = 93%). This could have occurred via the land connection between the Gymnesic Islands during glacial periods of the Plio-Pleistocene, allowing the dispersal of specimens from Menorca to Mallorca and the Cabrera archipelago. Later on, during interglacial intervals, isolation of the islands would have promoted the allopatric speciation of *A. c. campanyonii*. Dispersal between Menorca and Mallorca during the Plio-Pleistocene has also been documented in other organisms (e.g. Bover *et al.*, 2008; Brown *et al.*, 2008; Quintana & Moncunill-Solé, 2014). After the colonization and subsequent range expansion of *A. c. campanyonii* in Mallorca, secondary contact with the *A. c.* ssp. phylogroup would have led to hybridization between the two lineages. Our data assign the specimens collected from the Tarragona city walls to the *campanyonii* clade without significant genetic differences, confirming the hypothesis of its introduction in historical times (Gasull, 1963). The presence of specimens belonging to the *minoricensis* clade in the city of Ibiza suggests their unintentional anthropogenic introduction.

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APPENDIX S1 Study material, list of primers used for sequencing and PCR conditions.

Table S1 Specimens used for the study of the land snail genus *Allognathus* (Helicidae) from the Balearic Islands, with their identification codes, localities, coordinates, voucher numbers and GenBank accession numbers. Superscript numbers indicate that the GenBank accession number was published in another study (1, Wade *et al*., 2006; 2, Razkin *et al*., 2015; 3, unpublished). EHUMC, collection at the Zoology and Animal Cell Biology department, University of the Basque Country (Spain); MVHN, Museu Valencià d'Historia Natural (Spain).

					GenBank accession numbers		
Taxa	Locality	Distribution	UTM grid cell	Voucher	*COI*	16S rRNA	5.8S–ITS2–28S
Allognathus (Allognathus) graellsianus	Ma 10 km 26 road. Escorca	Mallorca	31SDE8608	EHUMC-1044	KM592534	KM592624	KM592709
	Finca Mortitx. Escorca	Mallorca	31SDE9313	EHUMC-1045	KM592535	KM592625	KM592710
	Caimari to Sóller road.	Mallorca	31SDE9005	MVHN-1789-1	KM592532	^{2}KJ458485	^{2}KJ458583
	Caimari to Sóller road.	Mallorca	31SDE9005	MVHN-1789-2	KM592533	KM592623	KM592708
	Sa Calobra	Mallorca	31SDE8311	EHUMC-1046	KM592536	KM592626	KM592711
Allognathus (Iberellus) hispanicus hispanicus	Puíg de la Verge, Pollença	Mallorca	31SEE9912	EHUMC-1047	KM592537	KM592627	KM592712
	Puíg de la Verge, Pollença	Mallorca	31SEE9912	EHUMC-1048	KM592538	KM592628	KM592713
	Puíg de la Verge, Pollença	Mallorca	31SEE9912	EHUMC-1049	KM592539	KM592629	KM592714
	Sóller	Mallorca	31SDE7400	MVHN-1732-1	KM592554	KM592643	KM592728
	Sóller	Mallorca	31SDE7400	MVHN-1732-2	KM592555	KM592644	KM592729
	Finca Mortitx. Escorca	Mallorca	31SDE9313	EHUMC-1050	KM592540	KM592630	KM592715
	Lluc Monastery, Escorca	Mallorca	31SDE9008	EHUMC-1051	KM592541	KM592631	KM592716
	Finca Mortitx. Escorca	Mallorca	31SDE9313	EHUMC-1052	KM592542	KM592632	KM592717
	Ma 10 km 26 road. Escorca	Mallorca	31SDE8608	EHUMC-1053	KM592543	KM592633	KM592718
	Caló d'en Monjo, Peguera	Mallorca	31SDD5175	EHUMC-1054	KM592545	KM592634	KM592719
	La Granja de Esporles	Mallorca	31SDD6291	EHUMC-1055	KM592546	KM592635	KM592720
	La Granja de Esporles	Mallorca	31SDD6291	EHUMC-1056	KM592547	KM592636	KM592721
	Clot de S'Aigua Banyalbufar	Mallorca	31SDD6192	EHUMC-1057	KM592548	KM592637	KM592722
	Ma 10 km 96 road. Es Grau lookout point. Estellencs	Mallorca	31SDD5288	EHUMC-1058	KM592549	KM592638	KM592723
	Sa Guixería. Andratx	Mallorca	31SDD4984	EHUMC-1059	KM592550	KM592639	KM592724
	Golf course "Camp de Mar"	Mallorca	31SDD4977	EHUMC-1060	KM592551	KM592640	KM592725
	Alaró castle	Mallorca	31SDD8298	EHUMC-1063	KM592552	KM592641	KM592726
	Ma 10 km 45 road. Fornalutx	Mallorca	31SDE7604	EHUMC-1064	KM592553	KM592642	KM592727

Taxa	Locality	Distribution	UTM grid cell	Voucher	GenBank accession numbers		
					COI	16S rRNA	5.8S–ITS2–28S
Allognathus (Iberellus) hispanicus campanyonii	Progreso square, Palma de Mallorca	Mallorca	31SDD6291	EHUMC-1065	KM592556	KM592645	KM592730
	Clot de S'Aigua. Banyalbufar	Mallorca	31SDD6192	EHUMC-1066	KM592557	KM592646	KM592731
	Clot de S'Aigua. Banyalbufar	Mallorca	31SDD6192	EHUMC-1067	KM592558	KM592647	KM592732
	Puig na Morisca. Santa Ponça	Mallorca	31SDD5573	EHUMC-1068	KM592559	KM592648	KM592733
	Puig na Morisca. Santa Ponça	Mallorca	31SDD5573	EHUMC-1069	KM592560	KM592649	KM592734
	Tarragona city walls	Iberian Peninsula	31TCF5353	EHUMC-1070	KM592561	KM592650	KM592735
	Tarragona city walls	Iberian Peninsula	31TCF5353	EHUMC-1071	KM592562	KM592651	KM592736
	Cabrera	Cabrera	31SDD9533	EHUMC-1127	KM592563	KM592652	KM592737
	Es Port	Cabrera	31SDD9433	EHUMC-1072	KM592564	KM592653	KM592738
	Es Port	Cabrera	31SDD9433	EHUMC-1073	KM592565	KM592654	KM592739
	French Prisioner's Monument	Cabrera	31SDD9432	EHUMC-1074	KM592566	KM592655	KM592740
	French Prisioner's Monument	Cabrera	31SDD9432S	EHUMC-1075	KM592567	KM592656	KM592741
	Ramón Muntaner street, Palma de Mallorca	Mallorca	31SDD6981	EHUMC-1076	KM592568	KM592657	KM592742
	Illa dels Conills	Cabrera	31SDD9637	EHUMC-1077	KM592569	KM592658	KM592743
	Illa dels Conills	Cabrera	31SDD9637	EHUMC-1078	KM592570	KM592659	KM592744
	Punta N'Ensiola	Cabrera	31SDD9331	EHUMC-1079	KM592571	KM592660	KM592745
Allognathus (Iberellus) hispanicus horadadae	Illa Na Foradada	Cabrera	31SDD9839	EHUMC-1080	KM592572	KM592661	KM592746
	Illa Na Foradada	Cabrera	31SDD9839	EHUMC-1081	KM592573	KM592662	KM592747
Allognathus (Iberellus) hispanicus pythiusensis	Illa na Plana	Ses Bledes	31SCD4016	EHUMC-1082	KM592574	KM592663	KM592748
	Illa na Plana	Ses Bledes	31SCD4016	EHUMC-1083	KM592575	KM592664	KM592749
	Cala Sant Vicent	Ibiza	31SCD7826	EHUMC-1084	KM592576	KM592665	KM592750
	Cala Sant Vicent	Ibiza	31SCD7826	EHUMC-1085	KM592577	KM592666	KM592751
Allognathus (Iberellus) hispanicus tanintianus	Cala Salada	Ibiza	31SCD5219	EHUMC-1086	KM592578	KM592667	KM592752
	Cala Salada	Ibiza	31SCD5219	EHUMC-1087	KM592579	KM592668	KM592753
	Cala Salada	Ibiza	31SCD5219	EHUMC-1088	KM592580	KM592669	KM592754
	Cala Salada	Ibiza	31SCD5219	EHUMC-1089	KM592581	KM592670	KM592755
	Cala Salada	Ibiza	31SCD5219	EHUMC-1090	KM592582	KM592671	KM592756
	Col de Sa Creu	Ibiza	31SCD4909	EHUMC-1091	KM592583	KM592672	KM592757
	Col de Sa Creu	Ibiza	31SCD4909	EHUMC-1092	KM592584	KM592673	KM592758

Taxa	Locality	Distribution	UTM grid cell	Voucher	GenBank accession numbers		
					COI	16S rRNA	5.8S–ITS2–28S
Allognathus (Iberellus) hispanicus minoricensis	Ses Mongetes, Ciutadella	Menorca	31SEE7221	EHUMC-1093	KM592585	KM592674	KM592759
	Ses Mongetes, Ciutadella	Menorca	31SEE7221	EHUMC-1094	KM592586	KM592675	KM592760
	Ciutadella city	Menorca	31TEE7128	EHUMC-1095	KM592587	KM592676	KM592761
	Ciutadella city	Menorca	31TEE7128	EHUMC-1096	KM592588	KM592677	KM592762
	Ciutadella city	Menorca	31TEE7128	EHUMC-1097	KM592588	KM592678	KM592763
	Ciutadella Port	Menorca	31TEE7128	EHUMC-1098	KM592589	KM592679	KM592764
	Ciutadella city	Menorca	31SEE7128	EHUMC-1099	KM592590	KM592680	KM592765
	Ciutadella city	Menorca	31SEE7128	EHUMC-1100	KM592591	KM592681	KM592766
	Ses Mongetes, Ciutadella	Menorca	31SEE7221	EHUMC-1101	KM592592	KM592682	KM592767
	Ses Mongetes, Ciutadella	Menorca	31SEE7221	EHUMC-1102	KM592593	KM592683	KM592768
	Ciutadella city	Menorca	31TEE7128	EHUMC-1103	KM592594	KM592684	KM592769
	Son Febrer, Ciutadella	Menorca	31SEE7926	EHUMC-1104	KM592595	KM592685	KM592770
	Son Febrer, Ciutadella	Menorca	31SEE7926	EHUMC-1105	KM592596	KM592686	KM592771
	Son Febrer 2, Ciutadella	Menorca	31SEE7926	EHUMC-1106	KM592597	KM592687	KM592772
	Son Febrer 2, Ciutadella	Menorca	31SEE7926	EHUMC-1107	KM592598	KM592688	KM592773
	Between Son Saura and Es Talaier, Ciutadella	Menorca	31SEE7620	EHUMC-1108	KM592599	KM592689	KM592774
	Cala En Bastó, Ciutadella	Menorca	31SEE7023	EHUMC-1109	KM592600	KM592690	KM592775
	Ibiza city	Ibiza	31SCD6307	EHUMC-1110	KM592601	KM592691	KM592776
	Between Es Tancats and Es Bolt, Algairens, Ciutadella	Menorca	31TEE7833	EHUMC-1111	KM592602	KM592692	KM592777
	Between Es Tancats and Es Bolt, Algairens, Ciutadella	Menorca	31TEE7833	EHUMC-1112	KM592603	KM592693	KM592778
	Algendar 1, Ferreires	Menorca	31SEE8325	EHUMC-1113	KM592604	KM592694	KM592779
	Algendar 1, Ferreires	Menorca	31SEE8325	EHUMC-1114	KM592605	KM592695	KM592780
	Algendar 2, Ferreires	Menorca	31SEE8325	EHUMC-1115	KM592606	KM592696	KM592781
	Algendar 2, Ferreires	Menorca	31SEE8325	EHUMC-1116	KM592607	KM592697	KM592782
	Es Lleó, Alaior	Menorca	31SEE9720	EHUMC-1009	KM592616	[2]KJ458531	[2]KJ458618
	Cap de Cavalleria, Es Mercadal	Menorca	31TEE9338	EHUMC-1117	KM592608	KM592698	KM592783
	Cala de S'Enclusa, Es Mercadal	Menorca	31TEE9733	EHUMC-1118	KM592609	KM592699	KM592784
	Ses Olles, Es Mercadall	Menorca	31TEE9932	EHUMC-1119	KM5926010	KM592700	KM592785
	Ses Olles, Es Mercadall	Menorca	31TEE9932	EHUMC-1120	KM592611	KM592701	KM592786

Taxa	Locality	Distribution	UTM grid cell	Voucher	GenBank accession numbers		
					COI	16S rRNA	5.8S–ITS2–28S
Allognathus (Iberellus) hispanicus minoricensis	Old road to St. Climent, Maó	Menorca	31SFE0214	EHUMC-1121	KM592612	KM592702	KM592787
	Bona Nova quarry, Ciutadella	Menorca	31TEE7428	EHUMC-1122	KM592613	KM592703	KM592788
	Bona Nova quarry, Ciutadella	Menorca	31TEE7428	EHUMC-1123	KM592614	KM592704	KM592789
	Cala Es Pous, Ciutadella	Menorca	31TEE7033	EHUMC-1124	KM592615	KM592705	KM592790
Allognathus (Iberellus) hispanicus palumbariae	Illa d'en Colom	Menorca	31SFE0923	EHUMC-1125	KM592617	KM592706	KM592791
	Illa d'en Colom	Menorca	31SFE0923	EHUMC-1126	KM592618	KM592707	KM592792
Outgroups							
Hemicycla bidentalis	Anaya, Tenerife	Canary Islands		MVHN-2160	KM592619	[2]KJ458528	[2]KJ458615
Pseudotachea splendida	Sierra de Quibas, Murcia	Iberian Peninsula		MVHN-2270	KM592622	[2]KJ458552	[2]KJ458634
Iberus gualtieranus	Sierra Elvira, Granada	Iberian Peninsula		EHUMC-1014	KM592620	[2]KJ458530	[2]KJ458617
Cepaea nemoralis					[3]JX911293	[2]KJ458498	[1]AY014130
Helix pomatia	(*COI*): Unknown; (16S): Unknown; (5.8–ITS2–28S): Pulpit Down, Buckinghamshire, UK				[3]JX911304	[3]AF208297	[1]AY841333

Table S2 List of primers used for amplification and sequencing.

Gene	Name	Sense	Sequence	Reference
COI	LCO1490	F	GGT CAA CAA ATC ATA AAG ATA TTG G	Folmer *et al.* (1994)
	HCO2198	R	TAA ACT TCA GGG TGA CCA AAA AAT CA	Folmer *et al.* (1994)
16S rRNA	16sar	F	CGC CTG TTT ATC AAA AAC AT	Palumbi *et al.* (1991)
	16sbr	R	CCG GTC TGA ACT CAG ATC ACG T	Palumbi *et al.* (1991)
5.8S–ITS2	LSU-1	F	CTA GCT GCG AGA ATT AAT GTG A	Wade *et al.* (2006)
	LSU-3	R	ACT TTC CCT CAC GGT ACT TG	Wade *et al.* (2006)
28S	LSU-2	F	GGG TTG TTT GGG AAT GCA GC	Wade *et al.* (2006)
	LSU-5	R	GTT AGA CTC CTT GGT CCG TG	Wade *et al.* (2006)
	LSU-2mod	F	TCT CAG GAG TCG GGT TGT TT	Razkin *et al.* (2015)

Table S3 PCR conditions for mitochondrial and nuclear markers.

(a) PCR conditions for *COI* and 16S rRNA genes

Step	T (°C)	Time	Cycles
1	96.0	1 min	1
2	94.0	30 s	35
	55.0	30 s	
	72.0	1 min	
3	72.0	10 min	1
Hold	10.0	∞	

(b) PCR conditions for nuclear markers (5.8S – ITS2 – 28S)

Step	T (°C)	Time	Cycles
1	96.0	1 min	1
2	94.0	30 s	35
	50.0	30 s	
	72.0	1 min	
3	72.0	10 min	1
Hold	10.0	∞	

APPENDIX S2 Supplementary figures. Figs S1 & S2 show the phylogenetic trees from the Bayesian analyses (MrBayes) of the mtDNA and nuclear DNA dataset.

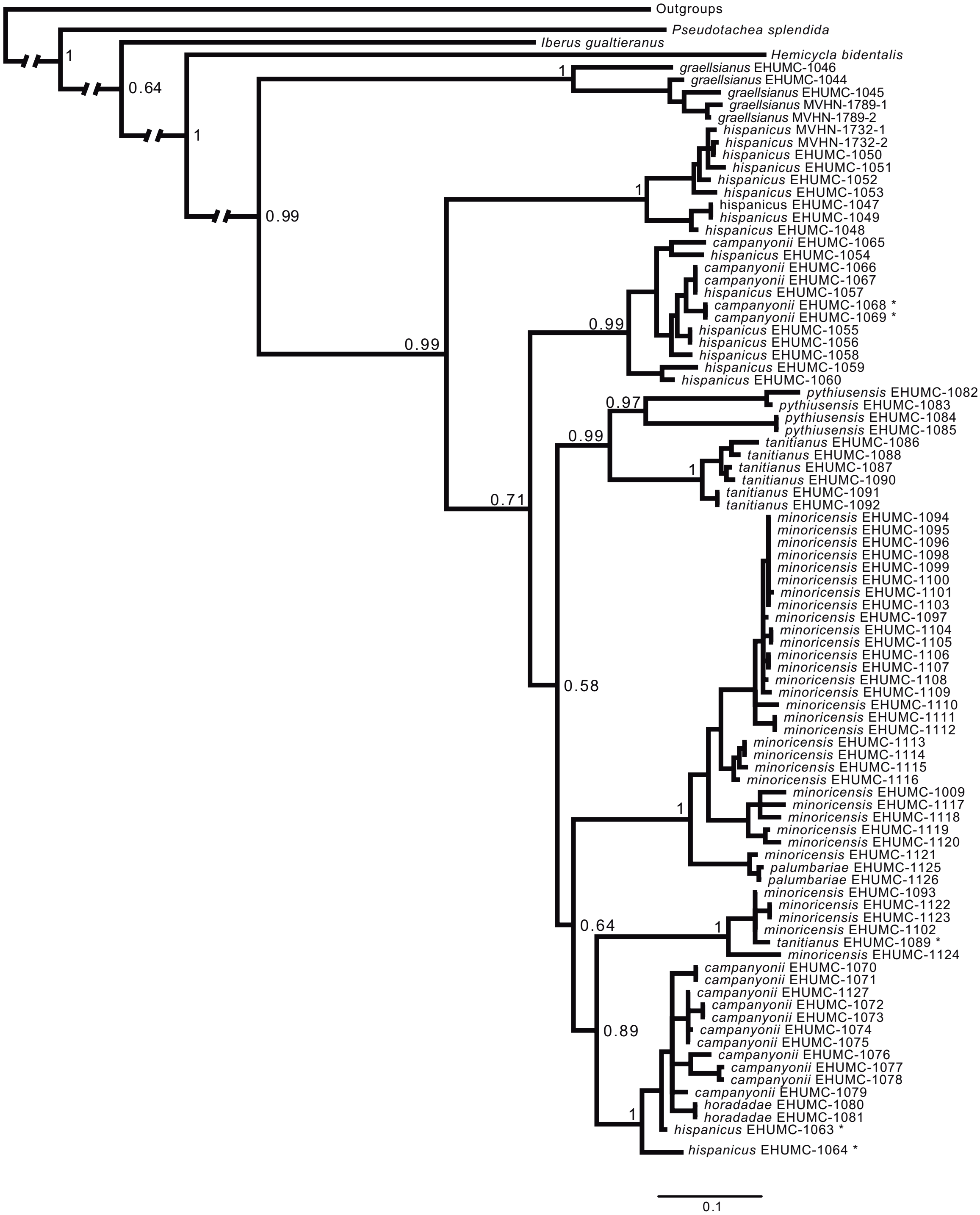

Figure S1 Bayesian-inference tree inferred based on concatenated dataset of mitochondrial gene fragments (COI, cytochrome *c* oxidase subunit I; 16S rRNA) of *Allognathus* species from the Balearic Islands and Tarragona (Iberian Peninsula). Numbers above branches correspond to posterior probability values in the BI analysis. Sampled with an asteriks indicated putative hybrid specimens.

Figure S2 Bayesian-inference (BI) tree inferred based on a concatenated dataset of nuclear DNA gene fragments (5.8S rRNA; ITS2, internal transcribed spacer 2; 28S rRNA) of *Allognathus* species form the Balearic Islands and Tarragona (Iberian Peninsula). Numbers above branches correspond to posterior probability values in the BI analysis. Sampled with an asterisks indicated putative hybrid specimens.

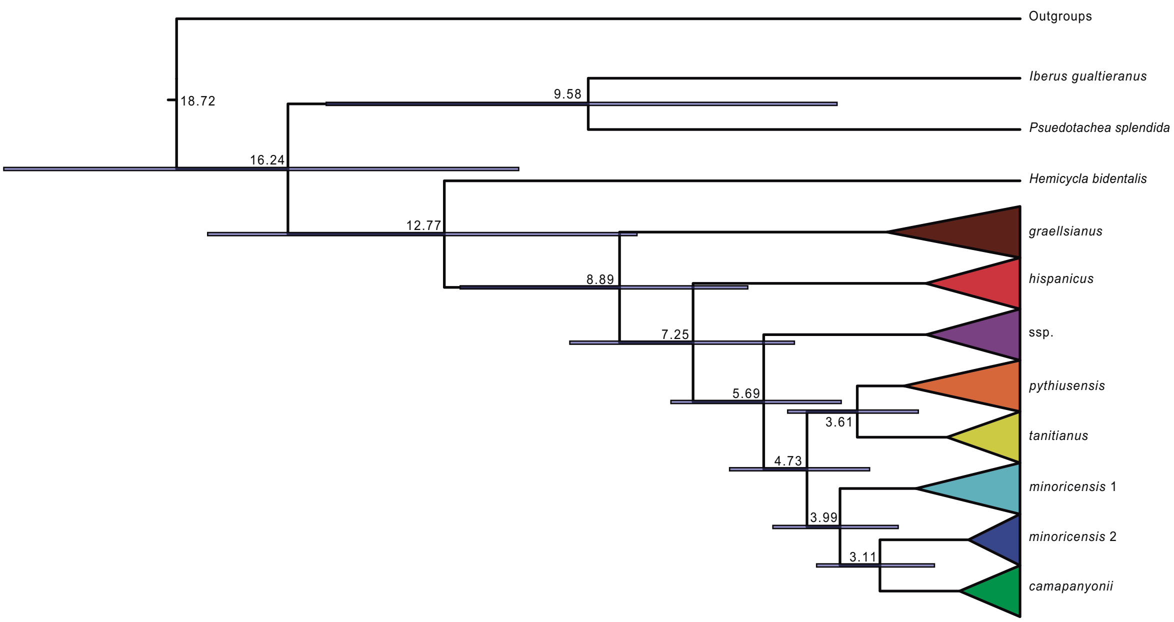

Figure S3 Timed calibrated multilocus tree for *Allognathus* obtained using BEAST based on substitution rates. Bars indicate 95% highest posterior densities of divergence dates, with means estimated in millions of years ago (Ma) given at nodes. The main clades are colour-coded to match to the phylogeny of Fig. 3.2.2.

APPENDIX S3 Genetic data information.

Table S4 Uncorrected average *p*-distance of *COI* (below) and 16S rRNA (top) among main *Allognathus* clades from the Balearic Islands.

	graellsianus	*hispanicus*	ssp	*campanyonii*	*minoricensis*	*pythiusensis*	*tanitianus*
graellsianus		10.91	7.76	9.57	9.72	10.20	9.32
hispanicus	13.35		5.88	5.69	6.90	6.88	5.67
ssp	12.91	9.87		4.95	5.53	6.03	5.30
campanyonii	13.37	9.88	7.29		4.65	5.50	4.99
minoricensis	13.52	9.96	7.45	6.25		6.70	6.35
pythiusensis	14.23	11.96	8.97	6.77	6.61		5.32
tanitianus	13.07	9.82	6.93	4.88	6.67	5.58	

Table S5 Uncorrected average *p*-distance of 5.8S–ITS2 (below) and 28S rRNA (top) among main *Allognathus* clades from the Balearic Islands.

	graellsianus	*hispanicus*	ssp	*campanyonii*	*minoricensis*	*pythiusensis*	*tanitianus*
graellsianus		0.24	0.24	0.25	0.35	0.24	0.12
hispanicus	0.91		0.24	0.25	0.35	0.24	0.12
ssp	1.18	0.87		0.01	0.11	0.00	0.12
campanyonii	1.04	0.73	0.54		0.10	0.01	0.13
minoricensis	1.24	0.93	0.74	0.16		0.11	0.23
pythiusensis	1.96	2.07	2.31	1.80	2.00		0.12
tanitianus	1.04	0.73	0.54	0.00	0.20	1.80	

Table S6 Genetic data information obtained for the three loci analysed in *Allognathus*.

	COI	16S rRNA	5.8S–ITS2–28S
Parsimony-informative sites	180 in 672 bp	94 in 435 bp	24 in 1345 bp
Evolutionary model selected	GTR+Γ+I	GTR+Γ+I	HKY

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CHAPTER 4

Xerocrassa

Paper III

Biogeography and radiation of the land snail genus *Xerocrassa* (Geomitridae) in the Balearic Islands

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ABSTRACT

Aim Our aim was to assess the evolutionary history of the land snail genus *Xerocrassa* on the Balearic Islands with a molecular phylogeny. We unravelled the current diversity of taxa within the archipelago and investigated the processes of radiation based on current distributions and palaeogeographical events in the western Mediterranean basin.

Location The Balearic Islands, Western Mediterranean.

Methods A data set of 2540-bp DNA sequences was generated from one nuclear and two mitochondrial gene fragments obtained from170 specimens representing112 populations. With maximum-likelihood and Bayesian phylogenetic methods and a Bayesian molecular clock, we examined the evolutionary history of the genus *Xerocrassa*. We also used genetic-based species delimitation models to clarify the taxonomy of the genus in the archipelago.

Results We provide the first molecular phylogeny of the endemic *Xerocrassa* of the Balearic Islands. The monophyly of several species currently recognized was not supported. The genus colonized the archipelago in a single event during the Messinian Salinity Crisis from the eastern part of the Iberian Peninsula. The hypothesis of a relatively recent origin for the evolutionary diversification within the group was supported by molecular clock analyses. Allopatric speciation occurred after the isolation of the Pityusic and Gymnesic island systems. Moreover, several intra-island speciation processes were detected.

Main conclusions Eleven species were delimited within the genus, some of them with several subspecies. Several nomenclatural changes are proposed, and several subspecies that were traditionally separated based on their geographical isolation and shell morphology were reassigned to their corresponding species. The radiation of the genus within the archipelago was influenced by the onset of the Mediterranean climate during the transition from the Pliocene to the Pleistocene and, the Pleistocene glacial cycles.

Keywords

Biogeography, colonization, Gastropoda, island endemism, Messinian salinity crisis, phylogeny, Pliocene, radiation, Western Mediterranean, *Xerocrassa*.

INTRODUCTION

The biota of islands provides unique opportunities for the study of evolutionary mechanisms and speciation processes (Losos & Ricklefs, 2009). With marine barriers that restrict dispersal and gene flow, for oceanic archipelagos, local evolutionary processes are the most important factor affecting endemism on islands (Emerson, 2002; Whittaker & Férnandez-Palacios, 2007; Warren *et al.*, 2015). Isolated oceanic islands provide new ecological opportunities for colonizers, which lead to the evolution of new species with different morphological and physiological features through adaptive radiation (Carlquist *et al.*, 2003; Grant & Grant, 2008). By contrast, local fauna on continental archipelagos experience different periods of connection among islands or to the mainland because of tectonic processes and sea level fluctuations, and therefore, island endemism is better explained by factors that affect immigration than local evolutionary processes. Generally, diversification on continental islands occurs through non-adaptive radiation, and genetic drift plays a major role in population divergence and allopatric speciation because of geographic isolation or competitive interactions (Gittenberger, 1991; Mayol *et al.*, 2012; Sauer *et al.*, 2013). Nevertheless, adaptive divergent speciation in response to different ecological conditions within an island is also postulated (Fiorentino *et al.*, 2013). The use of molecular tools and the increase in data acquisition produce deeper insights into the relative significance of dispersal and vicariance and into the effects of adaptive and non-adaptive components of diversification and speciation (Poulakakis *et al.*, 2014). The Mediterranean basin is a hotspot of biodiversity, and the islands of the basin are particularly rich in fauna and flora with many microendemisms (Médail & Quézel, 1999; Myers *et al.*, 2000). The Balearic Islands (Western Mediterranean) are a continental fragment archipelago that is the most isolated archipelago in the Mediterranean basin with as many as 90 small islets surrounding the four major islands. The palaeogeographical history of the archipelago is well known, and the connections to the mainland and among the major islands are placed within a well-defined time framework (Rosenbaum *et al.*, 2002; Rosenbaum & Lister, 2004; Jolivet *et al.*, 2006). The origins of several ancient biota endemic to the Balearic Islands are attributed to the Tyrrhenian vicariance hypothesis (Magri *et al.*, 2007; Pfenninger *et al.*, 2010; Bidegaray-Batista & Arnedo, 2011). Moreover, during the middle Miocene (16.0–11.6 Ma), several organisms colonized the archipelago across a landbridge that connected the Balearic Islands with the Iberian Peninsula (Adrover *et al.*, 1985; Quintana & Agustí, 2007; Bover *et al.*, 2008; Chueca *et al.*, 2015). Additionally, with the desiccation of the Mediterranean basin during the Messinian Salinity Crisis (MSC) 5.96–5.33Ma (Hsü *et al.*, 1973; Krijgsman *et al.*, 1999), several organisms colonized the Balearic Islands from the Iberian Peninsula (Fromhage *et al.*, 2004; Lalueza-Fox *et al.*, 2005; Delicado *etal.*, 2014). Since the refilling of the Mediterranean basin at the end of the MSC, the Balearic Islands have been completely isolated from the mainland. Similarly, the western (Pityusic) and eastern (Gymnesic) islands remained isolated following the end of the MSC, and allopatric speciation has occurred. Furthermore, the fluctuations in sea level caused by climatic changes during the Pleistocene (Sosdian & Rosenthal, 2009; Capraro *et al.*, 2011) highly modified the shape, size and connections of the emerged lands. With these oscillations in the sea level, species migrations and intraspecific gene flow occurred across the connections between the islands of Mallorca (including the Cabrera Archipelago) and Menorca and among Ibiza, Formentera and the coastal islets (Bover *et al.*, 2008; Brown *et al.*, 2008; Rodríguez *et al.*, 2013; Quintana & Moncunill-Solé, 2014; Chueca *et al.*, 2015).

Snails are among the most diverse groups of animals (Groombridge, 1992) and are a good model to study island biogeography and radiation processes (Parent & Crespi, 2006; Holland & Cowie, 2009; Greve *et al.*, 2010; Sauer *et al.*, 2013). Moreover, the radiation of land snails is addressed in several studies (Ketmaier *et al.*, 2006; Douris *et al.*, 2007; Fiorentino *et al.*, 2010; Pfenninger *et al.*, 2010; Sauer & Hausdorf, 2010; Bank *et al.*, 2013; Chueca *et al.*, 2015). Nevertheless, the phylogeography and speciation of land snails on the islands of the Mediterranean are studied far less than those of arthropods or vertebrates (Poulakakis *et al.*, 2014).

The land snail genus *Xerocrassa* is one of the most diverse genera within the Geomitridae in southern Europe, with approximately 50 currently recognized species (Bank, 2011). The distribution of the genus is disjunct within the Mediterranean basin with two centres of radiation, i.e., the eastern complex (Aegean region and Middle East) with 24 species (Heller, 2009; Bank, 2011) and the western complex (Iberian Peninsula and Balearic Islands) with at least 27 species (Beckmann, 2007; Bank, 2011). The genus is particularly well represented in the Balearic Archipelago, with species that inhabit the four main islands and most of the coastal islets and that occupy different habitats varying from dry, open sunny areas near the coastline to pine forests and shrublands along an altitudinal range.

In this study, we focused on the endemic *Xerocrassa* species of the Balearic Islands where the species of this genus of land snails have undergone extensive morphological diversification, from flattened-ribbed shells to high-conical or rounded shell forms of different sizes. The taxonomy of the Balearic species of the genus is controversial and has been subjected to several recent revisions (Graack, 2005; Quintana & Vilella, 2005; Beckmann, 2007; Forés, 2015). An adequate taxonomy remains to be proposed. Following the classification proposed by Beckmann (2007), several endemic species of *Xerocrassa* inhabit the archipelago (3 species in the western Pityusic Islands and 7 species in the eastern GymnesicIslands; Table 4.1). Moreover, several subspecies have been described within the archipelago because of the high shell variability and the isolation of many populations on small islets.

The determination of genetic diversity within *Xerocrassa* endemic to the Balearic Islands will contribute to our understanding of the historical biogeography of the Mediterranean basin. In this study, we included nearly all the currently recognized taxa of the genus that inhabit the archipelago. Our aims were to (1) investigate the radiation and diversity of the genus *Xerocrassa* in the Balearic Islands using a molecular phylogeny and species delimitation models and to (2) infer a phylogenetic framework to investigate the biogeographical patterns and evolutionary history of *Xerocrassa* in the Balearic Islands. Based on the new information, we proposed a taxonomic rearrangement of the *Xerocrassa* subspecies within the archipelago.

Table 4.1 Classification proposed for *Xerocrassa* of the Balearic Islands according to Beckmann, 2007. (1) Species considered by other authors, but not by Beckmann, 2007. (2) Species described after Beckmann, 2007.

Pityusic Islands		**Gymnesic Islands**	
X. ebusitana		*X. frater*	*X. f. frater*
X. caroli	*X. c. caroli*		*X. f. newka*
	X. c. alegriae		*X. f. pulaensis*
	X. c. espartariensis		*X. f. pollenzensis*
	X. c. formenterensis	*X. ferreri*	*X. f. ferreri*
	X. c. jaeckeli		*X. f. pobrensis*
X. cisternasi	*X. c. cisternasi*	*X. majoricensis*(1)	
	X. c. calasaladae	*X. prietoi*	*X. p. prietoi*
	X. c. calderensis		*X. p. muroensis*
	X. c. canae	*X. homeyeri*	
	X. c. conjungens	*X. ponsi*	
	X. c. hortae	*X. moraguesi*	
	X. c. margaritae	*X. claudinae*(1)	
	X. c. mesquidae	*X. ferrutxensis*(2)	
	X. c. muradae	*X. nyeli*	
	X. c. ortizi	*X. cardonae*	
	X. c. redonae		
	X. c. scopulicola		
	X. c. vedrae		
	X. c. vedranellensis		

MATERIALS AND METHODS

Sampling and molecular data collection

Within the Balearic Islands, 170 *Xerocrassa* specimens were collected at 112 sample sites (see Appendix S1 in Supporting Information). The samples included 36 of 38 subspecies recognized by different authors (Beckmann, 2007; Bank, 2011). Additionally, *X. subrogata*, *X. barceloi*, *X. montserratensis* and *X. ripacurcica* from the Iberian Peninsula and *X. molinae* from the Columbretes Islands were included in the analyses. *Hygromia limbata*, *Trochoidea pyramidata* and *Xerosecta adolfi* were included as outgroups. The total genomic DNA was extracted from tissue samples and preserved in 96% ethanol using DNeasy Tissue Kit (Qiagen, Hilden, Germany). Two mitochondrial gene fragments and a nuclear rDNA region were selected for multilocus analyses: cytochrome *c* oxidase subunit I (*COI*); the small subunit of ribosomal RNA (16S rRNA); and the cluster formed by the 3′ end of the 5.8S rRNA gene (*c*. 50 bp), the complete ITS2 region (*c*. 600 bp) and the 5′ end of the large subunit (28S rRNA) gene (*c*. 840 bp). All new sequences were deposited in GenBank (see Appendix S1 for PCR conditions, primers and GenBank accession numbers).

Phylogenetic analyses and delimitation of evolutionary lineages

The sequence alignments for the individual gene regions were performed using the online version of MAFFT 7 (Katoh *et al*., 2002) with the default settings. The Q-INS-i algorithm was applied to the 16S rRNA and 5.8S-ITS2-28S data, and the ‘auto’ strategy was used for the

COI data. The models of sequence evolution were chosen using jModelTest 2.1.6 (Darriba *et al*., 2012) according to the Akaike information criterion (AIC).

We conducted phylogenetic analyses of the concatenated dataset using maximum-likelihood (ML) and Bayesian inference (BI). Maximum-likelihood analyses were conducted using RAxML 8.1.11 (Stamatakis, 2014) under the GTRGAMMA model, with 1000 nonparametric bootstrap replicates to assess node support. Bayesian inference analyses were conducted with MrBayes 3.2.2 (Ronquist *et al*., 2012), using a partition scheme by gene: *COI* (for which the three codon positions were treated independently), 16S rRNA, 5.8S rRNA, ITS2 fragment and 28S rRNA. Two independent runs were conducted to check for convergence. Each analysis was run with 25 million iterations, sampling every 1000 generations. The evolutionary model GTR+G+I was considered for all partitions. The first 25% of trees was discarded as burn-in and a majority rule consensus tree was calculated from the remaining trees.

The information from single-locus genes is useful to generate preliminary hypotheses of species delineations (Kekkonen & Hebert, 2014). However, evolutionary processes such as introgression and incomplete lineage sorting and biological features such as dispersal ability and population size may compromise the ability of single markers to infer species boundaries (Kuo & Avise, 2005; Talavera *et al*., 2013). Therefore, candidate lineages must be subsequently validated using multilocus approaches and further corroborated with the addition of phenotypic/ecological information(Camargo *et al*., 2012). We conducted a primary species delimitation using two different methods, i.e., the automatic barcode gap discovery (abgd) method (Puillandre *et al*., 2012) and the tree-based general mixed Yule coalescent (GMYC) model (Pons *et al*., 2006). For the abgd method, the *COI* alignment was uploaded at http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html and was run under the default settings. An ultrametric mtDNA (*COI*+16S rRNA) tree was inferred using beast 1.8.1 (Drummond *et al*., 2012) to define species boundaries under the GMYC model. The GMYC tests were conducted using splits of the R statistical software package (Ezard et al., 2009) under the single-threshold model; this model performs better than the multiple-threshold model (Fujisawa & Barraclough, 2013). Finally, the Bayesian Markov chain Monte Carlo (MCMC) algorithms implemented in bpp (Yang & Rannala, 2010) were used to analyse the phylogenetic data using all the DNA fragments. A guide tree was constructed using *beast (Heled & Drummond, 2010) in beast 1.8.1. The species tree was tested on bpp. Reversible-jump MCMC was run for 500,000 generations (sampling interval of five) with a burn-in period of 50,000. Following Leaché & Fujita (2010), we selected algorithm 0 ($\varepsilon = 15.0$). Each species delimitation model was assigned equal prior probability. Given that the prior distributions of the ancestral population size (θ) and root age (t_0) can affect posterior probabilities of a model, two different combinations of priors were tested: (a) large ancestral population sizes and deep divergences among species: $\theta = G$ (1, 10) and $t_0 = G$ (1, 10); and (b) relatively small ancestral population sizes and shallow divergences among species: $\theta = G$ (2, 2000) and $t_0 = G$ (2, 2000).

Estimation of divergence times

We estimated absolute divergence times and substitution rates using a Bayesian approach as implemented in BEAST 1.8.1 (Drummond *et al*., 2012). The Yule model was selected as the speciation prior for all datasets, and an uncorrelated, exponential relaxed clock was employed. No fossils of *Xerocrassa* are known from the Balearic Islands before the Quaternary; therefore, we performed an analysis based on geographical calibration. We used a palaeogeographical event in the Mediterranean basin as a calibration point to estimate the absolute divergence times. For the primary inferred clades, we assumed that the most common ancestor of the three primary lineages (A, B and C) occurred during the MSC; therefore, 5.33 ± 0.3 Ma was assigned as the prior distribution (normal distribution) for the age of the root node of *Xerocrassa* in the Balearic Islands. Two independent runs were conducted for 7.5×10^7 million generations, with sampling every 1000 generations. Burn-in was determined with TRACER 1.6 (Rambaut *et al*., 2014). The two runs were combined, and the maximum-clade-credibility tree was identified using LOGCOMBINER 1.8.1 and TREEANNOTATOR 1.8.1 (Drummond *et al*., 2012).

RESULTS

Delimitation of evolutionary lineages and phylogenetic analyses

The topologies of the combined dataset from the BI and ML analyses were identical. All specimens from the Balearic Islands included in the analyses constituted a monophyletic group that was highly supported [posterior probability (BP) = 1.00; ML bootstrap (BS) = 96%] (Fig. 4.1). *Xerocrassa molinae*, endemic to the Columbretes Islands (a small archipelago between the Iberian Peninsula and the Balearic Islands), was the only species from outside the Balearic Archipelago that was recovered within this clade. This phylogroup, joining all the insular species, was recovered with full support (BP = 1.00; BS = 100%) as the sister group of the *Xerocrassa* species found on the eastern part of the Iberian Peninsula (*X. subrogata* and *X. barceloi*) (BP = 1.00; BS = 79%). A group formed by *X. montserratensis* and *X. ripacurcica*, from the northeasternpart of the Iberian Peninsula, was placed as the sister group of all other *Xerocrassa* species analysed (BP = 1.00; BS = 75%). The addition of more Iberian species to the mtDNA analyses (Fig. S1 in Appendix S2) also recovered the monophyly of the insular *Xerocrassa* species.

The Balearic species of *Xerocrassa* were recovered into three primary groups (A, B, and C). Clade A joined all specimens from the Pityusic Islands, which were grouped into 4 well-differentiated subclades (A1, A2, A3, and A4). Subclade A1 joined all *X. ebusitana* specimens from the Pityusic Islands, with *X. molinae* living on the Columbretes Archipelago as the sister group. The A1 subclade was highly supported (BP = 1.00; BS = 99%) as the sister group of subclades A2-A4. Subclade A2 grouped with strong support all the *X. caroli* taxa distributed along southwestern Ibiza and the nearby coastal islets (*X. caroli caroli*, *X. caroli alegriae*, *X. caroli espartariensis* and *X. caroli jaeckeli*). *Xerocrassa cisternasi conjungens* and *X. cisternasi scopulicola* from the Ses Bledes Archipelago and *X. cisternasi vedrae* and *X. cisternasi*

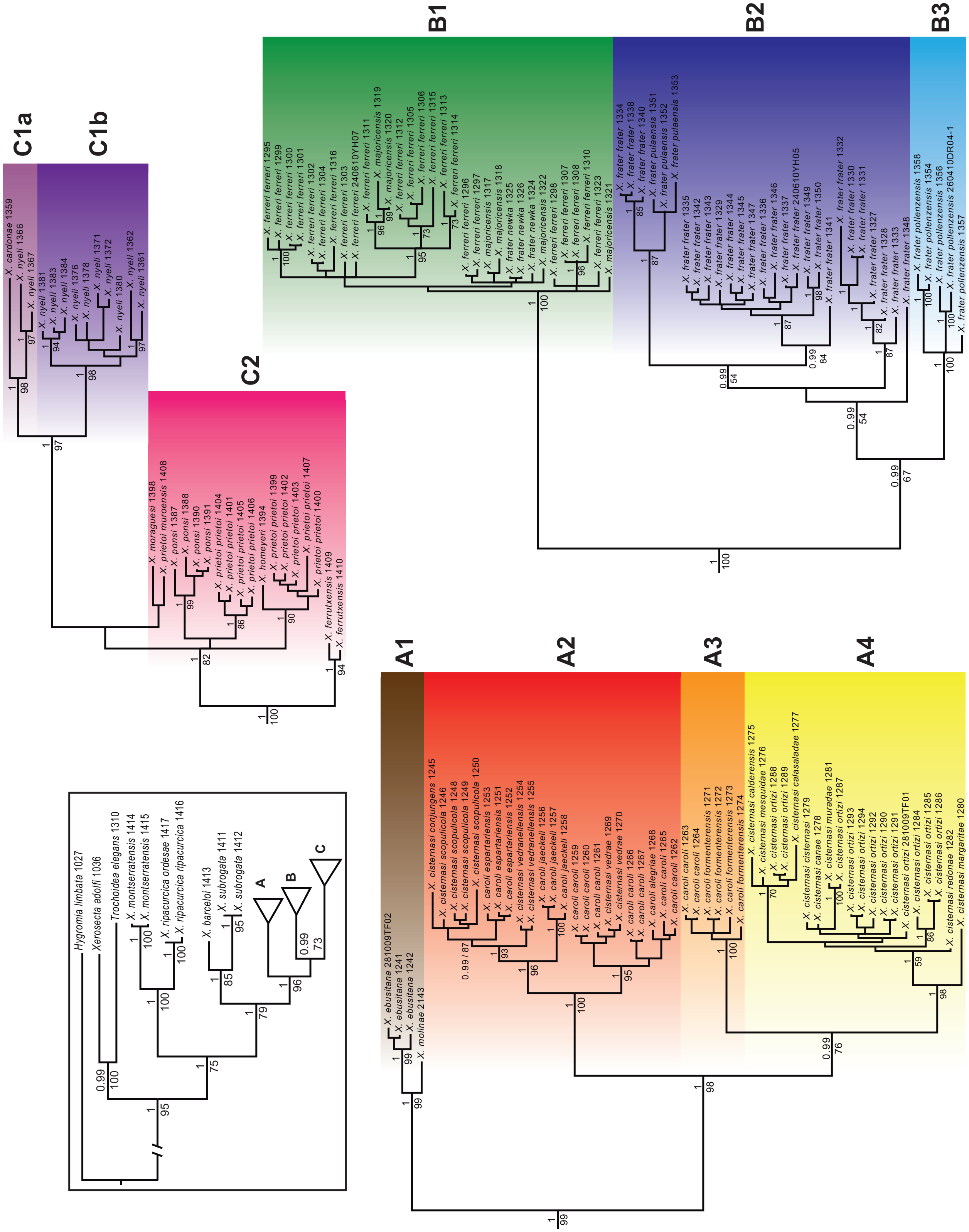

Figure 4.1 Bayesian-inference (BI) tree inferred based on a concatenated dataset (*COI*, cytochrome *c* oxidase subunit I; 16S rRNA, partial sequence; 5.8S rRNA, partial sequence; ITS2, internal transcribed spacer 2; 28S rRNA, partial sequence) of *Xerocrassa* species from the Balearic Islands. Numbers on the nodes are the posterior probabilities (BP) determined in the BI analysis and the bootstrap support values (BS) from the maximum-likelihood (ML) analysis.

vedranellensis from Es Vedrá and Es Vedranell islets were also grouped within subclade A2. Within this subclade, two phylogroups were recovered. The first phylogroup (BP = 1.00; BS = 96%) grouped the *X. caroli* specimens from eastern Ibiza and nearby coastal islets, including those from the Ses Bledes Archipelago and *X. cisternasi vedranellensis* from Es Vedranell Islet. The second phylogroup (BP = 1.00; BS = 95%) joined the *X. caroli* specimens from southern Ibiza and the southern Ibiza islets and *X. cisternasi vedrae* from Es Vedrá Islet. The A3subclade joined with full support *X. caroli formenterensis* from Formentera Island with specimens of *X. caroli* from S'Espardell Islet (Freus Islands). The remaining subspecies considered for *Xerocrassa cisternasi* (*X. c. cisternasi, X. c. calasaladae, X. c. calderensis, X. c. canae, X. c. margaritae, X. c. mesquidae, X. c. muradae, X. c. ortizi*, and *X. c. redonae*) were joined within subclade A4 (BP = 1.00; BS = 98%). The sister relationship of subclades A3 and A4 was strongly supported (BP = 0.99; BS = 76%).

With full support, clade B joined all specimens of *Xerocrassa ferreri*, *X. frater*, *X. majoricensis* from Mallorca Island and the Cabrera Archipelago. Subclade B1 joined *X. frater newka*, *X. majoricensis* and *X. ferreri ferreri* with full support. Subclade B2 (BP = 0.99; BS = 54%) grouped *X. frater frater* from Mallorca, primarily from the Tramuntana Mountains (northwestern Mallorca). *Xerocrassa frater pulaensis* was also placed within this group with the other *X. frater frater* specimens from northeastern Mallorca. Specimens of *X. frater pollenzensis* from the Formentor Peninsula (northwestern Mallorca) constituted subclade B3, which was grouped with full support and was recovered as the sister group of subclade B2 (BP = 0.99; BS = 67%).

With full support, clade C grouped all specimens from Menorca Island (*X. nyeli* and *X. cardonae*) with some other taxa found on Mallorca: *X. prietoi prietoi*, *X. prietoi muroensis*, *X. moraguesi*, *X. ponsi*, *X. homeyeri* and *X. ferrutxensis*. The basal relationships of this clade were not fully resolved. Nevertheless, all the specimens from Menorca Island were grouped together with strong support (BP = 1.00; BS = 97%) and formed two strongly supported clades. These clades are named in subsequent analyses as *nyeli* 1 and *nyeli* 2, and *X. cardonae* was placed within the *nyeli* 1 clade. *Xerocrassa ferrutxensis* was recovered as the sister group of the remaining taxa in clade C (BP = 1.00; BS = 82%). Although only the *COI* and 16S rRNA genes were amplified for *Xerocrassa claudinae*, which is endemic to the Formentor Peninsula, the species was recovered as an independent phylogroup within clade C by the mtDNA analysis. The species limits were tested using different approaches with varying results (Fig. 4.2). With the ABGD method, the initial partition reached stability at the distance of 0.013, which was the value at which both the initial and recursive partitions matched; considering this distance, the method revealed eleven groups (*ebusitana, caroli, formenterensis*, *cisternasi*, *newka*, *frater*, *pollenzensis*, *nyeli* 1, *nyeli* 2, *prietoi* and *claudinae*). The outcome of the GMYC analysis based on a *COI*+16S phylogenetic tree was significant and revealed 21 clusters and 6 entities. The choice of prior distributions in BPP analyses for (θ) and (τ_0) affected the results and the corresponding support values. From the analyses that assumed the combination of priors (a) ten putative species were strongly supported by speciation probabilities, whereas for the combination of priors (b), eight putative species

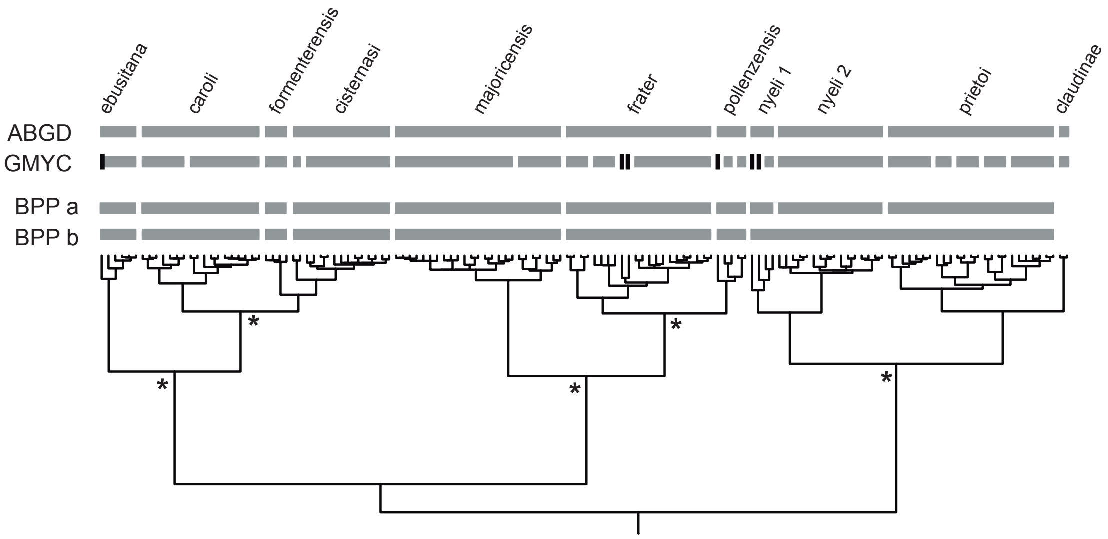

Figure 4.2 Summary results of the species delimitation analysis for Xerocrassa. Maximum-clade-credibility tree generated using all genetic data in *BEAST. Nodes with an asterisk indicate posterior probabilities > 0.95. Grey bars above the tree represent the putative species clusters recovered in the ABGD, GMYC and BPP (BPP-a: $\theta = 1/10$ and $\tau_0 = 1/10$; BPP-b: $\theta = 2/2000$ and $\tau_0 = 2/2000$) analyses.

were recognized in which phylogroups *prietoi*, *nyeli* 1 and *nyeli* 2 were one unique species. All the remaining groups revealed by ABGD were also recognized by the other three methods of species delimitation, although the *claudinae* group was only recovered with the methods limited to mtDNA trees.

Temporal framework of *Xerocrassa*

The estimated chronogram is shown in Fig. 4.3. The separation between the groups of *Xerocrassa* from the Balearic Islands and the Iberian Peninsula was estimated at 6.17 Ma (95% highest posterior density, HPD: 7.37–5.17 Ma). The age of the oldest split within the endemic *Xerocrassa* of the Balearic Islands was dated at 5.26 Ma (HPD: 5.84–4.66 Ma), separating the populations from the Gymnesic Islands from those of the Pityusic Islands. Within the Gymnesic Islands, an important split occurred at 4.60 Ma (HPD: 5.43–3.72 Ma), which corresponded to the separation of clades B and C. An approximately simultaneous split occurred within the three primary clades: in clade A at 2.69 Ma (HPD: 3.70–1.82 Ma); in clade B at 2.71 Ma (HPD: 3.63–1.85 Ma); and in clade C at 2.46 Ma (HPD: 3,32–1.69 Ma). Moreover, within the primary lineages, different split events (1.96–1.33 Ma) were dated during the Pleistocene. The estimated mean mtDNA substitution rate (2.925% divergence Myr^{-1}) was within the range of other rates calculated specifically for land snails on islands (2.8-6.0% divergence Myr^{-1}; Van Riel, 2005; Holland and Cowie; 2009).

DISCUSSION

Phylogeny and species delimitation

The study of the taxonomy and biogeography of land snails is aided by the application of molecular tools to investigate phylogenies and phylogeographical patterns (Parent & Crespi, 2006; Holland & Cowie, 2009; Greve et al., 2012; Sauer et al., 2013; Poulakakis et al., 2014; Neiber & Hausdorf, 2015; Psonis et al., 2015). The genus *Xerocrassa* has high phenotypic variation with many species and subspecies within the Balearic Islands. Many authors have discussed the classification of these numerous taxa and the specific or subspecific rank of each taxon (Graack, 2005; Quintana & Vilella, 2005; Beckmann, 2007; Forés, 2015). In this study, the first and most extensive phylogenetic analysis of these endemic invertebrate taxa in the Balearic Islands to incorporate information from DNA sequences is presented.

The monophyly of the *Xerocrassa* species in the Balearic Islands was highly supported by our results. All phylogenetic analyses supported the basal split of Balearic species from the *Xerocrassa* species group of the eastern part of the Iberian Peninsula. The three primary lineages corresponded to the primary island units: clade A with the Pityusic Islands, and clades B and C with the Gymnesic Islands. The results from the species delimitation models were similar and supported 8–11 putative species, which were consistent with those of the primary lineages obtained in the phylogeny. As many as four putative species were delimited for the Pityusic Islands and one or two were delimited for Menorca, with the remaining five delimited for Mallorca. Nevertheless, GMYC analysis revealed some single

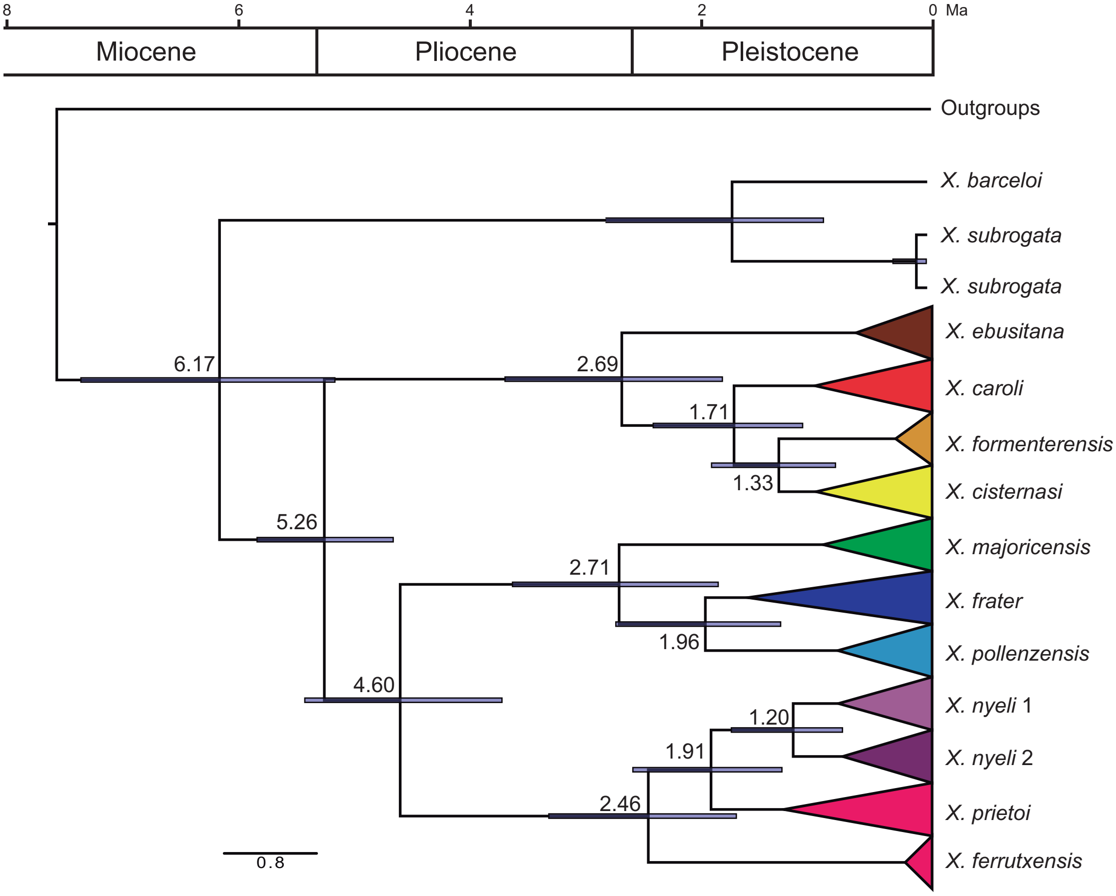

Figure 4.3 Time-calibrated multilocus tree for *Xerocrassa* obtained using BEAST. Bars indicate 95% highest posterior densities of divergence dates, with means estimated in million years ago (Ma) given at the nodes. The primary clades are colour-coded to match to the phylogeny in Fig. 4.1.

specimens to be putative species. However, GMYC analysis typically generates more clusters than other methods (Sauer & Hausdorf, 2012; Talavera *et al.*, 2013). In our case, these single specimens were grouped into the continuous primary clade.

Although we could not analyse *X. ebusitana* from Ibiza Island, we included some specimens of this species collected from the Freus Islands and Formentera. The three specimens of *X. ebusitana* constituted one monophyletic entity, with *X. molinae* recovered as the sister group within clade A1. *Xerocrassa ebusitana* is endemic to the Pityusic Islands, whereas *X. molinae* is endemic to the Columbretes Archipelago, which is 56 km from Ibiza (Puente, 1994). *Xerocrassa molinae* has been considered a different species, based on morphological and biogeographical data (Gasull, 1964; Puente, 1994); nevertheless, our species delimitation analyses joined both species in one unique taxon. Thus, with consideration of the morphological, biogeographical and the new molecular data, we propose two subspecies of one taxon, *X. ebusitana ebusitana* and *X. ebusitana molinae*, which is a classification that better summarizes the close phylogenetic relationship between the two taxa.

All subspecies of *Xerocrassa caroli* and *X. cisternasi* distributed along the southwestern part of Ibiza and on nearby islets constituted another monophyletic group (clade A2) recovered as a second putative species in the analyses. Shell morphology, particularly shell size and colour (Welter-Schultes, 2012), is the principal character to include *X. cisternasi conjungens* and *X. cisternasi scopulicola* from the Ses Bledes Archipelago, *X. c. vedrae* from Es Vedrá, and *X. c. vedranellensis* from Es Vedranell islets as subspecies of *X. cisternasi*. However, our results indicated that the *X. caroli* and *X. cisternasi* of previous authors (Gasull, 1964; Beckmann, 2007) were polyphyletic groups. The delimitation of closely related species is hampered by shared ancestral DNA polymorphisms, introgression, or inappropriate morphological taxonomy (Funk & Omland, 2003).Sauer and Hausdorf (2013) indicated that a frequent cause of non-monophyly of *Xerocrassa* species living in Crete was retention of an ancestral polymorphism. Nevertheless, the *Xerocrassa* clades recovered in the Pityusic Islands (clades A1-A4) were highly consistent with the biogeography, with almost all subspecies constituting monophyletic groups. With this result, the cases with retention of an ancestral DNA polymorphism or introgression were excluded in this study, which indicated that the subspecific classification of Beckmann (2007) for *X. caroli* and *X. cisternasi* based on shell size is not accurate. The size of animals on small islands can be very different from that of their closest relatives, which invalidates size alone as a taxonomic criterion. In this study, the large shell sizes of *Xerocrassa* that inhabitedthe Ses Bledes Archipelago and Es Vedrá and Es Vedranell islets could be explained by the 'island syndrome' (Whittaker & Férnandez-Palacios, 2007) in which the body size of animals on islands tends to increase. *Xerocrassa caroli* was described without the type locality, but the distribution is restricted to central-southern Ibiza and Formentera (Gasull, 1964; Beckmann, 2007), whereas *X. cisternasi* was created later to nominate the species from Santa Eulària Islet (north-eastern Ibiza). Clade A2 included *X. caroli* morphotypes from southern Ibiza; thus, all taxa, including those within clade A2, should be considered within *X. caroli*. Therefore, for the populations on the islets near Ibiza to maintain a nomenclatural differentiation based on morphological and biogeographical singularities, we propose the following classification: *X. caroli caroli*, *X. caroli conjungens*, *X. caroli espartariensis*, *X. caroli jaeckelii*, *X. caroli scopulicola*, *X. caroli vedrae*, and *X. caroli vedranellensis*.

The third putative species supported by our species delimitation analyses (clade A3) joined all specimens of *X. caroli formenterensis* from Formentera with the specimens of *X.caroli caroli* from S'Espardell Islet (Freus Islands). Our results suggested that *X. formenterensis*, including the populations from S'Espardell Islet, should be a separate species. Nevertheless, more samples from Formentera and the Freus Islands are required to fully resolve the intraspecific relationships within *X. formenterensis*.

The fourth putative species recognized in the Pityusic Islands (clade A4) grouped nine taxa classified within *X. cisternasi* in Beckman (2007), which were all distributed in northeastern Ibiza and on nearbycoastal islets. The subspecies of *X.cisternasi* of this area were described based on the isolation on small islets and by minor modifications of shell morphology (Beckmann, 2007). However, these minor changes could be the result of a founder principle or genetic drift in isolated populations (Frankham et al., 2002). Although the genetic differentiation of these populations from the species of the main island was not high, the geographical isolation on small islets and the differences in morphology could support maintaining subspecific status. Accordingly, we propose the following classification: *X. cisternasi calasaladae*, *X. cisternasi calderensis*, *X. cisternasi canae*, *X. cisternasi cisternasi*, *X. cisternasi margaritae*, *X. cisternasi mesquidae*, *X. cisternasi muradae*,*X. cisternasi ortizi* and *X. cisternasi redonae*. We considered that *X. cisternasi hortae*, from S'Hort Islet (northeastern Ibiza), which was not included in our analyses, was in this group based on geographical distribution and shell form.

Clade B1 is distributed along the south coast of Mallorca and in the Cabrera Archipelago and was formed by specimens belonging to *X. ferreri ferreri*, *X. frater newka* and *X. majoricensis*. The high conical shells characterize all three nominal taxa. *Xerocrassa majoricensis*is is considered synonymous with *X. frater* (Beckmann, 2007); however, based on our results, *X. majoricensis*is was not closely related to *X. frater*. Similarly, *X. majoricensis* and *X. newka* were considered synonymous by Gasull (1964). Our phylogenetic reconstruction did not recover the monophyly for these three taxa. Moreover, these three nominal taxa showed low genetic diversity for the markers selected in this study, and the ABGD and BPP species delimitation models also supported this clade as one species. According to these results, the three nominal taxa correspond to a single taxon. *Xerocrassa majoricensis* (Dohrn & Heynemann, 1862) and *X. newka* (Dohrn & Heynemann, 1862) have priority over *X. ferreri* (Jaeckel, 1952). The shell morphology of *X. majoricensis*is a better representation of the morphology of the entire group than *X. newka*, which has a specific morphology, and ranged from high conical to turriculate with two parallel, spiral keels along the last whorl (Gasull, 1964). Thus, we propose the name *X. majoricensis* (Dohrn & Heynemann, 1862) to nominate all the specimens included in clade B1. Although no specimens of *X. ferreri pobrensis* were included in the analyses, because the morphology is related to *X. ferreri ferreri* and the distribution is narrow (Na Pobra Islet of the Cabrera Archipelago) (Beckmann, 2007) and located inside the distribution range of *X. majoricensis*, we suggest that this name is also in *X. majoricensis*.

Xerocrassa frater (clade B2) is widespread on Mallorca Island, although most of the samples included in this work were collected in the Tramuntana Mountains region. The genetic polymorphism of this clade was high. Nevertheless, the species delimitation analyses revealed clade B2 as one unique putative species, including *X. f. pulaensis*, characterized by a hairy shell and known only from a reduced area in eastern Mallorca (Beckmann, 2007).

The taxon *X. f. pollenzensis* (Clade B3), from Formentor Peninsula (north of Mallorca),was recovered as a separate species in the different analyses of species delimitation. Therefore, we conclude the taxon should have full species status with the name *X. pollenzensis*.

The remaining taxa of the genus *Xerocrassa* from Mallorca were grouped with the specimens from Menorca in clade C. The species delimitation analyses provided different results, with recovery from one to four species within the clade. *Xerocrassa nyeli* from Menorca was considered two putative species (named *nyeli* 1 and *nyeli* 2 in this work) by the ABGD, GMYC and BPP-a analyses. Nevertheless, these were considered, with *X. prietoi*, as one species in the BPP-b analysis. However, we did not find any differences in shell morphology

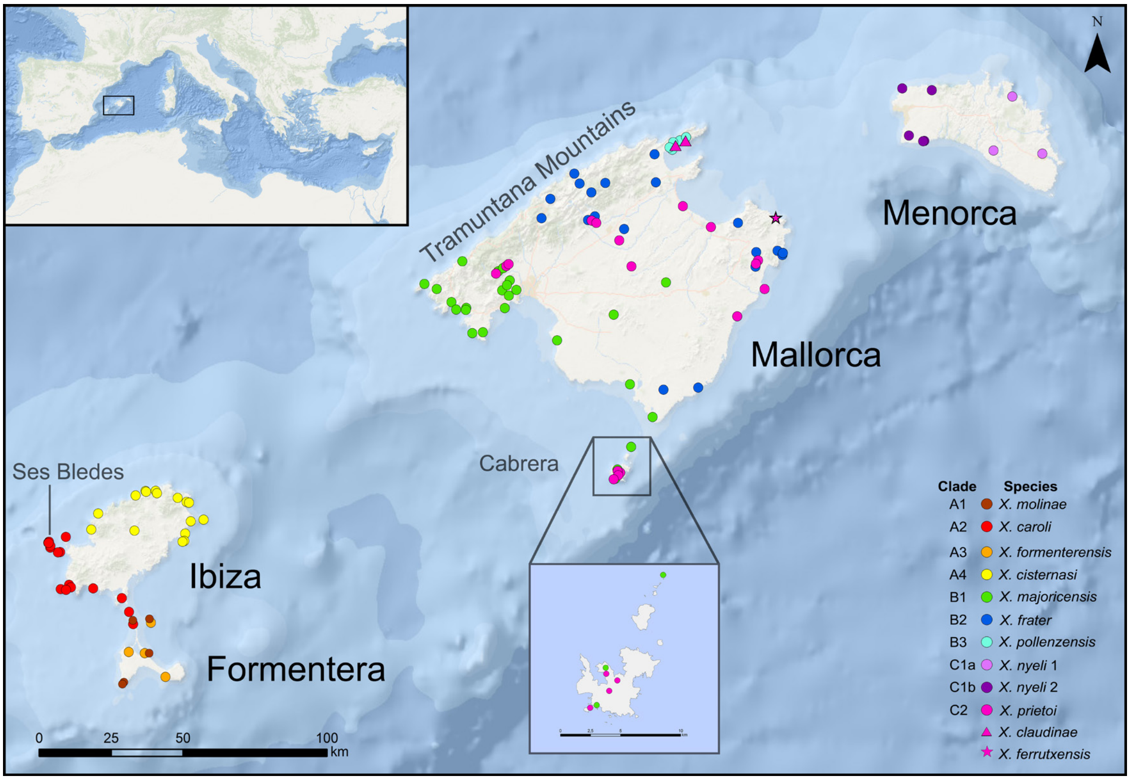

Figure 4.4 Map of the Balearic Islands (Western Mediterranean) with the collection localities of *Xerocrassa* species. Colours correspond to the clades obtained in the phylogeny (Fig. 4.1).

that could be attributed to a *nyeli* 1 or *nyeli* 2 groups. As a consequence, we considered that both phylogroups were a single species. *Xerocrassa cardonae*, characterized by a hairy shell (Quintana & Vilella, 2001), was grouped within the *nyeli* 1 clade. Only the GMYC analysis recovered *X. cardonae* as a different species. Different authors have discussed the validity of the status of *X. cardonae* as a species, with the taxon considered one valid species (Quintana & Vilella, 2001; Beckmann, 2007) or a junior synonym of *X. nyeli* (Gasull, 1964). The other species delimitation analyses indicated that *X. cardonae* was conspecific with *X. nyeli*, demonstrating that hairs on the shell was not sufficient to separate two species in the genus *Xerocrassa*, as neither is it in other genera of land snails (Elejalde *et al*., 2009; Bank *et al*., 2013). BPP-b analyses considered *X. nyeli* (including groups *nyeli* 1 and *nyeli* 2) and *X. prietoi* as conspecific. Nevertheless, based on the monophyly of *X. nyeli*, together with the high genetic distances among the populations of Menorca compared with those of Mallorca and the geographical isolation, we consider that *X. nyeli* (including the hairy *X. cardonae*) should be a separate species. More genetic studies are required to fully resolve the phylogenetic relationships of the *Xerocrassa* on Menorca, with the two phylogroups (*nyeli* 1 and *nyeli* 2) well separated in two different areas (Fig. 4.4). For the *Xerocrassa* specimens collected from Mallorca, all analyses, with the exception of GMYC, recovered *X. ferrutxensis*, *X. homeyeri*, *X. moraguesi*, *X. ponsi* and *X. prietoi* as a single species. Of these specimens, only *X. ferrutxensis* could be considered a different taxon because of the deepest ramification within the clade, although *X. ferrutxensis* was not recovered as a unique species by any species delimitation analysis. The monophyly of *X. ponsi* specimens, together with its genetic distances and its isolated distribution in the Cabrera Archipelago suggests that it could be considered as subspecies of *X. prietoi*. Moreover, more populations of *X. moraguesi* and *X. homeyeri* are needed to clarify their taxonomic status within the *X. prietoi* complex. Finally, within this clade C, *X. claudinae* from the Formentor Peninsula (north of Mallorca) was recovered as a single species by ABGD and GMYC analyses. Only the mitochondrial genes could be amplified for this species, and therefore, this taxon was not included in the concatenated phylogeny and BPP analyses. However, based on the mitochondrial genes, the taxon is well differentiated from the other taxa in the phylogeny (Fig. S1 in Appendix S2). According to our information, the taxon is a valid species because of the genetic differentiation and the morphological differences (Gasull, 1964; Forés, 2015).

Colonization of the Balearic Islands and radiation within the archipelago

The best strategy to test the monophyly of an island group is to include all the closely related species from nearby continental areas in the analyses (Emerson, 2002). Therefore, we included almost all *Xerocrassa* species from the eastern part of the Iberian Peninsula (Fig. S1 in Appendix S2). The monophyly of the endemic *Xerocrassa* of the Balearic Islands was well supported and indicated a single colonization event of the genus from the Iberian Peninsula to the archipelago.

The endemic biota of the Balearic Islands are attributed to three primary events: (1) the Tyrrhenian vicariance hypothesis in the Oligocene (Pfenninger *et al*., 2010; Bidegaray-Batista

& Arnedo, 2011; Lázaro *et al*., 2011); (2) the colonization across a landbridge connection during the middle Miocene (Bover et al., 2008; Chueca *et al*., 2015); and (3) the MSC (Fromhage *et al*., 2004; Lalueza-Fox *et al*., 2005; Delicado *et al*., 2014). In the absence of fossil records of *Xerocrassa* in the Balearic Islands before the Quaternary, we used a geographic point for the calibration of the phylogeny. We discarded an origin resulting from the split of the Hercynian belt into several microplates during the Oligocene, because under this mechanism, related taxa are found on some of the different Hercynian microplates that remained with the western Mediterranean islands and continental coasts (Fochetti *et al*., 2009; Pfenninger *et al*., 2010; Bidegaray-Batista & Arnedo, 2011). This origin was not the case for *Xerocrassa* because the genus is only found on the Iberian Peninsula and in the Balearic Islands in the western Mediterranean and is absent on the other microplates of the Hercynian belt. Colonization during the middle Miocene across a landbridge was also unlikely for this genus because it would result in divergence times of 0.85% per million years for mtDNA, which is a very low rate for land snails; however, in pulmonate gastropods, mitochondrial evolution can be exceptionally fast (e.g., up to 20%) (Thomaz et al., 1996). Moreover, no secondary contacts or gene flow were detected between *Xerocrassa* phylogroups in the Pityusic and Gymnesic islands, as was detected for the endemic land snail genus *Allognathus* (Chueca *et al*., 2015). This result suggested that the isolation of the two *Xerocrassa* unit groups began with the refilling of the Mediterranean Sea after the MSC, which is the last period when the entire archipelago was connected (Hsü *et al*., 1973; Krijgsman *et al*., 1999). The geographical distribution of *Xerocrassa* phylogroups supported the hypothesis for colonization of the archipelago during the MSC, which has also been suggested for other organisms (Fromhage *et al*., 2004; Lalueza-Fox *et al*., 2005; Brown *et al*., 2008; Delicado *et al*., 2014). With all this information, we considered the MSC as the geographical point for the calibration of the *Xerocrassa* phylogeny.

According to our findings, *Xerocrassa* likely colonized the Balearic Islands during the MSC when the archipelago was connected to the Iberian Peninsula because of the desiccation of the Mediterranean basin. Sister relationships of Balearic *Xerocrassa* with the species of this genus found on the eastern part of the Iberian Peninsula (*X. subrogata* and *X. barceloi*) demonstrated that the colonization of the archipelago originated from this region. After the Mediterranean basin refilled (5.33 Ma), a burst of diversification began in the Balearic Islands, originating the separation of the deepest Balearic clades by allopatric speciation processes between the Gymnesic and Pityusic islands. Parallel allopatric speciation processes that started at the end of the MSC with the split between the Pityusic and Gymnesic units are also described for the land snail genus *Allognathus* (Chueca *et al*., 2015) and the lizard genus *Podarcis* (Brown *et al*., 2008). Almost simultaneously, a further diversification process occurred within the Gymnesic Islands. More recently, two approximately simultaneous split events occurred within the three primary clades at approximately 2.7 and 1.9 Ma (Fig. 4.3). The first event is associated with the transition from the Pliocene to the Pleistocene, which is well known for the major climatic changes experienced worldwide (Lisiecki & Raymo, 2005; Herbert *et al*., 2015). During the late Pliocene (3.6–2.6 Ma), the climate was warm and humid (Willis *et al*., 1999), which became suddenly colder and drier during the early Pleistocene (2.6 – 1.8 Ma) (Webb & Bartlein, 1992) with the onset of glacial-interglacial cycles. In the northwestern Mediterranean, subtropical flora progressively diminished between 3.6–2.8 Ma with an increase of herbaceous plants linked to arid or subarid bioclimates (Fauquette *et al*., 1998; Suc & Popescu, 2005). This change in the vegetation could have promoted intra-island speciation processes in *Xerocrassa*. The transition between the Pliocene to the Pleistocene coincides with diversification events in other organisms (Dubey *et al*., 2006; Koepfli *et al*., 2008; Mouline *et al*., 2008; Kornilios *et al*., 2009; Planas *et al*., 2013). The onset of the Mediterranean climate is dated in this period (Suc, 1984; Thompson, 2005), which influenced *Xerocrassa* radiation, a genus with a strong Mediterranean character (Gasull, 1964). The split between *X. majoricensis* and *X. frater* + *X. pollenzensis* is placed within this period. A precipitation gradient is well-defined within Mallorca Island, with mean precipitation less than 600 mm yr^{-1} in the south coastal and central areas and between 800 and 1400 mm yr^{-1} along the Tramuntana Mountains and northern coastal regions (Ginés *et al*., 2012). The current distributions of *X. majoricensis*, *X. frater* and *X. pollenzensis* are also well-defined, with the first species inhabiting the drier areas of Mallorca Island and the other two occupying the wetter regions. Assuming that the precipitation range in the past was similar to the current one, wetter in high mountains and drier in southern areas of the island, the speciation between these taxa could be a consequence of an adaptive speciation to climatic conditions. A similar adaptive speciation to palaeoenvironmental conditions is observed in the land snail genus *Murella* (Fiorentino *et al*., 2013).

The second event is associated with the beginning of a period of strong glaciation in the northern hemisphere at 1.8 Ma, which was characterized by maximum values for aridity in the western Mediterranean and was correlated with the expansion of the steppe-like and cooler conditions (Agustí *et al*., 2001; Herbert *et al*., 2015). This diversification event is also observed in other organisms from the Palaearctic region (Dubey *et al*., 2006; Koepfli *et al*., 2008; Kornilios *et al*., 2009; Planas *et al*., 2013) and concretely from the Balearic Islands (Rodríguez *et al*., 2013). During this period, the split occurred between *X. frater* and *X. pollenzensis* in Mallorca and between *X. caroli* and *X. cisternasi* + *X. formenterensis* in Ibiza.

Although the major climatic changes that occurred during the Pliocene are correlated with the speciation processes that occurred on the different islands, non-adaptive speciation processes such as the restriction of gene flow by geographical barriers and the isolation by distance could also explain land snail speciation processes within the islands (Sauer *et al*., 2013).

The sea level fluctuations in the western Mediterranean caused by the Pleistocene glacial cycles (Sosdian & Rosenthal, 2009; Capraro *et al*., 2011) led to connections between Mallorca (including the Cabrera archipelago) and Menorca. With these connections, dispersion events occurred between Mallorca and Menorca, as it has been documented in other taxa (Bover *et al*., 2008; Brown *et al*., 2008; Quintana & Moncunill-Solé, 2014; Chueca *et al*., 2015). In the genus *Xerocrassa*, a close relationship between the species from Mallorca and those from Menorca was observed within clade C. The deeper node of clade C corresponded to the lineages of Mallorca, which suggested that the clade originated in Mallorca from which some populations colonized Menorca and originated the *X. nyeli* lineages. The high genetic distances recovered between *X. nyeli* from Menorca and the closest species on Mallorca could be the result of the founder principle followed by rapid genetic differentiation. With similar dispersion events, the connections during the Pleistocene between Mallorca and the Cabrera Archipelago resulted in the colonization of the archipelago with *X. majoricensis* (clade B1) and *X. prietoi* (populations named as *X. ponsi* in clade C2) from Mallorca. The largest increase in the level of the Mediterranean Sea occurred approximately 0.6 Ma (Emig & Geistdoerfer, 2004), which was followed by significant decreases in sea level, up to 120 m, during three periods approximately 0.5, 0.15 and 0.02 Ma (Emig & Geistdoerfer, 2004). Therefore, the colonization of coastal islets, including the Ses Bledes Archipelago, by the current taxa was apparently a recent event, probably occurred during the last glaciation, when the sea level dropped approximately 100 m (Emig & Geistdoerfer, 2004). The posterior isolation of *Xerocrassa* populations within the islets caused by an abrupt increase in sea level after the Last Glacial Maximum (0.019 Ma), resulted in some cases, in important morphological diversification but without significant genetic differentiation. A similar isolation pattern on the Ibiza islets is also observed in lizard populations of *Podarcis* (Rodríguez *et al*. 2013).

Finally, two primary mtDNA phylogroups of *X. nyeli* were found on Menorca Island. Although the shell morphology is highly variable from flattened to conical shells in *X. nyeli* (Quintana & Vilella, 2005), our results did not support any particular shell pattern to differentiate the two phylogroups. Two mtDNA phylogroups are also observed for the land snail species *Allognathus campanyonii minoricensis* on Menorca (Chueca *et al*., 2015), indicating the possibility of past speciation events within Menorca.

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SUPPORTING INFORMATION

APPENDIX S1 Study material, list of primers used for sequencing and PCR conditions.

Table S1. Specimens used for the study of the land snail genus *Xerocrassa* (Geomitridae) from the Balearic Islands, with their identification codes, localities, coordinates, voucher numbers and GenBank accession numbers. Superscript numbers indicate that the GenBank accession number was published in another study (1, Sauer & Hausdorf, 2009; 2, Razkin *et al*., 2015). EHUMC, collection at the Zoology and Animal Cell Biology department, University of the Basque Country (Spain); MVHN, Museu Valencià d'Historia Natural (Spain).

Taxa	Locality	Distribution	UTM grid cell	Voucher	GenBank accesions numbers		
					COI	16S rRNA	5.8-ITS2-28S
X. ebusitana	Racó des Forat	Formentera	31SCC6786	EHUMC-1241	KT968870	KT969067	KT969262
X. ebusitana	Cap de Barbaria	Formentera	31SCC5978	EHUMC-1242	KT968871	KT969068	KT969263
X. ebusitana	S'Espardell	Freus Islands	31SCC6795	EHUMC-1243	KT968872	KT969069	
X. ebusitana	S'Espalmador	Freus Islands	31SCC6395	EHUMC-1244	KT968873	KT969070	
X. ebusitana	Cap de Barbaria	Formentera	31SCC6078	MVHN-281009TF02	KT969064	KT969260	KT969416
X. caroli alegriae	Illa de Porroig	Ibiza	31SCD5203	EHUMC-1268	KT968897	KT969094	KT969286
X. caroli caroli	Cap des Jueu	Ibiza	31SCD4604	EHUMC-1259	KT968888	KT969085	KT969277
X. caroli caroli	Cap des Jueu	Ibiza	31SCD4504	EHUMC-1260	KT968889	KT969086	KT969278
X. caroli caroli	Cap des Jueu	Ibiza	31SCD4504	EHUMC-1261	KT968890	KT969087	KT969279
X. caroli caroli	Ses Salines	Ibiza	31SCD6001	EHUMC-1262	KT968891	KT969088	KT969280
X. caroli caroli	S'Espalmador	Freus Islands	31SCC6395	EHUMC-1265	KT968894	KT969091	KT969283
X. caroli caroli	Els Penjats	Freus Islands	31SCC6297	EHUMC-1266	KT968895	KT969092	KT969284
X. caroli caroli	Els Penjats	Freus Islands	31SCC6297	EHUMC-1267	KT968896	KT969093	KT969285
X. caroli espartariensis	Illa s'Espartar	Ibiza	31SCD4313	EHUMC-1251	KT968880	KT969077	KT969269
X. caroli espartariensis	Illa s'Espartar	Ibiza	31SCD4313	EHUMC-1252	KT968881	KT969078	KT969270
X. caroli espartariensis	Es Frare de s'Espartar	Ibiza	31SCD4313	EHUMC-1253	KT968882	KT969079	KT969271
X. caroli formenterensis	S'Espardell	Freus Islands	31SCC6795	EHUMC-1263	KT968892	KT969089	KT969281
X. caroli formenterensis	S'Espardell	Freus Islands	31SCC6795	EHUMC-1264	KT968893	KT969090	KT969282
X. caroli formenterensis	Can Marroig	Formentera	31SCC6186	EHUMC-1271	KT968900	KT969097	KT969289
X. caroli formenterensis	Can Marroig	Formentera	31SCC6186	EHUMC-1272	KT968901	KT969098	KT969290

Taxa	Locality	Distribution	UTM grid cell	Voucher	GenBank accesions numbers		
					COI	16S rRNA	5.8-ITS2-28S
X. caroli formenterensis	Es Caló des Mort	Formentera	31SCC7180	EHUMC-1273	KT968902	KT969099	KT969291
X. caroli formenterensis	Racó des Forat	Formentera	31SCC6786	EHUMC-1274	KT968903	KT969100	KT969292
X. caroli jaeckeli	Sa Conillera	Ibiza	31SCD4517	EHUMC-1256	KT968885	KT969082	KT969274
X. caroli jaeckeli	Sa Conillera	Ibiza	31SCD4517	EHUMC-1257	KT968886	KT969083	KT969275
X. caroli jaeckeli	Sa Conillera	Ibiza	31SCD4517	EHUMC-1258	KT968887	KT969084	KT969276
X. cisternasi calasaladae	Illa de Cala Salada	Ibiza	31SCD5219	EHUMC-1277	KT968906	KT969103	KT969295
X. cisternasi calderensis	Illa d'en Calders	Ibiza	31SCD6729	EHUMC-1275	KT968904	KT969101	KT969293
X. cisternasi canae	Illot des Canar	Ibiza	31SCD7717	EHUMC-1278	KT968907	KT969104	KT969296
X. cisternasi cisternasi	Illa de Santa Eulalia	Ibiza	31SCD7715	EHUMC-1279	KT968908	KT969105	KT969297
X. cisternasi conjungens	Escull Vermell	Ibiza	31SCD4016	EHUMC-1245	KT968874	KT969071	KT969264
X. cisternasi margaritae	Ses Margalides	Ibiza	31SCD5423	EHUMC-1280	KT968909	KT969106	KT969298
X. cisternasi mesquidae	Illot de Sa Mesquida	Ibiza	31SCD6929	EHUMC-1276	KT968905	KT969102	KT969294
X. cisternasi muradae	Illa Murada	Ibiza	31SCD6428	EHUMC-1281	KT968910	KT969107	KT969299
X. cisternasi ortizi	Punta Grossa, Cala Sant Vicent	Ibiza	31SCD7826	EHUMC-1284	KT968913	KT969110	KT969301
X. cisternasi ortizi	Cala Sant Vicent	Ibiza	31SCD7826	EHUMC-1285	KT968914	KT969111	KT969302
X. cisternasi ortizi	Cala Sant Vicent	Ibiza	31SCD7826	EHUMC-1286	KT968915	KT969112	KT969303
X. cisternasi ortizi	Sta Gertrudis	Ibiza	31SCD6318	EHUMC-1287	KT968916	KT969113	KT969304
X. cisternasi ortizi	Cala Xarraca	Ibiza	31SCD7028	EHUMC-1288	KT968917	KT969114	KT969305
X. cisternasi ortizi	Cala Xarraca	Ibiza	31SCD7028	EHUMC-1289	KT968918	KT969115	KT969306
X. cisternasi ortizi	Cala Boix, pine forest	Ibiza	31SCD7921	EHUMC-1290	KT968919	KT969116	KT969307
X. cisternasi ortizi	Cala Boix, pine forest	Ibiza	31SCD7921	EHUMC-1291	KT968920	KT969117	KT969308
X. cisternasi ortizi	Illa de Tagomago	Ibiza	31SCD8221	EHUMC-1292	KT968921	KT969118	KT969309
X. cisternasi ortizi	Punta Calders	Ibiza	31SCD6729	EHUMC-1293	KT968922	KT969119	KT969310
X. cisternasi ortizi	Punta Calders	Ibiza	31SCD6729	EHUMC-1294	KT968923	KT969120	KT969311
X. cisternasi ortizi	Port de Ses Caletes	Ibiza	31SCD7527	MVHN-281009TF01	KT969063	KT969259	KT969415
X. cisternasi redonae	Illa Redona	Ibiza	31SCD7615	EHUMC-1282	KT968911	KT969108	KT969300
X. cisternasi scopulicola	Na Gorra	Ses Bledes	31SCD4114	EHUMC-1246	KT968875	KT969072	KT969265

Taxa	Locality	Distribution	UTM grid cell	Voucher	GenBank accesions numbers		
					COI	16S rRNA	5.8-ITS2-28S
X. cisternasi scopulicola	Na Bosc	Ses Bledes	31SCD4115	EHUMC-1247	KT968876	KT969073	
X. cisternasi scopulicola	Na Bosc	Ses Bledes	31SCD4115	EHUMC-1248	KT968877	KT969074	KT969266
X. cisternasi scopulicola	Na Plana	Ses Bledes	31SCD4016	EHUMC-1249	KT968878	KT969075	KT969267
X. cisternasi scopulicola	Na Plana	Ses Bledes	31SCD4016	EHUMC-1250	KT968879	KT969076	KT969268
X. cisternasi vedrae	Es Vedra	Ses Bledes	31SCD4303	EHUMC-1269	KT968898	KT969095	KT969287
X. cisternasi vedrae	Es Vedra	Ses Bledes	31SCD4303	EHUMC-1270	KT968899	KT969096	KT969288
X. cisternasi vedranellensis	Es Vedranell	Ses Bledes	31SCD4503	EHUMC-1254	KT968883	KT969080	KT969272
X. cisternasi vedranellensis	Es Vedranell	Ibiza	31SCD4503	EHUMC-1255	KT968884	KT969081	KT969273
X. frater frater	Cala Romantica	Mallorca	31SED2674	EHUMC-1327	KT968955	KT969152	KT969343
X. frater frater	Cala Romantica	Mallorca	31SED2674	EHUMC-1328	KT968956	KT969153	KT969344
X. frater frater	Tossals Verds	Mallorca	31SDE8706	EHUMC-1329	KT968957	KT969154	KT969345
X. frater frater	Puig Santa Magdalena. Inca	Mallorca	31SDD9696	EHUMC-1330	KT968958	KT969155	KT969346
X. frater frater	Puig Santa Magdalena. Inca	Mallorca	31SDD9696	EHUMC-1331	KT968959	KT969156	KT969347
X. frater frater	Montdragó Natural Park	Mallorca	31SED1655	EHUMC-1332	KT968960	KT969157	KT969348
X. frater frater	Betlem hermitage. Artá	Mallorca	31SED2698	EHUMC-1333	KT968961	KT969158	KT969349
X. frater frater	Claper des Gegant. Artá	Mallorca	31SED3791	EHUMC-1334	KT968962	KT969159	KT969350
X. frater frater	Font des Padró	Mallorca	31SDD8799	EHUMC-1335	KT968963	KT969160	KT969351
X. frater frater	Coll de Sóller	Mallorca	31SDD7499	EHUMC-1336	KT968964	KT969161	KT969352
X. frater frater	Ses Barques lookout. Fornalutx	Mallorca	31SDE7604	EHUMC-1337	KT968965	KT969162	KT969353
X. frater frater	Artá cave	Mallorca	31SED3890	EHUMC-1338	KT968966	KT969163	KT969354
X. frater frater	Talaia Nova. Capdepera	Mallorca	31SED3890	EHUMC-1340	KT968967	KT969164	KT969355
X. frater frater	Pollença	Mallorca	31SEE0409	EHUMC-1341	KT968968	KT969165	KT969356
X. frater frater	Sta. Lucía. Manacor de laVall	Mallorca	31SDE8800	EHUMC-1342	KT968969	KT969166	KT969357
X. frater frater	Monaver Nou Hotel. Campanet	Mallorca	31SDE9505	EHUMC-1343	KT968970	KT969167	KT969358
X. frater frater	Pollença	Mallorca	31SEE0817	EHUMC-1344	KT968971	KT969168	KT969359
X. frater frater	Pollença	Mallorca	31SEE0817	EHUMC-1345	KT968972	KT969169	KT969360
X. frater frater	Font des Padró	Mallorca	31SEE8898	EHUMC-1346	KT968973	KT969170	KT969361

Taxa	Locality	Distribution	UTM grid cell	Voucher	GenBank accesions numbers		
					COI	16S rRNA	5.8-ITS2-28S
X. frater frater	Tossals Verds	Mallorca	31SDE8706	EHUMC-1347	KT968974	KT969171	KT969362
X. frater frater	Binifaldó. Escorça	Mallorca	31SDE9108	EHUMC-1348	KT968975	KT969172	KT969363
X. frater frater	Colls dels Reis. Escorça	Mallorca	31SDE8408	EHUMC-1349	KT968976	KT969173	KT969364
X. frater frater	Sa Calobra	Mallorca	31SDE8311	EHUMC-1350	KT968977	KT969174	KT969365
X. frater frater	Cala Llombards	Mallorca	31SED0654	MVHN-240610YH05	KT969060	KT969256	KT969412
X. frater newka	Na Burguesa. Génova	Mallorca	31SDD6780	EHUMC-1324	KT968952	KT969149	KT969340
X. frater newka	Génova	Mallorca	31SDD5175	EHUMC-1325	KT968953	KT969150	KT969341
X. frater newka	Génova	Mallorca	31SDD5175	EHUMC-1326	KT968954	KT969151	KT969342
X. frater pollenzensis	Formentor	Mallorca	31SEE1219	EHUMC-1354	KT968981	KT969178	KT969369
X. frater pollenzensis	Military base. Puerto de Pollença	Mallorca	31SEE0817	EHUMC-1356	KT968983	KT969180	KT969370
X. frater pollenzensis	Military base. Puerto de Pollença	Mallorca	31SEE0817	EHUMC-1357	KT968984	KT969181	KT969371
X. frater pollenzensis	Formentor Peninsula	Mallorca	31SEE1119	EHUMC-1358	KT968985	KT969182	KT969372
X. frater pollenzensis	Formentor lookout	Mallorca	31SEE0918	MVHN-260410DR04-1	KT969062	KT969258	KT969414
X. frater pulaensis	Camí de'n Rafalet. Son Servera	Mallorca	31SED3190	EHUMC-1351	KT968978	KT969175	KT969366
X. frater pulaensis	Puig de Son Sard	Mallorca	31SED3190	EHUMC-1352	KT968979	KT969176	KT969367
X. frater pulaensis	Pula golf course	Mallorca	31SED3188	EHUMC-1353	KT968980	KT969177	KT969368
X. ferreri ferreri	Path to French's monument	Cabrera	31SDD9433	EHUMC-1295	KT968924	KT969121	KT969312
X. ferreri ferreri	Paguera	Mallorca	31SDD5376	EHUMC-1296	KT968925	KT969122	KT969313
X. ferreri ferreri	Paguera	Mallorca	31SDD5376	EHUMC-1297	KT968926	KT969123	KT969314
X. ferreri ferreri	Paguera, fossil dunes	Mallorca	31SDD5375	EHUMC-1298	KT968927	KT969124	KT969315
X. ferreri ferreri	Punta de N'Ensiola	Cabrera	31SDD9331	EHUMC-1299	KT968928	KT969125	KT969316
X. ferreri ferreri	Na Foradada	Cabrera	31SDD9839	EHUMC-1300	KT968929	KT969126	KT969317
X. ferreri ferreri	Na Foradada	Cabrera	31SDD9839	EHUMC-1301	KT968930	KT969127	KT969318
X. ferreri ferreri	Ses Covetes	Mallorca	31SDD9756	EHUMC-1302	KT968931	KT969128	KT969319
X. ferreri ferreri	Platja dés Caragol	Mallorca	31SDD5869	EHUMC-1303	KT968932	KT969129	KT969320
X. ferreri ferreri	Cala Blava	Mallorca	31SDD5869	EHUMC-1304	KT968933	KT969130	KT969321
X. ferreri ferreri	Sant Elm	Mallorca	31SDD9374	EHUMC-1305	KT968934	KT969131	KT969322

Taxa	Locality	Distribution	UTM grid cell	Voucher	GenBank accesions numbers		
					COI	16S rRNA	5.8-ITS2-28S
X. ferreri ferreri	Sant Elm	Mallorca	31SDD6380	EHUMC-1306	KT968935	KT969132	KT969323
X. ferreri ferreri	Cala Portals	Mallorca	31SDD6285	EHUMC-1307	KT968936	KT969133	KT969324
X. ferreri ferreri	Cala Portals	Mallorca	31SDD5569	EHUMC-1308	KT968937	KT969134	KT969325
X. ferreri ferreri	Génova	Mallorca	31SDD5288	EHUMC-1310	KT968938	KT969135	KT969326
X. ferreri ferreri	Sont Cotoneret	Mallorca	31SDD4977	EHUMC-1311	KT968939	KT969136	KT969327
X. ferreri ferreri	Ma 10 km 98 road. Estellenchs	Mallorca	31SDD9756	EHUMC-1312	KT968940	KT969137	KT969328
X. ferreri ferreri	Camp de Mar golf course	Mallorca	31SDD5869	EHUMC-1313	KT968941	KT969138	KT969329
X. ferreri ferreri	Cala Fornells	Mallorca	31SDD5175	EHUMC-1314	KT968942	KT969139	KT969330
X. ferreri ferreri	Sa Dragonera	Mallorca	31SDD4282	EHUMC-1315	KT968943	KT969140	KT969331
X. ferreri ferreri	Vilafranca de Bonany	Mallorca	31SED0782	EHUMC-1316	KT968944	KT969141	KT969332
X. ferreri ferreri	El Toro	Mallorca	31SDD6579	EHUMC-1323	KT968951	KT969148	KT969339
X. ferreri ferreri	Santuario del Cura. Algaida	Mallorca	31SDD9374	MVHN-240610YH07	KT969061	KT969257	KT969413
X. majoricensis	Illetes. Calviá	Mallorca	31SDD6476	EHUMC-1317	KT968945	KT969142	KT969333
X. majoricensis	Illetes. Calviá	Mallorca	31SDD6476	EHUMC-1318	KT968946	KT969143	KT969334
X. majoricensis	Bunyolí. Establiments	Mallorca	31SDD6487	EHUMC-1319	KT968947	KT969144	KT969335
X. majoricensis	Bunyolí. Establiments	Mallorca	31SDD6487	EHUMC-1320	KT968948	KT969145	KT969336
X. majoricensis	Sont Quint Forest. Son Rapinya	Mallorca	31SDD6583	EHUMC-1321	KT968949	KT969146	KT969337
X. majoricensis	Son Vida	Mallorca	31SDD6482	EHUMC-1322	KT968950	KT969147	KT969338
X. prietoi muroensis	Platja del Muro	Mallorca	31SEE1102	EHUMC-1408	KT969033	KT969230	KT969401
X. prietoi prietoi	Bunyolí. Establiments	Mallorca	31SDD6487	EHUMC-1399	KT969024	KT969221	KT969392
X. prietoi prietoi	Sont Cotoneret	Mallorca	31SDD6285	EHUMC-1400	KT969025	KT969222	KT969393
X. prietoi prietoi	Inca	Mallorca	31SDD9493	EHUMC-1401	KT969026	KT969223	KT969394
X. prietoi prietoi	Sarriá. Establiments	Mallorca	31SDD6587	EHUMC-1402	KT969027	KT969224	KT969395
X. prietoi prietoi	Sarriá. Establiments	Mallorca	31SDD6587	EHUMC-1403	KT969028	KT969225	KT969396
X. prietoi prietoi	Son Joan Arnau	Mallorca	31SDD9887	EHUMC-1404	KT969029	KT969226	KT969397
X. prietoi prietoi	Path to Sa Font des Padró	Mallorca	31SDD8898	EHUMC-1405	KT969030	KT969227	KT969398
X. prietoi prietoi	Path to Sa Font des Padró	Mallorca	31SDD8898	EHUMC-1406	KT969031	KT969228	KT969399

Taxa	Locality	Distribution	UTM grid cell	Voucher	GenBank accesions numbers		
					COI	16S rRNA	5.8-ITS2-28S
X. prietoi prietoi	Son Serra de Marina	Mallorca	31SED1997	EHUMC-1407	KT969032	KT969229	KT969400
X. homeyeri	Cala Romantica	Mallorca	31SED2674	EHUMC-1392	KT969017	KT969214	
X. homeyeri	Pula Golf course	Mallorca	31SED3288	EHUMC-1393	KT969018	KT969215	
X. homeyeri	Punta de n'Amer	Mallorca	31SED3381	EHUMC-1394	KT969019	KT969216	KT969390
X. homeyeri	Puig de Son Sard	Mallorca	31SED3187	EHUMC-1395	KT969020	KT969217	
X. ponsi	Cabrera	Cabrera	31SDD9533	EHUMC-1385	KT969010	KT969207	
X. ponsi	Path to French's monument	Cabrera	31SDD9433	EHUMC-1386	KT969011	KT969208	
X. ponsi	Path to French's monument	Cabrera	31SDD9433	EHUMC-1387	KT969012	KT969209	KT969386
X. ponsi	French's monumet	Cabrera	31SDD9432	EHUMC-1388	KT969013	KT969210	KT969387
X. ponsi	French's monumet	Cabrera	31SDD9432	EHUMC-1389	KT969014	KT969211	
X. ponsi	Punta de N'Ensiola	Cabrera	31SDD9331	EHUMC-1390	KT969015	KT969212	KT969388
X. ponsi	Punta de N'Ensiola	Cabrera	31SDD9331	EHUMC-1391	KT969016	KT969213	KT969389
X. moraguesi	Font des Padró	Mallorca	31SDD8799	EHUMC-1398	KT969023	KT969220	KT969391
X. claudinae	Formentor Peninsula	Mallorca	31SEE1219	EHUMC-1396	KT969021	KT969218	
X. claudinae	Formentor viewpoint	Mallorca	31SEE0918	EHUMC-1397	KT969022	KT969219	
X. ferrutxensis	Cala Mesquida	Mallorca	31SED3699	EHUMC-1409	KT969034	KT969231	KT969402
X. ferrutxensis	Cala Mesquida	Mallorca	31SED3699	EHUMC-1410	KT969035	KT969232	KT969403
X. cardonae	Turdonell de Dalt. Maó	Menorca	31SFE0717	EHUMC-1359	KT968986	KT969183	KT969373
X. nyeli	Ses Mongetes	Menorca	31SEE7221	EHUMC-1361	KT968987	KT969184	KT969374
X. nyeli	Ses Mongetes	Menorca	31SEE7221	EHUMC-1362	KT968988	KT969185	KT969375
X. nyeli	Ses Mongetes	Menorca	31SEE7221	EHUMC-1363	KT968989	KT969186	
X. nyeli	Alaior	Menorca	31SEE9417	EHUMC-1365	KT968990	KT969187	
X. nyeli	Alaior	Menorca	31SEE9417	EHUMC-1366	KT968991	KT969188	KT969376
X. nyeli	Ses Olles. Es Mercadal	Menorca	31TEE9932	EHUMC-1367	KT968992	KT969189	KT969377
X. nyeli	Son Saura - Es Talaier	Menorca	31SEE7620	EHUMC-1368	KT968993	KT969190	
X. nyeli	Son Saura - Es Talaier	Menorca	31SEE7620	EHUMC-1369	KT968994	KT969191	
X. nyeli	Son Saura - Es Talaier	Menorca	31SEE7620	EHUMC-1370	KT968995	KT969192	

Taxa	Locality	Distribution	UTM grid cell	Voucher	GenBank accesions numbers		
					COI	16S rRNA	5.8-ITS2-28S
X. nyeli	Son Saura - Es Talaier	Menorca	31SEE7620	EHUMC-1371	KT968996	KT969193	KT969378
X. nyeli	Son Saura - Es Talaier	Menorca	31SEE7620	EHUMC-1372	KT968997	KT969194	KT969379
X. nyeli	Son Saura	Menorca	31SEE7620	EHUMC-1373	KT968998	KT969195	
X. nyeli	Son Saura	Menorca	31SEE7620	EHUMC-1374	KT968999	KT969196	
X. nyeli	Son Saura	Menorca	31SEE7620	EHUMC-1375	KT969000	KT969197	
X. nyeli	Cala Es Pous	Menorca	31TEE7033	EHUMC-1376	KT969001	KT969198	KT969380
X. nyeli	Cala Es Pous	Menorca	31TEE7033	EHUMC-1377	KT969002	KT969199	
X. nyeli	Cala Es Pous	Menorca	31TEE7033	EHUMC-1378	KT969003	KT969200	KT969381
X. nyeli	Cala Es Pous	Menorca	31TEE7033	EHUMC-1379	KT969004	KT969201	
X. nyeli	Cala Es Pous	Menorca	31TEE7033	EHUMC-1380	KT969005	KT969202	KT969382
X. nyeli	Cala d'Algariens	Menorca	31TEE7833	EHUMC-1381	KT969006	KT969203	KT969383
X. nyeli	Cala d'Algariens	Menorca	31TEE7833	EHUMC-1382	KT969007	KT969204	
X. nyeli	Cala d'Algariens	Menorca	31TEE7833	EHUMC-1383	KT969008	KT969205	KT969384
X. nyeli	Cala d'Algariens	Menorca	31TEE7833	EHUMC-1384	KT969009	KT969206	KT969385
X. barceloi	Orihuela	Alicante	30SXH8018	EHUMC-1413	KT969038	KT969235	KT969406
X. chiae	Roquesblanques	Girona	31TDG2985	EHUMC-1240	KT968869	KT969066	
X. chiae	Coll de Pal. Bagà	Barcelona	31TDG1184	EHUMC-1417	KT969042	KT969239	
X. chiae	Coll de Pal. Bagà	Barcelona	31TDG1184	EHUMC-1418	KT969043	KT969240	
X. cobosi	Enix	Almería	30SWF3878	EHUMC-1283	KT968912	KT969109	
X. derogata	Tàrbena	Alicante	30SYH5386	MVHN-2144	KT969051	KT969247	
X. geyeri	Penyagolosa	Castellón	30TYK2556	MVHN-29111JL03	KT969054	KT969250	
X. grabusana	Kaliviani 2 km towards Balo	Crete	34SGE3635	ZMH 29885-465	^{1}FJ627089	^{1}JN701847	
X. jimenensis	Sierra de Benajoan	Málaga	30STF9562	MVHN-1376	KT969048	KT969244	
X. jimenensis	Gaucín	Málaga	30STF8645	MVHN-1403	KT969049	KT969245	
X. meda	Mosta	Malta	33SVV47	MVHN-230412LR01	KT969058	KT969254	
X. molinae	Illa Grossa	Columbretes Islands	31SCE0219	MVHN-2143	KT969050	KT969246	KT969410
X. montserratensis	Santa Coloma de Gramanet	Barcelona	31TDF3490	EHUMC-1355	KT968982	KT969179	

Taxa	Locality	Distribution	UTM grid cell	Voucher	GenBank accesions numbers		
					COI	16S rRNA	5.8-ITS2-28S
X. montserratensis	Monistrol de Montserrat	Barcelona	31TDG0305	EHUMC-1414	KT969039	KT969236	KT969407
X. montserratensis	Castellar del Vallès	Barcelona	31TDG2110	EHUMC-1415	KT969040	KT969237	KT969408
X. montserratensis	Gallifa	Barcelona	31TDG2616	EHUMC-1432	KT969046	KT969243	
X. penchinati	Azagra	Navarra	30TWM9382	EHUMC-1419	KT969044	KT969241	
X. penchinati	Azagra	Navarra	30TWM9382	EHUMC-1420	KT969045	KT969242	
X. ripacurcica montsicciana	Pineta Valley	Hueca	31TBH6426	MVHN-210813FS02	KT969056	KT969252	
X. ripacurcica montsicciana	Congost de Ventamillo	Huesca	31TBH9006	MVHN-210813FS03	KT969057	KT969253	KT969411
X. ripacurcica ripacurcica	Circo de Armeña	Huesca	31TBH8111	EHUMC-1416	KT969041	KT969238	KT969409
X. ripacurcica ripacurcica	Cerler	Huesca	31TBH9817	MVHN-2146	KT969052	KT969248	
X. ripacurcica ripacurcica	Sopeira	Huesca	31TCG1487	MVHN-310713JH25	KT969065	KT969261	
X. roblesi	La Fonteta. Nàquera	Valencia	30SYJ1993	MVHN-080709DR06	KT969055	KT969251	
X. subrogata	Serra de la Borja. Pratdip	Tarragona	31TCF2345	EHUMC-1411	KT969036	KT969233	KT969404
X. subrogata	Serra de la Borja. Pratdip	Tarragona	31TCF2345	EHUMC-1412	KT969037	KT969234	KT969405
X. subrogata	El Bartolo	Castellón	31TBE4640	MVHN-2147	KT969053	KT969249	
X. turolensis	Puerto de Villaroya	Teruel	30TXK9488	MVHN-240610YH02	KT969059	KT969255	
Hygromia limbata	Queralbs. Daió	Girona	31TDG38	EHUMC-1027	KT968867	^{2}KJ458529	^{2}KJ458616
Trochoidea elegans	L'Alcudia	Valencia	30SYJ14	MVHN-1310	KT969047	^{2}KJ458564	^{2}KJ458642
Xerosecta adolfi	Nijar	Almería	30SWF79	EHUMC-1036	KT968868	^{2}KJ458567	^{2}KJ458645

Table S2 List of primers used for amplification and sequencing.

Gene	Name	Sense	Sequence	Reference
COI	LCO1490	F	GGT CAA CAA ATC ATA AAG ATA TTG G	Folmer *et al.* (1994)
	HCO2198	R	TAA ACT TCA GGG TGA CCA AAA AAT CA	Folmer *et al.* (1994)
16S rRNA	16sar	F	CGC CTG TTT ATC AAA AAC AT	Palumbi *et al.* (1991)
	16sbr	R	CCG GTC TGA ACT CAG ATC ACG T	Palumbi *et al.* (1991)
5.8S–ITS2	LSU-1	F	CTA GCT GCG AGA ATT AAT GTG A	Wade *et al.* (2006)
	LSU-3	R	ACT TTC CCT CAC GGT ACT TG	Wade *et al.* (2006)
28S	LSU-2	F	GGG TTG TTT GGG AAT GCA GC	Wade *et al.* (2006)
	LSU-5	R	GTT AGA CTC CTT GGT CCG TG	Wade *et al.* (2006)
	LSU-2mod	F	TCT CAG GAG TCG GGT TGT TT	Razkin *et al.* (2015)

Table S3 PCR conditions for mitochondrial and nuclear markers.

(a) PCR conditions for *COI* and 16S rRNA genes

Step	T (°C)	Time	Cycles
1	96.0	1 min	1
2	94.0	30 s	
	55.0	30 s	35
	72.0	1 min	
3	72.0	10 min	1
Hold	10.0	∞	

(b) PCR conditions for nuclear markers (5.8S – ITS2 – 28S)

Step	T (°C)	Time	Cycles
1	96.0	1 min	1
2	94.0	30 s	
	50.0	30 s	35
	72.0	1 min	
3	72.0	10 min	1
Hold	10.0	∞	

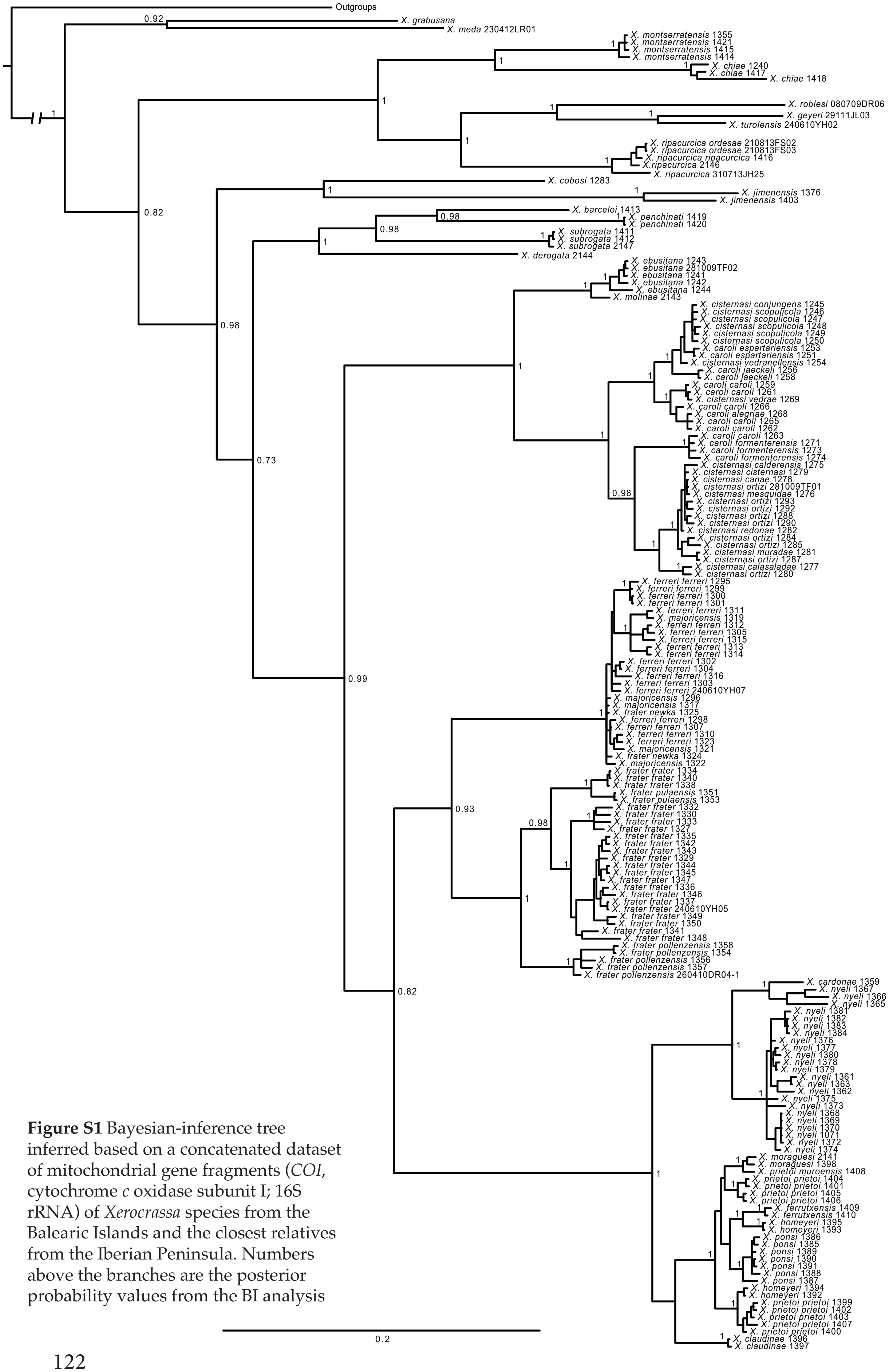

Figure S1 Bayesian-inference tree inferred based on a concatenated dataset of mitochondrial gene fragments (*COI*, cytochrome *c* oxidase subunit I; 16S rRNA) of *Xerocrassa* species from the Balearic Islands and the closest relatives from the Iberian Peninsula. Numbers above the branches are the posterior probability values from the BI analysis

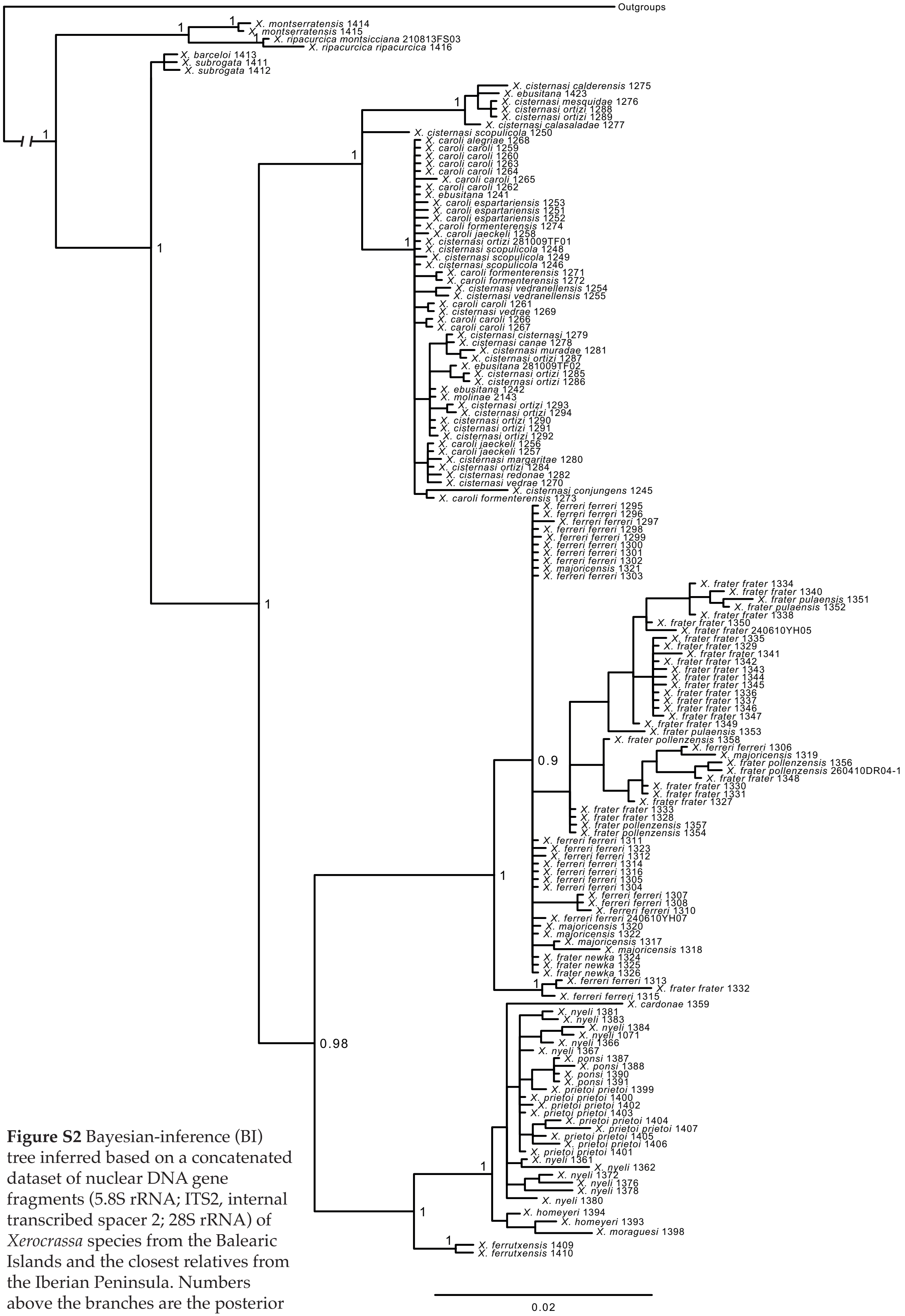

Figure S2 Bayesian-inference (BI) tree inferred based on a concatenated dataset of nuclear DNA gene fragments (5.8S rRNA; ITS2, internal transcribed spacer 2; 28S rRNA) of *Xerocrassa* species from the Balearic Islands and the closest relatives from the Iberian Peninsula. Numbers above the branches are the posterior probability values from the BI analysis.

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CHAPTER 5

Candidula

Paper IV

Molecular phylogeny of *Candidula* (Geomitridae) land snails inferred from mitochondrial and nuclear markers reveals the polyphyly of the genus

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In preparation

ABSTRACT

The genus *Candidula* (Geomitridae) consisting of 28 species in western Europe, is disjunctly distributed in the Iberian Peninsula, Italy, Balkan region, the Aegean Islands, and one species in the Canary Islands. Although the genus is well defined by characters of the reproductive system, the relationships within the genus are still unclear and some authors have indicated a possible subgeneric division based on the internal morphology of the dart sac. We present a molecular phylogeny of *Candidula* based on two mitochondrial genes (*COI* and 16S rRNA), the rDNA region (5.8S rNRA + ITS2 + 28S rRNA) and seven nuclear DNA regions isolated specifically to this genus (60SL13, 60SL17, 60SL7, RPL14, 40SS6, 60SL9, 60SL13a).

Two major clades were recovered with *Candidula* species, grouped into six monophyletic entities. One major clade grouped species from southern France and Italy with the widely distributed species *C. unifasciata*. The second major clade grouped all the species from the Iberian Peninsula, including *C. intersecta* and *C. gigaxii*. *C. ultima* from Canary Islands was recovered as one separated lineage within the latter clade. The incorporation of additional geomitrid genera allowed us to demonstrate the polyphyly of the genus *Candidula*. Moreover, we showed that similar structures of the stimulatory apparatus of the genital system in different taxa do not necessarily mean a close phylogenetic relationship in the Geomitridae. More genera of the family are needed to clarify their evolutionary relationships, and to fully understand the evolution of the stimulatory apparatus of the genital system within the Geomitridae.

Keywords

***Candidula*, dart sac, Geomitridae, Helicoidea, land snails, molecular phylogeny, nuclear markers, polyphyly.**

INTRODUCTION

Mediterranean basin is ranked among the 25 most important global biodiversity hotspots (Myers *et al*., 2000) and, particularly, land snails are one of the most diverse taxa within this region. However, as a result of the increment of human population, the Mediterranean basin has experienced important habitat losses and extinction processes, necessitating to set conservation priorities (Médail & Quézel, 1999; Cincotta *et al*., 2000; Brooks *et al*., 2002; Vogiatzakis *et al*., 2006). Therefore, in terms of conservation, comprehensive knowledge and an adequate classification of organisms is crucial. However, land snails are notorious for the existence of cryptic species (e.g. Parmarkelis *et al*., 2005; Dépraz *et al*., 2009; Pfenninger *et al*., 2010; Weigand *et al*., 2011), and so is *Candidula* (Pfenninger & Magnin, 2001). Traditional classification of land snail has been based on shell morphology and genital anatomy characters (Nordsieck, 1987; Puente, 1994; Schileyko, 2006). However, land snail shells variations are in many cases local adaptations to particular environmental conditions (Pfenninger *et al*., 2005; Schilthuizen *et al*., 2006; Elejalde *et al*., 2008; Fiorentino *et al*., 2013; Stankowski & Johnson, 2014; Köhler & Criscione, 2015). In Helicoidea superfamily, classification at supraspecific level is primarily based on the genital anatomy, and concretely on the morphology of the stimulatory genital system, composed by a dart sac, an accessory sac and vaginal glands. Independent modifications of genital anatomy occurred in different lineages, in particular dart sacs with related glands have repeatedly been lost or duplicated in several groups (Koene & Schulenburg, 2005; Wade *et al*., 2007; Hirano *et al*., 2014; Neiber & Hausdorf, 2015). This can led to misinterpretations about the phylogenetic relationships between different taxa indicating that the current classification of land snails may largely deviate from the natural system (Hirano *et al*., 2014). Thus, the general usefulness of these morphological structures as diagnostic characters should be revised for any group to ascertain which morphological features are indeed taxonomically informative and which are not (Köhler & Criscione, 2015). Using genetic data to resolve the evolutionary relationships of species is of major interest in evolutionary and systematic biology (Nater *et al*., 2015) and recent molecular phylogenies have improved the knowledge of relationships within several Helicoidea families (Hugall & Stanisic, 2011; Gómez-Moliner *et al*., 2013; Razkin *et al*., 2015).

In the present study, we focused on *Candidula* Kobelt 1871, a land snail genus with at least 28 currently recognised species (Bank, 2011; Welter-Schultes, 2012; Holyoak & Holyoak, 2014). The genus is defined by the presence of a large dart sac arising from the vagina (Holyoak & Holyoak, 2014), but see Hausdorf, 1989 and Puente, 1994. *Helicella*, with two dart sacs thought to present the ancestral character state, is considered the sister group of *Candidula*, where the atrophy of one of the dart sacs is considered an autapomorphy (Hausdorf, 1988).

The species of *Candidula* are widely distributed in Western Europe, from the eastern Canary Islands to the Balkans and northwards to Scotland and southernmost Sweden. The genus shows a disjunct distribution pattern with 18 species present in the Iberian

Peninsula (15 endemic), 5 species in Italy (4 endemic), 4 endemics in the Balkan region and finally, one species in Fuerteventura (Canary Islands) (Table 5.1). Most *Candidula* species are microendemics, with only three species having attained a somewhat larger distribution range (*C. intersecta*, *C. gigaxii* and *C. unifasciata*). The genus is present in many diverse habitats such as rocky limestone areas, sand dunes and grasslands, from dry Mediterranean shrublands to high mountain grasslands (Welter-Schultes, 2012; Holyoak and Holyoak, 2014) and probably adapted to the specific habitat and climate conditions encountered there (Pfenninger *et al*., 2007).

Here we present the first phylogenetic approach of *Candidula* which is based on the analyses of mitochondrial and nuclear sequences from 22 species that represent nearly all the currently known species within the Mediterranean basin. As for many other taxonomic groups, the most important development in molluscs genetics has been the accumulation of draft genome sequences and transcriptomes. Multilocus approaches based on analysis of both mitochondrial and nuclear markers are essential for an accurate reconstruction of the evolutionary processes underlying the history of species and their populations. In this regard, recent advances in next generation techniques, the decrease in the price and the increasing optimization of bioinformatics methods, have facilitated the routine use of genomic sequences from non-model organisms (Ellegren, 2013). Systematic searches of novel genome-wide genetic markers have increased recently in order to select the most appropriate markers to resolve phylogenetic hypotheses. Several studies have implemented the use of anonymous nuclear markers (ANM) to carry out multilocus approaches of species delimitation studies, phylogenetic inference and population structure and demographic history of species/populations studies (Lee & Edwards, 2008; Amaral *et al*., 2009; Polihronakis, 2009; Päckert *et al*., 2012; Satler *et al*., 2013). In this paper, we carried out the search and identification of anonymous nuclear markers (ANMs) from ESTs obtained de novo for *Candidula* genus with the aim to obtain new markers for a deeper study about phylogenetic relationships of this group.

The mains aims of the present study were to (1) discovery of new polymorphic markers from the transcriptomes of three *Candidula* species; (2) investigate the phylogenetic relationships of *Candidula* species; and (3) explore the systematic position of *Candidula* within the Geomitridae family.

MATERIAL AND METHODS

Taxon sampling and DNA isolation

We examined 22 species of *Candidula* from 87 populations (Fig. 5.1). Specimens were preserved and stored in 96% ethanol until DNA isolation. Additional specimens of each population were stored in 70% ethanol to study their anatomy. The collected specimens were confirmed to belong to *Candidula* and assigned into their corresponding species by the study of the genital anatomy. We also included several species of the closest genera to *Candidula* within the Geomitridae family (Razkin *et al*., 2015) and the genus *Pyrenaearia*

Table 5.1 Species belonging to *Candidula s.l.* genus and their distribution ranges

Species	Distribution
C. arganica (Servain, 1880)	N Iberian Peninsula
C. arrabidensis Holyoak & Holyoak, 2014	W Iberian Peninsula
C. belemensis (Servain 1880)	W Iberian Peninsula
C. camporroblensis (Fez, 1944)	Iberian Peninsula
C. carrapateirensis Holyoak & Holyoak, 2014	Iberian Peninsula
C. castriota Sóos, 1924	NE Albania
C. cavannae (Paulucci, 1881)	Italy
C. codia (Bourguignat, 1859)	SW Iberian Peninsula
C. corbellai Martínez-Ortí, 2011	NE Iberian Peninsula
C. coudensis Holyoak & Holyoak, 2010	W Iberian Peninsula
C. fiorii (Alzona & Alzona Bisachii, 1938)	Italy
C. gigaxii (Pfeiffer, 1847)	W Europe
C. grovesiana (Paulucci, 1881)	Italy
C. intersecta (Poiret, 1801)	W Europe
C. lernaea Haussdorf, 1991	Greece
C. najerensis (Ortiz de Zárate y López, 1950)	Iberian Peninsula
C. olisippensis (Servain, 1880)	W Iberian Peninsula
C. ponsulensis Holyoak & Holyoak, 2014	W Iberian Peninsula
C. rhabdotoides (Wagner, 1928)	Balkans
C. rocandioi (Ortiz de Zárate y López, 1950)	N Iberian Peninsula
C. rugosiuscula (Michaud, 1831)	SE France
C. scabiosula (Locard, 1899)	SW Iberian Peninsula
C. setubalensis (Pfeiffer, 1850)	W Iberian Peninsula
C. spadae (Calcara, 1845)	Italy
C. strucki (Maltzan 1886)	W Iberian Peninsula
C. syrensis (Pfeiffer, 1846)	Greece (Aegean Islands)
C. ultima (Mousson, 1872)	Canary Islands
C. unifasciata (Poiret, 1801)	C Europe

Figure 5.1. Map of Western Europe plotting collection localities of *Candidula* specimens analysed in this study (samples from Açores and Canary archipelagos, not shown).

(Hygromiidae family) was selected as outgroup taxa. The locations of voucher material are provided in Supplementary material. Total genomic DNA was extracted from foot muscle of specimens preserved in 96% ethanol, using the DNAeasy Tissue kit (Qiagen, Valencia, CA, USA).

Marker development strategy and Primer design
For this study we developed a set of multiple and applicable markers. We employed information of three EST data sets created for different species of *Candidula* (*C. unifasciata*, *C. gigaxii* and *C.rugosiuscula*) by Illumina sequencing of normalized transcriptomes (Haun, unpublished data). We used a four-step selection process to identify loci for suitability of amplifying the different species of *Candidula* included in this study.

Step 1: Comparison of read sequences derived from the EST developed for species representatives of *Candidula* with the publicly available cDNA of *Aplysia californica* and selection of loci that span introns and showed no sign of duplication in the *Aplysia californica* genome, i.e. that produced one single hit in the *BLAST* search. *Step 2*: for each loci selected we realized an aligment of the four *Candidula* species tested and *Aplysia californica* sequences. *Step 3*: Species-specific primers design in conservative regions and test of amplification using a sample congeneric to the species from which they were designed. We established general requirements for our primer sets design: *i*) amplicons length should be between 300 - 500 bp; *ii*) primers should have a maximal length of 24 bp; *iii*) all primers should have a similar melting point (Tm) at an optimum 57-60 ºC. *Step 4:* amplification of multiple samples from *Candidula* with primers able to amplify the samples in step 3 to verify the applicability to multiple species of this genus. *Step 5*: Optimization of PCRs and selection of primers. For primers design, the sequences from the ESTs were used as primer sequence. Primers design was performed using Primer3- Plus (Untergasser *et al.*, 2007) under manual control. For several loci, multiple primers were designed to achieve a higher coverage of the target gene and increase likelihood of positive amplification. All primers designed were initially tested in a temperature gradient ranging from 50ºC to 62ºC using a sample from the genus the EST was derived from. Optimal annealing temperatures were defined as the temperature where only one specific product was indicated after gel electrophoresis. Primers that showed no amplification product or more than one band in the gel electrophoresis over the whole range of temperature were discarded from subsequent steps. Primer pairs with positive amplification within the temperature gradient were tested as a PCR on all the remaining species using the optimal annealing temperature. Primers that were able to produce amplicons for the majority of the samples were chosen for further investigation.

PCR amplification

For the multi-locus analyses performed in this study, a set of seven new nuclear markers were selected, all of them developed specifically for this study. Besides, three loci commonly used in mollusc phylogenies were selected, two mitochondrial genes (*COI* and 16S ribosomal RNA gene) and approximately 850 bp of a nuclear rRNA cluster, including the 3′ end of the 5.8S gene, the complete ITS2 region and the 5′ end of the large subunit rRNA –(LSU; 28S). An overview of primers used for DNA amplifications are given in Table 5.2. General PCR cycling conditions used for DNA amplification were 1 min at 96 ºC, [30 s at 94 ºC, 30 s at 55–61 ºC (depending on the annealing temperature of the primer pair used), 1 min at 72 ºC] (repeated for 35 cycles) and 10 min at 72 ºC.

Amplicons were sequenced using dRhodamine Terminator Cycler Sequencing Ready reaction Kit (Applied Biosystems, Foster City, CA) run on ABI PRISM model 3100 Avant Genetic Analyzer and using the same primers as for PCR. The resulting forward and reverse sequences were assembled using Geneious v8.0.2 (Kearse *et al.*, 2012). Following automatic assembly, each contig was checked for errors/ambiguities. Double peaks with equally high intensities in the chromatograms were assigned as heterozygous sites (SNPs). These polymorphic sites were coded as ambiguous nucleotides following IUPAC-IUB code.

For new markers, exon boundaries were determined by BLAST searches of the sequences against the mRNA used for primer design. The definition of the open reading frame (ORF) of each gene was obtained using the ORF finder function at NCBI.

Phylogenetic analyses

Sequences were aligned with Mafft v.7 online version (Katoh *et al.*, 2002), using the Q-INS-i algorithm for the 16S rRNA and 5.8S-ITS2-28S, the G-INS-i for the rest of nuclear gene fragments and the ‘auto’ strategy for *COI* data. We aligned our sequences of the 16S rRNA

Table 5.2 List of 10 markers used in this study. The marker name and the sequences of each pair of primers.

!

Name	Primer name	Primer sequence	Reference
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer *et al*., 1994
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al*., 1994
16S rRNA	16Scs1	AAACATACCTTTTGCATAATGG	Chiba, 1999
	16Scs2	AGAAACTGACCTGGCTTACG	Chiba, 1999
5.8S-ITS2-28S	LSU-1	CTAGCTGC GAGAATTAATGTGA	Wade *et al*., 2006
	LSU-3	ACTTTCCCTCACGGTACTTG	Wade *et al*., 2006
60SL13	2Fw60SL13	GCAGCGCATGGTCAAAACAT	This study
	2Rv60SL13	CAGCTTGGCGTTGATTCGTG	This study
	30Fw60SL13	CCCATGAGAAAGAAGNGRAG	This study
	30Rv60SL13	CANAAGCGTTGTGGTTACGC	This study
60SL17	9Fw60SL17	TGGGTCATACCTGCGTGTTC	This study
	9Rv60SL17	CTTCTCGGCCAGGACAACTT	This study
	31Fw60SL17	AAATACYYGTGAAACTGCANA	This study
	31Rv60SL17	CATGTATGGRTTRAWGCGTCC	This study
60SL7	11Fw60SL7	AGCGAGCGGAGAAATATGCC	This study
	11Rv60SL7	TCTGTCTGACCAGGGCATCA	This study
	32Fw60SL7	AACTTCNATGTCCCNGCNGA	This study
	32Rv60SL7	CCRTARTCACCACCATCRTT	This study
RPL14	14FwRPL14	CCTACATTGCCCACGGAGAT	This study
	14RvRPL14	TTTCACGGCTTTCTTGGTTGG	This study
	33FwRPL14	AGATGACAAAGGCAAACTYGTK	This study
	33RvRPL14	CTTGGTTGGYTTCTTRGGCT	This study
40SS6	18Fw40SS6	GAAGGGCTACATTGTCCGCA	This study
	18Rv40SS6	TTCTTGAGAGCCAGTCGGTG	This study
	34Fw40SS6	RCAACRACWAGCAARGATTTC	This study
	34Rv40SS6	GRCTTTGCTTTTTGCCTTCTTA	This study
60SL9	19Fw60SL9	TGTCACTGTGAAGTCACGCA	This study
	19Rv60SL9	ACAGTTGTCTTCTCAGACACG	This study
	35Fw60SL9	GTNACTGTCAAAGRACCAAGG	This study
	35Rv60SL9	YGGRTRTCCTTCTTCTTCACA	This study
60SL13a	23Fw60SL13a	GGGTTTCTCCAACCAGCCAA	This study
	23Rv60SL13a	CAGATGCTTCTTGCGCTCAT	This study
	36Fw60SL13a	CAATTGTGATTGATGGCCGTG	This study
	36Rv60SL13a	CTGCTTGGATTTGACCTTTCTC	This study

and nuclear rRNA cluster genes with sequences published in GenBank. Thus, we analysed four different datasets: *i*) a matrix of *COI* sequences *ii*) the combination of mitochondrial gene fragments (*COI* + 16S rRNA); *iii*) the combination of 16S rRNA and nuclear rRNA gene cluster, and *iv*) the combined matrix for all genes.

Molecular characters statistics including parsimony informative sites, base frequency and A-T content were calculated with MEGA 6.05 (Tamura *et al*., 2013). Alignments of the complete dataset and rRNA dataset, outgroups included, are available at Supplementary material

The evolutionary model for each gene partition was estimated prior to analysis with jModelTest v3.7 (Darriba *et al*., 2012) according to the Akaike information criterion (AIC). The evolutionary models obtained for each partition are showed in Table 5.3.

Phylogenetic analyses of the different datasets were conducted using both Bayesian Inference (BI) and maximum likelihood (ML) methods with *Pyrenaeria carascalopsis* (Hygromiidae) as outgroup. A Bayesian Chain Monte Carlo search of tree space was conducted using MrBayes v.3.2.2 (Ronquist *et al*., 2012) on the CIPRES supercomputing cluster (Miller *et al*., 2010) applying a partition scheme by genes. MrBayes was programmed to run for 50 million generations in two parallel runs, sampling every 1000 generations with the first 25% of trees being discarded as burnin. The Bayesian search was determined to have reached stationarity when cold chains stopped increasing and randomly fluctuated within a stable –Ln range of values and when effective sample size for all metrics exceeded 200 as determined in the software Tracer v1.6 (Rambaut *et al*., 2014). Maximum likelihood analyses were conducted using RAxML v.8.0.24 (Stamatakis, 2014) under the GTRGAMMA model, with 1000 nonparametric bootstrap replicates to assess node support. For the different topologies obtained, we interpreted as significant statistical support values above 70% for bootstrapping procedures (BS) and 0.95 for Posterior Probability (BP) in the BI analyses.

RESULTS

PCR amplification and sequencing

In total, 32 primer pairs were designed corresponding to 23 loci: 9 pairs exhibited no product and 5 pairs exhibited multiple products over the whole range of the temperature gradient and were therefore discarded from further analysis. 18 out of the 32 primer pairs successfully amplified a clear distinct product using a sample congeneric to the EST. Of these, 14 were able to amplify at least four of the other species investigated in the last step.

Sequence variation and alignment

The alignments sites, parsimony informative sites, average base frequency and A-T content are listed in Table 5.3. Among the 5585 characters of the complete combined data set, 1864 (33.38%) were parsimony-informative (Table 5.3). Phylogenetic signal analyses based on substitution saturation showed that all molecular markers should posses enough information to infer phylogenetic relationships among the taxa considered (Supplementary material S1).

Candidula phylogeny

The phylogenetic reconstructions obtained by both dataset analyses, the complete concatenated-gene tree and the two rRNA concatenated-gene tree (16S rRNA + nuclear rRNA gene cluster), are shown in Fig 5.2 and Fig. 5.3 respectively. Other gene trees: *COI* and mitochondrial DNA (*COI*+16S rRNA) have been included as supplementary material (Supplementary material S2 and S3). These mtDNA topologies include a higher number of specimens because for some specimens all nuclear genes did not amplify.

For the concatenated dataset of all genes, phylogenetic topologies (Fig. 5.2) obtained with ML and BI methods were identical and they differed only on the support values, being generally higher in the BI tree. All the taxa included in the analyses, with *Pyrenaearia carascalensis* as outgroup, were recovered in two basal clades, here named A and B (BP =1: BS = 88%) with all the *Candidula* species being grouped into six monophyletic lineages (C1 – C6). The former basal clade grouped three subclades. One of them (A1) joined with full support (BP =1: BS = 100%) *Candidula ultima* (C1), the unique species of the genus living in the Canary Islands, as the sister group of *Microxeromagna lowei*. This group was basal with respect to the remaining taxa grouped within clade A. Another subclade (A2 = C2) joined exclusively several species of *Candidula* from the Iberian Peninsula. *C. najerensis* (BP = 1; BS = 99%) and *C. corbellai* (BP = 1; BS = 100%) were recovered with high support as two monophyletic lineages. Nevertheless, *C. camporroblensis* was recovered into four different lineages, constituting a paraphyletic entity. The close relationship between *C. camporroblensis*, *C. corbellai* and *C. najerensis* was fully supported and recovered as the sister clade of *C. gigaxii*. (BP = 1; BS = 87%). The monophyly of *C. gigaxii*, including populations from south Iberian Peninsula and central Europe was fully supported. The third subclade (A3) grouped several *Candidula* species form the Iberian Peninsula, joined in two different lineages (clades C3 and C4), together with the species of the genus *Helicella*, and with *Xerotricha gonzalezi* as the basal group. The lineage C3 grouped all the species from western Iberian Peninsula and *C. intersecta* with full support. The populations of *C. intersecta* from northern Iberian Peninsula (Basque Country and Galicia) clustered in two different lineages (BP = 1; BS = 96%). *C. olisippensis* was recovered as the sister group of *C. coudensis* (BP = 1; BS = 76%). These two species together with *C. belemensis* formed a monophyletic clade (BP = 1; BS = 77%) with *C. intersecta* as the sister group (BP = 0.98; BS = 69%). Nevertheless, *C. belemensis* did not constitute a monophyletic entity. The close relationship of these species with *C. arrabidensis* was only supported by BI analysis (BP = 0.98; BS = 63).

Table 5.3 Summary of the sequence information.

	Taxa	Length (bp)	Pi	Average base frequency (%)				A-T content (%)	Evolutionary model
				A	C	G	T		
COI	88	618	249	24.5	16.9	20.0	38.6	63.1	TVM+G+I
16S rRNA	90	893	470	34.5	13.5	17.6	34.4	68.9	GTR+G+I
5.8S-ITS2	84	593	196	14.0	34.3	33.5	18.2	32.2	TPM1uf+G+I
28S	84	325	1	23.4	25.8	31.4	19.4	42.8	TrN
60SL13	73	456	185	28.2	26.4	26.3	19.1	47.3	TIM3+G
60SL17	77	370	145	30.1	24.1	25.9	19.9	50.0	TIM2+G
60SL7	66	556	149	27.7	25.4	25.9	21.0	48.7	TrN+I
RPL14	83	392	123	31.7	21.5	26.4	20.4	52.1	TrN+I
40SS6	56	425	114	26.1	28.2	27.3	18.4	44.5	TPM1uf+G+I
60SL9	67	476	139	28.1	21.7	25.2	25.0	53.1	TIM3+G
60SL13a	43	481	93	27.1	22.1	26.7	24.1	51.2	TrNef+G

Pi means parsimony informative sites.

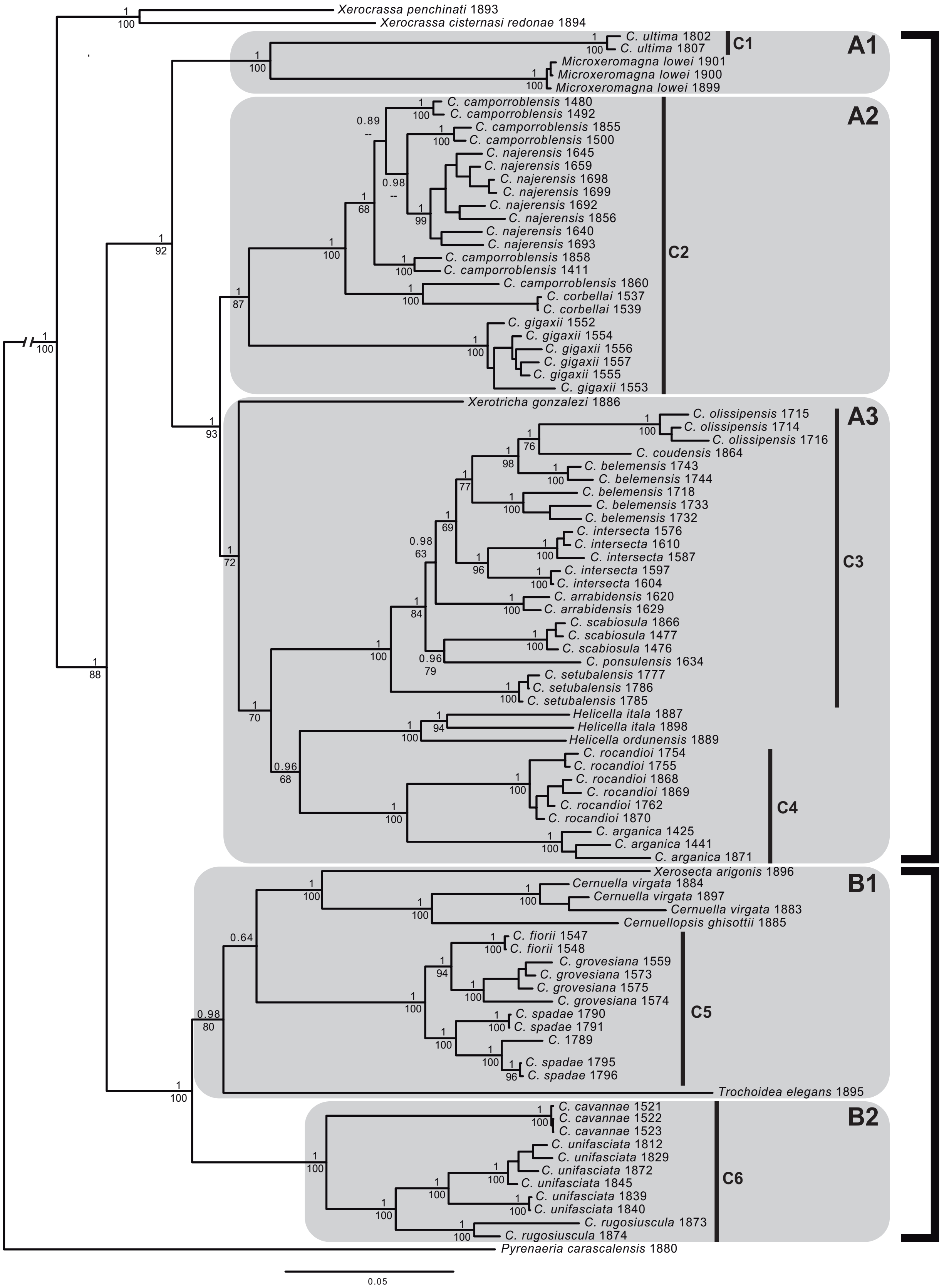

Figure 5.2. Phylogenetic relationships of *Candidula* species based on Bayesian analysis of a 5585 base pair alignment consisting of two mitochondrial (*COI*, 16S rRNA) and ten nuclear loci (5.8S rRNA + ITS2 + 28S rRNA; 60SL13; 60SL17; 60SL7; RPL14; 40SS6; 60SL9; 60SL13a). Node numbers correspond to Bayesian posterior probability (BP) and maximum likelihood (ML) support values.

On the other hand, *C. scabiosula* was recovered as the sister group of *C. ponsulensis* with strong support (BP = 1; BS = 84%), with *C. setubalensis* constituting the most basal group of C3 clade. *C. arganica* and *C. rocandioi* were recovered with full support within the lineage C4 with the species of *Helicella* genus as sister group.

The second main clade contained two subclades. The first one (B1) joined with high support (BP = 0.98; BS = 80%) three *Candidula* species from Italy. Here, *C. fiorii* and *C. grovesiana* were grouped together with high support (BP = 1; BS = 94%) with *C. spadae* as sister group. They were recovered as the sister group of a clade joining *Cernuella virgata*, *Cernuellopsis ghisottii* and *Xerosecta arigonis*. *Trochoidea elegans* was the basal group within B1. The second subclade (B2) grouped with full support *C. rugosiuscula*, endemic to southern France, as the sister group of *C. unifasciata*, widely distributed from Italy to central Europe. These two species were recovered with full support as the sister group of *C. cavannae*, endemic to the Apennines.

The analysis of two concatenated-genes (16S rRNA + nuclear gene cluster) (Fig. 5.3) shower the same tree topology for clades A1-A3 and B1-B2, although nodes supports were somewhat lower and the monophyly of clade B1 was not supported. It also recovered the same phylogenetic relationships for *Candidula* clades (C1, C6) fully supported for the BI analyses. This ribosomal clade included some additional taxa. Thereby, *Helicopsis turcica* and *Xerotricha apicina* were recovered within subclade A1, with the former joined with full support as the sister taxon of *Candidula ultima*. *Candidula codia*, endemic to southern Portugal, not included in the completed dataset tree, was recovered within subclade A3 without statistical support, forming a polytomy with the groups C3 (joining the other *Candidula* species from Portugal and *C. intersecta*) and C4. *Xerotricha gonzalezi* also belonged to subclade A3.

COI and mtDNA trees (Supplementary Material) included some more specimens of the different species of *Candidula*. MtDNA analyses recovered with high support the clades C1, C3, C4, C5 and C6, whereas *COI* analyses only recovered the clades C1, C3 and C5. Both trees showed that all populations of *Candidula intersecta* from the Basque Country, the Netherlands and the British Isles constituted a monophyletic group. The populations of Santa Maria Island (Açores) were recovered closely related to the populations of *C. intersecta* from Galicia (north-western Iberian Peninsula). However, the populations from Sao Miguel Island (Açores) were recovered, with full support, within the lineage formed by the populations of *C. intersecta* from central Europe and the Basque Country.

DISCUSSION

Several molecular studies have analysed evolutionary processes and distribution patterns within land snail genus endemic to the Mediterranean basin (Fiorentino *et al*., 2010; Sauer & Hausdorf, 2010; Chueca *et al*., 2015; Psonis *et al*., 2015). Many of those studies are focused in genera with reduced distribution ranges but with a high number of species (Douris *et al*., 2007; Fiorentino *et al*., 2010). Besides, a recent molecular phylogeny revealed the polyphyly of the genus *Cepaea* (Neiber & Hausdorf, 2015b) indicating that morphological characters are not always valid for the delimitation of genera. Although *Candidula* is one of the most diverse genera within the Mediterranean basin with several speciation centres, only one molecular phylogeny based on 6 species (Pfenninger *et al*., 2003) has been performed. This phylogeny was not enough to resolve the relationships within *Candidula*, a genus with at least 28 species. Compared to prior studies, the increased number of taxa sampled and the use of a higher number of loci, revealed interesting relationships within *Candidula*.

Moreover the selection of DNA regions commonly used in molecular phylogenies in land snails, allowed a comparison with other taxa within Geomitridae family, particularly to the Helicellini *sensu* Razkin *et al*. (2015).

Phylogeny of *Candidula*

Our study reveals that *Candidula* genus did not constitute a monophyletic lineage within the Geomitridae family. Although *Candidula* did not constitute a monophyletic group, the analyses revealed interesting relationships between the different species. All ML and BI analyses of the two data sets consistently indicated that there were six clades strongly supported (C1 – C6 in Fig. 5.2 and Fig. 5.3) within this genus.

The first *Candidula* lineage (C1 in Fig. 5.2) contained only one species of this genus: *C. ultima*, which was closely related to *Helicopsis turcica*, *Microxeromagna lowei*, *Xerotricha apicina* and *X. conspurcata* (Figs. 5.2 and 5.3). This species is endemic to Fuerteventura Island, eastern Canary Islands (Alonso *et al.*, 1996), located 100 km far away of western Africa. Due to its thick shell, considerably bigger than almost all *Candidula* species, and its geographical distribution, *C. ultima* was located within the *Sphincterochila* genus until the study of its anatomy (Alonso *et al.*, 1996). *C. camporroblensis*, *C. corbellai* and *C. najerensis* were grouped together within clade C2. This is in agreement with morphology, since their identification based on shell form is troublesome being very similar (Puente, 1994; Martínez-Ortí, 2011) and making it necessary the study of the genital anatomy. All the specimens of *C. najerensis*, are characterised by the presence of a short penial flagellum (Puente, 1994), and they really constituted one monophyletic group. On the contrary, the specimens with a long penial flagellum (*C. camporroblensis* and *C. corbellai*) formed a paraphyletic group. *C. corbellai* is a microendemism of the north-eastern Iberian Peninsula (Martínez-Ortí, 2011; Welter-Schultes, 2012), and formed also a distinct monophyletic lineage within C2 clade. Nevertheless, all the specimens belonging to the morphospecies *C. camporroblensis* were grouped in five different lineages, with *C. najerensis* as a derived group. These results indicated that an exhaustive study of this group is needed to elucidate if all taxa should be grouped as a single species (invalidating the flagellum length as a taxonomic character for these three species) or if there could be several cryptic species within *C. camporroblensis*. Specimens of *C. gigaxii*, including populations from southern Iberian Peninsula and central Europe joined together within clade C2. Genetic differences between populations of this widely distributed species were very low. This could be explained by a rapid expansion along Western Europe as has been suggested by Pfenninger *et al*. (2006). Nevertheless, the species is currently absent from the north Iberian Peninsula (Welter-Schultes, 2012), where an important geographic gap exists between the Iberian and European populations.

Candidula species living in western Iberian Peninsula and the widely distributed *C. intersecta* joined at lineage C3. *C. intersecta* complex was considered as a single species (Nobre, 1941) due to only subtle differences in shell characters and little or none in genital anatomy. However, Gittenberger (1993) recovered the validity of *C. olisippensis* and *C. belemensis* within the *C. intersecta* complex. Moreover, Holyoak & Holyoak (2014) described new cryptic *Candidula* species of this complex from Portugal. The analysis of complete dataset revealed the monophyly of many of the Portuguese species (*C. arrabidensis*, *C. olisippensis*, *C. ponsulensis* and *C. scabiosula*), which was in agreement with the species proposed by Holyoak and Holyoak (2014). The only morphospecies not recovered as a monophyletic entity was *C. belemensis*, which was recovered in two different lineages. This indicated that more than one species could be involved within *C. belemensis* being necessary a new revision of this taxon. Classification of *Candidula* specimens from Galicia has also been controversial, being classified as *C. intersecta* by some authors (Castillejo, 1986; Puente, 1994) although they present a long penial flagellum differing from the short penial flagellum of *C. intersecta s.str*. Nevertheless, Holyoak and Holyoak (2014) suggested that the material from Galicia corresponded to *C. olisippensis* due to similarities in its genital anatomy. In the same way, Ortiz de Zárate (1991) figured specimens from Galicia with a long penial flagellum suggesting that populations from Galicia should be considered a different taxon from *C. intersecta*. Our results showed that the populations from Galicia are more related to *C. intersecta* from the Basque Country (northern Iberian Peninsula) than to *C. olisippensis* from Portugal. According to all the information, the populations from Galicia should not be considered co-specific with *C. olissipensis*, but closely related to *C. intersecta*, instead, being probably, a different species. *C. intersecta* is one of the *Candidula* species widely distributed along central Europe. In the Iberian Peninsula the species is present in northern and western regions (Puente, 1994). However, Holyoak and Holyoak (2014), after the revision of the *Candidula* from Portugal, suggested that only the population from near Lisbon corresponds to *C. intersecta* (probably introduced), having been confused with *C. ponsulensis* (short penial flagellum) in Portugal and with *C. olissipensis* (long penial flagellum) in Galicia (north-western Iberian Peninsula). The keeled species *C. coudensis* (long penial flagellum) was recovered as valid species within the *C. intersecta* complex. This species is endemic to central Portugal, inhabiting only an area of 13.5 km^2 (Moreira *et al*., 2015). *C. setubalensis* is another keeled species endemic to Serra da Arrabida, characterized by a short penial flagellum which allows to differentiate it from *C. coudensis*. Besides, *C. setubalensis* constituted a monophyletic clade, being the sister group of the rest of *Candidula* species belonging to the *C. intersecta* complex. *C. codia*, endemic to south Portugal, was also grouped within clade A3 in the ribosomal analysis. Nevertheless, deep nodes of this clades were not resolved being necessary more studies to determine the relationships of *C. codia* with other species within clade C3.

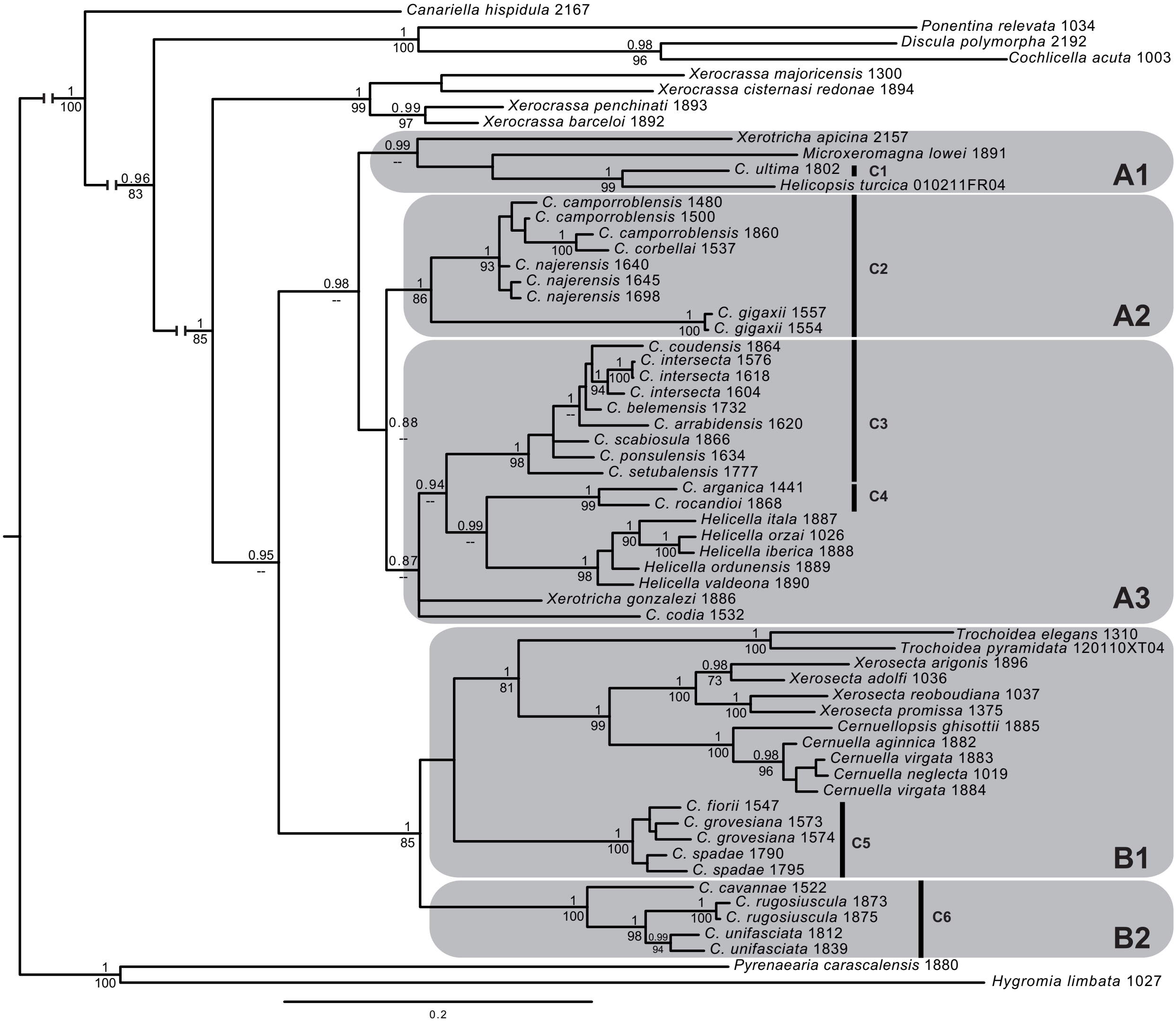

Figure 5.3. Bayesian 50% majority consensus tree based on the combined analysis of the rRNA dataset (16S rRNA; 5.8S rRNA + ITS2 + 28S rRNA). Posterior probabilities from Bayesian inference and bootstrap support values from maximum likelihood analyses are indicated at the nodes.

Clade C4 joined the species *C. arganica* and *C. rocandioi* with the genus *Helicella* as sister group (Fig. 5.2). Both *Candidula* species are endemic to the north Iberian Peninsula. They show nearly vicariant distribution ranges, being sympatric only in a few localities (Prieto, 1986). Besides, they show differences in anatomy, with *C. arganica* having a short penial flagellum and *C. rocandioi* a long one (Puente, 1994). Here, there was a high congruence of molecular phylogeny with shell and anatomical characters, and biogeography.

Four *Candidula* endemic species have been described from Italy, *C. cavannae*, *C. fiorii*, *C. grovesiana* and *C. spadae* (Welter-Schultes, 2012). Although all of them are endemic to Italy, they were not recovered as a monophyletic group. Three of them: *C. fiorii*, *C. grovesiana* and *C. spadae* constituted one monophylephic entity at clade C5 whereas *C. cavannae* was recovered at clade C6, joined together with *C. unifasciata* and *C. rugosiouscula*. The close relationships of *C. rugosiuscula*, endemic to south-eastern France, and the widely distributed species *C. unifasciata* was also observed by Pfenninger *et al.*, (2006). These results showed that *Candidula* species living in Italy are not phylogenetically related with the species of this genus living in the Iberian peninsula and that *C. rugosiuscula* and *C. unifasciata* are more closely related to the Italian group.

Our results recovered *Candidula* genus as polyphyletic. Pfenninger *et al.*, (2003) reconstructed the unique previous molecular phylogeny of *Candidula*, including the species *C. olisippensis*, *C. intersecta*, *C. gigaxii*, *C. unifasciata*, *C. rugosiuscula* and *C. spadae*, and recovering this genus as monophyletic. They could not find the polyphyly of the genus because they used *Xerocrassa geyery* (named as *Trochoidea geyeri*) as outgroup. We have included several *Xerocrassa* species from the Iberian Peninsula and Balearic Islands in the rRNA analysis, and they were recovered as the basal group for all the clades C1-C6 considered. So, *Xerocrassa* may therefore be an inappropriate outgroup to study the position s of *Candidula* within the Helicellinae. The recent molecular phylogeny of the western Helicoidea (Razkin *et al.*, 2015) included only two species of *Candidula*: *C. najerensis* and *C. corbellai*, both grouped within the same clade (C2 in Fig 5.2) in our study. Therefore, due to the absence of additional *Candidula* species belonging to other clades Razkin *et al.*, (2015) also failed to reveal non-monophyletic relationships within *Candidula*. Manganelli *et al.* (2005) published a molecular phylogeny approach of western Helicoidea where three *Candidula* species, *C. intersecta*, *C. spadae* and *C. unifasciata* were analysed. The three *Candidula* species did not constitute a monophyletic group. However, Manganelli *et al.* (2005) suggested that the molecular results did not reflect the true taxonomic relationships due to 16S rRNA gene could be evolving faster in *Candidula* than in other helicoid species analysed. On the other hand, Hausdorf (1988) found differences in the mucus glands and dart sac between *C. gigaxii* (clade C2) and *C. unifasciata* (clade C6), and suggested that two subgenera could be considered within *Candidula*. Unfortunately, we could not include any species of the Balkan Peninsula and Greek Islands in our analyses. So, their phylogenetic relationships with the different phylogenetic groups we found, remains unclear.

Biogeographical patterns of *Candidula*

C. ultima is endemic to Fuerteventura Island, eastern Canary Islands, located 100 km far away from the nearest western Africa coasts. Being *Candidula* a genus eminently distributed in Europe, one might think that *C. ultima* could have arrived to Fuerteventura Island by transcontinental transport, as it has been demonstrated for *Hemicycla* (Chueca *et al.*, 2015). Nevertheless, its close relationship with *Helicopsis turcica*, one species widely distributed throughout north-western Africa, indicated an African origin for *C. ultima*. In consequence, it shares an African origin with many other organisms endemic to Canary Islands, including plants (Santos Guerra, 2002), vertebrates (Carranza *et al.*, 2002), arthropods (Opatova & Arnedo, 2014; Planas & Ribera, 2014), or molluscs (Greve *et al.*, 2010). Thus, *C. ultima* seems to be a palaeoendemism restricted to a single island, in contrast of other land snail genera (*Canariella*, *Hemicycla*, *Napaeus* or *Theba*) widely distributed in the archipelago.

Within the Iberian Peninsula two main diversification centres of *Candidula* were observed. The Western Iberian Peninsula showed the first important speciation centre with at least 8 *Candidula* species distributed from Galicia to southern Portugal. A high intrageneric speciation process in this area has also been recently described for the land snail genus *Ponentina* (Holyoak & Holyoak, 2012). The Iberian System and The Cantabrian Mountains is the second main diversification centre of the genus, with five species currently recognized, and where one of them, *C. camporroblensis*, contains a high genetic diversity.

We have included in the *COI* and mtDNA analyses some specimens of *C. intersecta* from the British Isles and the Netherlands. They joined together with *C. intersecta* specimens from the Basque Country. This result indicated that the origin of *C. intersecta* was in the Iberian Peninsula from where it expanded along Western Europe. According with literature, the presence of most of the flora and fauna of the British Isles is consequence of a recolonization after the Last Glacial Maximum (LGM, c 18-21 ka) (Forbes, 1846; Montgomery *et al*., 2014). Nevertheless, it could also have been the result of more recent anthropic introductions, as it has been demonstrated for several organisms (Chytrý *et al*., 2009; Rowson *et al*., 2014). In this regard, several introduced populations of *C. intersecta* have been detected in several countries: New Zeeland (Barker, 1999), Australia (Quick, 1952; 1953), Colombia (Robinson, 1999), U.S.A. and Chile (idtools website [2015]). Besides, this species has also been cited in several islands from the Açores archipelago (northern Atlantic Ocean), presumably introduced from Portugal (Backhuys, 1975; Bank *et al*., 2002). We also included in *COI* and mtDNA analyses populations from two islands of the Açores archipelago, São Miguel and Santa Maria. Populations from São Miguel Island corresponded to the clade joining *C. intersecta* specimens from the Basque Country and Western Europe, including the British Isles. Besides, they are characterized by having a short penial flagellum. However, populations from Santa Maria Island showed long penial flagellum as observed by (Holyoak & Holyoak, 2014) and they were grouped with *C. intersecta* populations from Galicia. These results demonstrated two independent introduction processes into the Açores islands. Concerning *C. gigaxii*, another species widely distributed throughout Western Europe to British Isles (Welter-Schultes, 2012), its origin seemed also to be placed in the Iberian Peninsula from which it could also have expanded throughout France to Western Europe. The low genetic distances between specimens collected in central Europe and those collected in the Iberian Peninsula suggested that this expansion could have occurred after the last glacial maximum.

On the other hand, the Italian Peninsula, and in particular the Appennine Mountains, is another important speciation centre for *Candidula*. The Apennine Mountains joined four endemic species, *C. cavannae*, *C. fiorii*, *C. grovesiana* and *C. spadae*. Only *C. spadae* shows a wider distribution range along the Apennines, notwithstanding human activity and global change are reducing its habitat (Welter-Schultes, 2012), whereas the other three species show narrow distribution ranges. Moreover, *C. unifasciata* is also present in Italy, being the southern limit of its distribution range. Origin of *C. unifasciata* and *C. rugosiuscula* has been placed in southeastern France (Pfenninger *et al*., 2003). However, *C. unifasciata* expanded along central Europe after the onset of the climate warming after the last Pleistocene glaciation (Pfenninger & Posada, 2002), whereas *C. rugosiuscula* is restricted to southeastern France. Our results are concordant with these considerations about the origin of both species.

Additional remarks

After the last molecular studies (Razkin *et al*., 2015), the Helicellinae subfamily grouped four tribes: Cernuellini, Helicellini, Plentuisini and Trochoideini. *Candidula* lineages C1, C2, C3 and C4 were placed within the Helicellini, and the lineages C5 and C6 were placed as two different tribes but both more related to Trochoideini and Cernuellini than to Helicellini. Moreover, the closest relationship of *C. unifasciata* and *C. spadae* (clades C5 and C6) with Trochoideini and Cernuellini tribes was recovered by Manganelli *et al*. (2005) but without statistical support. Hausdorf (1988) considered *Candidula* as the sister genus of *Helicella* due to the autapomorphic absence of a penis nerve branching off the cerebral ganglion, whereas Schileyko (2006) suggested that *Candidula* is more related to *Cernuella*.

Excluding the polyphyly of *Candidula*, our results are in agreement with other molecular phylogenies proposed for Helicoidea. The two main groups that we have obtained in our analyses (clades A and B) corresponded to the clades Helicellini and Cernuellini + Trochoideini (sensu Razkin *et al*., 2015), respectively. Clade A joined species mainly distributed in western Mediterranean, whereas clade B grouped species with an Italian origin, suggesting for the Helicellinae different speciation centres, in the Iberian Peninsula (Clade A), Italy (Clade B) and, probably, in the Balkan region.

Due to the presence of two small accessory sacs arising symmetrically from the vagina wall, *Trochoidea* and *Xerocrassa* have been considered closely related genera (Puente, 1994; Bank, 2011; Welter-Schultes, 2012). *Xerocrassa* shows a disjunct distribution range within the Mediterranean basin, with two radiation centres: the eastern complex (Aegean region and Middle East) and the western complex (Iberian Peninsula and Balearic Islands). Our results based on the rRNA analyses indicated that, regarding at the species from the western complex, *Xerocrassa* is not the sister group of *Trochoidea*. Conversely, it has been recovered as the basal group of Helicellini and Cernuellini+Trochoideini. This seems to be another example that similar structures of the stimulatory apparatus of the genital system, concerning the number and position of dart sac(s) and accessory sac(s) (see Razkin *et al*., 2015) can be homoplasic characters. We want also to remark that the genus *Xerotricha* appeared to be not monophyletic. We have included only two species: *Xerotricha apicina* and *X. gonzalezi* in the rRNA analysis, which were included in clades A1 and A3, respectively. *Xerotricha* has been considered a genus closely related to *Helicella* (Hausdorf, 1988) or even co-generic with its (Puente, 1994). The closest relationship between these genera have been established by the similiraties of the genital system, composed by a double stimulatory apparatus with each unit consisting of one accessory sac opening into a large dart sac and, two bifurcate mocous glands connected to the vagina (Puente, 1994; Razkin *et al*., 2015). Nevertheless, none of the two *Xerotricha* species analysed was the sister taxa of *Helicella*. We can see again that the presence of two big dart sacs with dart, placed simmetrically at both sides of the vagina wall is not either a valid character by itself to obtain monophyletic taxa. On the contrary, *Helicella* species did constitute a monophyletic clade, while we also obtained a close relationship between *Cernuella* (with *Cernuellopsis*) and *Xerosecta*. The monophyly of the tribe Cernuellini is in agreement with the morphology of the three genera, characterized by a single stimulatory apparatus with a dart sac and an accessory sac (Puente, 1994). Besides, Hausdorf (1988) proposed the close relationship of *Candidula* and *Helicella* based on morphological characters. However, this conclusion was made comparing *Helicella itala* with *C. unifasciata* and *C. gigaxii*, none of them included in clade C4, the closest relative to *Helicella*. Regarding the results of the molecular phylogeny, a more complete phylogenetic study, including, as many genera of the Geomitridae family as possible, would be necessary to resolve the relationships within the family and to fully understand the evolution of the stimulatory apparatus of the genital system.

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Table S1 Specimens used for the study of the land snail genus *Candidula*, with their identification codes, localities, coordinates and voucher numbers. Sequences obtained for each specimen are marked by a black square, where loci corresponds as follow: (1), COI; (2), 16S rRNA; (3), 5.8S-ITS2-28S; (4), 60SL13; (5), 60SL17; (6), 60SL7; (7), RPL14; (8), 40SS6; (9), 60SL9; (10), 60SL13a.

Species	Voucher	Locality	Province	Country	UTM 10 km	1	2	3	4	5	6	7	8	9	10
Candidula arganica	EHUMC-1422	Monastery of Leyre	Navarra	Spain	30TXN52	■									
Candidula arganica	EHUMC-1423	Monastery of Leyre	Navarra	Spain	30TXN52	■									
Candidula arganica	EHUMC-1424	Monastery of Leyre	Navarra	Spain	30TXN52	■									
Candidula arganica	EHUMC-1425	Monastery of Leyre	Navarra	Spain	30TXN52	■	■			■	■	■	■	■	■
Candidula arganica	EHUMC-1426	Monastery of Leyre	Navarra	Spain	30TXN52	■	■								
Candidula arganica	EHUMC-1427	Monastery of Leyre	Navarra	Spain	30TXN52	■									
Candidula arganica	EHUMC-1428	Monastery of Leyre	Navarra	Spain	30TXN52	■									
Candidula arganica	EHUMC-1429	Monastery of Leyre	Navarra	Spain	30TXN52	■									
Candidula arganica	EHUMC-1430	Sanctuary of San Miguel de Aralar	Navarra	Spain	30TWN85	■									
Candidula arganica	EHUMC-1431	Sanctuary of San Miguel de Aralar	Navarra	Spain	30TWN85	■									
Candidula arganica	EHUMC-1432	Sanctuary of San Miguel de Aralar	Navarra	Spain	30TWN85	■									
Candidula arganica	EHUMC-1433	Sanctuary of San Miguel de Aralar	Navarra	Spain	30TWN85	■									
Candidula arganica	EHUMC-1434	Sanctuary of San Miguel de Aralar	Navarra	Spain	30TWN85	■									
Candidula arganica	EHUMC-1435	Sanctuary of San Miguel de Aralar	Navarra	Spain	30TWN85	■									
Candidula arganica	EHUMC-1436	Sanctuary of San Miguel de Aralar	Navarra	Spain	30TWN85	■									
Candidula arganica	EHUMC-1437	Sanctuary of San Miguel de Aralar	Navarra	Spain	30TWN85	■									
Candidula arganica	EHUMC-1438	Sanctuary of San Miguel de Aralar	Navarra	Spain	30TWN85	■									
Candidula arganica	EHUMC-1439	Beriain mountain	Navarra	Spain	30TWN84	■									
Candidula arganica	EHUMC-1440	Beriain mountain	Navarra	Spain	30TWN84	■	■								
Candidula arganica	EHUMC-1441	Beriain mountain	Navarra	Spain	30TWN84	■	■	■		■		■	■	■	
Candidula arganica	EHUMC-1442	Beriain mountain	Navarra	Spain	30TWN84	■									
Candidula arganica	EHUMC-1443	Beriain mountain	Navarra	Spain	30TWN84	■									
Candidula arganica	EHUMC-1444	Beriain mountain	Navarra	Spain	30TWN84	■									
Candidula arganica	EHUMC-1445	Beriain mountain	Navarra	Spain	30TWN84	■									
Candidula arganica	EHUMC-1446	Mendizalai. Sierra de Urbasa	Navarra	Spain	30TWN74	■									
Candidula arganica	EHUMC-1447	Mendizalai. Sierra de Urbasa	Navarra	Spain	30TWN74	■									

Species	Voucher	Locality	Province	Country	UTM 10 km	1	2	3	4	5	6	7	8	9	10
Candidula arganica	EHUMC-1448	Mendizalai. Sierra de Urbasa	Navarra	Spain	30TWN74	■									
Candidula arganica	EHUMC-1449	Mendizalai. Sierra de Urbasa	Navarra	Spain	30TWN74	■									
Candidula arganica	EHUMC-1450	Mendizalai. Sierra de Urbasa	Navarra	Spain	30TWN74	■									
Candidula arganica	EHUMC-1451	Mendizalai. Sierra de Urbasa	Navarra	Spain	30TWN74	■									
Candidula arganica	EHUMC-1452	Mendizalai. Sierra de Urbasa	Navarra	Spain	30TWN74	■									
Candidula arganica	EHUMC-1453	Mendizalai. Sierra de Urbasa	Navarra	Spain	30TWN74	■									
Candidula arganica	EHUMC-1454	Orduña mountain pass	Burgos	Spain	30TVN95	■									
Candidula arganica	EHUMC-1455	Orduña mountain pass	Burgos	Spain	30TVN95	■									
Candidula arganica	EHUMC-1456	Orduña mountain pass	Burgos	Spain	30TVN95	■									
Candidula arganica	EHUMC-1457	Orduña mountain pass	Burgos	Spain	30TVN95	■									
Candidula arganica	EHUMC-1458	Orduña mountain pass	Burgos	Spain	30TVN95	■									
Candidula arganica	EHUMC-1459	Orduña mountain pass	Burgos	Spain	30TVN95	■									
Candidula arganica	EHUMC-1460	Orduña mountain pass	Burgos	Spain	30TVN95	■									
Candidula arganica	EHUMC-1461	Orduña mountain pass	Burgos	Spain	30TVN95	■									
Candidula arganica	EHUMC-1462	Orduña mountain pass	Burgos	Spain	30TVN95	■									
Candidula arganica	EHUMC-1463	Vallegrull. Valderejo	Álava	Spain	30TVN74	■									
Candidula arganica	EHUMC-1464	Vallegrull. Valderejo	Álava	Spain	30TVN74	■									
Candidula arganica	EHUMC-1465	Vallegrull. Valderejo	Álava	Spain	30TVN74	■									
Candidula arganica	EHUMC-1466	Berrostegieta	Álava	Spain	30TWN23	■									
Candidula arganica	EHUMC-1467	Berrostegieta	Álava	Spain	30TWN23	■									
Candidula arganica	EHUMC-1468	Berrostegieta	Álava	Spain	30TWN23	■									
Candidula arganica	EHUMC-1469	Berrostegieta	Álava	Spain	30TWN23	■									
Candidula arganica	EHUMC-1470	Berrostegieta	Álava	Spain	30TWN23	■									
Candidula arganica	EHUMC-1471	Loza	Navarra	Spain	30TXN04	■									
Candidula arganica	EHUMC-1472	Loza	Navarra	Spain	30TXN04	■	■								
Candidula arganica	EHUMC-1871	Loza	Navarra	Spain	30TXN04	■	■	■		■	■	■	■	■	
Candidula arganica	EHUMC-1473	Loza	Navarra	Spain	30TXN04	■									
Candidula arrabidensis	EHUMC-1620	Serra da Arrábida	Setúbal	Portugal	29SMC95	■	■	■	■	■	■	■		■	■
Candidula arrabidensis	EHUMC-1621	Serra da Arrábida	Setúbal	Portugal	29SMC95	■	■								

Species	Voucher	Locality	Province	Country	UTM 10 km	1	2	3	4	5	6	7	8	9	10
Candidula arrabidensis	EHUMC-1622	Serra da Arrábida	Setúbal	Portugal	29SMC95	■									
Candidula arrabidensis	EHUMC-1623	Serra da Arrábida	Setúbal	Portugal	29SMC95	■									
Candidula arrabidensis	EHUMC-1624	Serra da Arrábida	Setúbal	Portugal	29SMC95	■									
Candidula arrabidensis	EHUMC-1625	Serra da Arrábida	Setúbal	Portugal	29SMC95	■									
Candidula arrabidensis	EHUMC-1626	Serra da Arrábida	Setúbal	Portugal	29SMC95	■									
Candidula arrabidensis	EHUMC-1627	Serra da Arrábida	Setúbal	Portugal	29SMC95	■									
Candidula arrabidensis	EHUMC-1628	Serra da Arrábida	Setúbal	Portugal	29SNC06	■	■								
Candidula arrabidensis	EHUMC-1629	Serra da Arrábida	Setúbal	Portugal	29SNC06	■	■	■	■	■	■	■		■	■
Candidula arrabidensis	EHUMC-1630	Serra da Arrábida	Setúbal	Portugal	29SNC06	■									
Candidula arrabidensis	EHUMC-1631	Serra da Arrábida	Setúbal	Portugal	29SNC06	■									
Candidula arrabidensis	EHUMC-1632	Serra da Arrábida	Setúbal	Portugal	29SNC06	■									
Candidula arrabidensis	EHUMC-1633	Serra da Arrábida	Setúbal	Portugal	29SNC06	■									
Candidula belemensis	EHUMC-1732	Vale da Couda	Leiria	Portugal	29SNE41	■	■	■	■	■	■	■	■	■	■
Candidula belemensis	EHUMC-1733	Vale da Couda	Leiria	Portugal	29SNE41	■	■	■		■	■		■		■
Candidula belemensis	EHUMC-1734	Vale da Couda	Leiria	Portugal	29SNE41	■									
Candidula belemensis	EHUMC-1735	Vale da Couda	Leiria	Portugal	29SNE41	■									
Candidula belemensis	EHUMC-1736	Vale da Couda	Leiria	Portugal	29SNE41	■									
Candidula belemensis	EHUMC-1737	Vale da Couda	Leiria	Portugal	29SNE41	■									
Candidula belemensis	EHUMC-1738	Vale da Couda	Leiria	Portugal	29SNE41	■									
Candidula belemensis	EHUMC-1739	Vale da Couda	Leiria	Portugal	29SNE41	■									
Candidula belemensis	EHUMC-1740	Vale da Couda	Leiria	Portugal	29SNE41	■									
Candidula camporroblensis	EHUMC-1478	Zael	Burgos	Spain	30TVM36	■									
Candidula camporroblensis	EHUMC-1479	Zael	Burgos	Spain	30TVM36	■									
Candidula camporroblensis	EHUMC-1480	Zael	Burgos	Spain	30TVM36	■	■	■	■	■	■	■	■	■	■
Candidula camporroblensis	EHUMC-1481	Zael	Burgos	Spain	30TVM36	■									
Candidula camporroblensis	EHUMC-1482	Zael	Burgos	Spain	30TVM36	■									
Candidula camporroblensis	EHUMC-1483	Zael	Burgos	Spain	30TVM36	■									
Candidula camporroblensis	EHUMC-1484	Zael	Burgos	Spain	30TVM36	■									
Candidula camporroblensis	EHUMC-1485	Zael	Burgos	Spain	30TVM36	■									

Species	**Voucher**	**Locality**	**Province**	**Country**	**UTM 10 km**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Candidula camporroblensis	EHUMC-1486	Zael	Burgos	Spain	30TVM36	■									
Candidula camporroblensis	EHUMC-1487	Zael	Burgos	Spain	30TVM36	■									
Candidula camporroblensis	EHUMC-1488	Paules del Agua	Burgos	Spain	30TVM35	■									
Candidula camporroblensis	EHUMC-1489	Paules del Agua	Burgos	Spain	30TVM35	■									
Candidula camporroblensis	EHUMC-1490	Paules del Agua	Burgos	Spain	30TVM35	■									
Candidula camporroblensis	EHUMC-1491	Paules del Agua	Burgos	Spain	30TVM35	■									
Candidula camporroblensis	EHUMC-1492	Paules del Agua	Burgos	Spain	30TVM35	■	■			■	■	■	■	■	■
Candidula camporroblensis	EHUMC-1493	Paules del Agua	Burgos	Spain	30TVM35	■									
Candidula camporroblensis	EHUMC-1494	Paules del Agua	Burgos	Spain	30TVM35	■									
Candidula camporroblensis	EHUMC-1495	Paules del Agua	Burgos	Spain	30TVM35	■									
Candidula camporroblensis	EHUMC-1854	Ciudad encantanda	Cuenca	Spain	30TWK85	■									
Candidula camporroblensis	EHUMC-1496	Ciudad encantanda	Cuenca	Spain	30TWK85	■									
Candidula camporroblensis	EHUMC-1497	Ciudad encantanda	Cuenca	Spain	30TWK85	■									
Candidula camporroblensis	EHUMC-1498	Ciudad encantanda	Cuenca	Spain	30TWK85	■									
Candidula camporroblensis	EHUMC-1499	Ciudad encantanda	Cuenca	Spain	30TWK85	■									
Candidula camporroblensis	EHUMC-1855	Ciudad encantanda	Cuenca	Spain	30TWK85	■	■	■		■	■	■	■		■
Candidula camporroblensis	EHUMC-1500	Ciudad encantanda	Cuenca	Spain	30TWK85	■	■	■		■	■	■			■
Candidula camporroblensis	EHUMC-1501	Camporrobles	Castellón	Spain	30SXJ38	■	■	■		■	■	■	■		■
Candidula camporroblensis	EHUMC-1502	Camporrobles	Castellón	Spain	30SXJ38	■									
Candidula camporroblensis	EHUMC-1503	Camporrobles	Castellón	Spain	30SXJ38	■									
Candidula camporroblensis	EHUMC-1859	Camporrobles	Castellón	Spain	30SXJ38	■									
Candidula camporroblensis	EHUMC-1860	Camporrobles	Castellón	Spain	30SXJ38	■	■	■							
Candidula camporroblensis	EHUMC-1861	Camporrobles	Castellón	Spain	30SXJ38	■									
Candidula camporroblensis	EHUMC-1504	Camporrobles	Castellón	Spain	30SXJ38	■									
Candidula camporroblensis	EHUMC-1862	Camporrobles	Castellón	Spain	30SXJ38	■									
Candidula camporroblensis	EHUMC-1863	Camporrobles	Castellón	Spain	30SXJ38	■									
Candidula camporroblensis	EHUMC-1508	Peñalcázar	Soria	Spain	30TWM70	■									
Candidula camporroblensis	EHUMC-1858	Peñalcázar	Soria	Spain	30TWM70	■		■	■	■	■	■		■	■
Candidula camporroblensis	EHUMC-1509	Peñalcázar	Soria	Spain	30TWM70	■									

Species	**Voucher**	**Locality**	**Province**	**Country**	**UTM 10 km**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Candidula camporroblensis	EHUMC-1510	Peñalcázar	Soria	Spain	30TWM70	■									
Candidula camporroblensis	EHUMC-1505	Peñalcázar	Soria	Spain	30TWM70	■									
Candidula camporroblensis	EHUMC-1506	Peñalcázar	Soria	Spain	30TWM70	■									
Candidula camporroblensis	EHUMC-1507	Peñalcázar	Soria	Spain	30TWM70	■									
Candidula camporroblensis	EHUMC-1411	Sierra de Miñana, Deza	Soria	Spain	30TWL79	■	■		■	■	■	■	■	■	■
Candidula cavannae	EHUMC-1512	Strada per Campitello Matese	Campobasso	Italy	33TVF68	■									
Candidula cavannae	EHUMC-1513	Strada per Campitello Matese	Campobasso	Italy	33TVF68	■									
Candidula cavannae	EHUMC-1514	Strada per Campitello Matese	Campobasso	Italy	33TVF68	■									
Candidula cavannae	EHUMC-1515	Strada per Campitello Matese	Campobasso	Italy	33TVF68	■									
Candidula cavannae	EHUMC-1516	Strada per Campitello Matese	Campobasso	Italy	33TVF68	■									
Candidula cavannae	EHUMC-1517	Strada per Campitello Matese	Campobasso	Italy	33TVF68	■									
Candidula cavannae	EHUMC-1518	Strada per Campitello Matese	Campobasso	Italy	33TVF68	■									
Candidula cavannae	EHUMC-1519	Strada per Campitello Matese	Campobasso	Italy	33TVF68	■									
Candidula cavannae	EHUMC-1520	Strada per Campitello Matese	Campobasso	Italy	33TVF68	■	■								
Candidula cavannae	EHUMC-1521	Strada per Campitello Matese	Campobasso	Italy	33TVF68	■	■	■	■					■	
Candidula cavannae	EHUMC-1522	Campitello Matese	Campobasso	Italy	33TVF49	■	■	■	■					■	
Candidula cavannae	EHUMC-1523	Campitello Matese	Campobasso	Italy	33TVF49	■	■	■	■					■	
Candidula cavannae	EHUMC-1524	Campitello Matese	Campobasso	Italy	33TVF49	■									
Candidula cavannae	EHUMC-1525	Campitello Matese	Campobasso	Italy	33TVF49	■									
Candidula cavannae	EHUMC-1526	Campitello Matese	Campobasso	Italy	33TVF49	■									
Candidula cavannae	EHUMC-1527	Campitello Matese	Campobasso	Italy	33TVF49	■									
Candidula cavannae	EHUMC-1528	Campitello Matese	Campobasso	Italy	33TVF49	■									
Candidula cavannae	EHUMC-1529	Campitello Matese	Campobasso	Italy	33TVF49	■									
Candidula cavannae	EHUMC-1530	Campitello Matese	Campobasso	Italy	33TVF49	■									
Candidula cavannae	EHUMC-1531	Campitello Matese	Campobasso	Italy	33TVF49	■									
Candidula codia	EHUMC-1532	NE Boliqueime	Faro	Portugal	29SNB71	■	■	■	■						■
Candidula codia	EHUMC-1533	NE Boliqueime	Faro	Portugal	29SNB71	■									
Candidula codia	EHUMC-1534	NE Boliqueime	Faro	Portugal	29SNB71	■									
Candidula codia	EHUMC-1535	NE Boliqueime	Faro	Portugal	29SNB71	■									

Species	Voucher	Locality	Province	Country	UTM 10 km	1	2	3	4	5	6	7	8	9	10
Candidula corbellai	EHUMC-1536	Pla de Busa. Navés	Lleida	Spain	31TCG86	■									
Candidula corbellai	EHUMC-1537	Pla de Busa. Navés	Lleida	Spain	31TCG86	■	■	■		■	■	■	■	■	
Candidula corbellai	EHUMC-1538	Pla de Busa. Navés	Lleida	Spain	31TCG86	■									
Candidula corbellai	EHUMC-1539	Sierra de Busa. Navés	Lleida	Spain	31TCG86	■	■	■		■	■	■	■	■	
Candidula corbellai	EHUMC-1540	Sierra de Busa. Navés	Lleida	Spain	31TCG86	■	■								
Candidula corbellai	EHUMC-1541	Sierra de Busa. Navés	Lleida	Spain	31TCG86	■									
Candidula corbellai	EHUMC-1542	Sant Cristòfol de Busa hermitage. Navés	Lleida	Spain	31TCG86	■									
Candidula corbellai	EHUMC-1543	Sant Cristòfol de Busa hermitage. Navés	Lleida	Spain	31TCG86	■									
Candidula corbellai	EHUMC-1544	Sant Cristòfol de Busa hermitage. Navés	Lleida	Spain	31TCG86	■									
Candidula coudensis	EHUMC-1864	Vale da Couda	Leiria	Portugal	29SNE41		■	■		■	■	■	■	■	■
Candidula fiorii	EHUMC-1546	Grans Sasso. Campo Pericoli	L'Aquila	Italy	33TUH80	■									
Candidula fiorii	EHUMC-1547	Grans Sasso. Campo Pericoli	L'Aquila	Italy	33TUH80	■	■	■	■	■	■	■		■	
Candidula fiorii	EHUMC-1548	Grans Sasso. Campo Pericoli	L'Aquila	Italy	33TUH80	■	■	■	■	■	■	■		■	
Candidula fiorii	EHUMC-1549	Grans Sasso. Campo Pericoli	L'Aquila	Italy	33TUH80	■									
Candidula fiorii	EHUMC-1550	Grans Sasso. Campo Pericoli	L'Aquila	Italy	33TUH80	■									
Candidula fiorii	EHUMC-1551	Grans Sasso. Campo Pericoli	L'Aquila	Italy	33TUH80	■									
Candidula gigaxii	EHUMC-1552	Valdepeñas de Jaén	Jaén	Spain	30SVG25	■	■		■		■	■	■	■	■
Candidula gigaxii	EHUMC-1553	Río Bogarra, Bogarra	Albacete	Spain	30SWH67				■			■	■		
Candidula gigaxii	EHUMC-1873	Coudoux	Bouches-du-Rhône	France	31TFJ82		■	■		■	■		■	■	■
Candidula gigaxii	EHUMC-1554	M-514 km 4 road. N Moncarapacho	Faro	Portugal	29SPB00	■	■	■	■		■		■	■	
Candidula gigaxii	EHUMC-1557	Montagne de la Loube	Var	France	31TGJ40	■	■	■	■	■		■	■	■	■
Candidula gigaxii	EHUMC-1555	Saint-Paul-de-Vence	Alpes-Maritimes	France	32TLP43		■	■				■	■	■	
Candidula grovesiana	EHUMC-1558	Castrovalva, Gole de Sagittario (Riserva Regionale)	L'Aquila	Italy	33TVG04	■	■								
Candidula grovesiana	EHUMC-1559	Castrovalva, Gole de Sagittario (Riserva Regionale)	L'Aquila	Italy	33TVG04	■	■	■	■	■	■	■	■	■	
Candidula grovesiana	EHUMC-1560	Castrovalva, Gole de Sagittario (Riserva Regionale)	L'Aquila	Italy	33TVG04	■									
Candidula grovesiana	EHUMC-1561	Castrovalva, Gole de Sagittario (Riserva Regionale)	L'Aquila	Italy	33TVG04	■									
Candidula grovesiana	EHUMC-1562	Castrovalva, Gole de Sagittario (Riserva Regionale)	L'Aquila	Italy	33TVG04	■									
Candidula grovesiana	EHUMC-1563	Castrovalva, Gole de Sagittario (Riserva Regionale)	L'Aquila	Italy	33TVG04	■									
Candidula grovesiana	EHUMC-1564	Castrovalva, Gole de Sagittario (Riserva Regionale)	L'Aquila	Italy	33TVG04	■									

Species	Voucher	Locality	Province	Country	UTM 10 km	1	2	3	4	5	6	7	8	9	10
Candidula grovesiana	EHUMC-1565	Castrovalva, Gole de Sagittario (Riserva Regionale)	L'Aquila	Italy	33TVG04	■									
Candidula grovesiana	EHUMC-1566	Monte Genzana	L'Aquila	Italy	33TVG14	■									
Candidula grovesiana	EHUMC-1567	Monte Genzana	L'Aquila	Italy	33TVG14	■									
Candidula grovesiana	EHUMC-1568	Monte Genzana	L'Aquila	Italy	33TVG14	■									
Candidula grovesiana	EHUMC-1569	Monte Genzana	L'Aquila	Italy	33TVG14	■									
Candidula grovesiana	EHUMC-1570	Monte Genzana	L'Aquila	Italy	33TVG14	■									
Candidula grovesiana	EHUMC-1571	Monte Genzana	L'Aquila	Italy	33TVG14	■									
Candidula grovesiana	EHUMC-1572	Monte Genzana	L'Aquila	Italy	33TVG14	■	■	■							
Candidula grovesiana	EHUMC-1573	Monte Genzana	L'Aquila	Italy	33TVG14	■	■	■	■	■	■	■	■	■	
Candidula grovesiana	EHUMC-1574	Monte Marsicano	L'Aquila	Italy	33TVG02	■	■	■	■	■	■	■	■	■	
Candidula grovesiana	EHUMC-1575	Monte Marsicano	L'Aquila	Italy	33TVG02	■	■	■		■	■	■	■		
Candidula intersecta	EHUMC-1576	Artxanda, Bilbao	Bizkaia	Spain	30TWN18	■	■	■		■	■	■	■	■	■
Candidula intersecta	EHUMC-1577	Artxanda, Bilbao	Bizkaia	Spain	30TWN18	■	■								
Candidula intersecta	EHUMC-1578	Artxanda, Bilbao	Bizkaia	Spain	30TWN18	■									
Candidula intersecta	EHUMC-1579	Artxanda, Bilbao	Bizkaia	Spain	30TWN18	■									
Candidula intersecta	EHUMC-1580	Artxanda, Bilbao	Bizkaia	Spain	30TWN18	■									
Candidula intersecta	EHUMC-1581	Artxanda, Bilbao	Bizkaia	Spain	30TWN18	■									
Candidula intersecta	EHUMC-1582	Artxanda, Bilbao	Bizkaia	Spain	30TWN18	■									
Candidula intersecta	EHUMC-1583	Artxanda, Bilbao	Bizkaia	Spain	30TWN18	■									
Candidula intersecta	EHUMC-1584	Artxanda, Bilbao	Bizkaia	Spain	30TWN18	■									
Candidula intersecta	EHUMC-1585	Sanctuary of Arantzazu. Oñati	Gipuzkoa	Spain	30TWN46	■									
Candidula intersecta	EHUMC-1586	Sanctuary of Arantzazu. Oñati	Gipuzkoa	Spain	30TWN46	■									
Candidula intersecta	EHUMC-1587	Sanctuary of Arantzazu. Oñati	Gipuzkoa	Spain	30TWN46	■	■	■	■		■	■		■	■
Candidula intersecta	EHUMC-1588	Sanctuary of Arantzazu. Oñati	Gipuzkoa	Spain	30TWN46	■	■								
Candidula intersecta	EHUMC-1589	Sanctuary of Arantzazu. Oñati	Gipuzkoa	Spain	30TWN46	■									
Candidula intersecta	EHUMC-1590	Sanctuary of Arantzazu. Oñati	Gipuzkoa	Spain	30TWN46	■									
Candidula intersecta	EHUMC-1591	Sanctuary of Arantzazu. Oñati	Gipuzkoa	Spain	30TWN46	■									
Candidula intersecta	EHUMC-1592	Santimamiñe cave	Bizkaia	Spain	30TWN29	■									
Candidula intersecta	EHUMC-1593	Oia	Pontevedra	Spain	29TGN04	■									

Species	Voucher	Locality	Province	Country	UTM 10 km	1	2	3	4	5	6	7	8	9	10
Candidula intersecta	EHUMC-1594	Oia	Pontevedra	Spain	29TGN04	■									
Candidula intersecta	EHUMC-1595	Oia	Pontevedra	Spain	29TGN04	■									
Candidula intersecta	EHUMC-1596	Oia	Pontevedra	Spain	29TGN04	■									
Candidula intersecta	EHUMC-1597	Oia	Pontevedra	Spain	29TGN04	■	■	■	■	■	■	■			
Candidula intersecta	EHUMC-1598	Oia	Pontevedra	Spain	29TGN04	■	■								
Candidula intersecta	EHUMC-1599	Oia	Pontevedra	Spain	29TGN04	■									
Candidula intersecta	EHUMC-1600	Oia	Pontevedra	Spain	29TGN04	■									
Candidula intersecta	EHUMC-1601	Pico Sacro	A Coruña	Spain	29TNH43	■									
Candidula intersecta	EHUMC-1602	Pico Sacro	A Coruña	Spain	29TNH43	■									
Candidula intersecta	EHUMC-1603	Pico Sacro	A Coruña	Spain	29TNH43	■									
Candidula intersecta	EHUMC-1604	Pico Sacro	A Coruña	Spain	29TNH43	■	■	■			■	■	■		■
Candidula intersecta	EHUMC-1605	Pico Sacro	A Coruña	Spain	29TNH43	■	■								
Candidula intersecta	EHUMC-1606	N-240 km 22 road. S Ubidea	Álava	Spain	30TWN29	■									
Candidula intersecta	EHUMC-1607	N-240 km 22 road. S Ubidea	Álava	Spain	30TWN29	■									
Candidula intersecta	EHUMC-1608	N-240 km 22 road. S Ubidea	Álava	Spain	30TWN29	■									
Candidula intersecta	EHUMC-1609	N-240 km 22 road. S Ubidea	Álava	Spain	30TWN29	■									
Candidula intersecta	EHUMC-1610	N-240 km 22 road. S Ubidea	Álava	Spain	30TWN29	■	■	■	■		■	■		■	
Candidula intersecta	EHUMC-1611	N-240 km 22 road. S Ubidea	Álava	Spain	30TWN29	■	■								
Candidula intersecta	EHUMC-1612	N-240 km 22 road. S Ubidea	Álava	Spain	30TWN29	■									
Candidula intersecta	EHUMC-1613	N-240 km 22 road. S Ubidea	Álava	Spain	30TWN29	■									
Candidula intersecta	EHUMC-1614	University of Cambridge Botanic Gardens. Cambridge	England	England	31UCT08	■	■								
Candidula intersecta	EHUMC-1615	University of Cambridge Botanic Gardens. Cambridge	England	England	31UCT08	■									
Candidula intersecta	EHUMC-1616	Vila Faia, Livramento. Ilha de Sao Miguel	Açores	Portugal	26SPG28	■	■								
Candidula intersecta	EHUMC-1617	4.5 km W of Castricum	Noord-Holland	Netherlands	31UFU02	■									
Candidula intersecta	EHUMC-1618	3 km WNW of Heemskerk	Noord-Holland	Netherlands	31UFU02	■	■	■							
Candidula intersecta	EHUMC-1619	2 km NW of Castricum	Noord-Holland	Netherlands	31UFU02	■									
Candidula najerensis	EHUMC-1637	Miño de Medinaceli	Soria	Spain	30TWL45	■									
Candidula najerensis	EHUMC-1638	Miño de Medinaceli	Soria	Spain	30TWL45	■									
Candidula najerensis	EHUMC-1639	Miño de Medinaceli	Soria	Spain	30TWL45	■									

Species	**Voucher**	**Locality**	**Province**	**Country**	**UTM 10 km**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Candidula najerensis	EHUMC-1640	Miño de Medinaceli	Soria	Spain	30TWL45	■	■	■	■	■		■	■	■	■
Candidula najerensis	EHUMC-1641	Miño de Medinaceli	Soria	Spain	30TWL45	■	■								
Candidula najerensis	EHUMC-1642	Miño de Medinaceli	Soria	Spain	30TWL45	■									
Candidula najerensis	EHUMC-1643	Miño de Medinaceli	Soria	Spain	30TWL45	■									
Candidula najerensis	EHUMC-1644	Nájera	La Rioja	Spain	30TWM29	■									
Candidula najerensis	EHUMC-1645	Nájera	La Rioja	Spain	30TWM29	■	■	■		■	■	■		■	■
Candidula najerensis	EHUMC-1646	Nájera	La Rioja	Spain	30TWM29	■	■								
Candidula najerensis	EHUMC-1647	Uncastillo	Zaragoza	Spain	30TXM58	■									
Candidula najerensis	EHUMC-1648	Magaña	Soria	Spain	30TWM73	■									
Candidula najerensis	EHUMC-1649	Magaña	Soria	Spain	30TWM73	■									
Candidula najerensis	EHUMC-1650	Magaña	Soria	Spain	30TWM73	■									
Candidula najerensis	EHUMC-1651	Magaña	Soria	Spain	30TWM73	■									
Candidula najerensis	EHUMC-1652	Magaña	Soria	Spain	30TWM73	■									
Candidula najerensis	EHUMC-1653	Magaña	Soria	Spain	30TWM73	■									
Candidula najerensis	EHUMC-1654	Magaña	Soria	Spain	30TWM73	■									
Candidula najerensis	EHUMC-1655	Magaña	Soria	Spain	30TWM73	■									
Candidula najerensis	EHUMC-1656	Valdemadera	La Rioja	Spain	30TWM74	■									
Candidula najerensis	EHUMC-1657	Calcena	Zaragoza	Spain	30TXM01	■									
Candidula najerensis	EHUMC-1658	Alto de los Almudejos, Beratón	Soria	Spain	30TXM02	■	■								
Candidula najerensis	EHUMC-1659	Alto de los Almudejos, Beratón	Soria	Spain	30TXM02	■	■	■	■	■	■	■	■	■	■
Candidula najerensis	EHUMC-1660	Alto de los Almudejos, Beratón	Soria	Spain	30TXM02	■									
Candidula najerensis	EHUMC-1661	Alto de los Almudejos, Beratón	Soria	Spain	30TXM02	■									
Candidula najerensis	EHUMC-1662	Alto de los Almudejos, Beratón	Soria	Spain	30TXM02	■									
Candidula najerensis	EHUMC-1663	Alto de los Almudejos, Beratón	Soria	Spain	30TXM02	■									
Candidula najerensis	EHUMC-1664	Alto de los Almudejos, Beratón	Soria	Spain	30TXM02	■									
Candidula najerensis	EHUMC-1665	Alto de los Almudejos, Beratón	Soria	Spain	30TXM02	■									
Candidula najerensis	EHUMC-1666	Alto de los Almudejos, Beratón	Soria	Spain	30TXM02	■									
Candidula najerensis	EHUMC-1667	Alto de los Almudejos, Beratón	Soria	Spain	30TXM02	■									
Candidula najerensis	EHUMC-1668	Muela de Beratón	Soria	Spain	30TXM01	■									

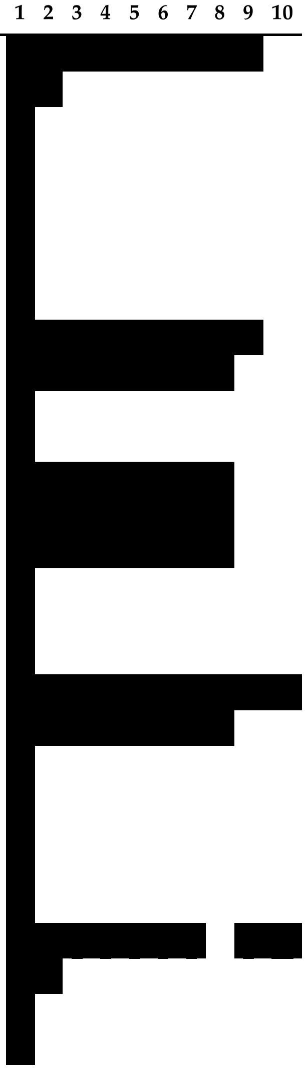
Species	Voucher	Locality	Province	Country	UTM 10 km	1	2	3	4	5	6	7	8	9	10
Candidula najerensis	EHUMC-1669	Muela de Beratón	Soria	Spain	30TXM01	■									
Candidula najerensis	EHUMC-1670	Muela de Beratón	Soria	Spain	30TXM01	■									
Candidula najerensis	EHUMC-1671	Muela de Beratón	Soria	Spain	30TXM01	■									
Candidula najerensis	EHUMC-1672	Muela de Beratón	Soria	Spain	30TXM01	■									
Candidula najerensis	EHUMC-1673	Muela de Beratón	Soria	Spain	30TXM01	■									
Candidula najerensis	EHUMC-1674	Muela de Beratón	Soria	Spain	30TXM01	■									
Candidula najerensis	EHUMC-1675	Muela de Beratón	Soria	Spain	30TXM01	■									
Candidula najerensis	EHUMC-1676	Muela de Beratón	Soria	Spain	30TXM01	■									
Candidula najerensis	EHUMC-1677	Ciria	Soria	Spain	30TWM80	■									
Candidula najerensis	EHUMC-1678	Ciria	Soria	Spain	30TWM80	■									
Candidula najerensis	EHUMC-1679	Ciria	Soria	Spain	30TWM80	■									
Candidula najerensis	EHUMC-1680	Ciria	Soria	Spain	30TWM80	■									
Candidula najerensis	EHUMC-1681	Ciria	Soria	Spain	30TWM80	■									
Candidula najerensis	EHUMC-1682	Ciria	Soria	Spain	30TWM80	■									
Candidula najerensis	EHUMC-1683	Ciria	Soria	Spain	30TWM80	■									
Candidula najerensis	EHUMC-1684	Ciria	Soria	Spain	30TWM80	■									
Candidula najerensis	EHUMC-1685	Ciria	Soria	Spain	30TWM80	■									
Candidula najerensis	EHUMC-1686	Ciria	Soria	Spain	30TWM80	■									
Candidula najerensis	EHUMC-1691	Cardejón, Almenas de Soria	Soria	Spain	30TWM71	■									
Candidula najerensis	EHUMC-1857	Cardejón, Almenas de Soria	Soria	Spain	30TWM71	■									
Candidula najerensis	EHUMC-1692	Cardejón, Almenas de Soria	Soria	Spain	30TWM71	■	■	■	■	■		■	■	■	■
Candidula najerensis	EHUMC-1856	Cardejón, Almenas de Soria	Soria	Spain	30TWM71	■	■	■	■	■	■	■	■	■	■
Candidula najerensis	EHUMC-1687	Cardejón, Almenas de Soria	Soria	Spain	30TWM71	■									
Candidula najerensis	EHUMC-1688	Cardejón, Almenas de Soria	Soria	Spain	30TWM71	■									
Candidula najerensis	EHUMC-1689	Cardejón, Almenas de Soria	Soria	Spain	30TWM71	■									
Candidula najerensis	EHUMC-1690	Cardejón, Almenas de Soria	Soria	Spain	30TWM71	■									
Candidula najerensis	EHUMC-1693	Sierra de Miñana, Deza	Soria	Spain	30TWL79	■	■	■	■	■	■	■		■	■
Candidula najerensis	EHUMC-1694	Nájera	La Rioja	Spain	30TWM29	■									
Candidula najerensis	EHUMC-1695	Azagra	Navarra	Spain	30TWM98	■									

Species	Voucher	Locality	Province	Country	UTM 10 km	1	2	3	4	5	6	7	8	9	10
Candidula najerensis	EHUMC-1696	Azagra	Navarra	Spain	30TWM98	■									
Candidula najerensis	EHUMC-1697	Azagra	Navarra	Spain	30TWM98	■									
Candidula najerensis	EHUMC-1698	Azagra	Navarra	Spain	30TWM98	■	■	■	■	■	■	■	■	■	■
Candidula najerensis	EHUMC-1699	Azagra	Navarra	Spain	30TWM98	■	■	■	■	■	■	■	■	■	■
Candidula najerensis	EHUMC-1700	Azagra	Navarra	Spain	30TWM98	■									
Candidula najerensis	EHUMC-1701	Azagra	Navarra	Spain	30TWM98	■									
Candidula najerensis	EHUMC-1702	Azagra	Navarra	Spain	30TWM98	■									
Candidula najerensis	EHUMC-1703	Azagra	Navarra	Spain	30TWM98	■									
Candidula najerensis	EHUMC-1704	Ablitas	Navarra	Spain	30TXM14	■									
Candidula najerensis	EHUMC-1705	Ablitas	Navarra	Spain	30TXM14	■									
Candidula najerensis	EHUMC-1706	Ablitas	Navarra	Spain	30TXM14	■									
Candidula najerensis	EHUMC-1707	Ablitas	Navarra	Spain	30TXM14	■									
Candidula najerensis	EHUMC-1708	Ablitas	Navarra	Spain	30TXM14	■									
Candidula najerensis	EHUMC-1709	Ablitas	Navarra	Spain	30TXM14	■									
Candidula najerensis	EHUMC-1710	Ablitas	Navarra	Spain	30TXM14	■									
Candidula najerensis	EHUMC-1711	Ablitas	Navarra	Spain	30TXM14	■									
Candidula najerensis	EHUMC-1712	Ablitas	Navarra	Spain	30TXM14	■									
Candidula najerensis	EHUMC-1713	Ablitas	Navarra	Spain	30TXM14	■									
Candidula olisippensis	EHUMC-1714	Lagoa da Vela	Coímbra	Portugal	29TNE15	■	■		■	■	■	■		■	
Candidula olisippensis	EHUMC-1879	Lagoa da Vela	Coímbra	Portugal	29TNE15	■	■								
Candidula olisippensis	EHUMC-1715	Lagoa da Vela	Coímbra	Portugal	29TNE15	■	■		■	■	■	■		■	■
Candidula olisippensis	EHUMC-1716	Lagoa das Braças	Coímbra	Portugal	29TNE15				■	■	■	■			
Candidula olisippensis	EHUMC-1717	Alfarelos	Coímbra	Portugal	29TNE24	■									
Candidula olisippensis	EHUMC-1718	Alfarelos	Coímbra	Portugal	29TNE24	■	■	■	■		■	■	■	■	■
Candidula olisippensis	EHUMC-1719	Alfarelos	Coímbra	Portugal	29TNE24	■	■								
Candidula olisippensis	EHUMC-1720	Alfarelos	Coímbra	Portugal	29TNE24	■									
Candidula olisippensis	EHUMC-1721	Alfarelos	Coímbra	Portugal	29TNE24	■									
Candidula olisippensis	EHUMC-1722	Alfarelos	Coímbra	Portugal	29TNE24	■									
Candidula olisippensis	EHUMC-1723	Alfarelos	Coímbra	Portugal	29TNE24	■									

Species	Voucher	Locality	Province	Country	UTM 10 km	1	2	3	4	5	6	7	8	9	10
Candidula olisippensis	EHUMC-1724	Alfarelos	Coímbra	Portugal	29TNE24	■									
Candidula olisippensis	EHUMC-1725	Alfarelos	Coímbra	Portugal	29TNE24	■									
Candidula olisippensis	EHUMC-1726	Conímbriga Roman ruins	Coímbra	Portugal	29TNE43	■									
Candidula olisippensis	EHUMC-1727	Conímbriga Roman ruins	Coímbra	Portugal	29TNE43	■									
Candidula olisippensis	EHUMC-1728	Conímbriga Roman ruins	Coímbra	Portugal	29TNE43	■									
Candidula olisippensis	EHUMC-1729	Conímbriga Roman ruins	Coímbra	Portugal	29TNE43	■									
Candidula olisippensis	EHUMC-1730	Conímbriga Roman ruins	Coímbra	Portugal	29TNE43	■									
Candidula olisippensis	EHUMC-1731	Conímbriga Roman ruins	Coímbra	Portugal	29TNE43	■									
Candidula olisippensis	EHUMC-1741	Arrimal	Leiria	Portugal	29SND17	■									
Candidula olisippensis	EHUMC-1742	Arrimal	Leiria	Portugal	29SND17	■									
Candidula olisippensis	EHUMC-1743	Arrimal	Leiria	Portugal	29SND17	■	■	■	■		■	■	■	■	■
Candidula olisippensis	EHUMC-1744	Arrimal	Leiria	Portugal	29SND17	■	■	■	■	■	■	■	■	■	■
Candidula olisippensis	EHUMC-1745	Arrimal	Leiria	Portugal	29SND17	■									
Candidula olisippensis	EHUMC-1746	Vinha Velha. Ilha de Santa Maria	Açores	Portugal	26SPF79	■	■								
Candidula olisippensis	EHUMC-1747	Vinha Velha. Ilha de Santa Maria	Açores	Portugal	26SPF79	■									
Candidula olisippensis	EHUMC-1748	Vinha Velha. Ilha de Santa Maria	Açores	Portugal	26SPF79	■									
Candidula olisippensis	EHUMC-1749	Figueiral. Ilha de Santa Maria	Açores	Portugal	26SPF69	■	■								
Candidula olisippensis	EHUMC-1750	Figueiral. Ilha de Santa Maria	Açores	Portugal	26SPF69	■									
Candidula ponsulensis	EHUMC-1634	Pías	Zamora	Spain	29TPG66	■	■	■			■		■	■	
Candidula ponsulensis	EHUMC-1635	Pías	Zamora	Spain	29TPG66	■	■								
Candidula ponsulensis	EHUMC-1636	Pías	Zamora	Spain	29TPG66	■									
Candidula rocandioi	EHUMC-1751	Renedo de Valdetuéjar	León	Spain	30TUN34	■									
Candidula rocandioi	EHUMC-1752	Renedo de Valdetuéjar	León	Spain	30TUN34	■									
Candidula rocandioi	EHUMC-1753	Renedo de Valdetuéjar	León	Spain	30TUN34	■									
Candidula rocandioi	EHUMC-1754	Renedo de Valdetuéjar	León	Spain	30TUN34	■	■	■	■	■		■			
Candidula rocandioi	EHUMC-1755	Renedo de Valdetuéjar	León	Spain	30TUN34	■	■	■	■	■		■			
Candidula rocandioi	EHUMC-1756	Renedo de Valdetuéjar	León	Spain	30TUN34	■									
Candidula rocandioi	EHUMC-1757	Renedo de Valdetuéjar	León	Spain	30TUN34	■									
Candidula rocandioi	EHUMC-1868	Muela de Beratón	Soria	Spain	30TXM01	■	■	■	■	■		■		■	

Species	Voucher	Locality	Province	Country	UTM 10 km	1	2	3	4	5	6	7	8	9	10
Candidula rocandioi	EHUMC-1869	Muela de Beratón	Soria	Spain	30TXM01	■	■	■	■	■		■			
Candidula rocandioi	EHUMC-1758	Muela de Beratón	Soria	Spain	30TXM01	■									
Candidula rocandioi	EHUMC-1759	Muela de Beratón	Soria	Spain	30TXM01	■									
Candidula rocandioi	EHUMC-1760	Muela de Beratón	Soria	Spain	30TXM01	■									
Candidula rocandioi	EHUMC-1761	Mansilla de la Sierra	La Rioja	Spain	30TWM06	■									
Candidula rocandioi	EHUMC-1762	Mansilla de la Sierra	La Rioja	Spain	30TWM06	■	■	■	■	■		■		■	
Candidula rocandioi	EHUMC-1763	Mansilla de la Sierra	La Rioja	Spain	30TWM06	■	■								
Candidula rocandioi	EHUMC-1764	Pancorbo	Burgos	Spain	30TVN92	■									
Candidula rocandioi	EHUMC-1765	Pancorbo	Burgos	Spain	30TVN92	■									
Candidula rocandioi	EHUMC-1766	Pancorbo	Burgos	Spain	30TVN92	■									
Candidula rocandioi	EHUMC-1767	Villanueva de Cameros	La Rioja	Spain	30TWM26	■									
Candidula rocandioi	EHUMC-1768	Villanueva de Cameros	La Rioja	Spain	30TWM26	■									
Candidula rocandioi	EHUMC-1870	Villanueva de Cameros	La Rioja	Spain	30TWM26	■	■	■		■		■		■	
Candidula rocandioi	EHUMC-1769	Villanueva de Cameros	La Rioja	Spain	30TWM26	■	■								
Candidula rocandioi	EHUMC-1770	Villanueva de Cameros	La Rioja	Spain	30TWM26	■									
Candidula rocandioi	EHUMC-1771	Villanueva de Cameros	La Rioja	Spain	30TWM26	■									
Candidula rugosiuscula	EHUMC-1772	Le Thoronet	Var	France	32TKP81	■	■								
Candidula rugosiuscula	EHUMC-1875	Le Thoronet	Var	France	32TKP81	■	■	■							
Candidula rugosiuscula	EHUMC-1773	Le Thoronet	Var	France	32TKP81	■									
Candidula rugosiuscula	EHUMC-1774	Le Thoronet	Var	France	32TKP81	■									
Candidula rugosiuscula	EHUMC-1775	Le Thoronet	Var	France	32TKP81	■									
Candidula rugosiuscula	EHUMC-1776	Le Thoronet	Var	France	32TKP81	■									
Candidula rugosiuscula	EHUMC-1874	Thoronet	Var	France	32TKP82		■		■	■		■	■	■	■
Candidula scabiosula	EHUMC-1474	M-514 km 4 road. N Moncarapacho	Faro	Portugal	29SPB00	■									
Candidula scabiosula	EHUMC-1475	M-514 km 4 road. N Moncarapacho	Faro	Portugal	29SPB00	■									
Candidula scabiosula	EHUMC-1866	M-514 km 4 road. N Moncarapacho	Faro	Portugal	29SPB00	■	■	■	■			■		■	
Candidula scabiosula	EHUMC-1867	M-514 km 4 road. N Moncarapacho	Faro	Portugal	29SPB00	■									
Candidula scabiosula	EHUMC-1476	M-514 km 4 road. N Moncarapacho	Faro	Portugal	29SPB00	■	■	■	■		■	■		■	
Candidula scabiosula	EHUMC-1477	M-514 km 4 road. N Moncarapacho	Faro	Portugal	29SPB00	■	■	■	■			■		■	

Species	Voucher	Locality	Province	Country	UTM 10 km
Candidula setubalensis	EHUMC-1777	Serra da Arrabida	Setúbal	Portugal	29SMC95
Candidula setubalensis	EHUMC-1778	Serra da Arrabida	Setúbal	Portugal	29SMC95
Candidula setubalensis	EHUMC-1779	Serra da Arrabida	Setúbal	Portugal	29SMC95
Candidula setubalensis	EHUMC-1780	Serra da Arrabida	Setúbal	Portugal	29SMC95
Candidula setubalensis	EHUMC-1781	Serra da Arrabida	Setúbal	Portugal	29SMC95
Candidula setubalensis	EHUMC-1782	Serra da Arrabida	Setúbal	Portugal	29SMC95
Candidula setubalensis	EHUMC-1783	Serra da Arrabida	Setúbal	Portugal	29SNC06
Candidula setubalensis	EHUMC-1784	Serra da Arrabida	Setúbal	Portugal	29SNC06
Candidula setubalensis	EHUMC-1785	Serra da Arrabida	Setúbal	Portugal	29SNC06
Candidula setubalensis	EHUMC-1786	Serra da Arrabida	Setúbal	Portugal	29SNC06
Candidula setubalensis	EHUMC-1787	Serra da Arrabida	Setúbal	Portugal	29SNC06
Candidula setubalensis	EHUMC-1788	Serra da Arrabida	Setúbal	Portugal	29SNC06
Candidula spadae	EHUMC-1789	Monte Vettore sopra Forca di Presta	Ascoli Piceno	Italy	33TUH53
Candidula spadae	EHUMC-1790	Presso Forca d'Acero	L'Aquila	Italy	33TVG02
Candidula spadae	EHUMC-1791	Presso Forca d'Acero	L'Aquila	Italy	33TVG02
Candidula spadae	EHUMC-1792	Presso Forca d'Acero	L'Aquila	Italy	33TVG02
Candidula spadae	EHUMC-1793	Presso Forca d'Acero	L'Aquila	Italy	33TVG02
Candidula spadae	EHUMC-1794	Presso Forca d'Acero	L'Aquila	Italy	33TVG02
Candidula spadae	EHUMC-1795	Gran Sasso, Campo Pericoli	L'Aquila	Italaia	33TUH80
Candidula spadae	EHUMC-1796	Gran Sasso, Campo Pericoli	L'Aquila	Italaia	33TUH80
Candidula spadae	EHUMC-1797	Gran Sasso, Campo Pericoli	L'Aquila	Italaia	33TUH80
Candidula spadae	EHUMC-1798	Gran Sasso, Campo Pericoli	L'Aquila	Italaia	33TUH80
Candidula spadae	EHUMC-1799	Gran Sasso, Campo Pericoli	L'Aquila	Italaia	33TUH80
Candidula ultima	EHUMC-1800	Between Betancuria and Pájara. Fuerteventura Island	Las Palmas	Spain	28RES83
Candidula ultima	EHUMC-1801	Between Betancuria and Pájara. Fuerteventura Island	Las Palmas	Spain	28RES83
Candidula ultima	EHUMC-1802	Between Betancuria and Pájara. Fuerteventura Island	Las Palmas	Spain	28RES83
Candidula ultima	EHUMC-1803	Between Betancuria and Pájara. Fuerteventura Island	Las Palmas	Spain	28RES83
Candidula ultima	EHUMC-1804	Between Betancuria and Pájara. Fuerteventura Island	Las Palmas	Spain	28RES83
Candidula ultima	EHUMC-1805	Between Betancuria and Pájara. Fuerteventura Island	Las Palmas	Spain	28RES83



Species	Voucher	Locality	Province	Country	UTM 10 km	1	2	3	4	5	6	7	8	9	10
Candidula ultima	EHUMC-1806	Between Betancuria and Pájara. Fuerteventura Island	Las Palmas	Spain	28RES83	■									
Candidula ultima	EHUMC-1807	Pájara. Fuerteventura Island	Las Palmas	Spain	28RES83	■	■	■	■	■	■	■			■
Candidula unifasciata	EHUMC-1808	Strada per Campo Felice Lucoli	L'Aquila	Italy	33TUG68	■									
Candidula unifasciata	EHUMC-1809	Strada per Campo Felice Lucoli	L'Aquila	Italy	33TUG68	■									
Candidula unifasciata	EHUMC-1810	Strada per Campo Felice Lucoli	L'Aquila	Italy	33TUG68	■									
Candidula unifasciata	EHUMC-1811	Strada per Campo Felice Lucoli	L'Aquila	Italy	33TUG68	■									
Candidula unifasciata	EHUMC-1812	Chiapporato	Bologna	Italy	32TPP68	■	■	■	■	■		■			
Candidula unifasciata	EHUMC-1813	Chiapporato	Bologna	Italy	32TPP68	■	■								
Candidula unifasciata	EHUMC-1814	Chiapporato	Bologna	Italy	32TPP68	■									
Candidula unifasciata	EHUMC-1815	Chiapporato	Bologna	Italy	32TPP68	■									
Candidula unifasciata	EHUMC-1816	Chiapporato	Bologna	Italy	32TPP68	■									
Candidula unifasciata	EHUMC-1817	Campo Cecina. Versante Nord	Massa-Carrara	Italy	32TNP88	■									
Candidula unifasciata	EHUMC-1818	Campo Cecina. Versante Nord	Massa-Carrara	Italy	32TNP88	■									
Candidula unifasciata	EHUMC-1819	Campo Cecina. Versante Nord	Massa-Carrara	Italy	32TNP88	■									
Candidula unifasciata	EHUMC-1820	Campo Cecina. Versante Nord	Massa-Carrara	Italy	32TNP88	■									
Candidula unifasciata	EHUMC-1821	Strada per Monte Sagro. Versante Est	Massa-Carrara	Italy	32TNP98	■									
Candidula unifasciata	EHUMC-1822	Strada per Monte Sagro. Versante Est	Massa-Carrara	Italy	32TNP98	■									
Candidula unifasciata	EHUMC-1823	Strada per Monte Sagro. Versante Est	Massa-Carrara	Italy	32TNP98	■									
Candidula unifasciata	EHUMC-1824	Strada per Monte Sagro. Versante Est	Massa-Carrara	Italy	32TNP98	■									
Candidula unifasciata	EHUMC-1825	Strada per Monte Sagro. Versante Est	Massa-Carrara	Italy	32TNP98	■									
Candidula unifasciata	EHUMC-1826	Strada per Monte Sagro. Versante Est	Massa-Carrara	Italy	32TNP98	■									
Candidula unifasciata	EHUMC-1827	Strada per Monte Sagro. Versante Est	Massa-Carrara	Italy	32TNP98	■									
Candidula unifasciata	EHUMC-1828	Punta Corvo, Montemarcello. Ameglia	La Spezia	Italy	32TNP77	■	■								
Candidula unifasciata	EHUMC-1829	Punta Corvo, Montemarcello. Ameglia	La Spezia	Italy	32TNP77	■	■	■	■	■	■	■	■		
Candidula unifasciata	EHUMC-1830	Castle Fieschi, Savignone	Genova	Italy	32TMQ93	■									
Candidula unifasciata	EHUMC-1872	Foce di Fianza, Campo Cecina, Apuan Alps. Carrara	Massa-Carrara	Italy	32TNP98	■	■	■	■	■		■			
Candidula unifasciata	EHUMC-1831	Foce di Fianza, Campo Cecina, Apuan Alps. Carrara	Massa-Carrara	Italy	32TNP98	■	■								
Candidula unifasciata	EHUMC-1832	Foce di Fianza, Campo Cecina, Apuan Alps. Carrara	Massa-Carrara	Italy	32TNP98	■									
Candidula unifasciata	EHUMC-1833	Foce di Fianza, Campo Cecina, Apuan Alps. Carrara	Massa-Carrara	Italy	32TNP98	■									

Species	Voucher	Locality	Province	Country	UTM 10 km	1	2	3	4	5	6	7	8	9	10
Candidula unifasciata	EHUMC-1834	Between Entrelevie and La Villette. Courmayeur	Aosta	Italy	32TLR47	■									
Candidula unifasciata	EHUMC-1835	Between Entrelevie and La Villette. Courmayeur	Aosta	Italy	32TLR47	■									
Candidula unifasciata	EHUMC-1836	Between Entrelevie and La Villette. Courmayeur	Aosta	Italy	32TLR47	■									
Candidula unifasciata	EHUMC-1837	Between Entrelevie and La Villette. Courmayeur	Aosta	Italy	32TLR47	■									
Candidula unifasciata	EHUMC-1838	Near Crocefieschi. Busalla	Liguria	Italy	32TNQ03	■									
Candidula unifasciata	EHUMC-1839	Near Crocefieschi. Busalla	Liguria	Italy	32TNQ03	■	■	■	■	■	■		■	■	
Candidula unifasciata	EHUMC-1840	Near Crocefieschi. Busalla	Liguria	Italy	32TNQ03	■	■	■	■	■	■		■	■	
Candidula unifasciata	EHUMC-1841	Bosco della Panfilia. Sant Agostino	Ferrara	Italy	32TPQ86	■									
Candidula unifasciata	EHUMC-1842	Bosco della Panfilia. Sant Agostino	Ferrara	Italy	32TPQ86	■									
Candidula unifasciata	EHUMC-1843	Bosco della Panfilia. Sant Agostino	Ferrara	Italy	32TPQ86	■									
Candidula unifasciata	EHUMC-1878	Saint-Jurs	Alpes-de-Haute-Provence	France	32TKP76	■	■								
Candidula unifasciata	EHUMC-1844	Saint-Jurs	Alpes-de-Haute-Provence	France	32TKP76	■									
Candidula unifasciata	EHUMC-1865	Col d'Orme	Alpes-de-Haute-Provence	France	32TLP66	■	■								
Candidula unifasciata	EHUMC-1845	Col d'Orme	Alpes-de-Haute-Provence	France	32TLP66	■	■	■		■		■		■	
Candidula unifasciata	EHUMC-1846	Col d'Orme	Alpes-de-Haute-Provence	France	32TLP66	■									
Candidula unifasciata	EHUMC-1847	Col d'Orme	Alpes-de-Haute-Provence	France	32TLP66	■									
Candidula unifasciata	EHUMC-1556	Grasse	Alpes-Maritimes	France	32TLP33	■	■	■	■	■	■	■	■	■	■
Candidula unifasciata	EHUMC-1876	Winterscheid	Rheinland-Pfalz	Germany	32ULA06	■	■								
Candidula unifasciata	EHUMC-1877	Winterscheid	Rheinland-Pfalz	Germany	32ULA06	■	■								
Candidula unifasciata	EHUMC-1848	Winterscheid	Rheinland-Pfalz	Germany	32ULA06	■									
Candidula unifasciata	EHUMC-1849	Winterscheid	Rheinland-Pfalz	Germany	32ULA06	■									
Candidula unifasciata	EHUMC-1850	Winterscheid	Rheinland-Pfalz	Germany	32ULA06	■									
Candidula unifasciata	EHUMC-1851	Near Cantalupo Ligure (Rocchetta Ligure)	Alessandria	Italy	32TNQ05	■	■								
Candidula unifasciata	EHUMC-1852	Near Cantalupo Ligure (Rocchetta Ligure)	Alessandria	Italy	32TNQ05	■	■								
Candidula unifasciata	EHUMC-1853	Near Cantalupo Ligure (Rocchetta Ligure)	Alessandria	Italy	32TNQ05	■	■								
Cernuella aginnica	EHUMC-1882	Praia As Catedrais, Ribadeo	Lugo	Spain	29TPJ52		■	■							
Cernuella virgata	EHUMC-1883	Leioa	Bizkaia	Spain	30TWN09	■	■	■	■	■					■
Cernuella virgata	EHUMC-1884	Stymfalia lake. Korinthias	Peloponnisos	Greece	34SFG29	■	■	■	■	■		■	■	■	
Cernuella virgata	EHUMC-1897	Tripoli - Sparti 30 km. Lakonias	Peloponnisos	Greece	34SFG22	■	■		■	■			■	■	

Species	Voucher	Locality	Province	Country	UTM 10 km	1	2	3	4	5	6	7	8	9	10
Cernuellopsis ghisottii	EHUMC-1885	Frontignano (MC) Monte Bove - Sibillini	Ascoli Piceno	Italy	33TUH55	■	■	■		■	■		■		■
Helicella iberica	EHUMC-1888	Monastery of Leyre	Navarra	Spain	30TXN52		■	■							
Helicella itala	EHUMC-1887	Aizkorri	Gipuzkoa	Spain	30TWN55	■	■	■	■	■	■	■		■	■
Helicella itala	EHUMC-1898	Gopegi	Álava	Spain	30TWN25	■	■	■		■	■	■	■	■	■
Helicella ordunesis	EHUMC-1889	Orduña mountain pass	Burgos	Spain	30TVN95	■	■	■	■	■		■	■	■	
Helicella valdeona	EHUMC-1890	Canal del Perro	León	Spain	30TUN48		■	■							
Microxeromagna lowei	EHUMC-1891	Fuentemilanos	Segovia	Spain	30TUL92	■	■	■							
Microxeromagna lowei	EHUMC-1901	Fuentemilanos	Segovia	Spain	30TUL92	■	■	■		■	■	■	■		
Microxeromagna lowei	EHUMC-1899	Valdestillas	Valladolid	Spain	30TUL59	■	■	■		■	■	■			
Microxeromagna lowei	EHUMC-1900	Valdestillas	Valladolid	Spain	30TUL59	■	■	■		■	■	■			
Pyrenaearia carascalensis	EHUMC-1880	Aigüestortes, Rocablanca	Lleida	Spain	31TCH31	■	■	■	■	■	■	■	■		■
Trochoidea elegans	EHUMC-1895	Vitoria-Gastiez	Álava	Spain	30TWN24	■		■	■	■		■			■
Xerocrassa barceloi	EHUMC-1413	Orihuela	Alicante	Spain	30SXH81		■	■							
Xerocrassa cisternasi redonae	EHUMC-1894	Illa Redona	Ibiza	Spain	31SCD71	■	■	■	■	■	■	■	■	■	
Xerocrassa penchinati	EHUMC-1893	Borja	Zaragoza	Spain	30TXM23	■	■	■	■	■		■		■	■
Xerosecta arigonis	EHUMC-1896	Vitoria-Gastiez	Álava	Spain	30TWN24	■	■	■		■		■	■	■	
Xerotricha gonzalezi	EHUMC-1886	Urbasa	Navarra	Spain	30TWN64	■	■	■	■		■		■	■	■

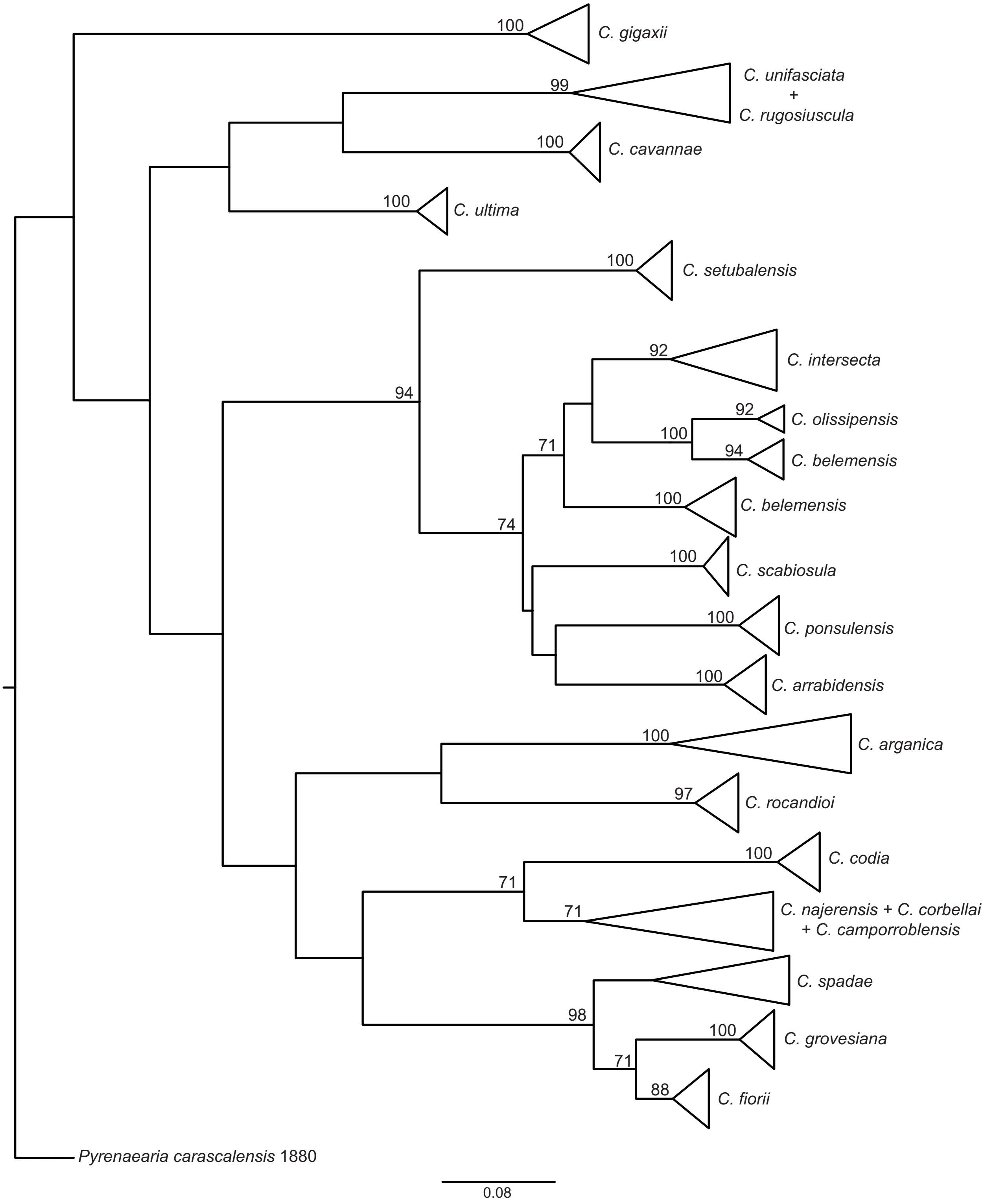


Figure S1. Phylogenetic tree of *Candidula* based on maximum likelihood (ML) analyses of *COI* (cytochrome *c* oxidasae subunit I) gene. Numbers correspond to ML bootstrap values.

Figure S2. Phylogenetic tree of *Candidula* based on Bayesian inference (BI) and maximum likelihood (ML) analyses of mtDNA (*COI* + 16S rRNA) gene fragments. Numbers correspond to BI posterior probabilities, ML bootstrap values, respectively. Asterisks (*) indicate full support of nodes: BI posterior probabilities = 1.00 and ML bootstrap values = 100%

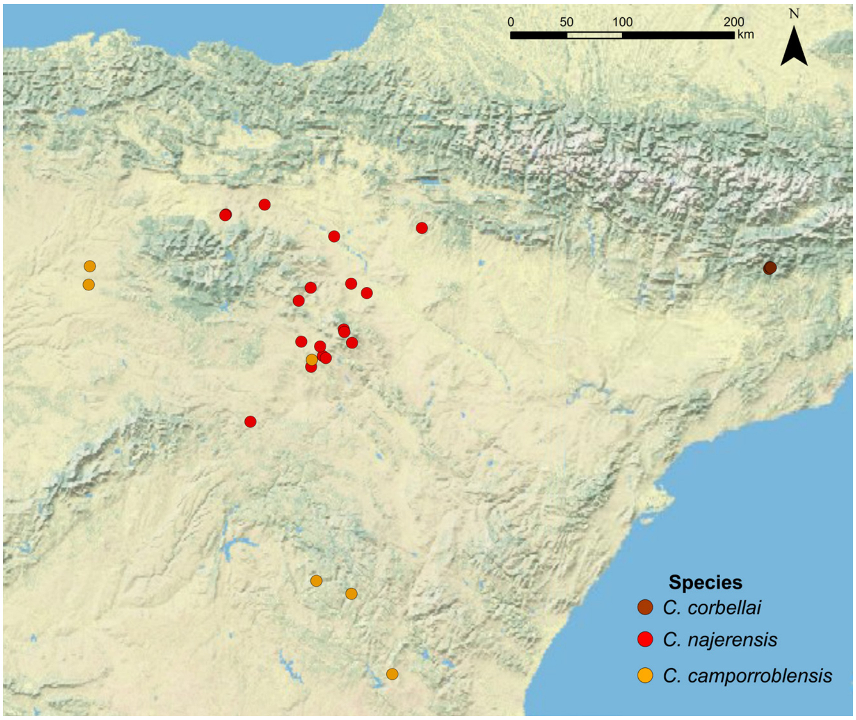


Figure S3a. Map plotting collection localities of *Candidula* species recovered at clade C2 in the molecular phylogeny (Fig. 5.2). Samples belonging to *C. gigaxii*, not shown.

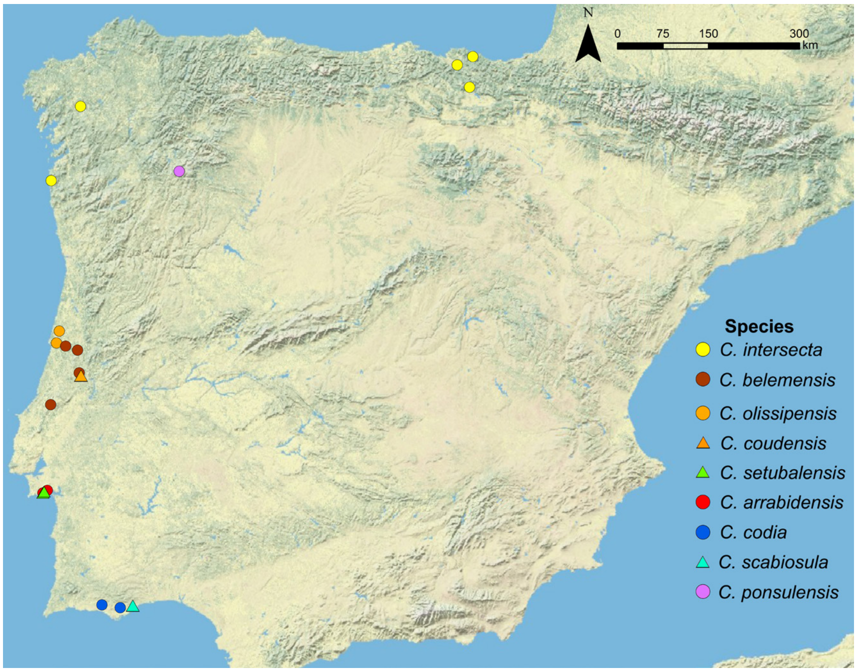


Figure S3b. Map plotting collection localities of *Candidula* species recovered at clade C3 in the molecular phylogeny (Fig. 5.2).

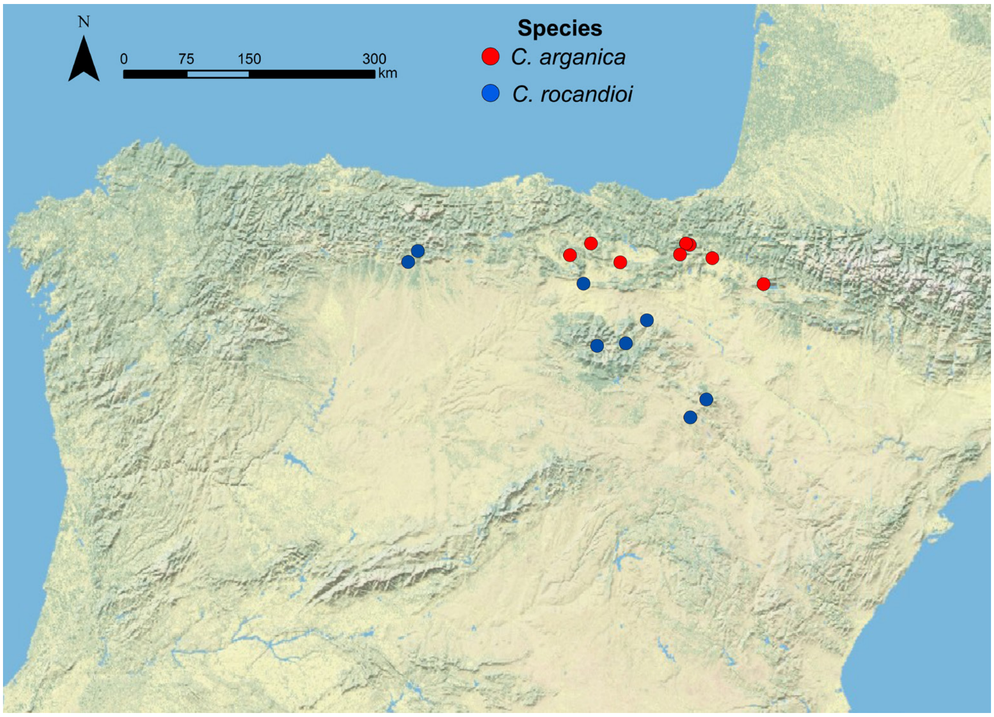


Figure S3c. Map plotting collection localities of *Candidula* species recovered at clade C4 in the molecular phylogeny (Fig. 5.2).

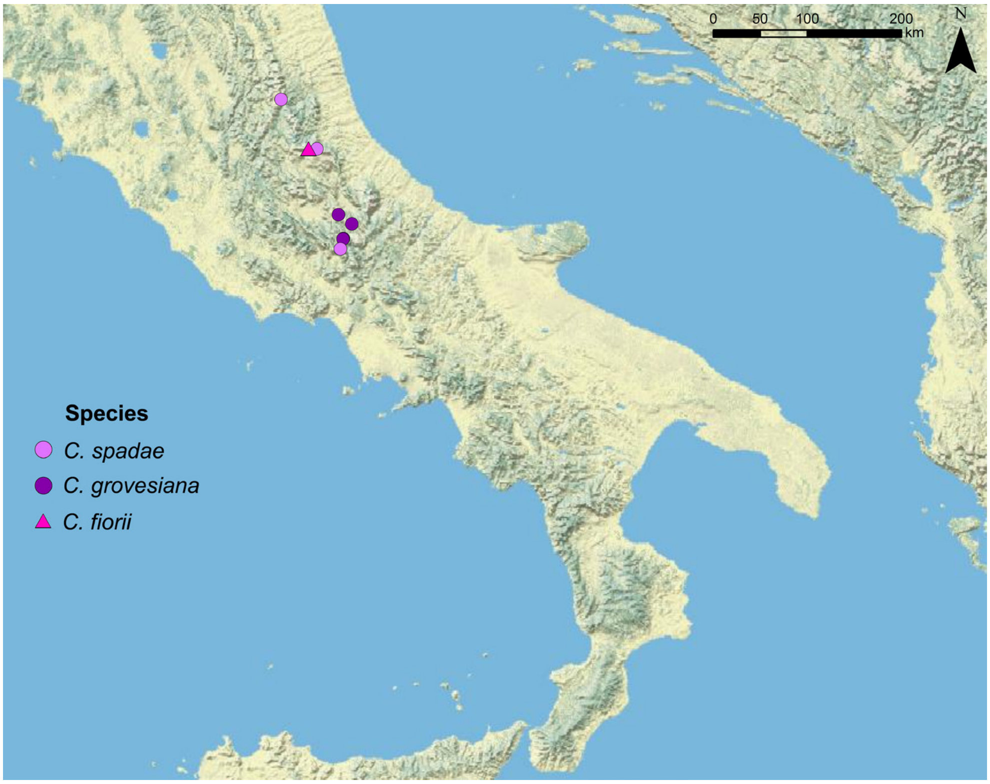


Figure S3d. Map plotting collection localities of *Candidula* species recovered at clade C5 in the molecular phylogeny (Fig. 5.2).

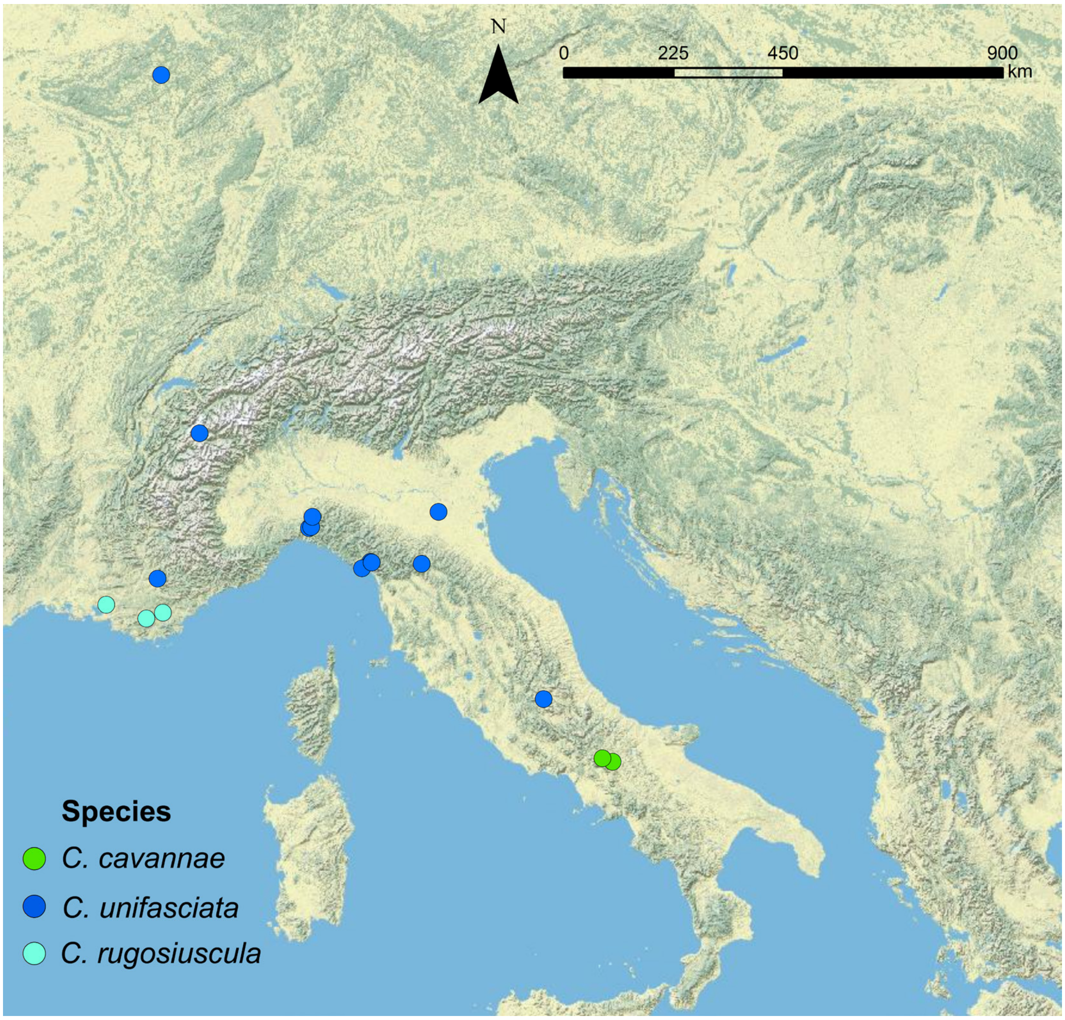


Figure S3e. Map plotting collection localities of *Candidula* species recovered at clade C6 in the molecular phylogeny (Fig. 5.2).

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CHAPTER 6

Taxonomic Implications

In the light of the results obtained and the discussions exposed in this work, new taxonomic reorganizations and some nomenclatural changes are proposed. Thus, taxonomic status of several taxa studied in this thesis has been updated. Major changes of the new classification proposed are showed below.

Allognathus Pilsbry, 1888

According to morphological, geographical and molecular information we propose an updated classification for the genus *Allognathus* (Table 6.1).

We decided to preserve the taxonomic status of the subgenera *Allognathus* Pislbry, 1888 and *Iberellus* Hesse, 1908 because there exist important differences in shell morphology and genetic distances between the taxa belonging to both subgenera. Nevertheless, genetic distances are considerably lower than those separating genera in the Allognathini. The taxa *A. campanayonii* (Rossmässler, 1839) from Mallorca, *A. minoricensis* (Mitree, 1842) from Menorca, *A. pythiusensis* (Bofill & Aguilar-Amat, 1924) from Ses Bledes archipelago and Ibiza, and *A. tanitianus* (Forés & Vilella, 1993) from Ibiza are considered subspecies of *A. (I.) campanyonii* (Rossmässler, 1839). A different lineage have been identified in the southern Tramuntana Mountains, which is morphologically similar to *A. (I.) hispanicus* but, genetically, is more related to *A. (I.) campanyonii*. This cryptic taxon that appears under the name of *A. (I) campanyonii* ssp. hybridizes with *A. (I.) campanyonii campanyonii*. Hybrids between *A. (I.) campanyonii minoricensis* and *A. (I.) campanyonii pythiusensis* have also been detected in Ibiza Island. All these hybrids indicate that there is not reproductive isolation between *A. (I.) campanyonii* lineages, supporting their consideration as subspecies of the same species under the biological species concept.

Due to the low genetic differences, *A. (I.) palumbariae* (Aguilar-Amat, 1933) and *A. (I.) horadadae* (Jaeckel, 1952) are considered junior synonyms of *A. (I.) campanyonii minoricensis* and *A. (I.) campanyonii campanyonii*, respectively. *A. (I.) palumbariae* is the name used by some authors to the populations of *Allognathus* living in Colom Islet, a small islet close to Menorca, but its validity has not been generally accepted. Besides, there are not morphological differences with the populations of *Allognathus* living in Menorca. *A. (I.) horadadae* is the name used for the populations of *Allognathus* living in Na Foradada, one small islet of the Cabrera Archipelago. The remaining populations of *Allognathus* from Cabrera Archipelago

Table 6.1. Taxonomic proposal for the genus *Alllognathus* Pilsbry 1888

Taxon	Author
Allognathus (Allognathus) graellsianus	(Pfeiffer, 1848)
Allognathus (Iberellus) hispanicus	(Rossmässler, 1838)
Allognathus (Iberellus) campanyonii campanyonii	(Rossmässler, 1839)
Allognathus (Iberellus) campanyonii minoricensis	(Mitrre, 1842)
Allognathus (Iberellus) campanyonii pythiusensis	(Bofill & Aguilar-Amat, 1924)
Allognathus (Iberellus) campanyonii tanitianus	(Forés & Vilella, 1993)
Allognathus (Iberellus) campanyonii ssp.	

included in our analyses, belong to *A. (I.) campanyonii campanyonii*. There are not genetic, morphological or biogeographical reasons to consider *A. (I.) horadadae* as one different taxon. After Chueca *et al.*, 2013, the name *Allognathus grateloupi* (Graells, 1846) was proposed to the endemic taxon to the Tramuntana Mountains. However, posterior revisions (Altaba *in litt*) indicated that the name *Helix grateloupi* Graells, 1846, was previously used for a gastropod from Madagascar *Helix grateloupii* Pffeiffer, 1842, so the first name available is *Helix graellsiana*, Pfeiffer, 1848 and by derivation *Allognathus graellsianus* (Pfeiffer, 1848).

The consideration of *A. (I.) campanyonii campanyonii* as one subspecies implies that *A. (I.) camapanyonii minoricensis* is paraphyletic. Both subspecies are confined to Mallorca and Menorca islands, respectively. We have to emphasize that there are two mitochondrial lineages well differentiated within *A. (I.) c. minoricensis*, but both lineages are intermixed in many populations and they do not show differences in nuclear DNA, constituting a single biological entity. Therefore, they should not be considered as different subspecies.

A. (A.) graellsianus and *A. (I.) hispanicus* are distributed in the northwestern Tramuntana Mountains, cohabiting in many populations. *A. (I.) campanyonii* ssp. is distributed in the southern Tramuntana mountains whereas *A. (I.) campanyonii campanyonii* is mainly present in the lower areas from Mallorca and in Cabrera Archipelago. *A. (I.) campanyonii minoricensis* is present in Menorca Island whereas *A. campanyonii tanitianus* is distributed in Ibiza Island. *A. campanyonii pythiusensis* is present in Ses Bledes archipelago but also in some localities in Ibiza Island. The populations of *A. (I.) campanyonii campanyonii* living in Tarragona city walls are the result of recent introductions from Mallorca. Besides, the populations of *A. (I.) campanyonii minoricensis* living in Ibiza city are also the result of recent introductions from Menorca Island.

Xerocrassa Monterosato, 1892

The analyses of the species of *Xerocrassa* living in the Balearic Islands suggest that the current classification should be updated. Therefore a new taxonomic reorganization is here proposed (Table 6.2).

The traditional names of the three species living in the Pytiusic Islands are corroborated: *X. ebusitana* (Hidalgo, 1869), *X. caroli* (Dohrn & Heynemann, 1862) and *X. cisternasi* (Hidalgo, 1883). Additionally, the species *X. formenterensis* (Schröder, 1984), classified by previous authors as one subspecies of *X. caroli*, should be considered with full species status. Furthermore, several subspecies traditionally considered as subspecies of *X. cisternasi* should be reassigned to *X. caroli*. This is the case of *X. caroli conjungens*, *X. caroli scopulicola*, *X. caroli vedrae* and *X. caroli vedranellensis*. This new classification shows a higher biogeographical consistence, where *X. caroli* is present in southern Ibiza and coastal islets, including Ses Bledes archipelago, and western Freus Islets, *X. cisternasi* is present in northern Ibiza and coastal islets, *X. formenterensis* in Fomentera and eastern Freus Islets and *X. ebusitana* in Ibiza, Formentera and Freus Islets. The low genetic differentiation between the populations of *X. caroli* and *X. cisternasi* from Ibiza and coastal islets could be explained by recent separation between populations, probably after the Last Glacial Maximum. The preservation of the subspecies status for these taxa responds to their isolation in small islets around Ibiza, but not on the basis of genetic differentiation. Nevertheless, some of these taxa also show important morphological differences such as *X. caroli conjungens* or *X. caroli vedranellensis*. *Xerocrassa molinae,* one endemism of the Columbretes Islands (an archipelago located 100 km far away from Pytiusic Islands) is genetically very closely related to *X. ebusitana* from Ibiza. Based on these results we consider that it should be classified as one subspecies of the latter. As a consequence, in our proposal, *X. ebusitana* includes two subspecies: *X. ebusitana ebusitana* and *X. ebusitana molinae*.

Regarding the species from the Gymensic Islands, *X. frater newka* (Dohrn & Heynemann, 1862) and *X. ferreri ferreri* (Jaeckel, 1952) are considered junior synonyms of *X. majoricensis* (Dohrn & Heynemann, 1862) because they do not constitute monophyletic clades, showing very low genetic differences and inhabiting along southern Mallorca Island

Table 6.2. Taxonomic proposal for the species of the genus *Xerocrassa* Monterosato 1892 from the Balearic Islands

Xerocrassa ebusitana ebusitana (Hidalgo, 1869)
Xerocrassa ebusitana molinae (Hidalgo, 1883)
Xerocrassa caroli alegriae Schröder, 1984
Xerocrassa caroli caroli (Dohrn & Heynemann, 1862)
Xerocrassa caroli conjungens (Jaeckel, 1952)
Xerocrassa caroli espartariensis Schröder, 1984
Xerocrassa caroli jaeckeli (Altimira, 1965)
Xerocrassa caroli scopulicola (Bofill & Aguilar-Amat, 1924)
Xerocrassa caroli vedrae (Jaeckel, 1952)
Xerocrassa caroli vedranellensis (Jaeckel, 1952)
Xerocrassa cisternasi calasaladae (Jaeckel, 1952)
Xerocrassa cisternasi calderensis (Gasull 1964)
Xerocrassa cisternasi canae (Jaeckel, 1952)
Xerocrassa cisternasi cisternasi (Hidalgo, 1883)
Xerocrassa cisternasi hortae (Schröder, 1978)*
Xerocrassa cisternasi margaritae (Jaeckel, 1952)
Xerocrassa cisternasi mesquidae (Schröder 1978)
Xerocrassa cisternasi muradae (Jaeckel, 1952)
Xerocrassa cisternasi ortizi (Gasull, 1964)
Xerocrassa cisternasi redonae (Jaeckel, 1952)
Xerocrassa formenterensis Schröder, 1984
Xerocrassa majoricensis (Dohrn & Heynemann, 1862)
Xerocrassa frater (Dohrn & Heynemann, 1862)
Xerocrassa pollenzensis (Hidalgo, 1878)
Xerocrassa prietoi prietoi (Hidalgo, 1878)
Xerocrassa prietoi ponsi (Hidalgo, 1878)
Xerocrassa ferrutxensis Forés & Altaba, 2014
Xerocrassa nyeli (Mittre, 1842)
Xerocrassa claudinae (Gasull, 1964)

and Cabrera Archipelago. *Xerocrassa ferreri pobrensis* (Gasull, 1964) from Na Pobra Islet (Cabrera Archipelago), was not included in the molecular analyses. However, due to all *X. ferreri* specimens form Cabrera Archipelago and Mallorca do not show relevant genetic differences, we think that this taxon is a junior synonym of *X. ferreri ferreri* and therefore, a junior synonym of *X. majoricensis*. In the same way, *X. frater pulaensis* Beckmann, 2007, is considered a junior synonym of *X. frater* (Dohrn & Heynemann, 1862) because the species delimitation analyses did not recover it as a valid species and genetic differences with *X. frater* are very low. *Xerocrassa pollenzensis* is considered a valid species, as it is recovered in all species delimitation analyses conducted. Its populations seem to be restricted to Formentor Peninsula.

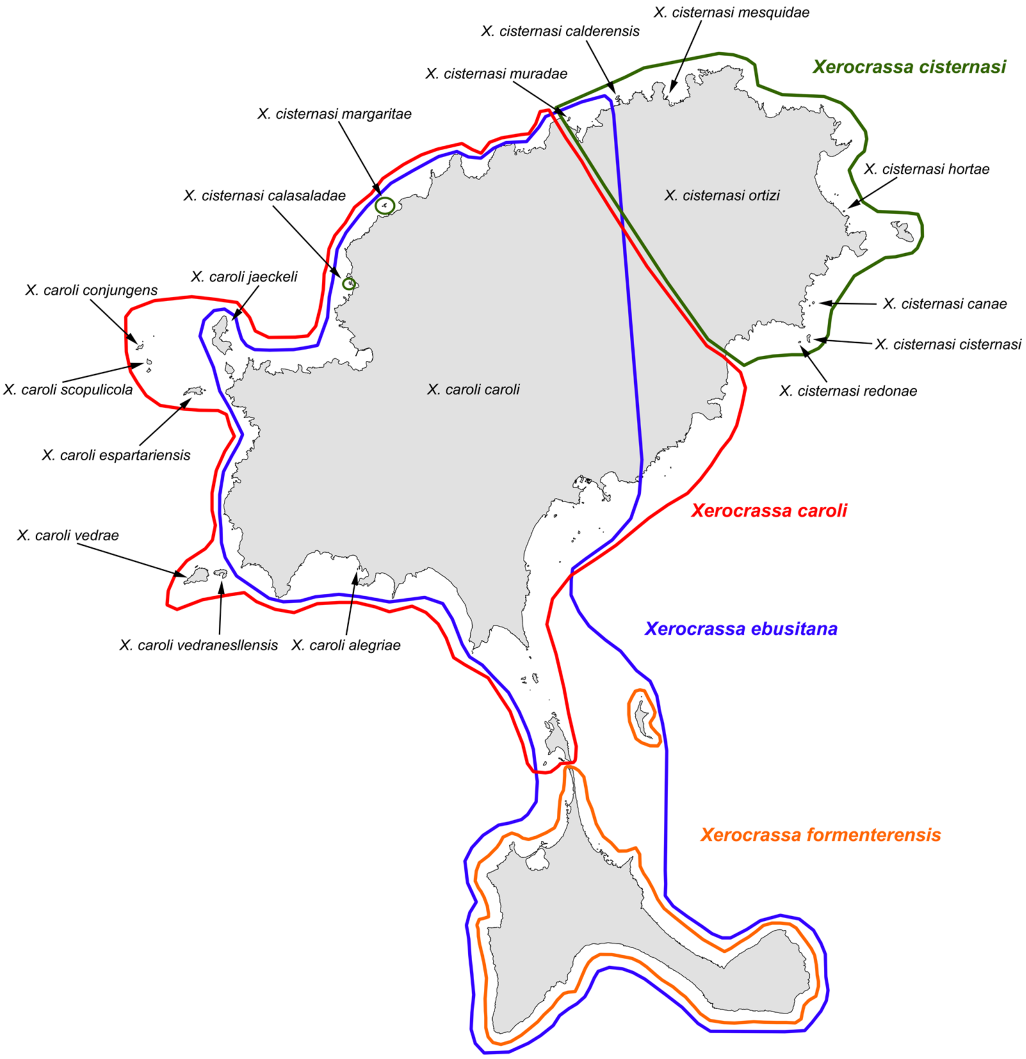

Figure 6.1. Approximate distribution of *Xerocrassa* species, delimited by coloured lines, and subspecies, indicated by arrows, of the Pityusic Islands.

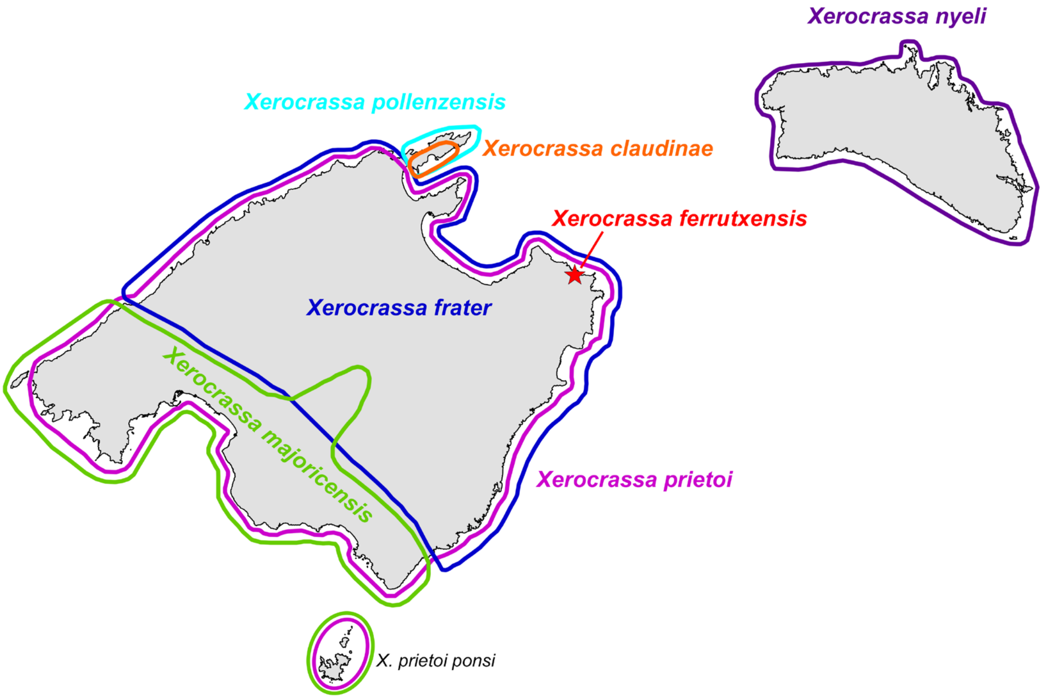

Figure 6.1. Approximate distribution of *Xerocrassa* species of the Gymnesic Islands.

Within *X. prietoi* complex, *X. homeyeri* (Dohrn & Heynemann, 1862), *X. moraguesi* (Kobelt, 1883) and *X. prietoi muroensis* Graack, 2005, should be included in the synonymy of *X. prietoi* (Hidalgo, 1878). In our opinion, *X. ponsi* (Hidalgo, 1878) could be considered a subspecies of *X. prietoi* because it formed a monophyletic group and its distribution range is restricted to the main island of Cabrera Archipelago. *X. prietoi* complex formed a polytomy with the taxon *X. nyeli*. We decided to preserve *X. nyeli* as a valid species because the species delimitation analyses recovered it as valid species and its distribution is restricted to Menorca Island. Nevertheless, *X. cardonae* (Hidalgo, 1867) is considered as a synonym of *X. nyeli* (Mitrre, 1892) due to no genetic differences have been detected between both taxa. Moreover, *X. ferrutxensis*, is considered as valid species. Although this taxon has not been recovered as one species in any species delimitation analyses performed, the high genetic differences recovered in the molecular phylogeny suggested that it could be considered a different species from *X. prietoi*. Following the observations of Forés (2015), we considered that *X. moraguesi* (Kobelt, 1883) and *X. claudinae* (Gasull, 1964) are two different nominal taxa. As it has been pointed above, topotypes of *X. moraguesi* from Palma de Mallorca belong to *X. prietoi*. On the contrary, topotypes of *X. claudinae* from Formentor Peninsula, showed important genetic differences (only information of mtDNA) with respect to *X. prietoi*. According to this information, we consider that *X. claudinae* is a valid species from Formentor Peninsula.

Candidula Kobelt, 1871

The phylogenetic results identified the polyphyly of the genus *Candidula* within the Geomitridae family. The analyses revealed six primary groups, with each one joining several species, except for the group of *C. ultima*, which is monospecific (Table 6.3). The presence of a single large dart sac arising from the vagina is therefore not enough to make a generic diagnosis in *Candidula*. We did not identify any morphological character to describe these six groups as six independent genera, thus further and detailed anatomical investigations should be done to find synapomorphies that could be diagnostic for each group. *Candidula unifasciata* is the type species of the genus. Thus, the name *Candidula* should be restricted to species belonging to clade 1 of this study. An exhaustive investigation with more Geomitridae genera is needed to resolve the relationships within the family. The resolution of sister relationships of other geomitrid taxa to the remaining five groups of *Candidula* should be fulfilled before setting up new names for these taxa.

The molecular results recovered the validity of most *Candidula* species traditionally considered (*C. arganica*, *C. cavannae*, *C. codia*, *C. fiorii*, *C. gigaxii*, *C. grovesiana*, *C. intersecta*, *C. najerensis*, *C. olisippensis*, *C. rocandioi*, *C. rugosiuscula*, *C. setubalensis*, *C. spadae*, *C. unifasciata* and *C. ultima*) as well as the validity of several recently described species (*C. arrabidensis*, *C. coudensis*, *C. corbellai* and *C. ponsulensis*). The validity of *C. scabiosula*, recently recovered by Holyoak & Holyoak, 2014, was also recovered in our study. Moreover, probably, the

Table 6.3. *Candidula* species joined into the six main groups. Superscript asterisks (*) mean that these species were not included in the molecular analyses.

	Species
Group 1	*Candidula cavannae* (Paulucci, 1881) *Candidula rugosiuscula* (Michaud, 1831) *Candidula unifasciata* (Poiret, 1801)
Group 2	*Candidula fiorii* (Alzona & Alzona Bisacchi, 1938) *Candidula grovesiana* (Paulucci, 1881) *Candidula spadae* (Calcara, 1845)
Group 3	*Candidula ultima* (Mousson, 1872)
Group 4	*Candidula camporroblensis* (Fez, 1944) *Candidula corbellai* Martínez-Ortí, 2011 *Candidula gigaxii* (Pfeiffer, 1847) *Candidula najerensis* (Ortiz de Zárate y López, 1950)
Group 5	*Candidula arrabidensis* Holyoak & Holyoak, 2014 *Candidula belemensis* (Servain, 1880) *Candidula carrapateirensis* Holyoak & Holyoak, 2014 * *Candidula coudensis* Holyoak & Holyoak, 2010 *Candidula intersecta* (Poiret, 1801) *Candidula olisippensis* (Servain, 1880) *Candidula ponsulensis* Holyoak & Holyoak, 2014 *Candidula scabiosula* (Locard, 1899) *Candidula setubalensis* (Pfeiffer, 1850) *Candidula strucki* (Maltzan, 1886) *
Group 6	*Candidula arganica* (Servain, 1880) *Candidula rocandioi* (Ortiz de Zárate y López, 1950)

populations of *C. intersecta* from Galicia could be a new species different from *C. intersecta* and *C. olisippensis*. Furthermore, *C. belemensis* and *C. camporroblensis* are paraphyletic, being necessary to analyse more populations to resolve the taxonomy of these taxa.

C. carrapateirensis and *C. strucki* were not included in the molecular analyses. However, due to its reduced distribution ranges in west Portugal together with their morphological similarities to many Portuguese species of *Candidula*, we consider that both taxa belong to the Group 5. C*andidula codia* was recovered also as a different species and closely related to clade 5. Finally, it is necessary to study the species living in the Balkan region (*C. castriota, C. lernaea, C. rhabdotoides* and *C. syrensis*) to determine which group they belong to.

CHAPTER 7

Concluding remarks

From the studies performed in this PhD thesis the following conclusions can be drawn:

1. *Allognathus*

1.1. According to the molecular phylogeny and species delimitation analyses, three species were delimited within the genus, that after our revision of the nomenclature, should be named: *Allognathus (Allognathus) graellsianus*, *Allognathus (Iberellus) hispanicus* and *Allognathus (Iberellus) campanyonii*, the last one containing at least five subspecies.

1.2. *Allognathus horadadae* and *A. palumbariae* are junior synonyms of *A. campanyonii campanyonii* and *A. campanyonii minoricensis,* respectively. The genital anatomy of *A. campanyonii tanitianus* was redescribed.

1.3. The genus appears to have colonized Mallorca from the south-east Iberian Peninsula during the middle Miocene (15.97 – 11.62 Ma) through a land-bridge connection. After this colonization, several speciation processes occurred within Mallorca that originated *A. graellsianus*, *A. hispanicus* and *A. campanyonii ssp*.

1.4. During the Messinian Salinity Crisis (5.96 – 5.33 Ma), *Allognathus* colonized the entire archipelago from Mallorca. After the MSC it started an allopatric speciation process, originating *A. campanyonii pythiusensis* and *A. campanyonii tanitianus* in the Pytiusic Islands, and *A. campanyonii campanyonii* and *A. campanyonii minoricensis* in the Gymnesic Islands.

1.5. Sea level fluctuations that took place in the Western Mediterranean from the Messinian to the present, explained the diversification and secondary contacts of the phylogroups of *Allognathus* within the Gymnesic or within the Pytiusic Islands and islets, as well as their distributions ranges. *Allognathus campanyonii* colonized Mallorca from Menorca Island, giving rise to *A. c. campanyonii*.

1.6. After the Messinian, no gene-flow has been detected between Gymnesic and Pytiusic Islands in *Allognathus*.

1.7. The presence of *A. campanyonii campanyonii* in the Iberian Peninsula, and *A. campanyonii minoricensis* in Ibiza, are the result of introductions in historic times from Mallorca and Menorca, respectively.

2. *Xerocrassa*

2.1. *Xerocrassa* colonized the archipelago in a single event during the Messinian salinity crisis (5.96 – 5.33 Ma) from the eastern Iberian Peninsula due to the desiccation of the Mediterranean Basin. After the Messinian, Gymnesic and Pytiusic populations have been fully isolated, without secondary contacts. As in *Allognathus*, secondary contacts have occurred between Mallorca and Menorca after the Messinian, but in *Xerocrassa*, the colonization occurred from Mallorca to Menorca.

2.2. The onset of the Mediterranean climate during the transition of the Pliocene to the Pleistocene, and Pleistocene glacial cycles could have influenced the radiation of the genus within the archipelago.

2.3. Molecular species delimitation analyses revealed eleven species of *Xerocrassa* within the Balearic Islands: *X. ebusitana*, *X. caroli*, *X. formenterensis*, *X. cisternasi*, *X. majoricensis*, *X. frater*, *X. pollenzensis*, *X. ferrutxensis*, *X. prietoi* and *X. nyeli*. Although there is not information for nuclear markers, mitochondrial analyses suggested that *X. claudinae* is also a valid species.

2.4. The origin of the populations of *Xerocrassa* living in the different islets surrounding Ibiza, (including Bledes Archipelago) probably postdated the Last Glacial Maximum. Subspecific rank consideration for all of them is mostly based on shell morphology and biogeographical criteria, due to their isolation in islets (*X. caroli*, 8 subspecies; *X. cisternasi*, 9 subspecies). We propose a new classification for all these taxa, based on our molecular results, to be used in case they were named as subspecies.

2.5. *X. formenterensis* and *X. pollenzensis* were elevated to the rank of species. On the other hand, *X. newka* and *X. ferreri*, were considered as junior synonyms of *X. majoricensis*. *X. molinae*, from the Columbretes Islands, is very closely related to *X. ebusitana*, and we conclude that based on morphology, biogeography and differences in DNA sequences, they should be considered subspecies of *X. ebusitana*.

3. *Candidula*

3.1. Seven new polymorphic nuclear markers amplified successfully for many species belonging to the Hellicellinae. Moreover, these new nuclear markers successfully amplified several other species of Geomitridae and Hygromiidae.

3.2. *Candidula* is polyphyletic within the Geomitridae. The presence of a single large dart sac is, probably, a character that has appeared independently several times within the Geomitridae. Preliminary studies also indicate that *Xerotricha* is polyphyletic and that the presence of two dart sacs on opposite sides of the vagina is also a homoplasy. Moreover, the Western Mediterranean *Xerocrassa* species and the genus *Trochoidea* are not sister groups. Further studies are needed to resolve the relationships between the genera of the Geomitridae to propose an updated classification of the family.

3.3. Almost all *Candidula* species analyzed were monophyletic. *Candidula belemensis* and *C. camporroblensis* were paraphyletic, being necessary the study of more populations to resolve their taxonomy. *C. intersecta* could include more than one species.

3.4. *Candidula* species were recovered in six primary monophyletic clades with high biogeographical consistence. Western groups were included within the Helicellini whereas the central-eastern groups were closely related to the Trochoideini and Cernuellini.

3.5. The main regions recovered for the primary clades were: *i*) the Canary Islands; *ii*) the Atlantic-Lusitanian region; *iii*) north Iberian Peninsula + Iberian System; *iv*) Mediterranean region of the Iberian Peninsula; *v*) the French Mediterranean region and *vi*) the Italian Peninsula.