

ORIGINAL  
ARTICLE



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

Luis J. Chueca<sup>1,2\*</sup>, María José Madeira<sup>1,2</sup> and Benjamín J. Gómez-Moliner<sup>1,2</sup>

<sup>1</sup>Department of Zoology and Animal Cell Biology, Faculty of Pharmacy, University of the Basque Country, 01006 Vitoria-Gasteiz, Álava, Spain, <sup>2</sup>Biodiversity Research Group CIEA Lucio Lascaray, 01006 Vitoria-Gasteiz, Álava, Spain

## 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.

\*Correspondence: Luis J. Chueca, Department of Zoology and Animal Cell Biology, Faculty of Pharmacy, University of the Basque Country, Paseo de la Universidad 7, 01006 Vitoria-Gasteiz, Álava, Spain.  
E-mail: luisjavier.chueca@ehu.es

## INTRODUCTION

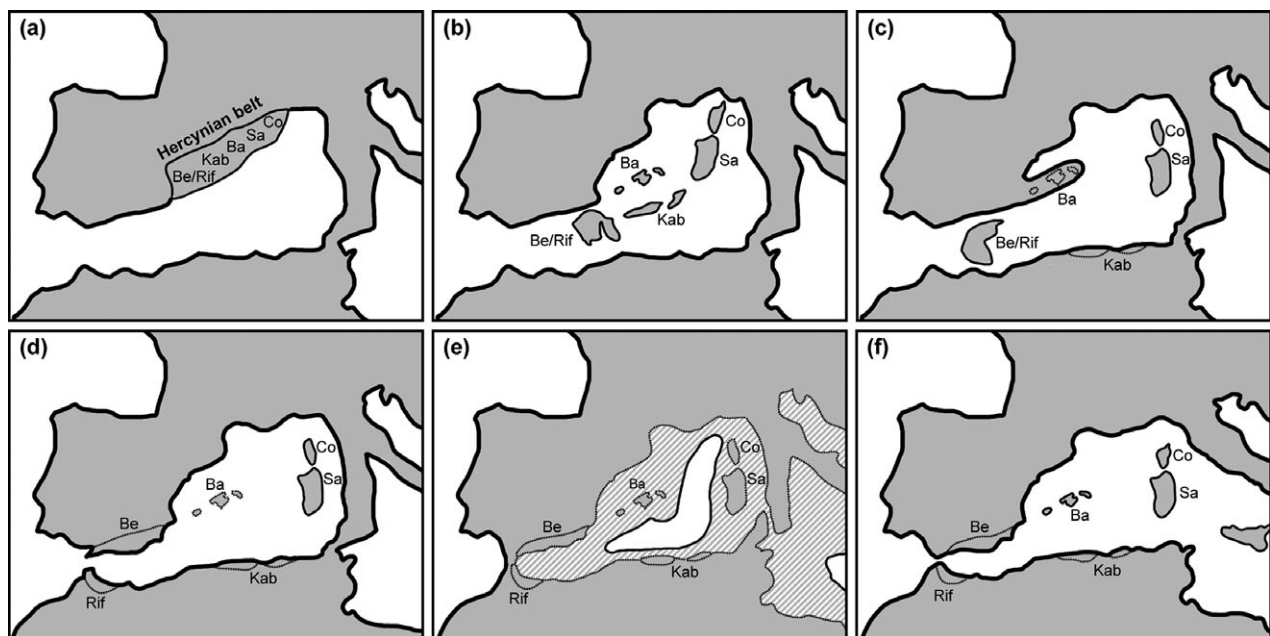
The Mediterranean Basin has been described as a biodiversity hotspot and is home to many endemic species (Myers, 1990; de Jong, 1998; Blondel & Aronson, 1999; Médail & Diadema,

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 endemism 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. 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 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



**Figure 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, Baetic–Rif system; Kab, Kabylies; Ba, Balearic Islands; Sa, Sardinia; Co, Corsica.

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 the 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 (Katoch *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+ $\Gamma$ +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://www.wabi.snv.jussieu.fr/public/abgd/abgd-web.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 ( $\epsilon = 15.0$ ). Each species-delimitation model was assigned equal prior probability. Given that the prior distributions of the ancestral population size ( $\theta$ ) and root age ( $\tau_0$ ) 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:  $\theta = G(2, 2000)$  and  $\tau_0 = G(2, 2000)$ ; and (3) large ancestral population sizes,  $\theta = G(1, 10)$ , and relatively shallow divergences among species,  $\tau_0 = G(2, 2000)$ .

### Estimation of divergence times

Divergence times within *Allognathus* were estimated using a Bayesian relaxed-clock approach implemented in BEAST 1.8.0, using  $7.5 \times 10^7$  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. 2) occurred during the MSC, so 5.33 Ma  $\pm$  0.1 Myr 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

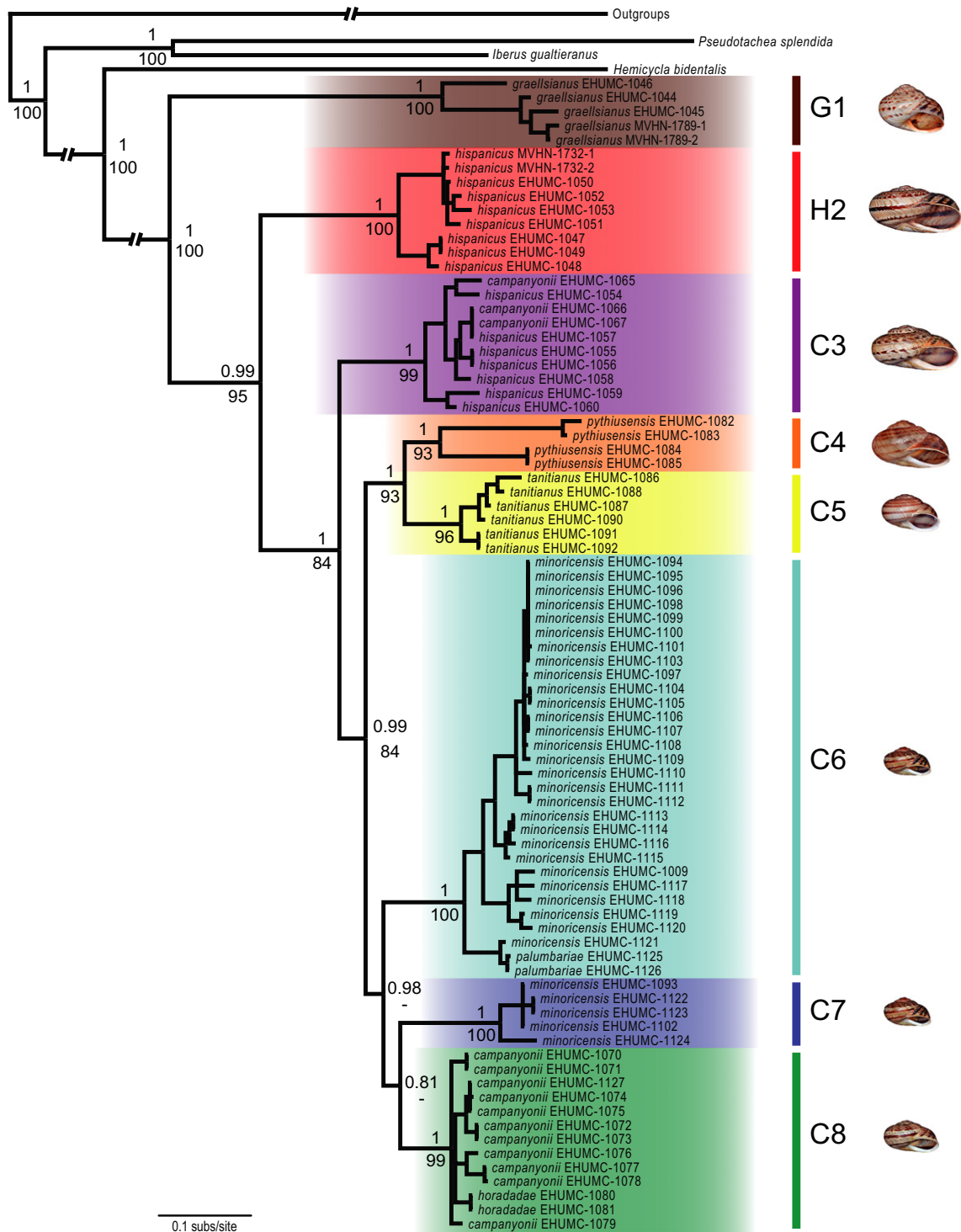
The ancestral range at each divergence event was reconstructed using Bayesian binary MCMC analysis (BBM) as implemented in RASP 3.0 (Yu *et al.*, 2014). This program determines the probability of a given ancestral range at a node by averaging over a posterior set of trees, thereby accounting for phylogenetic 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 the basal nodes (Fig. 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





**Figure 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 Peninsula). 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.

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). Under the 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  $\theta$  and  $\tau_0$  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

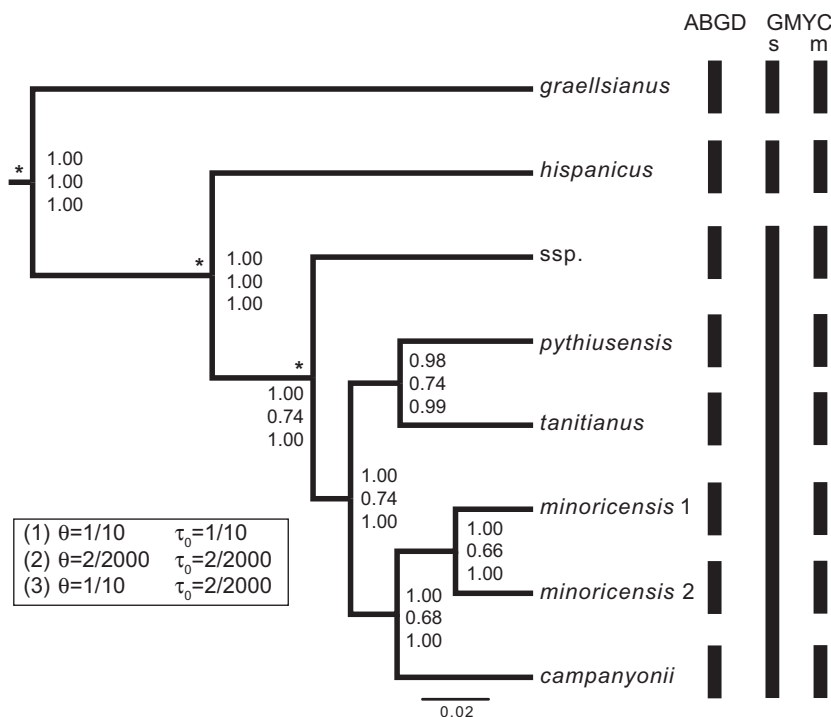
putative species were recognized: the phylogroups denoted *graellsianus* and *hispanicus*, and a third formed by the remaining phylogroups.

### Temporal framework of *Allognathus* diversification

The different analyses each resulted in similar node-age estimates (Table 1, Fig. 4, and Fig. S3 in Appendix S2). The median age for the split between the group formed by *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 than 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. 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. 4) supported an ancestral range for the *Allognathus* complex (node II) in the north-eastern Tramuntana Mountains (Mallorca). The marginal probability for this



**Figure 3** Summary results of the species-delimitation analysis for *Allognathus*. Maximum-clade-credibility tree generated using all genetic data in \*BEAST. Nodes with an asterisk indicate posterior probabilities > 0.95. Numbers adjacent to nodes represent speciation 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.

**Table 1** Main node-age estimates (Ma) from calibrated BEAST analyses of Balearic-endemic *Allognathus* and related genera. Values are means, with highest posterior density intervals (95% HPD) in parentheses. Nodes are indicated in Fig. 4.

	Geographical calibration	Substitution rates
<i>Iberus</i> + <i>Pseudotacheal</i> <i>Hemicycla</i> + <i>Allognathus</i> 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)

basal node reconstruction ( $P = 96.5\%$ ) was much higher than for the alternative area (Iberian Peninsula + north-eastern 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 (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

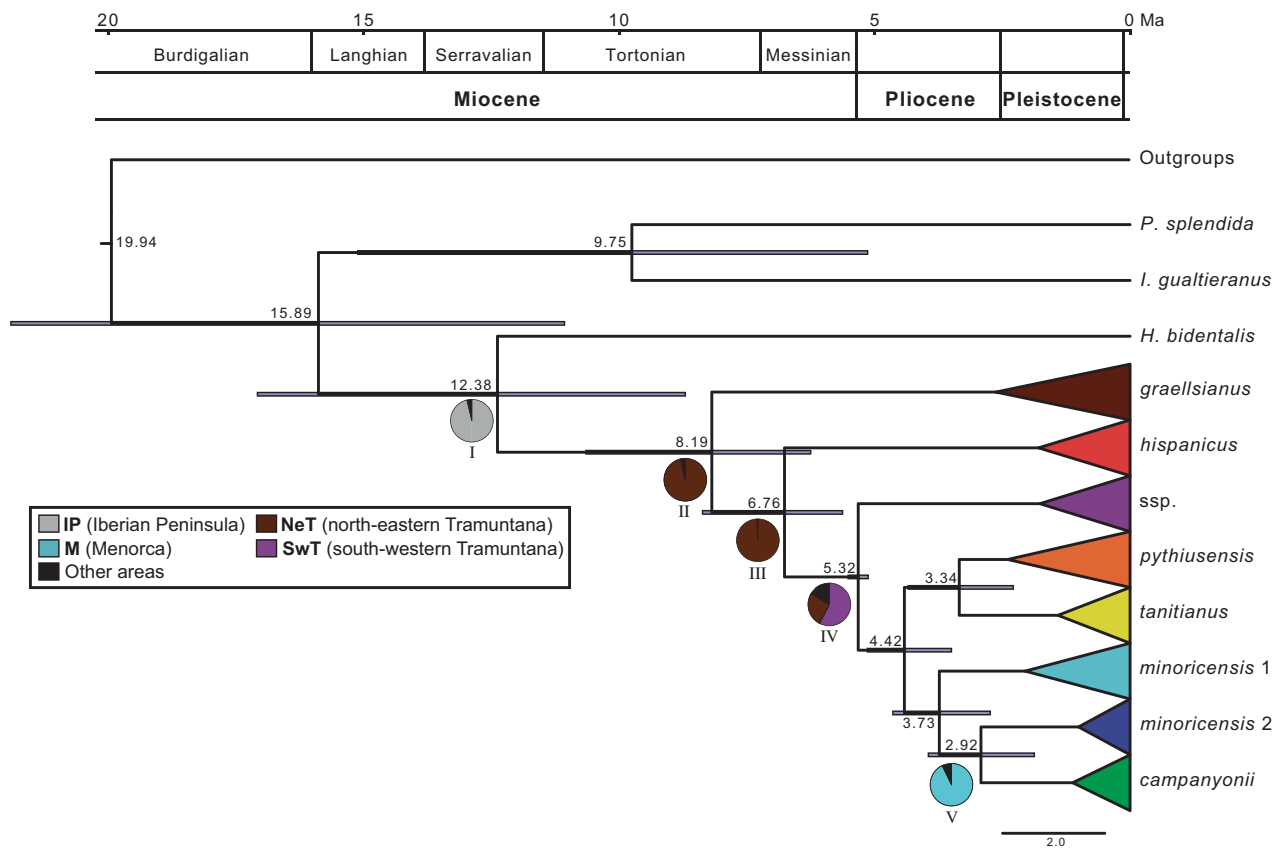
### Phylogeny and species delimitation

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. 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 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. companyonii*, 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. companyonii* (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. companyonii* 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 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. companyonii* 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 (*companyonii* × *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.) companyonii*. The last of these species con-



**Figure 4** Time-calibrated multilocus tree for *Allognathus* obtained using BEAST. Bars indicate 95% highest posterior densities 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. 2. Pie charts indicate the probability of ancestral areas for nodes with > 0.50 posterior probability support. Colours of ancestral areas displayed on pie charts are defined in the box (bottom left).

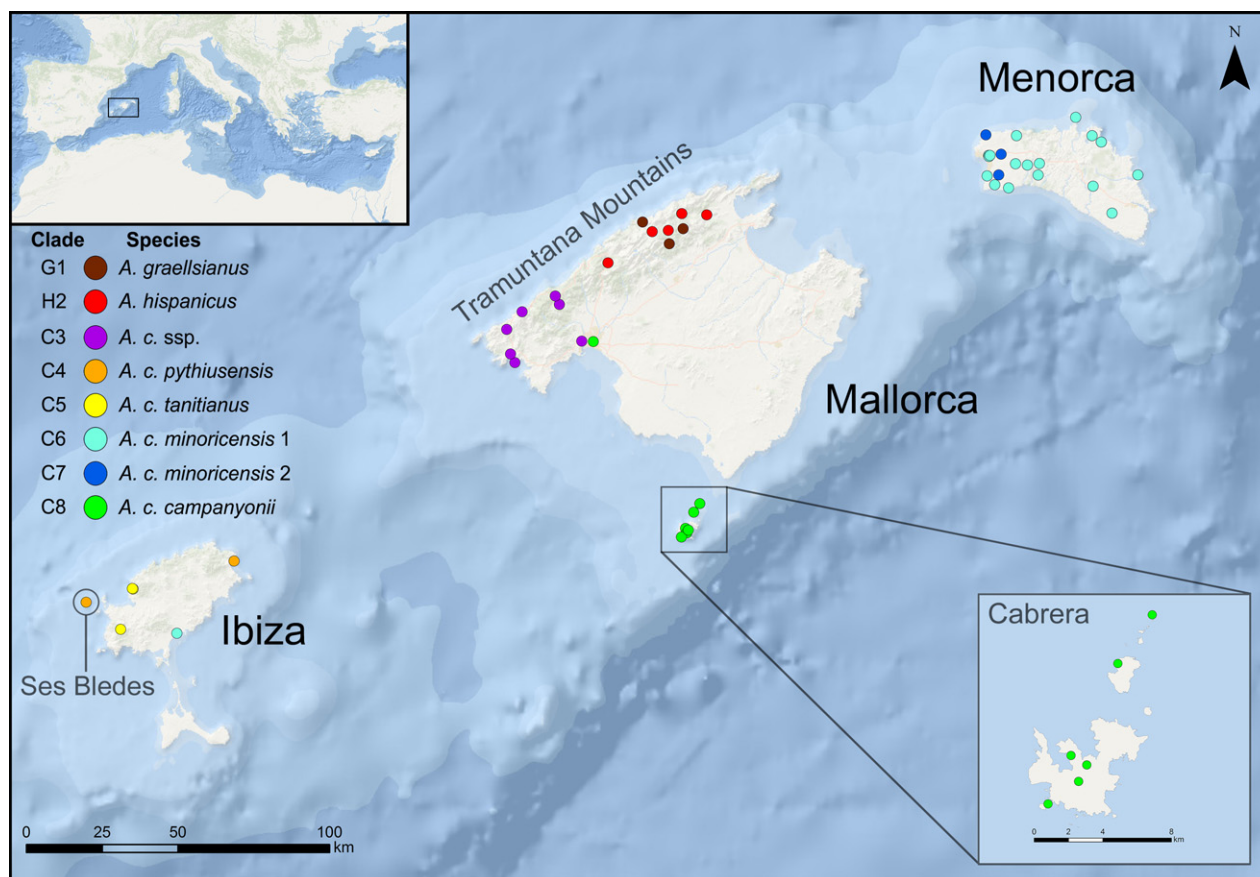
tains at most five subspecies, mainly defined by biogeographical criteria: (1) *A. c. campanyonii* from the Cabrera archipelago and low areas of Mallorca, also present in Tarragona; (2) *A. c. ssp.*, living in the south-western Tramuntana Mountains, which is cryptic to *A. hispanicus* and hybridizes 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. An 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* simultaneous split that occurred between the clades from Ibiza (C4–C5) and the 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).





**Figure 5** Map of the Balearic Islands (Western Mediterranean) plotting collection localities of *Allognathus* species (samples from Tarragona city, Iberian Peninsula, not shown). Colours correspond to clades obtained in the phylogeny (Fig. 2).

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 attrib-

uted 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; Bauzá-Ribot *et al.*, 2011). This could explain the diversification of different *Allognathus* lineages within Mallorca, giving rise to *A. 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; Bauzá-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.

## ACKNOWLEDGEMENTS

The authors thank M. Forés, J. Quintana and V. Bros for collecting indispensable material for this work; A. Martínez-Ortí for providing material from the Museo Valenciano de Historia Natural (MVHN); and the Park Service of Illes Balears for granting permits for sampling on the archipelago. Thanks also to K. Triantis and three anonymous referees for their suggestions during the review process. This work was funded by the Basque Government through the research projects (GIC10/76; IT575/13); L.J.C. was supported by a predoctoral fellowship from the University of the Basque Country.

## REFERENCES

- Adrover, R., Agustí i Ballester, J., Pons-Moyà, J. & Moyà Solà, S. (1985) Nueva localidad de micromamíferos insulares del Mioceno medio en las proximidades de San Lorenzo en la isla de Mallorca. *Paleontologia i Evolució*, **18**, 121–130.
- Altaba, C.R. (1997) Phylogeny and biogeography of midwife toads (*Alytes*, Discoglossidae): a reappraisal. *Contributions to Zoology*, **66**, 257–262.
- Bank, R.A. (2011) *Checklist of the land and freshwater Gastropoda of the Iberian Peninsula (Spain, Portugal, Andorra, Gibraltar)*. Fauna Europaea Project. Available at: [http://www.nmbe.ch/sites/default/files/uploads/pubinv/fauna\\_europaea\\_-\\_gastropoda\\_of\\_iberian\\_peninsula.pdf](http://www.nmbe.ch/sites/default/files/uploads/pubinv/fauna_europaea_-_gastropoda_of_iberian_peninsula.pdf).
- Bauzá-Ribot, M.M., Jaume, D., Fornós, J.J., Juan, C. & Pons, J. (2011) Islands beneath islands: phylogeography of a groundwater amphipod crustacean in the Balearic archipelago. *BMC Evolutionary Biology*, **11**, 221.
- Beckmann, K.-H. (2007) *Die Land- und Süßwassermollusken der balearischen Inseln*, 1st edn. ConchBooks, Hackenheim.
- Bidegaray-Batista, L. & Arnedo, M.A. (2011) Gone with the plate: the opening of the Western Mediterranean basin drove the diversification of ground-dweller spiders. *BMC Evolutionary Biology*, **11**, 317.
- Blondel, J.B. & Aronson, J. (1999) *Biology and wildlife of the Mediterranean region*, 1st edn. Oxford University Press, Oxford.
- Bover, P., Quintana, J. & Alcover, J.A. (2008) Three islands, three worlds: paleogeography and evolution of the vertebrate fauna from the Balearic Islands. *Quaternary International*, **182**, 135–144.
- Brown, R.P., Terrasa, B., Pérez-Mellado, V., Castro, J.A., Hoskisson, P.A., Picornell, A. & Ramon, M.M. (2008)

- Bayesian estimation of post-Messinian divergence times in Balearic Island lizards. *Molecular Phylogenetics and Evolution*, **48**, 350–358.
- Capraro, L., Massari, F., Rio, D., Fornaciari, E., Backman, J., Channell, J.E.T., Macrì, P., Prosser, G. & Speranza, F. (2011) Chronology of the Lower–Middle Pleistocene succession of the south-western part of the Crotona Basin (Calabria, Southern Italy). *Quaternary Science Reviews*, **30**, 1185–1200.
- Chueca, L.J., Forés, M. & Gómez-Moliner, B.J. (2013) Consideraciones nomenclaturales sobre las especies del género *Allognathus* (Gastropoda: Pulmonata: Helicidae) y estudio anatómico de *Allognathus hispanicus tanitianus*. *Iberus*, **31**, 63–74.
- Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, **9**, 772.
- Delicado, D., Machordom, A. & Ramos, M.A. (2014) Vicariant versus dispersal processes in the settlement of *Pseudamnicola* (Caenogastropoda, Hydrobiidae) in the Mediterranean Balearic Islands. *Zoological Journal of the Linnean Society*, **171**, 38–71.
- Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.
- Eljalde, M.A., Madeira, M.J., Arrébola, J.R., Muñoz, B. & Gómez-Moliner, B.J. (2008) Molecular phylogeny, taxonomy and evolution of the land snail genus *Iberus* (Pulmonata: Helicidae). *Journal of Zoological Systematics and Evolutionary Research*, **46**, 193–202.
- Exzard, T., Fujisawa, T. & Barraclough, T. (2009) *SPLITS: Species' Limits by Threshold Statistics*. Available at: <http://r-forge.r-project.org/projects/splits/> (accessed June 2013).
- Fochetti, R., Sezzi, E., Tierno de Figueroa, J.M., Modica, M.V. & Oliverio, M. (2009) Molecular systematics and biogeography of the western Mediterranean stonefly genus *Tyrrenoleuctra* (Insecta, Plecoptera). *Journal of Zoological Systematics and Evolutionary Research*, **47**, 328–336.
- Fontboté, J.M., Guimerà, J., Roca, E., Sàbat, F., Santanach, P. & Fernández-Ortigosa, F. (1990) The Cenozoic geodynamic evolution of the Valencia Trough (western Mediterranean). *Revista de la Sociedad Geológica de España*, **3**, 249–259.
- Fromhage, L., Vences, M. & Veith, M. (2004) Testing alternative vicariance scenarios in Western Mediterranean discoglossid frogs. *Molecular Phylogenetics and Evolution*, **31**, 308–322.
- Gasull, L. (1963) Algunos moluscos terrestres y de agua dulce de Baleares. *Bolletí de la Societat d'Història Natural de les Balears*, **9**, 3–80.
- Heled, J. & Drummond, A.J. (2010) Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*, **27**, 570–580.
- Hsü, K.J., Ryan, W.B.F. & Cita, M.B. (1973) Late Miocene desiccation of the Mediterranean. *Nature*, **242**, 240–244.
- Jesse, R., Véla, E. & Pfenninger, M. (2011) Phylogeography of a land snail suggests trans-Mediterranean Neolithic transport. *PLoS ONE*, **6**, e20734.
- Jolivet, L., Augier, R., Robin, C., Suc, J.-P. & Rouchy, J.M. (2006) Lithospheric-scale geodynamic context of the Messinian salinity crisis. *Sedimentary Geology*, **188–189**, 9–33.
- de Jong, H. (1998) In search of historical biogeographic patterns in the western Mediterranean terrestrial fauna. *Biological Journal of the Linnean Society*, **65**, 99–164.
- Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**, 3059–3066.
- Krijgsman, W., Hilgen, F.J., Raffi, I., Sierro, F.J. & Wilson, D.S. (1999) Chronology, causes and progression of the Messinian salinity crisis. *Nature*, **400**, 652–655.
- Lalueza-Fox, C., Castresana, J., Sampietro, L., Marquès-Bonet, T., Alcover, J.A. & Bertranpetit, J. (2005) Molecular dating of caprines using ancient DNA sequences of *Myotragus balearicus*, an extinct endemic Balearic mammal. *BMC Evolutionary Biology*, **5**, 70.
- Lázaro, E.M., Harrath, A.H., Stocchino, G.A., Pala, M., Bagnuà, J. & Riutort, M. (2011) *Schmidtea mediterranea* phylogeography: an old species surviving on a few Mediterranean islands? *BMC Evolutionary Biology*, **11**, 274.
- Magri, D., Fineschi, S., Bellarosa, R., Buonamici, A., Sebastiani, F., Schirone, B., Simeone, M.C. & Vendramin, G.G. (2007) The distribution of *Quercus suber* chloroplast haplotypes matches the palaeogeographical history of the western Mediterranean. *Molecular Ecology*, **16**, 5259–5266.
- Martínez-Solano, I., Gonçalves, H.A., Arntzen, J.W. & García-París, M. (2004) Phylogenetic relationships and biogeography of midwife toads (Discoglossidae: *Alytes*). *Journal of Biogeography*, **31**, 603–618.
- Martín-Suárez, E., Freudenthal, M. & Agustí, J. (1993) Micromammals from the Middle Miocene of the Granada Basin (Spain). *Geobios*, **26**, 377–387.
- Mayol, M., Palau, C., Rosselló, J.A., González-Martínez, S.C., Molins, A. & Riba, M. (2012) Patterns of genetic variability and habitat occupancy in *Crepis triasii* (Asteraceae) at different spatial scales: insights on evolutionary processes leading to diversification in continental islands. *Annals of Botany*, **109**, 429–441.
- Mayr, E. (1942) *Systematics and the origin of species*, 1st edn. Columbia University Press, New York.
- Médail, F. & Diadema, K. (2009) Glacial refugia influence plant diversity patterns in the Mediterranean Basin. *Journal of Biogeography*, **36**, 1333–1345.
- Moore, W.S. (1995) Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution*, **49**, 718–726.
- Myers, N. (1990) The biodiversity challenge: expanded hotspots analysis. *The Environmentalist*, **10**, 243–256.
- Nordsieck, H. (2014) Annotated check-list of the genera of fossil land snails (Gastropoda: Stylommatophora) of



- western and central Europe (Cretaceous–Pliocene). *Archiv für Molluskenkunde*, **143**, 153–185.
- Pamilo, P. & Nei, M. (1988) Relationships between gene trees and species trees. *Molecular Biology and Evolution*, **5**, 568–583.
- Pérez-Losada, M., Breinholt, J.W., Porto, P.G., Aira, M. & Domínguez, J. (2011) An earthworm riddle: systematics and phylogeography of the Spanish lumbricid *Postandrilus*. *PLoS ONE*, **6**, e28153.
- Pfenninger, M., Véla, E., Jesse, R., Elejalde, M.A., Liberto, F., Magnin, F. & Martínez-Ortí, A. (2010) Temporal speciation pattern in the western Mediterranean genus *Tudorella* P. Fischer, 1885 (Gastropoda, Pomatiidae) supports the Tyrrhenian vicariance hypothesis. *Molecular Phylogenetics and Evolution*, **54**, 427–436.
- Pomar, L. (1991) Reef geometries, erosion surfaces and high-frequency sea-level changes, upper Miocene Reef Complex, Mallorca, Spain. *Sedimentology*, **38**, 243–269.
- Pons, G.X. & Palmer, M. (1996) *Fauna endèmica de les illes Balears*. Institut d'Estudis Balearics, Palma, Spain.
- Pons, J., Barraclough, T.G., Gomez-Zurita, J., Cardoso, A., Duran, D.P., Hazell, S., Kamoun, S., Sumlin, W.D. & Vogler, A.P. (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology*, **55**, 595–609.
- Puente, A.I. (1994) *Estudio taxonómico y biogeográfico de la superfamilia Helicoidea Rafinesque, 1815 (Gastropoda: Pulmonata: Stylommatophora) de la Península Ibérica e Islas Baleares*. PhD Thesis, University of the Basque Country, Spain.
- Puillandre, N., Lambert, A., Brouillet, S. & Achaz, G. (2012) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology*, **21**, 1864–1877.
- Quintana, J. & Agustí, J. (2007) Los mamíferos insulares del Mioceno medio y superior de Menorca (islas Baleares, Mediterráneo occidental) [The insular mammals of the middle and upper Miocene from Minorca (Balearic Islands, Western Mediterranean)]. *Geobios*, **40**, 677–687.
- Quintana, J. & Moncunill-Solé, B. (2014) *Hypolagus balearicus* Quintana, Bover, Alcover, Agustí & Bailon, 2010 (Mammalia: Leporidae): new data from the Neogene of Eivissa (Balearic Islands, Western Mediterranean). *Geodiversitas*, **36**, 283–310.
- Rambaut, A., Suchard, M.A., Xie, D. & Drummond, A.J. (2014) *Tracer v1.6*. University of Edinburgh, Edinburgh, UK. Available at: <http://beast.bio.ed.ac.uk/Tracer>.
- Razkin, O., Gómez-Moliner, B.J., Prieto, C.E., Martínez-Ortí, A., Arrébola, J.R., Muñoz, B., Chueca, L.J. & Madeira, M.J. (2015) Molecular phylogeny of the western Palaearctic Helicoidea (Gastropoda, Stylommatophora). *Molecular Phylogenetics and Evolution*, **83**, 99–117.
- Riba, O. (1981) Aspectes de la geologia marina de la conca mediterrània balear durant el Neogen. *Memòries de la Real Acadèmia de Ciències i Arts de Barcelona*, **805**, 1–116.
- Roca, E. (1996) La evolución geodinámica de la Cuenca Catalano-Balear y áreas adyacentes desde el Mesozoico hasta la actualidad. *Acta Geológica Hispánica*, **29**, 3–25.
- Rodríguez, V., Brown, R.P., Terrasa, B., Pérez-Mellado, V., Castro, J.A., Picornell, A. & Ramon, M.M. (2013) Multilocus genetic diversity and historical biogeography of the endemic wall lizard from Ibiza and Formentera, *Podarcis pityusensis* (Squamata: Lacertidae). *Molecular Ecology*, **22**, 4829–4841.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**, 539–542.
- Rosenbaum, G. & Lister, G.S. (2004) Formation of arcuate orogenic belts in the western Mediterranean region. *Geological Society of America Special Papers*, **383**, 41–56.
- Rosenbaum, G., Lister, G.S. & Duboz, C. (2002) Reconstruction of the tectonic evolution of the western Mediterranean since the Oligocene. *Journal of the Virtual Explorer*, **8**, 107–130.
- Santos-Gally, R., Vargas, P. & Arroyo, J. (2012) Insights into Neogene Mediterranean biogeography based on phylogenetic relationships of mountain and lowland lineages of *Narcissus* (Amaryllidaceae). *Journal of Biogeography*, **39**, 782–798.
- Shchilevko, A.A. (2006) Treatise on recent terrestrial pulmonate molluscs, Part 13: Helicidae, Pleurodontidae, Polygyridae, Ammonitellidae, Oreohelicidae, Thysanophoridae. *Ruthenica*, **2**, 1765–1906.
- Sosdian, S. & Rosenthal, Y. (2009) Deep-sea temperature and ice volume changes across the Pliocene-Pleistocene climate transitions. *Science*, **325**, 306–310.
- Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**, 1312–1313.
- Uit de Weerd, D.R., Schneider, D., Gittenberger, E. (2005) The provenance of the Greek land snail species *Isabellaria pharsalica*: molecular evidence of recent passive long-distance dispersal. *Journal of Biogeography*, **32**, 1571–1581.
- Wu, C.I. (1991) Inferences of species phylogeny in relation to segregation of ancient polymorphism. *Genetics*, **127**, 429–435.
- Yang, Z. & Rannala, B. (2010) Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences USA*, **107**, 9264–9269.
- Yu, Y., Harris, A. & He, X.-J. (2014) RASP (reconstruct ancestral state in phylogenies) 3.0. Available at: <http://mnh.scu.edu.cn/soft/blog/RASP/>.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** Study material and PCR conditions.

**Appendix S2** Supplementary figures (Figs S1–S3).

**Appendix S3** Genetic data information.

## BIOSKETCHES

**Luis J. Chueca** is a PhD student at the University of the Basque Country. His research interests lie in the field of the biogeography and systematics of land snails in the Mediterranean Basin, particularly in the Balearic Islands.

**María José Madeira** is a researcher of the Department of Zoology at the University of the Basque Country with expertise in the biogeography and population dynamics of several groups of invertebrates and vertebrates, with special emphasis on the systematics of land snails.

**Benjamín J. Gómez-Moliner** is Professor of Zoology and Fauna Management at the University of the Basque Country. His current research projects include the study of the evolution, biogeography and population dynamics of several groups of invertebrates and vertebrates, and he is particularly interested in the systematics of land snails.

---

Editor: Kostas Triantis