



Molecular phylogeny and trait evolution of Madeiran land snails: radiation of the Geomitriini (Stylommatophora: Helicoidea: Geomitridae)

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Abstract

The Geomitriini is the most species-rich group of land snails in the Madeiran Archipelago. The phylogeny of the group is reconstructed based on mitochondrial and nuclear genetic markers. The timing of diversification, the colonisation history of the islands of the Madeiran Archipelago and the evolution of characters of the dart apparatus are studied. The results of the phylogenetic analyses confirm the sister group relationship of Geomitriini and Cochlicellini, but also show that several previously accepted genus-group taxa are not monophyletic. A new classification for the Geomitriini is proposed, including the description of two new genera, *Domunculifex* Brozzo, De Mattia, Harl & Neiber, n. gen. and *Testudodiscula* Brozzo, De Mattia, Harl & Neiber, n. gen. The onset of diversification of Geomitriini was dated in our analysis at 13 Ma, which largely coincides with the emergence of the present-day islands. The ancestral state estimation recovered the presence of two appendiculae in the reproductive system as the ancestral state in Geomitriini. One appendicula was lost three times independently within the tribe and is even missing completely in one group. The ancestral area estimation suggests recurrent colonisations of Madeira (and the Ilhas Desertas) from the older island Porto Santo.

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Introduction

The Madeiran Archipelago (Fig. 1) is located about 800 km west of Morocco and 900 km southwest of the Iberian Peninsula. It includes the main island Madeira

(741 km²) with an estimated age of 4.6 Ma and Porto Santo (69 km²), which has an estimated age of 14.3 Ma and is surrounded by various satellite islets, as well as the three lesser islands 10 km to the southeast of Madeira, the uninhabited Ilhas Desertas (Ilhéu Chão, Deserta Grande and Bugio; together approximately 15 km²) with an estimated age of around 3.6 Ma (Geldmacher et al., 2000).

In contrast to the currently emerged islands, the Madeiran Archipelago has its origins about 65–67 Ma

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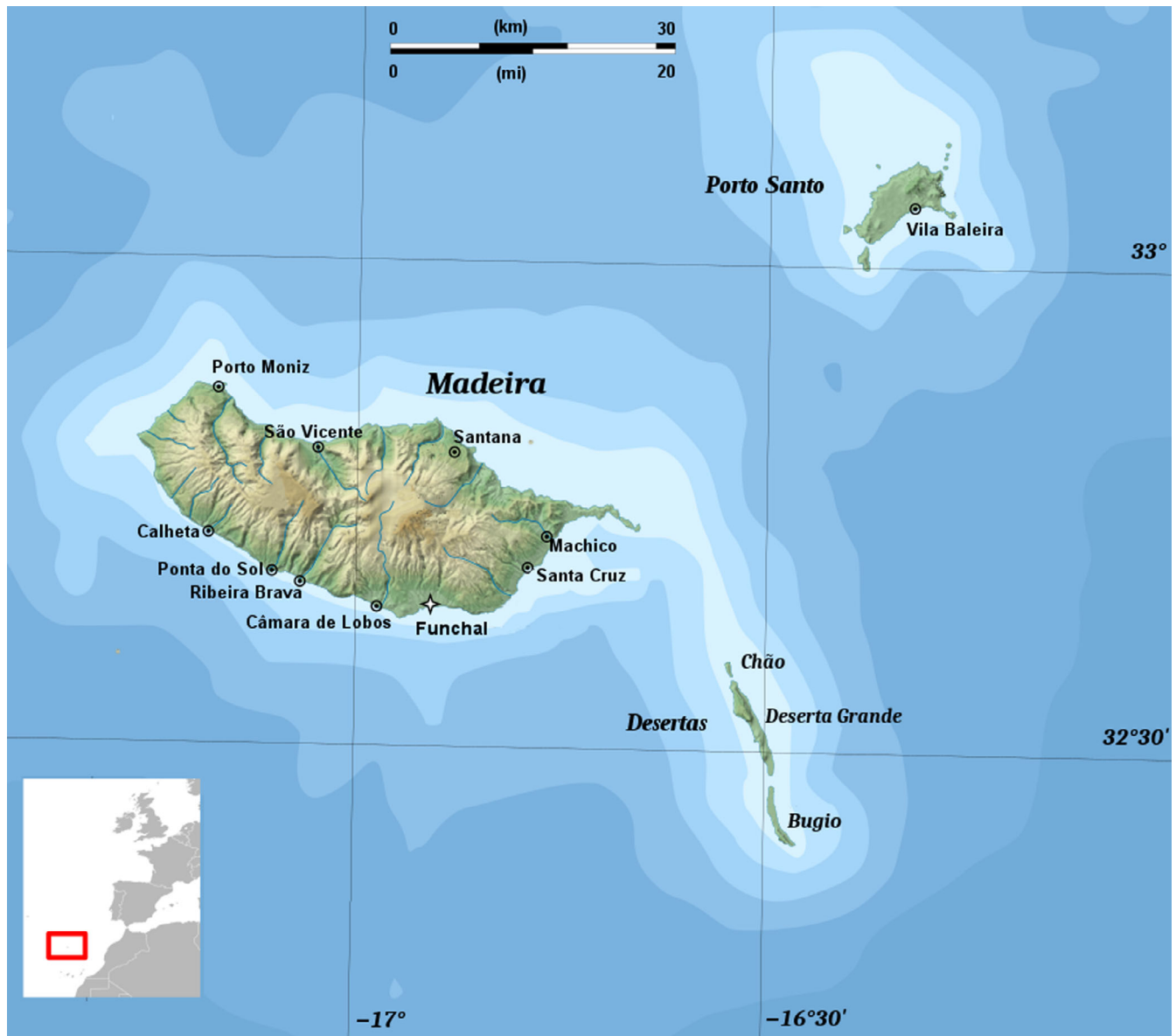


Fig. 1. Map of the Madeiran Archipelago with the main islands Madeira and Porto Santo as well as the Ilhas Desertas and the satellite islets. The insert shows the position of the archipelago in the Atlantic Ocean. Modified from the creative commons map “Madeira topographic map-fr” under CC BY-SA 3.0-license (<https://creativecommons.org/licenses/by-sa/2.0/de/legalcode>; Bourrichon). [Colour figure can be viewed at wileyonlinelibrary.com]

ago, close to the Cretaceous/Paleogene boundary. The Madeiran Archipelago forms the end of a volcanic hot-spot chain that connects the archipelago with the Iberian Peninsula to the north-east by a chain of isolated volcanic seamounts, which probably also formed islands and submerged in the course of time due to erosion (Geldmacher et al., 2000, 2005; Czajkowski, 2002; Klügel et al., 2005). Within the region forming the Canary Islands, van den Bogaard (2013) found even older volcanic structures from the beginning of the Cretaceous. Since that time, a continuous production of new

as well as a constant loss of older islands within the archipelagos is assumed (Fernández-Palacios et al., 2011), which presumably made the process of colonisation for both, the land flora and fauna (from, e.g., the Iberian Peninsula or other islands) much easier than today. Fernández-Palacios et al. (2011) emphasise the potential role of the archipelagos of Palaeo-Macaronesia as refuges and radiation centres of palaeo-endemic taxa of plant and animal groups and as stepping stones for the colonisation of the geologically younger Madeiran Islands and Canary Islands.

In the Madeiran Archipelago, a closer biogeographical connection to Europe rather than Africa is supported by the presence of many lineages that can be traced back to the European mainland, but which often almost vanished there with the onset of the Pleistocene climate cooling (Baez, 1993; Fernández-Palacios et al., 2011; Caro et al., 2019). Endemism in the Madeiran Archipelago is particularly high in animal groups that have a low capacity for dispersal, such as Diplopoda (Cook, 2008) and land snails (Waldén, 1983).

The Madeiran Archipelago harbours an astonishingly rich land snail fauna. The high number of endemic taxa is remarkable, and many of these endemic taxa are only found on one single island within the archipelago, in a small part of an island or even a single locality (Bank et al., 2002; De Mattia et al., 2018a). From the 19th to the beginning of the 21st century, this rich land snail fauna has caught the interest of many biologists, leading to various descriptions, monographs, revisions and checklists (e.g. Lowe, 1831; de Paiva, 1867; Wollaston, 1878; Mandahl-Barth, 1950; Waldén, 1983; Bank et al., 2002; Seddon, 2008; Bank, 2009; Groh et al., 2009; De Mattia et al., 2018a, b).

A total of approximately 190 extant land-snail species are recorded from the Madeiran Archipelago so far, including about 140 endemic species (Bank, 2009; De Mattia et al., 2018a,b; Teixeira et al., 2019). The Geomitridae Boettger, 1909 (in the sense of Razkin et al., 2015; Neiber et al., 2017) comprise approximately 65 recent species in the Madeiran Archipelago (Bank, 2009; De Mattia et al., 2018a,b; Teixeira et al., 2019). The family is composed of two subfamilies, the Geomitrinae Boettger, 1909 (containing the tribes Geomitriini Boettger, 1909, Cochlicellini Schileyko, 1991 and Ponentinini Schileyko, 1991) and the Helicellinae Ihering, 1909 (containing the tribes Trochoideini Nordsieck, 1987, Helicopsini Nordsieck, 1987, Helicellini Ihering, 1909, Cernuellini Schileyko, 1991 and Plentuisini Razkin, Gómez-Moliner, Prieto, Martínez-Ortí, Arrébola, Muñoz, Chueca & Madeira, 2015). Except for two introduced Cochlicellini and five introduced Helicellinae (Bank, 2009), all Geomitridae from the Madeiran Archipelago belong to the Geomitriini. The Geomitriini are endemic to the Madeiran Archipelago and the Azores (the records from the Canary Islands may be non-autochthonous), most of them to the Madeiran Archipelago (Backhuys, 1975; Bank et al., 2002; Razkin et al., 2015). Members of the Geomitriini have evolved various shell shapes, which is unusual among helicoid land snails (Fig. 2). There is also an exceptional variation in shell size among species, varying from *Steenbergia paupercula* (Lowe, 1831) with a small shell of approximately 3.5 mm in diameter to *Pseudocampylaea lowii* (Férussac, 1835) (a

species that has probably gone extinct early in the 20th century) with a shell diameter of 48–55 mm (Fig. 2). Shell shapes range from the flat, discoidal shell of, e.g., *Discula (Mandahlia) tectiformis* (Sowerby, 1824) and the globular shell of *Caseolus (Helicomela) punctulatus* (Sowerby, 1824) to the turreted shell of *Wollastonaria turricula* (Lowe, 1831) (Fig. 2). The shell surface can be distinctly ornamented, including granulated, ribbed or even hirsute forms (Fig. 2). The snails are mostly found on or under rocks or in xeric grassland habitats, which are the primary environments on Porto Santo since the colonisation of the islands by humans in the 15th century (Goodfriend et al., 1994), but also occur in more humid habitat from the sea level to higher mountainous areas on Madeira (Seddon, 2008).

Family-group and genus-group systematics of Helicoidea Rafinesque, 1815, to which Geomitridae belong, traditionally rested on the morphology of the reproductive organs, especially the presence or absence and morphology of accessory genital appendages such as the dart apparatus (Hesse, 1921, 1931, 1934; Schileyko, 1970, 1972a,b, 1978a,b, 1991, 2004, 1991; Giusti and Manganelli, 1987; Nordsieck, 1987, 1993). Molecular analyses in the past 15 years have led to numerous systematic rearrangements in helicoid land snails (Wade et al., 2007; Hugall and Stanisic, 2011; Gómez-Moliner et al., 2013; Razkin et al., 2015; Neiber et al., 2017; Sei et al., 2017). Moreover, it could be shown that reconfigurations, transformations and reductions of the dart apparatus have evolved many times in parallel in this superfamily (Hirano et al., 2014; Köhler and Criscione, 2015; Walther et al., 2016, 2018; Neiber and Hausdorf, 2017; Neiber et al., 2017, 2018a; Chueca et al., 2018), and it may even differ considerably among closely related taxa or within a single species (Korábek et al., 2015; Neiber and Hausdorf, 2015; Kruckenhauser et al., 2017; Zopp et al., 2017; Neiber et al., 2018b). In Geomitriini, the calcareous dart has been lost and the dart sac or the accessory sac has been transformed to a hollow tube, the appendicula. In some groups of Geomitriini, two appendiculae are present, while in others only a single appendicula is developed or appendiculae are entirely missing (Fig. 3; see also Mandahl-Barth, 1950; Schileyko, 2006b). De Mattia et al. (2018a) noted that molecular genetic analyses did not always support the separation of genera based on shell characters and to some extent on genital anatomy. These authors also started to investigate the genital anatomy of Geomitriini in detail, resulting in a revised taxonomy of several genera and the description of a new genus and several (sub-)species. Internal structures of the genital organs proved useful for distinguishing closely related species, but in some cases also allowed the distinction of genus-group taxa. A comprehensive molecular analysis of the

Geomitriini is, however, currently lacking. Therefore, the present contribution aims to (i) reconstruct a backbone phylogeny of the tribe based on of a representative sample of species covering nearly the entire generic diversity of the group as currently accepted, (ii) shed light on the evolution of the dart apparatus in this group, (iii) date the radiation of Geomitriini in the Madeiran Archipelago based on a molecular clock approach and (iv) use the molecular genetic analyses to discuss the role of in situ radiations on single islands or groups of islands versus recurrent inter-island dispersal events.

Material and methods

DNA sampling

DNA extractions of specimens previously used in the study of De Mattia et al. (2018a), as well as additional tissue samples of other Geomitriini taxa, were used for the analyses. Specimens were chosen to cover almost all described genera in the Geomitriini. *Craspedaria* Lowe, 1852 is possibly extinct (Seddon, 2008, 2011; Cuttelod et al., 2011; Neubert et al., 2019) and *Moreletina* de Frias Martins, 2002 from the Azores (de Frias Martins, 2002) could not be included in the analyses. Altogether, 41 specimens of the Geomitriini were used, plus eight individuals belonging to other genera representing the related subfamilies/tribes within the Geomitridae as well as one specimen, *Hygromia* (*Hygromia*) *cinctella* (Draparnaud, 1801), as a representative of the Hygromiidae Tryon, 1866, and one specimen, *Canariella* (*Canariella*) *giustii* Ibáñez & Alonso, 2006, as a representative of the Canariellidae Schileyko, 1991 as an outgroup. The classification, sampling sites and extraction numbers of specimens are compiled in Table 1.

DNA extraction and amplification

For the DNA extraction, foot muscle tissue stored in 100% isopropanol was used. Whenever fresh material was unavailable, foot muscle tissue of museum specimens stored in 70% ethanol was used for DNA extraction.

Total genomic DNA was extracted from the foot tissue using a slightly modified version of the protocol of Sokolov (2000) as described in Scheel and Hausdorf (2012) with additional modifications: Tissue samples were incubated in 500 μ L lysis buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 10 mM EDTA, 1% sodium dodecyl sulphate) and 20 μ L proteinase K at 56 °C until complete digestion. Then, 50 μ L saturated KCl solution was added to the lysate. The samples were centrifuged at 16 000 g for 15 min. The supernatant was transferred to a clean tube and 500 μ L of icecold isopropanol (100%) and 50 μ L 3 M sodium acetate solution was added. DNA was precipitated overnight at -20 °C. The samples were then centrifuged at 16 000 g for 15 min, and the pellet was washed in 70% ethanol and air-dried. The pellet was subsequently re-suspended in 80 μ L of ddH₂O. For older specimens, stored in 70% ethanol, 300 μ L lysis buffer, 30 μ L KCl and 30 μ L sodium acetate was used instead.

Partial sequences of the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) and of the 16S rRNA (16S) gene, as well as a part of the nuclear ribosomal RNA gene cluster including the 3' end of the 5.8S rRNA (5.8S) gene, the complete internal transcribed spacer 2 (ITS2) and the 5' end of the 28S rRNA (28S) gene, were amplified

by polymerase chain reaction (PCR). The primer pairs used were LCO1490 plus HCO2198 (Folmer et al., 1994) for *cox1*, 16Scl1 (Chiba, 1999) plus 16S_MN3R (Neiber et al., 2017) for 16S and LSU1 plus LSU3 (Wade and Mordan, 2000) as well as LSU2 plus LSU4 (Wade and Mordan, 2000) for the nuclear ribosomal rRNA gene cluster, respectively. For a subset of samples, especially older museum material stored in 70% ethanol, other primer combinations were used, since the amplification had been difficult and/or incomplete. In these cases, previously published (Palumbi, 1991; Uit de Weerd, 2008) as well as newly designed internal forward and reverse primers were used in combination with one of the above-listed primers (see Supporting Information, Table S1).

Amplifications were performed in 25 μ L volumes containing 18.3 μ L ddH₂O, 2.5 μ L Dream Taq™ Green Buffer (Thermo Fisher Scientific, Waltham, MA, USA), 1.0 μ L of a dNTP mix (5 mM each), 1.0 μ L of each primer (10 μ M), 0.2 μ L DreamTaq™ DNA polymerase (Thermo Fisher Scientific) and 1.0 μ L of the template DNA under the following reaction conditions: an initial denaturation step at 94 °C for 2 min, 35–45 PCR cycles (94 °C for 30 s, primer specific annealing temperature for 30 s, 72 °C for 30 s) and a final extension step at 72 °C for 5 min. Both strands of the amplified products were sequenced at Macrogen Europe Laboratory (Amsterdam, The Netherlands).

Phylogenetic analyses

Forward and reverse sequences were assembled using ChromasPro 1.7.4 (Technelysium, Tewantin, Australia). The protein-coding mitochondrial sequences were aligned with MUSCLE (Edgar, 2004) as implemented in MEGA X (Kumar et al., 2018) with the default settings. The nuclear sequences and the sequences coding for the 16S rRNA gene were aligned with MAFFT 7 (Katoh et al., 2017), using the Q-INS-i strategy and otherwise default settings (see Supplementary Files, Data S1). Bayesian inference (BI), maximum likelihood (ML) and maximum parsimony (MP) analyses were used to reconstruct phylogenetic relationships.

Partitions and evolutionary models were evaluated separately for mitochondrial and nuclear sequences. The *cox1* sequences were initially divided into three partitions based on codon positions (1st, 2nd and 3rd position), while the 16S sequences were not further subdivided. The nuclear sequences were initially divided into three partitions (5.8S, ITS2 and 28S). PartitionFinder 2.1.1 (Lanfear et al., 2017) was used to search for the best evolutionary models and partitioning schemes, conducting exhaustive searches on the basis of the mitochondrial and nuclear data sets, respectively, with a separate estimation of branch lengths for each partition and with the Bayesian information criterion to select among models and partitions. The models were limited to those available in MrBayes 3.2.6 (Ronquist et al., 2012). For the *cox1* and 16S sequences, the PartitionFinder analysis suggested three partitions, the first containing the 1st and 2nd codon positions of *cox1* (GTR + G model), the second containing the 3rd codon positions of *cox1* (GTR + I + G model) and the third containing the 16S sequences (HKY + I + G model). For the nuclear sequences, the PartitionFinder analyses suggested a single partition and the HKY + I + G model.

The BI analysis was performed using MrBayes. Metropolis-coupled Monte Carlo Markov chain (MC³) searches were run with four chains in two separate runs with 50 000 000 generations with default priors, trees sampled every 1000 generations under default heating using the partitions and evolutionary models for the mitochondrial and nuclear data sets as suggested by the PartitionFinder analyses. The first 500 000 generations of each run were discarded as a burn-in.

The ML analysis was performed using GARLI 2.1 (Zwickl, 2006) with the partitions and models suggested by PartitionFinder and otherwise default settings from the standard configuration file

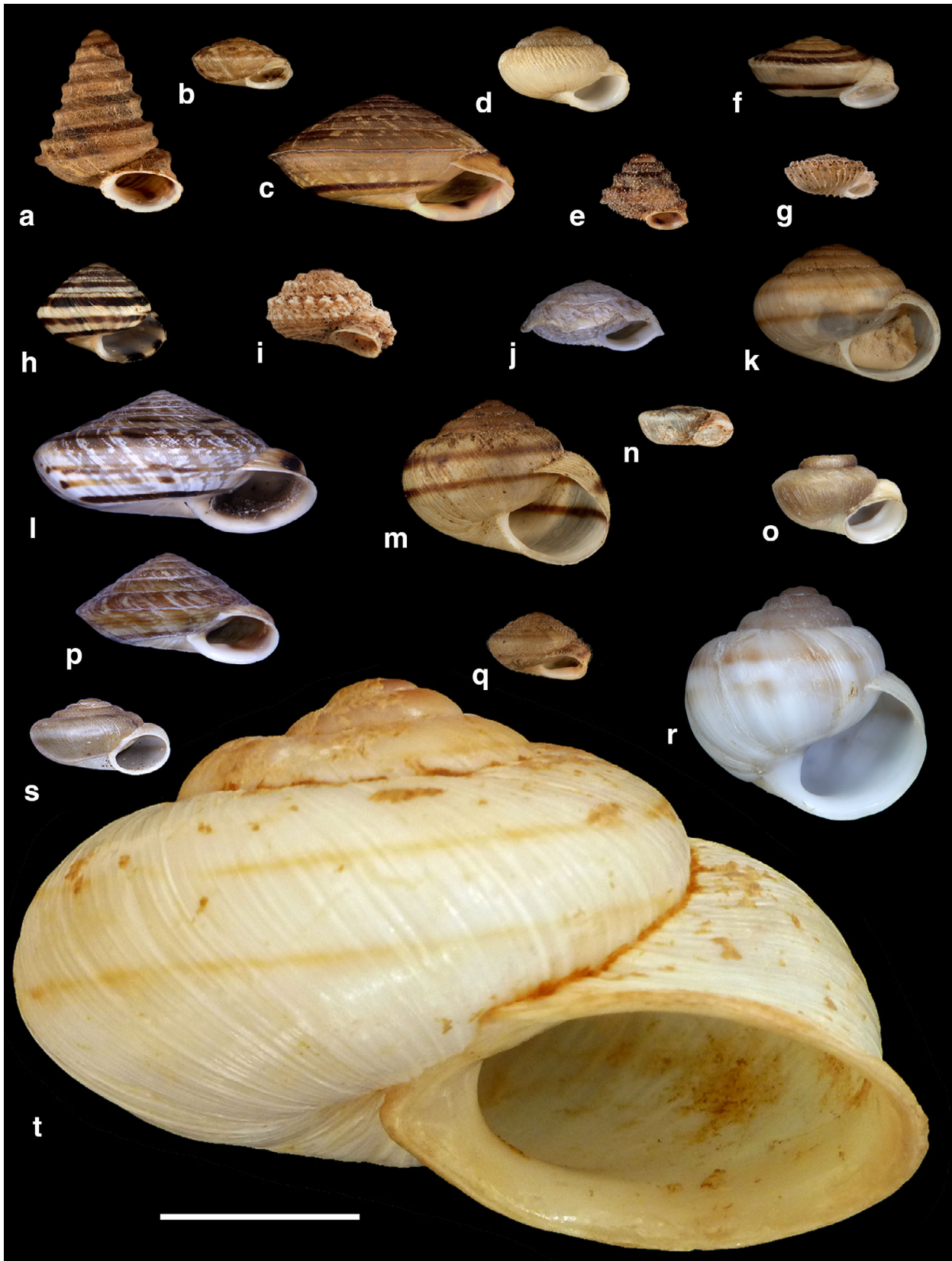


Fig. 2. Diversity of shells of Geomitridae from the Madeiran Archipelago. (a) *Wollastonia turricula* (Lowe, 1831), Ilhéu de Cima, top plateau. (b) *Actinella (Actinella) lentiginosa* (Lowe, 1831), Madeira, gorge of Ribeira da Janela 1 km from the sea. (c) *Callina bulverii* (Wood, 1828), Porto Santo, 200 m SW of Zimbreiro near the road serpentine. (d) *Caseolus (Caseolus) i. innominatus* (Gray, 1825), Ilhéu de Cima, top plateau. (e) *Hystricella bicarinata* (Sowerby, 1824), Porto Santo, Pico do Castelo, summit. (f) *Disculella m. madeirensis* (Wood, 1828), Madeira, Jardim do Mar. (g) *Serratorotula aff. acarinata* (Hemmen & Groh, 1985), Ilhéu de Baixo. (h) *Lemniscia michaudi* (Deshayes, 1831), Porto Santo, Pico de Baixo, northern slope just below the summit. (i) *Geomitra watsoni* (Johnson, 1897), Ilhéu do Farol. (j) *Discula (Mandahlia) t. tectiformis* (Sowerby, 1824), Porto Santo, Pico de Baixo. (k) *Domunculifex littorinella* (Mabille, 1883), Porto Santo, summit of Pico do Castelo. (l) *Testudodiscula testudinalis* (Lowe, 1852), Porto Santo, Porto do Pedregal. (m) *Plebocula nitidiuscula* (Sowerby, 1824), Madeira, gorge of Ribeira da Janela 1 km from the sea. (n) *Steenbergia pauperula* (Lowe, 1831), Madeira, Porto da Cruz towards Caniçal. (o) *Spirorbula oblecta* (Lowe, 1831), Porto Santo, Zimbral da Areia. (p) *Discula (Discula) discina* (Lowe, 1852), Porto Santo, Porto da Morena. (q) *Actinella (Faustella) fausta* (Lowe, 1831), Madeira, gorge of Ribeira da Janela 1 km from the sea. (r) *Helicomela p. punctulata* (Sowerby, 1824), Porto Santo, Fonte da Areia. (s) *Caseolus (Leptosticta) leptostictus* (Lowe, 1831), Madeira, Ponta do Garajau. (t) *Pseudocampylaea lowii* (Férussac, 1835), Porto Santo, probably NW-coast. Scale bar: 10 mm. [Colour figure can be viewed at wileyonlinelibrary.com]

(<http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html>), except for setting the number of replicates to 100. Support values were calculated by bootstrapping with 1000 replications. For comparison of support values, an additional bootstrap analysis (1000 non-parametric bootstrap replications) was conducted using IQ-TREE (Chernomor et al., 2016; Minh et al., 2020) using the same partitions and evolutionary models as in the analysis with GARLI.

Heuristic MP searches were conducted with PAUP* 4.0b10 (Swofford, 2002) with unordered characters, 100 random sequence addition replicates, tree bisection reconnection (TBR) branch swapping, and gaps treated as missing data. Support for internal branches was assessed in PAUP* by bootstrapping with 1000 replications, using full heuristic searches with 10 random addition sequence replicates, TBR branch swapping, and one tree held at each step during stepwise addition. For comparison of support values, an additional bootstrap analysis (1000 non-parametric bootstrap replications) was conducted using TNT (Goloboff et al., 2008) with 10 random addition sequence replicates, TBR branch swapping, and one tree held at each step during stepwise addition.

Bootstrap support (BS) values from the ML and MP analyses as well as posterior probabilities (PP) from the Bayesian analysis were mapped on the BI 50% majority-rule consensus tree with SumTrees 3.3.1, which is part of the DendroPy 3.8.0 package (Sukumaran and Holder, 2010). PP ≥ 0.95 and BS ≥ 70 were interpreted as positive support for nodes.

Molecular dating

To infer a time frame for diversification patterns of Geomitridae, we used the Bayesian algorithm implemented in Beast 2.5.2 (Bouckaert et al., 2019) based on mitochondrial and nuclear sequence data assuming the same partitions and nucleotide substitution models as in the ML and BI analyses. A strict molecular clock was rejected at $\alpha = 0.05$ by the test implemented in MEGA X for each of the different partitions. Therefore, a linked uncorrelated relaxed lognormal molecular clock was used for the Beast analysis assuming the birth-death model as tree prior. The analysis was run for 50 000 000 generations with a sampling frequency set to 10 000. Tracer v1.7.1 (Rambaut et al., 2018) was used to assess convergence and to check that the estimated effective population sizes (ESS) for all estimated parameters were above 200. 10% of generations were discarded as burn-in. A maximum clade credibility tree with median node heights was calculated with Treeannotator 2.1.3 (included in the Beast 2.5.2 software suite) from the post-burn-in samples.

We used *Loganiopharynx rarus* (Boissy, 1840) from Ypresian deposits (Early Eocene, 56–47.8 Ma) in France for the calibration of the tree. Nordsieck (2017) classified this taxon in Hygromiidae. However, it cannot be assigned unambiguously to one of the subfamilies of the crown group of Hygromiidae. We assume that it is a representative of the stem group taxon of Hygromiidae. Actually, it was assigned to the stem group of Hygromiidae in the analysis of

Razkin et al. (2015: fig. 3). Thus, this taxon can be used to put a minimum age on the divergence of Hygromiidae and their sister group, Geomitridae plus Canariellidae, according to Ho and Phillips (2009: fig. 1). We used a lognormal-distributed prior for this calibration.

Ancestral character state and ancestral area estimation

For the ancestral state estimation, Mesquite v.3.4 (Maddison and Maddison, 2018) was used, tracing the state of the appendicular (missing, single or double) over the MP tree calculated in the previous step by using the parsimony approach and otherwise default settings.

The biogeographic history of the group, especially with regard to inter-island dispersal events within the Madeiran Archipelago (island hopping) was estimated on the basis of our mitochondrial and nuclear sequence data set using the Bayesian binary MCMC method implemented in RASP 3.02 (Yu et al., 2015). For the analysis, the calibrated maximum clade credibility tree from the Beast analysis was used together with a matrix, in which the distribution of the sequenced species in the following seven geographical regions was listed: (A) Madeira, (B) Porto Santo and its satellite islets, (C) Ilhas Desertas, (D) Canary Islands, (E) Iberian Peninsula, (F) Europe (excl. the Iberian Peninsula), (G) North Africa. The analysis was run on 100 randomly selected post-burn-in trees from the Beast analysis to account for statistical uncertainty and otherwise default settings.

Results

Phylogenetic analyses

The concatenated alignment of mitochondrial and nuclear sequences obtained from 51 individuals comprised a total number 2698 base pairs (bp) (655 bp *cox1*, 515 bp 16S and 1528 bp 5.8S + ITS2 + 28S). The three different methods (BI, ML and MP) for the reconstruction of phylogenetic relationships resulted in trees with identical topologies. The results of the bootstrap analyses with IQ-Tree (ML) and TNT (MP) were very similar to the results of the analyses with GARLI (ML) and PAUP* (MP) (Fig. 4). For brevity, only the results obtained with GARLI and PAUP* are presented in the following, but see Fig. 4 for all support values.

The Geomitridae in the sense of Razkin et al. (2015) and Neiber et al. (2017) were recovered in our

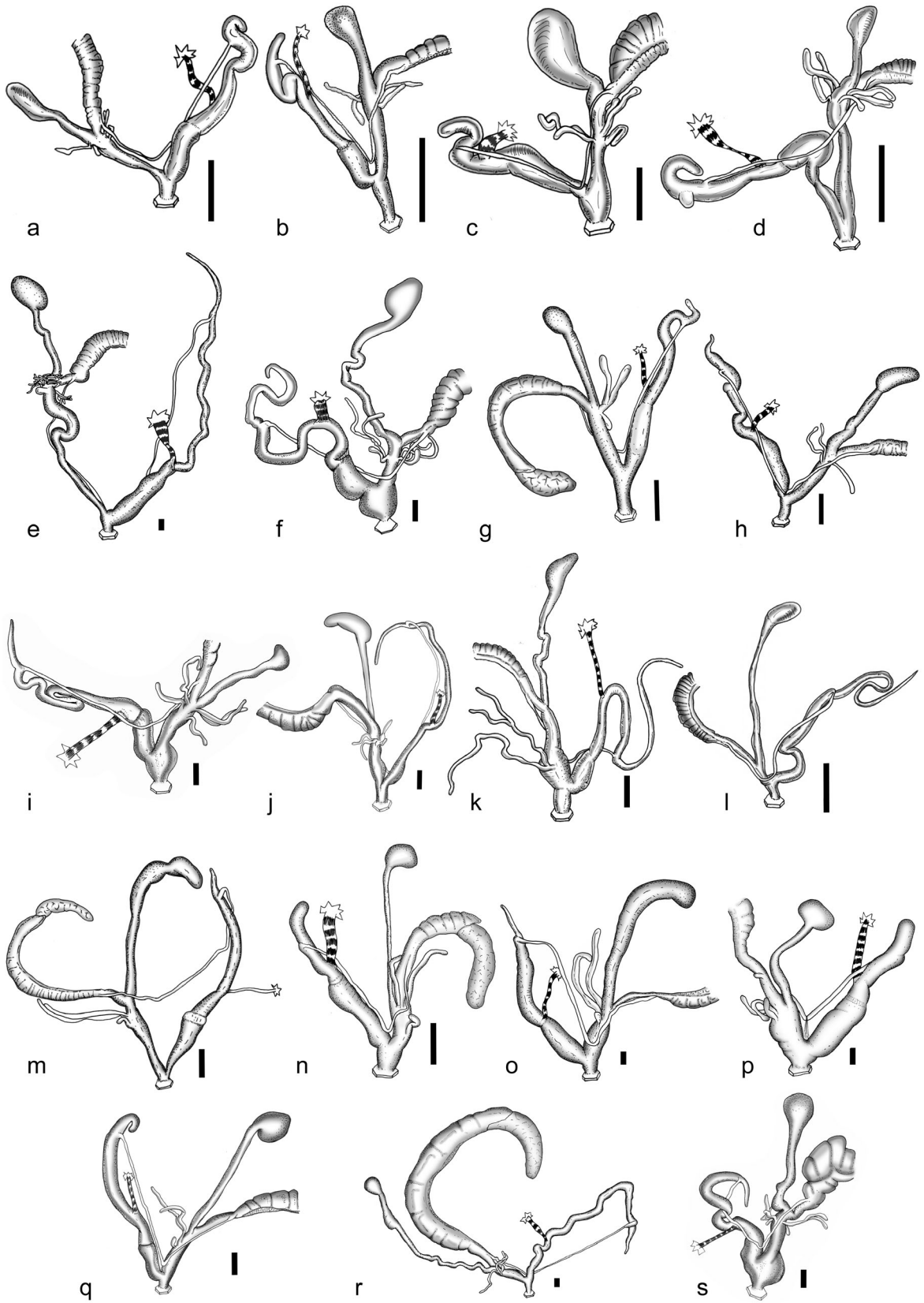


Fig. 3. Distal genitalia of Geomitriini from the Madeiran Archipelago. (a) *Geomitra coronula* (Lowe, 1852), Deserta Grande, Pedregal. (b) *Actinella (Actinella) arcta* (Lowe, 1831), Madeira, Porto Novo. (c) *Actinella (Hispidella) armitageana* (Lowe, 1852), Madeira, Pico Ruivo. (d) *Actinella (Faustella) fausta* (Lowe, 1831), Madeira, Ribeira da Metade. (e) *Pseudocampylaea portosanctana* (Sowerby, 1824), Ilhéu de Cima. (f) *Helicomela p. punctulata* (Sowerby, 1824), Porto Santo, Fonte da Areia. (g) *Serratorotula juliformis* (Lowe, 1852), Porto Santo, Pico da Ana Ferreira. (h) *Caseolus (Caseolus) i. innominatus* (Gray, 1825), Porto Santo, Ribeira da Areia. (i) *Caseolus (Leptostictea) h. hartungi* (Albers, 1852), Porto Santo, Pico Branco. (j) *Disculella m. madeirensis* (Wood, 1828), Madeira, Porto Novo. (k) *Spirorbula oblecta* (Lowe, 1831), Porto Santo, Zimbral da Areia. (l) *Steenbergia paupercula* (Lowe, 1831), Porto Santo, Fonte da Areia. (m) *Lemniscia michaudi* (Deshayes, 1831), Porto Santo, Terra Chã. (n) *Hystricella bicarinata* (Sowerby, 1824), Porto Santo, Pico do Facho, south slope, along the path. (o) *Callina rotula* (Lowe, 1831), Porto Santo, Cabeço dos Bodes. (p) *Wollastonaria turricula* (Lowe, 1831), Ilhéu de Cima. (q) *Discula (Discula) discina* (Lowe, 1852), Porto Santo, Morenos, road to Ponta Canaveira. (r) *Discula (Mandahlia) t. tectiformis* (Sowerby, 1824), Porto Santo, Pico de Baixo. (s) *Plebecula nitidiuscula* (Sowerby, 1824), Scale bars: 1 mm. f–h, m, o–p: Modified from De Mattia et al. (2018a). For the designation of the different sections of the distal genitalia, see Figs 7 and 8.

phylogenetic analyses with strong support (PP: 1.00; BS (ML): 79; BS (MP): 88). The subfamilies Geomitriinae (PP: 1.00; BS (ML): 92; BS (MP): 82) and Helicellinae (PP: 1.00; BS (ML): 82; BS (MP): 81) in the sense of Razkin et al. (2015) and Neiber et al. (2017) were also recovered with strong support. Within the Helicellinae, the relationships of the representatives of the Cernuellini, Helicellini, Helicopsini and Trochoideini were only supported in the BI analysis but not in the ML and MP analyses (Fig. 4).

Within the Geomitriinae, the Ponentinini represented by *Ponentina* cf. *revelata* (Michaud, 1831) was recovered as the sister group of a maximally supported clade including the Cochlicellini and the Geomitriini. The Cochlicellini, represented by *Cochlicella (Cochlicella) conoidea* (Draparnaud, 1801) and *Monilearia monilifera* (Webb & Berthelot, 1833), were recovered as a maximally supported monophyletic group in all three analyses, whereas the monophyly of the Geomitriini was supported in the BI and MP analyses but not in the ML analysis (PP: 1.00; BS (ML): 65; BS (MP): 91).

Within the Geomitriini, the genera *Geomitra* Swainson, 1840, *Pseudocampylaea* Pfeiffer, 1877, *Serratorotula* Groh & Hemmen, 1986 and *Disculella* Pilsbry, 1895 in the sense of Bank (2009) formed maximally supported groups in our phylogenetic analyses. Furthermore, the genera *Hystricella* Lowe, 1855, *Callina* Lowe, 1855 and *Wollastonaria* De Mattia, Neiber & Groh, 2018 in the sense of De Mattia et al. (2018a,b) were also recovered as monophyletic groups with high support (Fig. 4), with *Wollastonaria* as the sister group of a maximally supported clade including *Hystricella* and *Callina*. However, some genera in the sense of, e.g., Seddon (2008) and/or Bank (2009) were recovered as non-monophyletic, i.e. *Actinella* Lowe, 1852, *Caseolus* Lowe, 1852 and *Discula* Lowe, 1852.

The representative of *Caseolus* s. str. was joined with the representative of the subgenus *Leptostictea* Mandahl-Barth, 1950 with maximal support in all three analyses. However, individuals representing the subgenus *Helicomela* Lowe, 1855 formed a clade with *Pseudocampylaea*, albeit only significantly supported in the BI analysis.

The representatives of *Actinella* s. str. and the subgenera *Hispidella* Lowe, 1852 and *Faustella* Mandahl-Barth, 1950 were joined with strong support (PP: 1.00; BS (ML): 100; BS (MP): 99) as the sister group of *Geomitra* Swainson, 1840, although only supported in the BI analysis (PP: 1.00; BS (ML): 56; BS (MP): 53). Representatives of the subgenus *Plebecula* Lowe, 1852 in the sense of Bank (2009), i.e. *Actinella (Plebecula) nitidiuscula* (Sowerby, 1824) from Madeira and *Actinella (Plebecula) littorinella* (Mabille, 1883) from Porto Santo, were not recovered as a monophyletic group and placed on different branches of the phylogenetic tree (Fig. 4). The two individuals of *A. (Plebecula) nitidiuscula* formed a maximally supported clade that branched off first, albeit only supported in the BI analysis, in the Geomitriini clade, while *A. (Plebecula) littorinella* was recovered as the sister taxon of *Serratorotula*, although likewise only supported in the BI analysis. For the latter taxon, the new genus *Domunculifex* is formally introduced below.

Representatives of *Discula* in the sense of De Mattia et al. (2018a), except for *Discula testudinalis* (Lowe, 1852), formed a well-supported clade, with all nodes within this clade with positive support in all analyses (Fig. 4). The representative of the subgenus *Mandahlia* Forcart, 1965, *D. (Mandahlia) tectiformis* (Sowerby, 1824), was recovered as the sister group of the remaining *Discula* s. str. species within this clade. *Discula testudinalis* formed a strongly supported clade with the conchologically very distinct species *Lemniscia michaudi* (Deshayes, 1831) (PP: 1.00; BS (ML): 82; BS (MP): 89), which in turn was recovered as the sister group of the clade including the genera *Hystricella*, *Callina* and *Wollastonaria*. For the nominal taxon *Helix (Discula) testudinalis* Lowe, 1852 the new genus *Testudodiscula* is formally described below.

Finally, *Steenbergia* Mandahl-Barth, 1950 and *Spirorbula* Lowe, 1852, represented by *Steenbergia paupercula* (Lowe, 1831) (in the sense of Lace, 1992) and *Spirorbula oblecta* (Lowe, 1831) respectively, formed a strongly supported clade in all three analyses (PP: 1.00; BS (ML): 79; BS (MP): 81), although its position as the sister group to the clade containing *Lemniscia*, *Testudodiscula*, *Hystricella*, *Callina* and

Table 1
Collection numbers, voucher numbers, GenBank accession numbers and geographical coordinates for the specimens used in the molecular phylogenetic analyses

Taxon	Collection No.	DNA Voucher No.	GenBank accession No.		Latitude	Longitude
			<i>cox1</i>	5.8S rRNA + ITS2 + 28S rRNA		
Hygromiidae Tryon, 1866						
<i>Hygromia (Hygromia) cinctella</i> (Draparnaud, 1801)	ZMH 96006	1825	KX622025*	KX622001*	47°21'17"N	08°33'08"E
Canariellidae Schileyko, 1991						
<i>Canariella (Canariella) giustii</i> Ibáñez & Alonso, 2006	MN	3654	KY818423*	KY818447*	28°19'50"N	16°51'25"W
Geomitridae Boettger, 1909						
Geomitridae Boettger, 1909						
Cochlicellini Schileyko, 1991						
<i>Cochlicella (Cochlicella) conoidea</i> (Draparnaud, 1801)	EHUMC 1004	SP165	KY818425*	KY818452*	41°08'15"N	08°40'00"W
<i>Monilearia (Monilearia) monilifera</i> (Webb & Berthelot, 1833)	EHUMC 1906	SP769	KY818426*	KY818514*	28°22'20"N	14°06'06"W
Geomitridae Boettger, 1909						
<i>Actinella (Actinella) lentiginosa lentiginosa</i> (Lowe, 1831)	MN	MN3079	MT772362	MT764852	32°50'57"N	17°09'42"W
<i>Actinella (Faustella) fausta</i> (Lowe, 1831)	MN	MN3080	MT772363	MT764853	32°50'57"N	17°09'42"W
<i>Actinella (Faustella) armitageana</i> (Lowe, 1852)	MN	MN3060	MT772364	MT764854	32°44'24"N	16°56'19"W
<i>Callina bulverii</i> (Wood, 1828)	NHMW	DI15_2	MG575131‡	MT764855	33°04'16"N	16°18'53"W
<i>Callina rotula</i> (Lowe, 1831)	NHMW	DI44_2	MG575145‡	MT764856	33°04'16"N	16°18'53"W
<i>Caseolus (Caseolus) cf. abjectus candidatus</i> (Pfeiffer, 1853)	NHMW	CA08_1	MG575187‡	MT764857	32°52'03"N	17°09'56"W
<i>Caseolus (Leptosticta) hartungi ficitilis</i> (Lowe, 1852)	NHMW	CA10_1	MG575188‡	MT764858	33°05'15"N	16°21'16"W
<i>Discula (Discula) atrita</i> (Lowe, 1831)	NHMW	DI05_1	MG575147‡	MT764859	33°04'44"N	16°17'58"W
<i>Discula (Discula) calceigena</i> (Lowe, 1831)	NHMW	DI42_1	MG575163‡	MT764860	33°06'15"N	16°19'15"W
<i>Discula (Discula) cheiranthicola</i> (Lowe, 1831)	MN	MN3056	MT772365	MT764861	33°05'48"N	16°18'54"W
<i>Discula (Discula) discina</i> (Lowe, 1852)	NHMW	DI24_2	MG575177‡	MT764862	33°05'07"N	16°21'21"W
<i>Discula (Discula) pulvinata</i> (Lowe, 1831)	NHMW	DI30_1	MG575181‡	MT764863	32°44'41"N	16°41'58"W
<i>Discula (Discula) polymorpha arenicola</i> (Lowe, 1831)	NHMW	DI06_1	MG575179‡	MT764863	32°44'41"N	16°41'58"W
<i>Discula (Discula) polymorpha nebulata</i> (Lowe, 1855)	MN	MN3071	MT772366	MT764864	32°50'57"N	17°09'42"W
<i>Discula (Mandahlia) tectiformis tectiformis</i> (Sowerby, 1824)	MN	MN3066	MT772367	MT764866	33°03'46"N	16°17'57"W
<i>Disculella madeirensis taeniata</i> (Webb & Berthelot, 1833)	MN	MN3070	MT772369	MT764868	32°44'10"N	17°12'38"W
<i>Disculella cf. spirulina</i> (Cockerell, 1921)	MN	MN3069	MT772368	MT764867	32°48'49"N	17°15'45"W
<i>Domunculifex littorinella</i> (Mabille, 1883)	MN	MN3068	MT772370	MT764898	33°04'55"N	16°20'07"W
<i>Geomitra coronula</i> (Lowe, 1852)	MN	MN3081	MT772371	MT764870	32°32'25"N	16°31'49"W
<i>Geomitra watsoni</i> (Johnson, 1897)	MN	MN3082	MT772372	MT764871	32°44'11"N	16°40'36"W
<i>Helicomea punctulata punctulata</i> (Sowerby, 1824)	MN	MN3059	MT772373	MT764872	33°02'24"N	16°23'41"W
<i>Helicomea punctulata punctulata</i> (Sowerby, 1824)	MN	MN3063	MT772374	MT764873	33°03'13"N	16°18'53"W
<i>Steenbergia paupercula</i> (Lowe, 1831)	MN	MN3061	MT772381	MT764884	32°45'41"N	16°47'21"W
<i>Hystriocella bicarinata</i> (Sowerby, 1824)	MN	HY03_2	MG575049‡	MT764874	33°04'16"N	16°18'53"W
<i>Hystriocella echimulata</i> (Lowe, 1831)	MN	MN3010	MG575093‡	MT764875	33°05'38"N	16°18'01"W
<i>Lemnisca michaudi</i> (Deshayes, 1832)	MN	MN3055	MT772375	MT764876	33°05'35"N	16°18'09"W
<i>Plebecula nitidiuscula</i> (Sowerby, 1824)	MN	MN3074	MT772376	MT764877	32°41'37"N	17°05'18"W
<i>Plebecula nitidiuscula</i> (Sowerby, 1824)	MN	MN3078	MT765152	MT764908	32°50'57"N	17°09'42"W
<i>Pseudocampylaea portosanciana</i> (Sowerby, 1824)	MN	MN3057	MT772377	MT764879	33°04'43"N	16°18'06"W
<i>Pseudocampylaea portosanciana</i> (Sowerby, 1824)	MN	MN3062	MT772378	MT764880	33°04'43"N	16°18'06"W
<i>Serratorotula aff. acarinata</i> (Hemmen & Groh, 1985)	MN	MN 3083	MT772379	MT764881	33°00'04"N	16°23'03"W
<i>Serratorotula juliformis</i> (Lowe, 1852)	MN	MN3033	MT765151	MT764882	33°00'25"N	16°17'57"W
<i>Serratorotula juliformis</i> (Lowe, 1852)	MN	MN3036	MG575210‡	MT764913	33°03'45"N	16°17'57"W
<i>Spirorbula obrecta</i> (Lowe, 1831)	MN	MN3058	MT772380	MT764883	33°06'08"N	16°19'32"W
<i>Testudodiscula testudinalis</i> (Lowe, 1852)	MN	MN3077	MG575186‡	MT764885	33°06'14"N	16°19'18"W

Table 1
(Continued)

Taxon	Collection No.	DNA Voucher No.	<i>cox1</i>	GenBank accession No.			Latitude	Longitude
				16S rRNA	5.8S rRNA + ITS2 + 28S rRNA	ITS2 + 28S rRNA		
<i>Wollastonia jessicae jessicae</i> (De Mattia, Neiber & Groh, 2018)	NHMW	HY05_1	MG575110‡	MT764886	MT764916	33°03'44"N	16°19'35"W	
<i>Wollastonia jessicae monticola</i> (De Mattia, Neiber & Groh, 2018)	NHMW	HY03_1	MG575118‡	MT764887	MT764917	33°04'16"N	16°18'53"W	
<i>Wollastonia klausgrohi</i> (De Mattia & Neiber, 2018)	NHMW	HY31_1	MG575117‡	MT764888	MG575196‡	33°04'06"N	16°18'52"W	
<i>Wollastonia leacockiana</i> (Wollaston, 1878)	NHMW	HY01_2	MG575122‡	MT764889	MG575197‡	33°02'57"N	16°22'04"W	
<i>Wollastonia oxytropis</i> (Lowe, 1831)	NHMW	HY38_1	MG575125‡	MT764890	MG575198‡	33°04'42"N	16°17'56"W	
<i>Wollastonia turricula</i> (Lowe, 1831)	MN	MN3000	MG575129‡	MT764891	MT764918	33°03'13"N	16°18'53"W	
Ponentini Schileyko, 1991								
<i>Ponentina</i> cf. <i>revelata</i> (Michaud, 1831)	EHUMC 1033	SP158	KY818428*	KY818529*	KJ458630†	42°51'28"N	03°35'56"W	
Helicellinae Ihering, 1909								
Cernuellini Schileyko, 1991								
<i>Cernuella (Cernuella) virgata</i> (da Costa, 1778)	EHUMC 1907	SP801	KY818429*	KY818449*	KY818563*	42°49'55"N	02°41'14"W	
Helicellini Ihering, 1909								
<i>Helicella itala itala</i> (Linnaeus, 1758)	ZMH 96999	1690	KY818430*	KY818475*	KY818584*	52°20'42"N	09°52'54"E	
Helicopsini Nordsteck, 1987								
<i>Helicopsis striata</i> (Müller, 1774)	ZMH 96682	2250	KY818431*	KY818476*	KY818585*	52°49'36"N	14°05'13"E	
Plentuisini Razkin, Gómez-Moliner, Prieto, Martínez-Ortí, Arrébola, Muñoz, Chueca & Madeira, 2015								
<i>Plentuisa vendia</i> Puente & Prieto, 1992	EHUMC 1032	SP407	KY818432*	KY818526*	KJ458629†	43°15'52"N	04°46'28"W	
Trochoideini Nordsteck, 1987								
<i>Trochoidea elegans</i> (Gmelin, 1791)	ZMH 37714	1066	KY818433*	KY818540*	KY818646*	37°10'13"N	10°11'54"E	

EHUMC: Zoology and Animal Cell Biology Department, University of the Basque Country, Vitoria-Gasteiz, Spain; MN: study collection of Marco T. Neiber, University Hamburg, Germany; NHMW: Natural History Museum, Vienna, Austria; ZMH: Zoological Museum of the Center for Natural History (CeNak), University Hamburg, Hamburg, Germany. Neiber et al. (2015).* Razkin et al. (2015).† De Mattia et al. (2018a,b).‡

Wollastonaria was only significantly supported in the BI analysis.

Molecular dating

Assuming an Early Eocene (47.8 Ma) split of Hygromiidae and the clade including Geomitridae plus Canariellidae, the split of the latter clade was dated at 38.9 Ma (95% highest posterior density interval (HPD): 27.5–48.7 Ma) in the Late Eocene (Fig. 5). The split of Geomitrinae and Helicellinae in the sense of Razkin et al. (2015) was dated to have occurred in the Early Oligocene at 32.6 Ma (HPD: 22.4–43.4 Ma), with the diversification of Helicellinae starting at 26.8 Ma (HPD: 17.4–36.8 Ma) in the Late Oligocene. The lineage representing the Ponentinini split off at 26.0 Ma (HPD: 17.4–36.1 Ma) from the remaining two tribes of the Geomitrinae, Geomitriini and Cochlicellini, the split of which was dated at 15.8 Ma (HPD: 10.2–21.7 Ma) in the Early Miocene (Fig. 5). The onset of diversification of the Geomitriini was dated to the Middle Miocene at 13.0 Ma (HPD: 8.4–17.0 Ma).

The basal relationships within Geomitriini were not well-resolved in the phylogenetic analyses. However, a sister group relationship of *Geomitra* and *Actinella*, *Helicomela* and *Pseudocampylaea*, as well as *Serratorotula* and *Domunculifex*, were supported in the Bayesian analysis (Fig. 5) and the respective splits of these sister groups were all dated at 9.3–9.4 Ma in the Late Miocene (Fig. 5). Similarly, the separation of *Caseolus* and a clade including *Disculella*, *Discula*, *Spirorbula*, *Steenbergia*, *Testudodiscula*, *Lemniscia*, *Hystricella*, *Callina* and *Wollastonaria* was dated at 9.2 Ma (HPD: 5.9–12.7 Ma). The split of *Disculella* from Madeira and the remaining genera in the latter clade was dated at 7.9 Ma (HPD: 5.1–11.0 Ma), and the split of *Discula* and the clade including *Spirorbula*, *Steenbergia*, *Testudodiscula*, *Lemniscia*, *Hystricella*, *Callina* and *Wollastonaria* was dated at 7.1 Ma (HPD: 4.6–9.9 Ma), with the onset of diversification of *Discula* dated at 3.3 Ma (HPD: 1.1–5.1 Ma). *Steenbergia* and *Spirorbula* were dated to have diverged 4.6 Ma (HPD: 2.7–6.7 Ma), with the two together separating from the lineage leading to *Testudodiscula*, *Lemniscia*, *Hystricella*, *Callina* and *Wollastonaria* 6.7 Ma (Ma: 4.2–9.3 Ma). *Testudodiscula* and *Lemniscia* were recovered to have split 2.1 Ma (HPD: 1.0–3.5 Ma) and the origin of the clade including *Hystricella*, *Callina* and *Wollastonaria* was dated at 2.9 Ma (HPD: 1.9–4.2 Ma), while the split of these two clades was dated at 3.8 Ma (HPD: 2.4–

5.6 Ma). *Hystricella*, *Callina* and *Wollastonaria* all originated according to our analysis within the last two million years (Fig. 5).

Ancestral character state and ancestral area estimation

The ancestral state estimation recovered the presence of two appendiculae as the ancestral state of the Geomitriini (Fig. 6). The analysis suggested that one appendicula was lost three times independently within the tribe. One occurred in *Pseudocampylaea*, a second in *Serratorotula* and a third loss in the lineage leading to the clade including *Disculella*, *Discula*, *Steenbergia*, *Spirorbula*, *Lemniscia*, *Testudodiscula*, *Callina*, *Hystricella* and *Wollastonaria*. In the lineage leading to *Steenbergia* and *Spirorbula*, the second appendicula was subsequently also lost, resulting in a complete absence of these vaginal appendages in these two taxa. However, glandulae mucosae are present in *Spirorbula*, while these are lost in *Steenbergia*.

The ancestral area estimation (Fig. 6) implies that the Madeiran Geomitriini originated most likely on Madeira. The analysis further suggests an inter-island dispersal event from Porto Santo to Madeira for the lineage leading to the clade including *Geomitra* and *Actinella* and another inter-island dispersal event for the lineage leading to *Disculella*, also from Porto Santo to Madeira (Fig. 6). Moreover, the ancestral area estimation suggests a dispersal event from Porto Santo to Madeira within *Discula*, i.e. the lineage leading to *D. polymorpha* probably colonised Madeira from Porto Santo (Fig. 6).

Systematic descriptions

Geomitridae Boettger, 1909

Geomitrinae Boettger, 1909

Geomitriini Boettger, 1909

Testudodiscula Brozzo, De Mattia, Harl & Neiber n. gen. (Figs 2l, 7a–e).

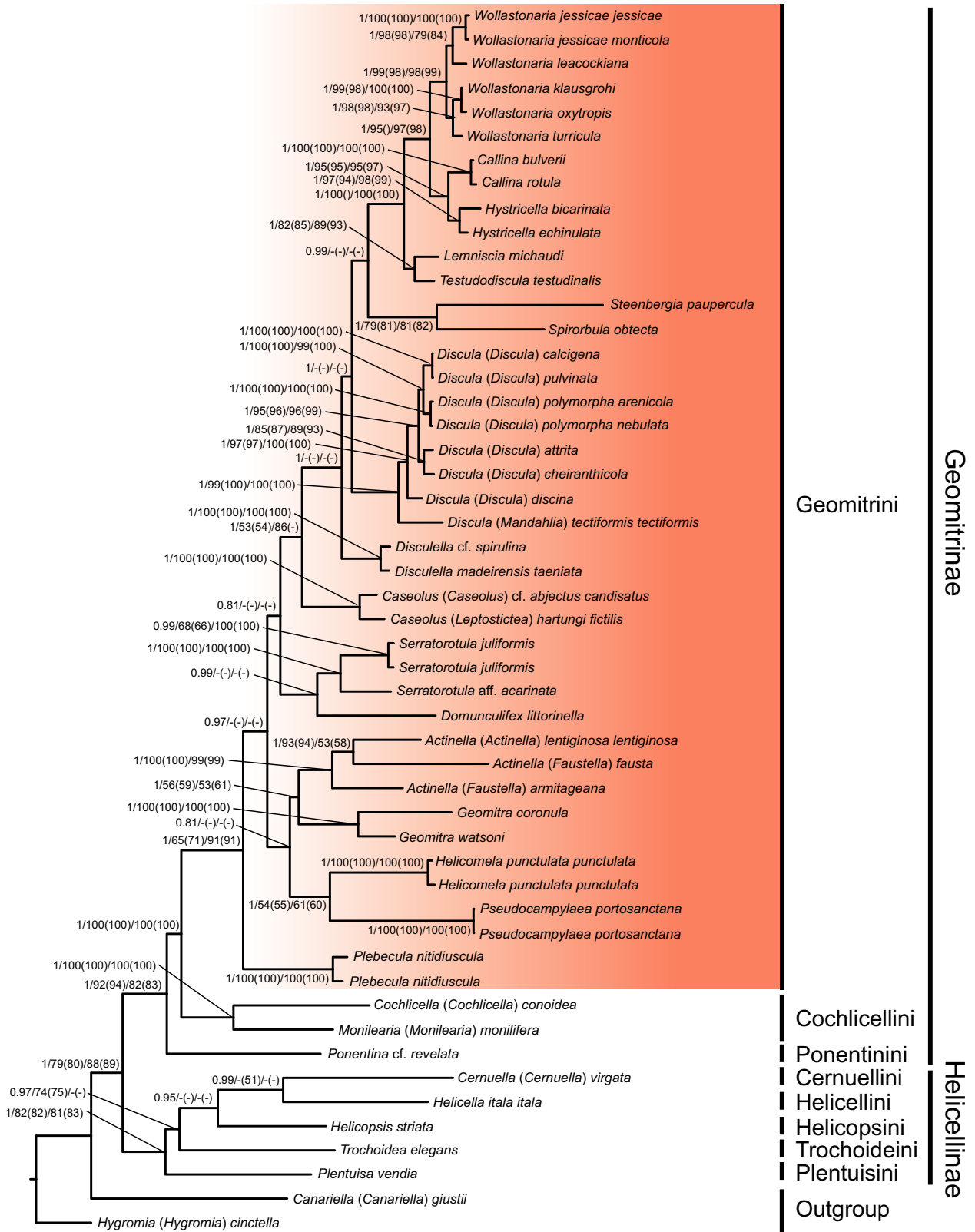
ZooBank registration: <http://zoobank.org/D54985D9-217A-4961-8F88-8AD49F6E5732>

Type species: *Helix (Discula) testudinalis* Lowe, 1852 (herewith designated).

Etymology: Compound word derived from the Latin *testudo* (= tortoise) and the generic name *Discula* Lowe, 1852.

Description: Shell discoidal, keeled (keel not bent downwards), 16–21 mm wide, with 6–7 finely granulated whorls; umbilicus open, perspectival. Vagina with

Fig. 4. Phylogeny of Geomitriini from the Madeiran Archipelago. Bayesian 50% majority-rule consensus tree based on the analysis of concatenated partial *cox1* and 16S and nuclear 5.8S + ITS2 + 28S sequences. The numbers at the nodes refer to posterior probabilities (PP) from the Bayesian analysis (left), bootstrap support (BS) values from the maximum likelihood analysis with GARLI (IQ-TREE) (middle) and BS values from the maximum parsimony analysis with PAUP* (TNT) (right). Only nodes with PP \geq 0.5 and/or BS \geq 50 are annotated. [Colour figure can be viewed at wileyonlinelibrary.com]



a single appendicula; much shorter than the single bunch of ramified glandulae mucosae; epiphallus very long, 5–6 times the length of the penis; flagellum short, distinctly thinner than adjacent parts of epiphallus.

Remarks: *Testudodiscula testudinalis* (Lowe, 1852) is very similar to *Callina bulverii* (Fig. 2c) with regard to the shell, the keel along the body whorl is however not bent downwards and usually situated higher on the body whorl. Anatomically it differs from *C. bulverii* by having a relatively shorter epiphallus with less numerous longitudinal folds on the inner wall. From the phylogenetically closely related *L. michaudi* (Fig. 2h), *T. testudinalis* is easily distinguished by size and shell form. Anatomically, *Testudodiscula* differs from *Lemniscia* (Fig. 3m) by the shorter (in relation to the appendicula), more strongly ramified glandulae mucosae.

Domunculifex Brozzo, De Mattia, Harl & Neiber n. gen. (Figs 2k, 8a–d).

ZooBank registration: <http://zoobank.org/41ABC045-8E66-4241-A7D9-433C0869E721>

Type species: *Helix littorinella* Mabille, 1883 (here-with designated).

Etymology: Masculine, from domuncula (= small house) and -fex (maker/builder); Latinisation of the German vernacular word “Häuslebauer” meaning constructor/builder of a small house.

Description: Shell depressed-globular, cream-coloured, usually with two narrow brown spiral bands, ornamented with irregular growth lines, without hairs, 9–10 mm wide, 6–7 mm high, with approximately 5 whorls; aperture elliptical, peristome simple, without thickened lip that is only slightly reflected on the columellar side, partly covering the narrow umbilicus. Vagina longer than penis, slightly shorter than epiphallus, with two appendiculae that are less than one quarter the length of the unramified glandulae mucosae; at the distal end of the vagina a distinct bulge is visible from the outside that corresponds to the location of a very conspicuous, fleshy fold that runs along the inner wall of the vagina from just distal of the level of the insertion points of the appendiculae to the distal end of the vagina, slightly reaching into the lumen of the genital atrium. Duct of the bursa copulatrix stout, approximately as long as vagina. Flagellum very short and slender.

Remarks: *Domunculifex* differs from *Serratorotula* (Fig. 2g), its sister taxon in our phylogenetic reconstructions, by the depressed-globular shell that is ornamented with irregular growth lines, whereas the shell of *Serratorotula* is depressed-lenticular with prominent, often wing-like expanded radial ribs. Anatomically *Domunculifex* differs from *Serratorotula* (Fig. 3g) by the presence of two appendiculae and the presence of a very conspicuous, fleshy fold on the inner wall of the vagina, which is completely lacking in *Serratorotula*.

With regard to shell shape, *Domunculifex* is similar to *Plebecula* and *Helicomela*. It differs from *Helicomela* (Figs 2r, 3f) in the smaller, less globular shell, the vestigial flagellum, a shorter duct of the bursa copulatrix in relation to the length of the vagina (much longer in *Helicomela*) and the presence of a very distinct, fleshy, longitudinal fold on the inner wall of the vagina. *Plebecula* (Figs 2m, 3s) differs from *Domunculifex* in the presence of hairs and a more globular, usually larger shell, the presence of a transverse fold in the atrium and a longer flagellum.

Discussion

Phylogeny, classification and character evolution

The phylogeny based on mitochondrial and nuclear sequence data presented here (Fig. 3) is the first to cover almost the entire genus-level diversity of the Geomitriini. It thus allows insights into the phylogenetic relationships of this morphological highly diverse group of land snails. Our results confirm incongruences between the traditional classification based on shell morphology and anatomy on the one hand and the molecular-based analysis on the other hand, as has also been observed in other groups of helicoid land snails (Hirano et al., 2014; Köhler and Criscione, 2015; Neiber and Hausdorf, 2015, Neiber and Hausdorf, 2017; Walther et al., 2016, 2018; Neiber et al., 2017, 2018a; Chueca et al., 2018).

The phylogenetic relationships obtained here were largely congruent with the results obtained by Razkin et al. (2015), Neiber et al. (2017) and De Mattia et al. (2018a) concerning family-group taxa, though within the Helicellinae the phylogenetic relationships were slightly different compared to the work of Razkin et al. (2015). Since these discrepancies only occurred in relation to lineages that were only supported in the BI analysis (Fig. 3), the classification of Razkin et al. (2015) remains, however, unopposed. Within a previous work, De Mattia et al. (2018a) were able to show the polyphyly of the genera *Discula* and *Hystricella* in the sense of Bank et al. (2002), Seddon (2008), Groh et al. (2009) or Bank (2009). The subgenus *Discula* (*Callina*) was raised to genus level, while *Hystricella* was split into *Hystricella* and *Wollastonaria* (De Mattia et al., 2018a,b). These results were confirmed here since the former subgenus *Callina* formed a clade with the two remaining *Hystricella* taxa and this clade was recovered as sister to *Wollastonaria* (Fig. 3). The sister-group relationship between *Testudodiscula* (Figs 2l, 7a–e) and *Lemniscia* (Figs 2h, 3m) was one of the most surprising results of this work because the shell morphology of the representatives of these two taxa is very different. *Testudodiscula testudinalis* exhibits a

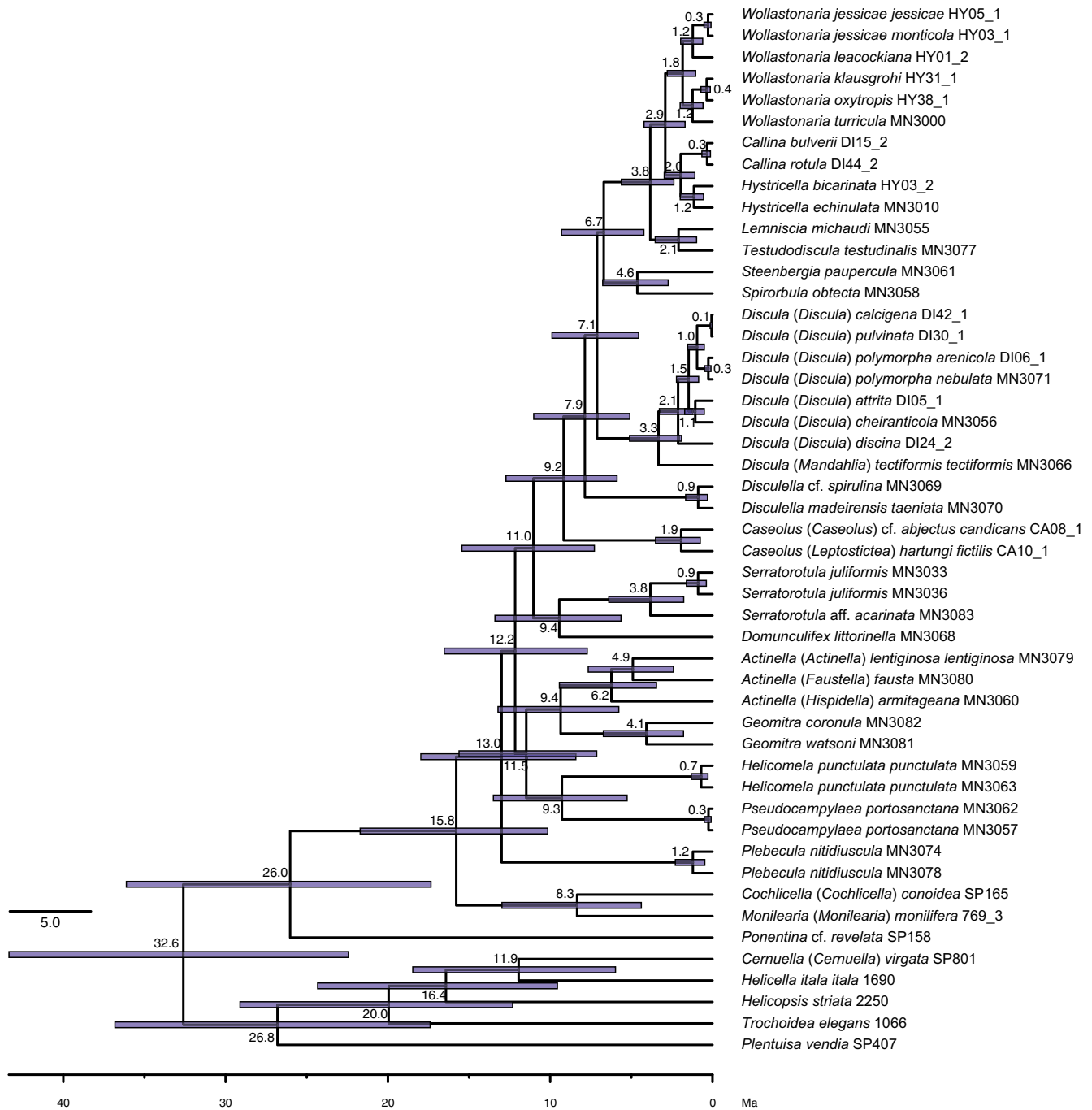


Fig. 5. Dated phylogeny of Geomitridae from the Madeiran Archipelago. Numbers at nodes represent median node ages in Ma and bars represent 95% highest posterior probability intervals. Only estimates for Geomitridae are shown. [Colour figure can be viewed at wileyonlinelibrary.com]

flatter, discoidal shell, while the shell of *L. michaudi* has a conical shape. Both species also differ markedly in size (Fig. 2h,l). Anatomically *L. michaudi* and *T. testudinalis* share some anatomical features like the short flagellum and the relatively long epiphallus, but *L. michaudi* differs, for example, by the less ramified glandulae mucosae (Figs 3m, 7b,c). *Testudodiscula*

testudinalis and *Callina bulverii* were both previously classified in *Discula* s. str. (Bank, 2009), the type species of which, *D. discina* (Lowe, 1852), is conchologically similar to both taxa, suggesting that either discoidal shell shapes evolved independently in several lineages of the Geomitridae or that the ancestor of *Hystricella*, *Callina*, *Lemniscia*, *Wollastonaria*, *Spirorbula*,

Steenbergia and *Discula* (incl. *Mandahlia*) possessed such a shell form (Fig. 3). It remains to be shown whether other taxa currently included in *Discula* and not studied here or by De Mattia et al. (2018a) such as *D. (D.) lyelliana* (Lowe, 1852) from Deserta Grande or *D. (D.) tetrica* (Lowe, 1862) from Bugio actually belong to *Discula* or instead to *Callina* or *Testudodiscula*.

Other genera, e.g. *Actinella* and *Caseolus* in the sense of Bank (2009), were also shown to be polyphyletic (Fig. 3). The two species that were studied here and classified by Bank et al. (2002), Seddon (2008), Groh et al. (2009) or Bank (2009) in the subgenus *Plebecula* of *Actinella* were not grouped within *Actinella*. Furthermore, they did not even form a clade together (Fig. 3). The sister-group relationship of *Domunculifex littorinella* (Mabille, 1883) (Fig. 2k) and *Serratorotula* (Fig. 2g) remains somewhat questionable because it only received significant support from the BI analysis (Fig. 3) and both groups differ in their number of appendiculae (Fig. 4). The nominal species *Helix littorinella* Mabille, 1883 differs from *P. nitidiuscula* in the structure of the genital organs, most markedly in the internal structure of the vagina, which is equipped with a very conspicuous and large, fleshy fold in *H. littorinella* (Fig. 8b–d). Taken together with the results of our phylogenetic analyses, the introduction of a new genus, *Domunculifex* Brozzo, De Mattia, Harl & Neiber, for *H. littorinella* appears therefore justified. A strongly developed vaginal fold is not known from any other Geomitriini and is here regarded as an apomorphy of the new genus.

Helicomela (Fig. 2r), which contains only one extant species, was hitherto regarded as a subgenus of *Caseolus*, from which it is distinguishable, e.g., by the much larger, globular shell. It was resolved, however, as the sister group of *Pseudocampylaea*, although only supported in the BI analysis. *Helicomela* differs from *Pseudocampylaea* not only by the more globular shell but also in the number of appendiculae inserting into the vagina. While *Helicomela* possesses two appendiculae, one appendicula has been lost in *Pseudocampylaea*, which suggests the previous classification of *Helicomela* based on anatomical characters (e.g. Seddon, 2008) was probably misled by the high variability of this trait (Fig. 6). It is noteworthy that species within the clade including *Helicomela* and *Pseudocampylaea* have evolved exceptionally large adult body sizes, with *P. lowii* and *H. bowdichiana* (Férussac, 1832) being the largest representatives of these lineages, respectively.

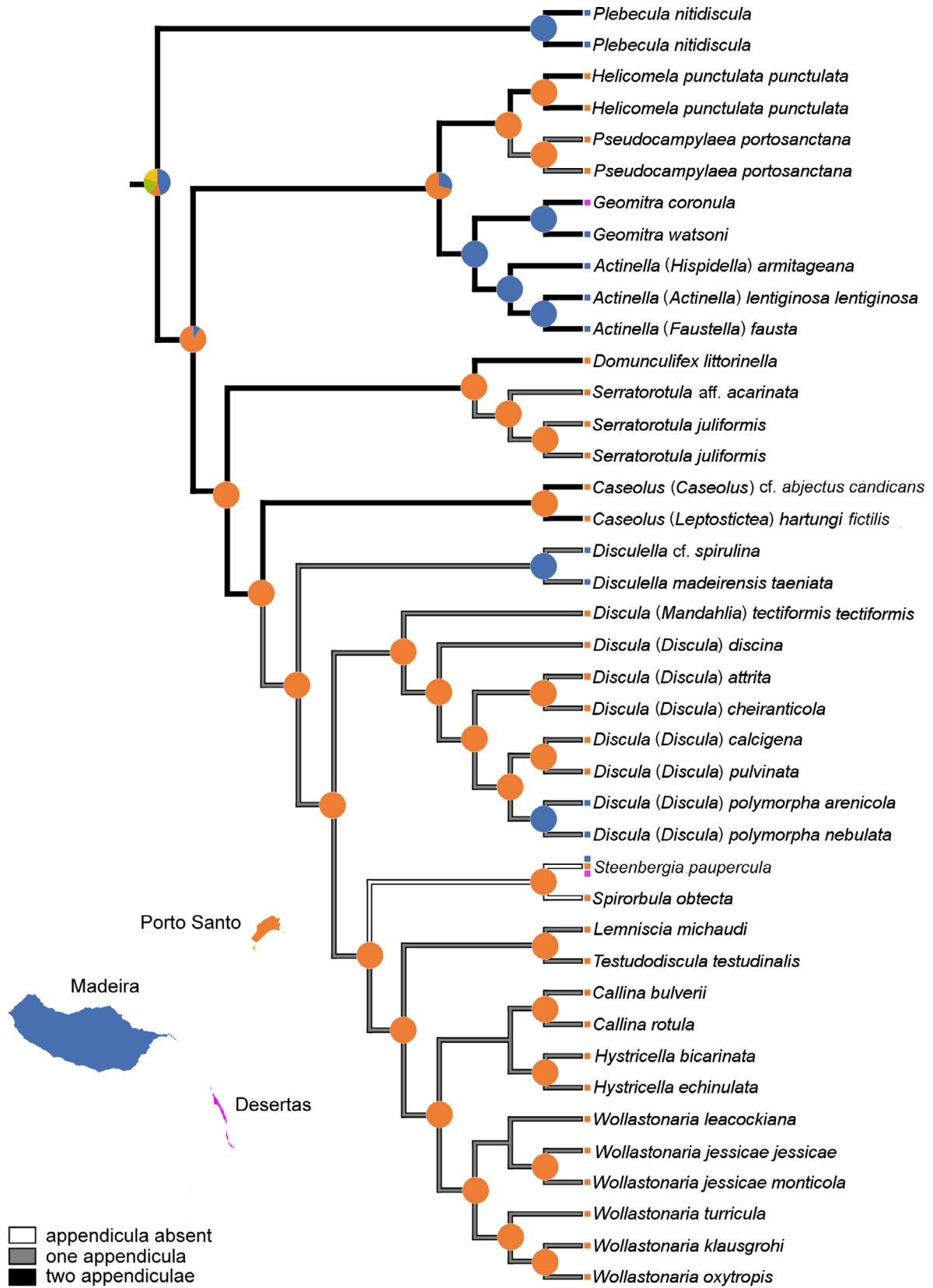
The absence of larger predators on Porto Santo and surrounding islets in the past may have favoured the evolution of larger body sizes in these snails as has also been put forward as an explanation for other cases of island gigantism (e.g. Barahona et al., 2000). Both taxa are meanwhile thought to be extinct. While *H. bowdichianus* probably went extinct shortly after human settlement in the 15th century, *P. lowii* survived at least until the end of the 19th century on the small islet Ilhéu de Cima off Porto Santo (Seddon, 2008, 2019). Causes for the decline of the species are unknown, but may possibly be the consequence of habitat destruction or alteration and the introduction of predators.

Aside from high variability of shell morphology and shell size, the estimation of the ancestral state of the number of appendiculae (Fig. 6) showed also a high variability of this trait within the Geomitriini, as it is also common within other Helicoidea (Razkin et al., 2015, Neiber et al., 2017 and Chueca et al., 2018). Of the three losses of one appendicula, one probably occurred along a branch which was positively supported in the MP analysis as well as in the BI analysis which is leading to a clade including *Disculella*, *Discula*, *Steenbergia*, *Spirorbula*, *Lemniscia*, *Testudodiscula*, *Callina*, *Hystericella* and *Wollastonaria* (Figs 3 and 6). Within this clade, the appendiculae were entirely lost along the branch leading to the positively supported clade including *Steenbergia* and *Spirorbula*, with the glandulae mucosae being also lost in *Steenbergia* (Figs 3 and 6). The other two losses of one appendicula occurred along branches that were only supported in the BI analysis, which therefore requires further research to confirm these results.

Timing of diversification and ancestral area estimation

The onset of diversification of Geomitriini was dated in our analysis at 13 Ma (Fig. 5). It has to be noted that the estimates of node ages have to be interpreted with caution because HPD intervals are often relatively large and in part overlapping. Accepting the fossil calibration, diversification patterns of Geomitriini largely coincide with the emergence of the present-day islands (Geldmacher et al., 2000). The oldest known fossil, referred to *Caseolus (Leptostictea)* sp. by Groh (1984) because of conchological similarity, originates from > 13 Ma old Miocene deposits of Ilhéu de Cima off the south-eastern coast of Porto Santo. Whether this fossil can be assigned to *Caseolus* or represents a

Fig. 6. Ancestral area estimation and ancestral state estimation of the number of appendiculae of the Geomitriini. Branches are shaded according to the presence of one (grey) or two (black) appendiculae or the absence of appendiculae (white). Pie charts at the nodes indicate the estimated ancestral areas: Madeira (blue), Ilhas Desertas (magenta), Porto Santo (orange), Iberian Peninsula (green) and the rest of Europe (yellow). Dots at the tips indicate the distribution of a species. Only results for the Geomitriini are shown. [Colour figure can be viewed at wileyonlinelibrary.com]



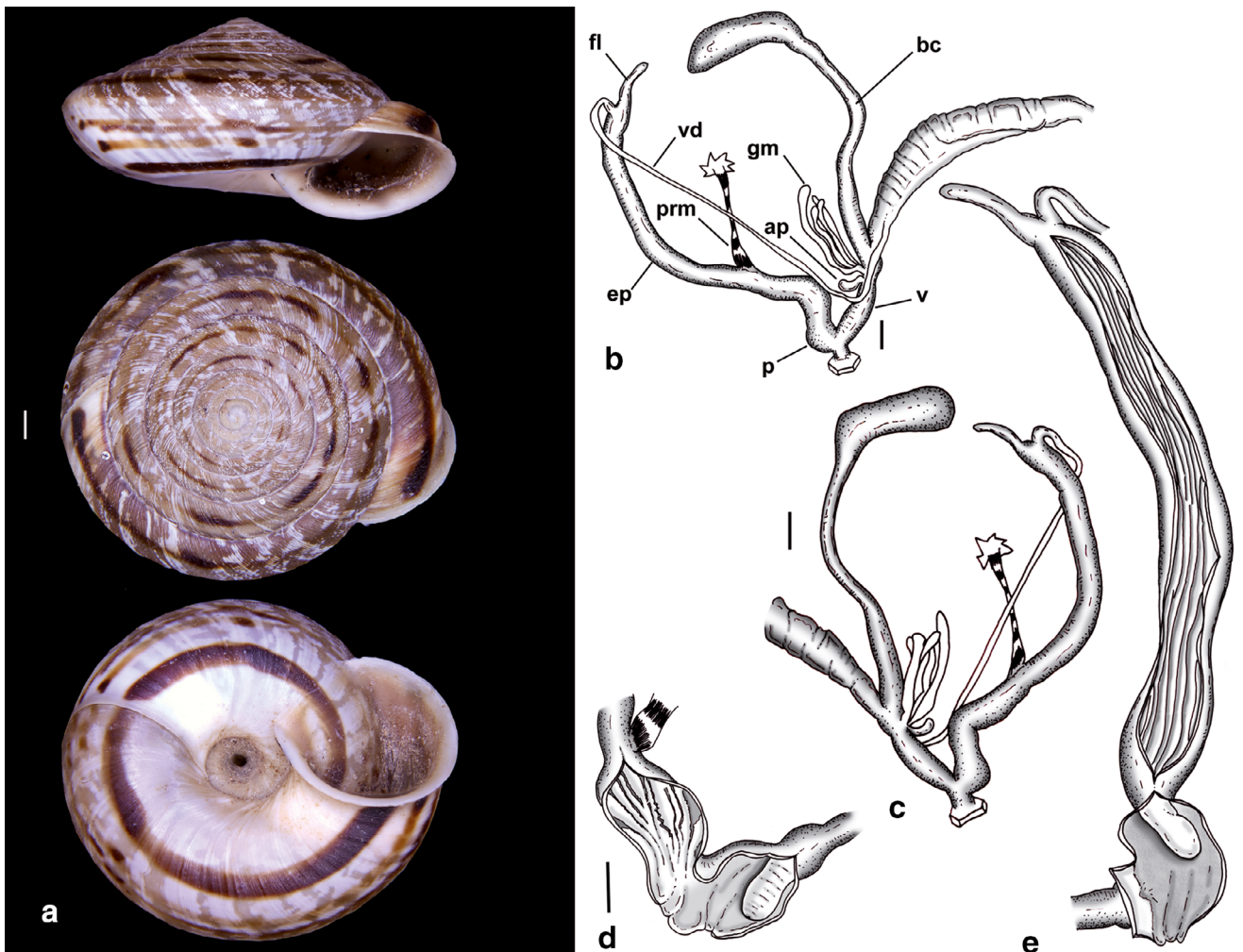


Fig. 7. Shell and genital system of *Testudodiscula testudinalis* (Lowe, 1852). (a) Shell. (b–c) Genital system. (d) Inner structure of vagina and penis with exposed penial papilla. (e) Inner structure of epiphallus and penis with exposed penial papilla. Scale bars: 1 mm. (a) Porto Santo, Porto do Pedregal. (b–e) Porto Santo, Cabeço dos Bodes. Abbreviations: ap, appendicula; bc, bursa copulatrix; ep, epiphallus; fl, flagellum; gm, glandulae mucosae; p, penis; prm, penis retractor muscle; v, vagina; vd, vas deferens. [Colour figure can be viewed at wileyonlinelibrary.com]

different, possibly extinct lineage close to the most recent common ancestor of Geomitriini can currently not be answered with certainty. This is due to the overlapping HPD intervals (Fig. 5) and the depressed-globular shell with a blunt keel which, according to Groh (1984), could represent the ancestral shell form of Geomitriini (“Grundplanvertreter”). The split of the representatives of *Cascolus* s. str. and *C. (Leptostictea)* was only dated at 1.9 Ma (HPD: 0.8–3.5 Ma) rendering an assignment of the fossil taxon to *Leptostictea* at least questionable.

The split of some lineages (*Plebecula* clade, *Actinella* + *Geomitra* clade, *Disculella* clade) occurring on Madeira (and on the Ilhas Desertas) from their respective sister groups on Porto Santo and surrounding satellite islets predates, however, the formation of the subaerial parts of these islands. Whether this

dating is the result of insufficient phylogenetic resolution, the uncertainty of age estimates or may be attributed to several independent dispersal events to the younger islands and subsequent extinctions of the respective lineages on the older islands cannot be answered with certainty at the moment. However, there are many species and subspecies only known as fossils and many examples of formerly somewhat wider geographic ranges exist suggesting that range contractions/expansions and extinctions must have been rather frequent during the evolution of Geomitriini (Waldén, 1983; Groh, 1984; Cook et al., 1993; Goodfriend et al., 1994, 1995; Cameron et al., 1996; Bank et al., 2002; Seddon, 2008; Groh et al., 2009; De Mattia et al., 2018a).

Although our ancestral area estimation places the origin of the present-day diversity of Geomitriini in the

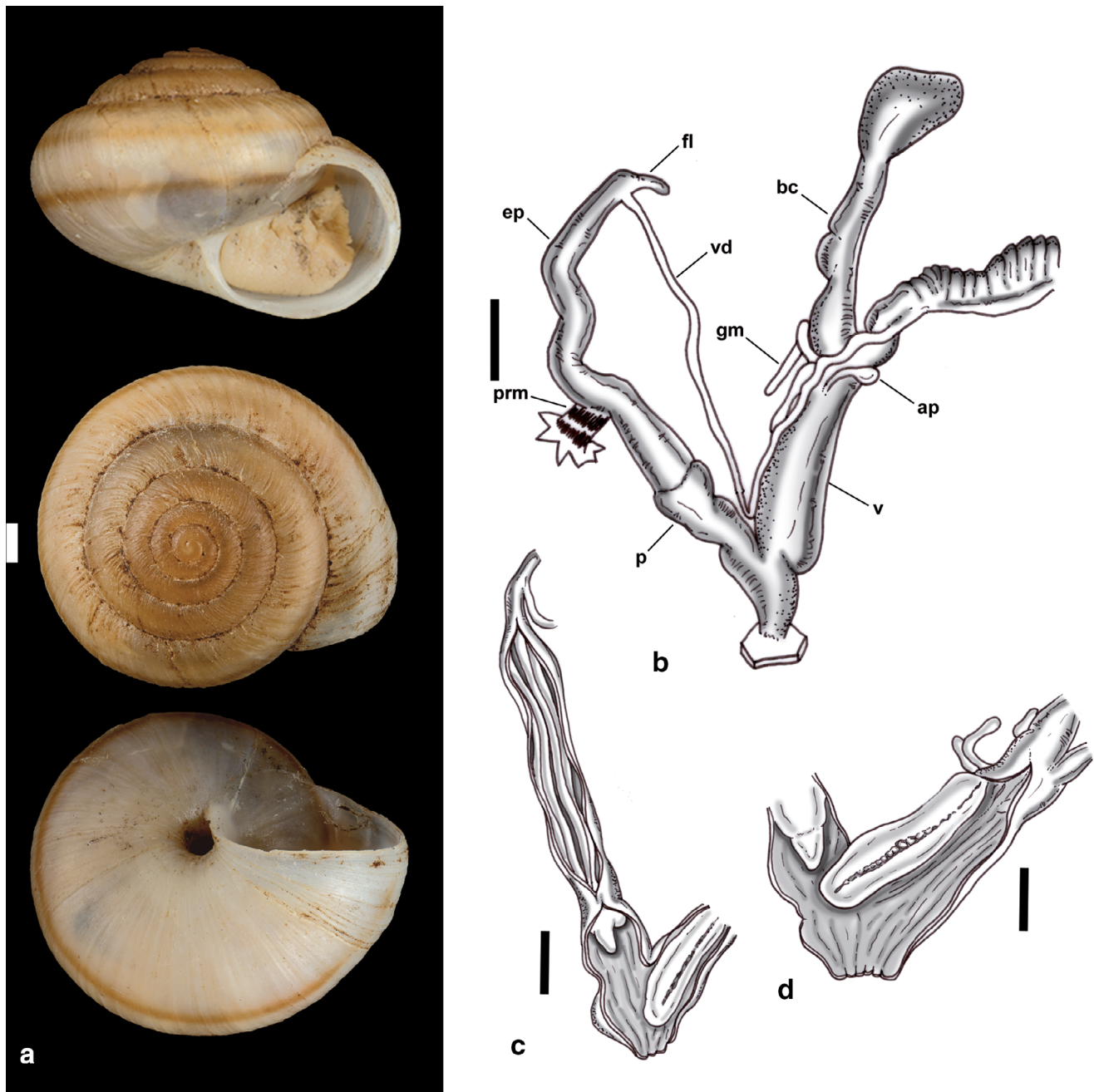


Fig. 8. Shell and genital system of *Domunculifex littorinella* (Mabille, 1883). (a) Shell. (b) Genital system. (c) Inner structure of vagina with vaginal fold, epiphallus and penis with exposed penial papilla. (d) Inner structure of vagina with exposed vaginal fold and penis with exposed penial papilla. Scale bars: 1 mm. (a) Porto Santo, north and east slopes and summit of Pico do Castelo. (b–d) Porto Santo, Pico do Facho, east side. Abbreviations: ap, appendicula; bc, bursa copulatrix; ep, epiphallus; fl, flagellum; gm, glandulae mucosae; p, penis; prm, penis retractor muscle; v, vagina; vd, vas deferens. [Colour figure can be viewed at wileyonlinelibrary.com]

Madeiran Archipelago (Fig. 6), the estimation of Madeira as the ancestral area is contradictory to the sequence of island emergence (Porto Santo–Madeira–Ilhas Desertas; Geldmacher et al., 2000). This is interpreted as an artefact of the analysis here as the algorithms implemented in RASP 3.02 do not allow temporal layering to be taken into account (Yu et al.,

2015). Furthermore, the decisive position of *Plebecula* from Madeira was only supported in the BI analysis (Fig. 4). However, it appears likely that extinction rates on Porto Santo were higher because of the smaller size of that island so those old lineages might have been lost on Porto Santo. Because of the lack of *Morletina* from the Azores in the phylogenetic analyses,

we can also not clarify whether the Madeiran Archipelago or the Azores was the ancestral area of Geomitriini. Our ancestral area estimation points towards an origin of Geomitriinae in Europe, more precisely the Iberian Peninsula (not shown) probably as a consequence of recovering Ponentinini as the sister group of Cochlicellini plus Geomitriini. Outgroup sampling is, however, not dense enough to allow a definite conclusion on whether the Geomitriini reached the Madeiran Archipelago from Europe or North Africa. The genus *Cochlicella* Férussac, 1821 occurs autochthonously in the Western Mediterranean region of Europe, Africa and the Canary Islands (Richter et al., 2019), while *Monilearia* Mousson, 1872 and *Ripkeniella* Hutterer & Gittenberger, 1998 are restricted to the Canary Islands and *Obelus* Hartmann, 1842 is known from the Canary Islands and the adjacent coastal regions of north-western Africa (Mousson, 1872; Wollaston, 1878; Schileyko and Menkhorst, 1997; Hutterer and Gittenberger, 1998; Ibáñez et al., 2003, 2006; Groh, 2014; Neiber, 2015; Neiber et al., 2016).

Further investigations will have to show, whether Cochlicellini originated in Europe or north-western Africa and colonised the Canary Islands from either of these regions. This will have implications for the origins of Geomitriini because an origin of Cochlicellini in north-western Africa would suggest an African affinity for Geomitriini as well. An origin of Cochlicellini in Europe, on the other hand, would also indicate an origin of Geomitriini in that region.

Affinities with north-western Africa have been suggested for the helicid genus *Theba* Risso, 1826. However, it is not entirely clear whether Africa and subsequently south-western Europe have been colonised by this genus from the Canary Islands or the other way around (Greve et al., 2010; Haase et al., 2014; Böckers et al., 2016). The colonisation of the Atlantic archipelagos from south-western Europe has been suggested for the helicid genus *Hemicycla* Swainson, 1840 (Canary Islands; Chueca et al., 2015) and the hygromiid genus *Leptaxis* Lowe, 1852 (Madeiran Archipelago, Azores, Cape Verde Islands and fossil also from the Canary Islands; Neiber et al., 2017; Caro et al., 2019). The analyses of Caro et al. (2019) suggest that *Leptaxis* have colonised the Azores from the Madeiran Archipelago. *Moreletina* from the Azores is the only taxon in the Geomitriini that is not known to occur in the Madeiran Archipelago. It would be interesting to test whether this taxon also reached the Azores from the Madeiran Archipelago as appears the case for *Leptaxis*. Unfortunately, no material was available for study.

Although taxon sampling is not complete, it is already clear from our analyses that inter-island dispersal must have played an essential role in shaping the present-day distribution of taxa in the Geomitriini

(Fig. 6) and especially that Madeira (and the Ilhas Desertas) has been colonised recurrently from Porto Santo (Fig. 6).

Radiation of the Geomitriini

Gittenberger (1991) defined radiation relatively vaguely as the evolution of a relatively large, monophyletic group of species or higher taxa. That author further distinguishes adaptive and non-adaptive radiations, with the latter being the evolutionary diversification from a single clade, not accompanied by relevant niche differentiation. This kind of radiation may, however, involve sexual selection (Sauer and Hausdorf, 2009; Sauer and Hausdorf, 2010). Fehér et al. (2018) showed that the rate of niche differentiation appears to have lagged behind compared to phylogenetic divergence in rock-dwelling door snails (Clausiliidae) and viewed this as strong support for the hypothesis that speciation in this group of land snails was driven primarily by non-adaptive processes. Contrarily, adaptive radiation in the sense of Schluter (2000) is the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage involving the differentiation of a single ancestor into an array of species that inhabit a variety of environments and that differ in the morphological and physiological traits used to exploit those environments.

Our phylogenetic analyses show that Geomitriini form a monophyletic group (Fig. 4), the diversification of which probably started shortly after the emergence of the present-day islands of the Madeiran Archipelago in the Miocene (Fig. 5). In that sense, the Madeiran Geomitriini represents a radiation according to the above-cited definition of Gittenberger (1991). The timing of the diversification of Geomitriini in the Madeiran Archipelago suggests a gradual pattern of diversification for the Geomitriini as a whole (Fig. 5) rather than only an initial burst of lineage diversification. It has to be noted, however, that extinctions have been frequent in the land snail fauna of the archipelago as evidenced by the numerous fossil taxa that have been described (Bank et al., 2002; Teixeira et al., 2019; Walther and Groh, 2020). Therefore, it cannot be ruled out that some of the comparatively long internal branches in the dated phylogeny (Fig. 5) may actually be the result of extinction rather than the absence of diversification.

Cook and Pettitt (1979) suggested for *Discula* from Madeira a direct adaptive component, i.e. they viewed the development of a keel and increased granulation as likely to increase shell strength and give protection from wear and conceivably predation. Somewhat in contrast, Cameron et al. (1996) concluded, based on data in part obtained from Geomitriini, that adaptive responses have occurred in the land snails from Porto

Santo, but that much of the species richness can be interpreted as non-adaptive in the sense of Gittenberger (1991). Although the broad range of shell sizes and different shell forms and ornamentations in Geomitridini (Fig. 2) points towards an adaptive component in the Geomitridini radiation, a detailed interpretation of the contribution of adaptive and/or non-adaptive processes in the sense of Gittenberger (1991) and Schluter (2000) is currently not possible as detailed data on phenotype–environment correlations or the utility of traits are not available for these land snails. The Geomitridini may, however, serve as an excellent model system to study the putatively complex interplay of adaptive and non-adaptive processes, for which the results presented here can provide the basis.

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Conflict of interest

All authors declare that they have no conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Alignment file in nexus format including *cox1*, 16S rRNA and 5.8S rRNA + ITS2 + 28S rRNA sequences of Geomitriini and related groups. For information on used specimens, see Table 1.

Table S1. Primers used for the PCR amplifications