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## Molecular phylogeny of the western Palaearctic Helicoidea (Gastropoda, Stylommatophora)



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## ABSTRACT

The Helicoidea is one of the most diverse superfamilies of terrestrial land snails. In this study we present a molecular phylogeny of the western Palaearctic Helicoidea obtained by means of neighbor joining, maximum likelihood and Bayesian analysis of the mitochondrial 16S rRNA gene fragment and the nuclear rRNA gene cluster including the 3' end of the 5.8S gene, the complete ITS2 region and 5' end of the large subunit 28S. Most of the morphologically-defined families were confirmed. We propose a revised phylogenetic classification so that families, subfamilies and tribes are monophyletic. The family Hygromiidae *sensu* Hausdorf and Bouchet (2005) is divided into three clades which are here given familial rank: Canariellidae and Geomitridae, which are recognized for the first time at familial rank, and Hygromiidae *s.str.* (including *Ciliella* and *Trochulus*) that is here restricted. The subfamilies Ciliellinae, Geomitrinae, Hygromiinae, Monachainae and Trochulinae recognized in current classifications were not recovered as monophyletic groups. The family Cochlicellidae is here given tribe rank (Cochlicellini) belonging to the Geomitridae. We describe a new tribe, Plentuisini. Three subfamilies are recognized within Helicidae: Ariantinae, Helicinae (including *Theba*) and Murellinae. New classification indicates that free right ommatophore retractor muscle arose only once within Geomitridae. The anatomy of the auxiliary copulatory organs of the reproductive system of families, subfamilies and tribes is highlighted. We estimate the origin of the Helicoidea at the end of the Early Cretaceous and its families as Late-Cretaceous to Paleogene. Western Palaearctic Helicoidea belongs to two different lineages that diverged around 86 Ma ago, both starting their diversification at the end of the Cretaceous (around 73–76 Ma). Radiation of some western Helicoidean families started during the Eocene.

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## 1. Introduction

After arthropods, molluscs are perhaps the most diverse group of metazoans with over 118,000 species (Zhang, 2013). The clade Stylommatophora (Gastropoda: Pulmonata) accounts for around 80% of 30,000–35,000 extant terrestrial molluscs (Solem, 1984)

classified into 103 families (Bouchet and Rocroi, 2005). Inferred phylogenetic relationships among the Stylommatophora have been much disputed (Schileyko, 1979; Nordsieck, 1985; Tillier, 1989), and the suborder is currently under revision according to the results of molecular techniques (Tillier et al., 1996; Wade et al., 2001, 2006; Madeira et al., 2010). Within the Stylommatophora the superfamily Helicoidea Rafinesque, 1815 is one of the most diverse groups of land snails and includes a number of large species of commercial value, as well as many microendemisms adapted to specific habitat conditions. The Helicoidea shows an almost worldwide distribution absent only from most of sub-Saharan Africa, and some islands of the South Pacific (Scott, 1997). Ecological, morphological and systematic studies have led to the proposal of several classification systems (Nordsieck,

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1987; Schileyko, 1989). The importance of the reproductive system in the classification of the Helicoidea was initiated during the XIX century (Moquin-Tandon, 1855; Pilsbry, 1893–1895) and highlighted by posterior authors (Hesse, 1931, 1934; Zilch, 1960). Recent reviews of the helicoidean classification have since focussed on anatomical characters (e.g. Nordsieck, 1987; Schileyko, 1991; Puente, 1994), particularly the presence of several appendages (diverticulum of the stalk of bursa copulatrix, flagellum, and penis caecum) and the number and morphology of organs comprising accessory copulatory organs, also referred to as stimulatory organs (dart sac, accessory sac, atrial appendages and glands). However, incongruences detected between new molecular-based phylogenies and traditional classifications point to high levels of homoplasy in genital characters (Mejía and Zúñiga, 2007; Hirano et al., 2014). As a result, the composition of the Helicoidea has remained controversial, and several questions remain largely unresolved including its phylogenetic position within the Stylommatophora, or the number and composition of its families and/or subfamilies. Schileyko (2004, 2006a, 2006b) proposed subdividing the Helicoidea into five different superfamilies: Helicoidea *s.str.*, Xanthonychoidea, Camaenoidea, Polygyroidea and Hygromioidea (Fig. 1). Nevertheless, recent molecular data obtained for the Stylommatophora (Wade et al., 2001, 2006) support the monophyly of the Helicoidea, indicating that subdivisions into separate superfamilies are not justified. According to the classification of Hausdorf and Bouchet (in Bouchet and Rocroi, 2005: 269–270), there are 19 families within the Helicoidea (see Fig. 1), eight of which inhabit the western Palaearctic region. The families Cochlicellidae, Elonidae, Helicodontidae, Sphincterochilidae and Trissexodontidae are restricted to the western Palaearctic; the Helicidae are found across the western Palaearctic and adjacent Arabia, while the range of the highly diverse Hygromiidae *sensu* Hausdorf and Bouchet (2005) (herein designated Hygromiidae *s.l.*) extends throughout the western Palaearctic, central Asia, northeastern Africa and Arabia (Falkner et al., 2001; Schileyko, 2004, 2006a, 2006b). The Asian family Bradybaenidae is represented with one species in Europe. Within the past decade, several authors have used molecular methods as rigorous tests of evolutionary relationships among helicoidean species. Some species have often been included in larger phylogenetic studies of gastropods (Wade et al., 2001, 2006, 2007; Davison et al., 2005, 2009; Holznagel et al., 2010; Dayrat et al., 2011). Recent helicoidean phylogenies based on molecular information have been extended to include representatives of several families and polytypic genera (Koene and Schulenburg, 2005: Helicidae, Hygromiidae, Helminthoglyptidae and Bradybaenidae; Mejía and Zúñiga, 2007: *Humboldtiana*, Humboldtianidae; Wade et al., 2007: Camaenidae; Elejalde et al., 2008: *Iberus*, Helicidae; Elejalde et al., 2009: *Pyrenaearia*, Hygromiidae; Fiorentino et al., 2010: *Marmorana* group, Helicidae; Greve et al., 2010: *Theba*, Helicidae; Hugall and Stanisc, 2011: Camaenidae; Kotsakiozi et al., 2012: *Codringtonia*, Helicidae; Groenenberg et al., 2011; Cadahía et al., 2014: Ariantinae, Helicidae; Hirano et al., 2014: Bradybaenidae). In addition, two studies have examined the molecular phylogeny of the western Palaearctic Helicoidea (Steinke et al., 2004; Manganelli et al., 2005), but unfortunately, the results of the former study have been questioned due to errors, methodological weaknesses and taxonomic misidentifications (critically reviewed in Groenenberg et al., 2011). Manganelli et al. (2005) included the widest representation of western helicoidean taxa but their study was restricted to the analysis of the mitochondrial 16S rRNA region. According to the studies of Wade et al. (2006, 2007) based on nuclear 28S rRNA gene sequences, the Helicidae (9 genera sequenced) is monophyletic. The monophyly of the Hygromiidae (only 4 genera sequenced) was also proposed by Wade et al. (2006, 2007) but without statistical support. Moreover, the Hygromiidae still emerged as monophyletic when shorter

sequences of nine additional hygromiid genera published by Koene and Schulenburg (2005) were incorporated in the analysis (Wade et al., 2007). Recently, Gómez-Moliner et al. (2013) confirmed in a molecular phylogeny of Helicodontidae and Trissexodontidae (23 species included in the study together with other Helicoidea taxa using mitochondrial and nuclear DNA sequences) the monophyly of these two families.

In the present study, we reconstruct the most exhaustive phylogeny of the western Palaearctic Helicoidea to date, based on molecular markers from the nuclear ribosomal RNA (rRNA) gene cluster (partial 5.8S, complete ITS2 and partial 28S sequences) and one mitochondrial gene fragment (16S rRNA). The nuclear DNA fragment was homologous to that used by Wade et al. (2001, 2006, 2007), thus allowing direct comparisons with their results. The mitochondrial gene fragment examined also enabled comparisons with the results of Manganelli et al. (2005) and Groenenberg et al. (2011), who both also focused their work on the western Palaearctic Helicoidea. The introduction of additional taxa can often resolve basal nodes and ambiguities, or increase support values in phylogenetic trees. With this goal in mind, we included sequences of 76 species belonging to 45 genera for the 16S analysis and 67 species of 44 genera for the nuclear rRNA tests along with GenBank sequences from various studies. This paper therefore revisits the phylogeny of the Helicoidea, with the following aims: (i) to construct a phylogenetic hypothesis for the Helicoidea based on both mitochondrial and nuclear rRNA gene sequences; (ii) to test the classifications proposed by Hausdorf and Bouchet (in Bouchet and Rocroi, 2005) and Schileyko (2004, 2006a, 2006b) and to evaluate the systematic genera arrangements of the European helicoideans adopted by the CLECOM system: Check List of European Continental Mollusca (Bank et al., 2001; Bank, 2011), and (iii) to estimate divergence times by fossil calibration of the resultant phylogeny using the program BEAST.

## 2. Materials and methods

### 2.1. Specimens

Representatives were included of the eight helicoidean families living in the western Palaearctic region (*sensu* Bouchet and Rocroi, 2005). We obtained 173 new sequences from 99 specimens covering 76 species. New sequences were restricted to the families Helicidae and Hygromiidae *s.l.*, the most diverse families of the western Palaearctic region (see Table 1). We also included recently published sequences from the work of Gómez-Moliner et al. (2013) mainly focused on Trissexodontidae and Helicodontidae. Published sequences of other representative taxa were obtained from GenBank, including some non-western helicoidean families to obtain a more complete phylogeny of the entire group (Table 1). Considering both the new and Genbank sequences, we here examine data for 15 out of 20 families of Helicoidea, including all families living in the western Palaearctic. The locations of voucher material are provided in Table 1.

### 2.2. DNA extraction, PCR amplification and sequencing

The material examined was preserved in 96% ethanol, and total genomic DNA was extracted from foot muscle using the DNeasy Tissue kit (Qiagen, Valencia, CA, USA). Four gene fragments were selected for multi-locus analyses: one mitochondrial marker, around 430 bp of the 16S ribosomal RNA gene; and three nuclear fragments, approximately 1445 bp of the rRNA gene cluster, including the 3' end of the 5.8S gene (–50 bp), the complete ITS2 region (–600 bp) and the 5' end (–840 bp) of the large subunit rRNA-(LSU; 28S) gene. General PCR cycling conditions used for

BOUCHET & ROCROI				SCHILEYKO					
.oidea	.idae	.inae	T	Type genus	T	.inae	.idae	.oidea	
Helic.	Hygromi.	Hygromi.	Leptaxini	<b>Leptaxis</b>	Hygromiini	Hygromi.	Hygromi.	Hygromi.	
			Hygromiini	<b>Hygromia +3</b>	Cernuellini				
			Trochulini	<b>Cernuella +1</b>	Trochulini	Trochul.			
			Helicellini	<b>Trochulus</b>	Helicellini				
			Archaicini	<b>Helicella +2</b>		Archaic.			
		Metafruticicolini	Archaica		Metafruticicol.				
		Monacha.		<b>Metafruticicola</b>		Monacha.			
				<b>Monacha +5</b>		Hesseol.			
				Hesseola					
				<b>Geomitri +3</b>		Geomitri.			
		Geomitri.		<b>Geomitri*</b>		Trochoideini			Geomitrin.
				<b>Trochoidea</b>					
		Ciliell.		<b>Paedhoplita</b>		Paedhoplit.			
				<b>Canariella</b>		Canariell.			
		Ponentin.		<b>Ciliella</b>		Ciliell.			
			<b>Ponentina</b>		Ponentin.				
	Halolimnohelic.		<b>Halolimnohelix</b>		Halolimnohelic.				
			<b>Vicariihelix</b>		Vicariihelic.				
			<b>Trissexodont</b>						
	Trissexodont.		<b>Trissexodontini</b>		Trissexodont.				
			<b>Mastigophallus</b>						
	Helicodont.	Helicodont.		<b>Caracollina +3</b>	Caracollin.	Helicodont.			
				<b>Oestophora</b>	Oestophor.				
				<b>Gittenbergeria</b>	Gittenbergeri.				
	Helicodont.	Helicodont.		<b>Helicodonta</b>	Helicodont.	Helicodont.			
				<b>Drepanostoma</b>	Drepanostomat.				
	Helic.	Helic.		<b>Lindholmiola +1</b>	Lindholmiol.	Helic.			
				<b>Helix +16</b>	Helic.				
	Helic.	Helic.		<b>Theba</b>		Helic.			
				<b>Murella</b>					
	Helic.	Ariant.		<b>Arianta +2</b>	Ariant.	Helic.			
				<b>Campylaea +1</b>					
	Cochlicell.	Cochlicell.		<b>Cylindrus</b>		Cochlicell.			
				<b>Lampadia</b>					
	Elon.	Elon.		<b>Cochlicella</b>		Elon.			
			<b>Elona</b>						
Sphincterochil.	Sphincterochil.		<b>Sphincterochila</b>		Sphincterochil.				
			<b>Epiphragmophor.</b>						
Epiphragmophor.	Epiphragmophor.		<b>Epiphragmophora</b>		Epiphragmophor.				
			<b>Monadeni.</b>						
Monadeni.	Monadeni.		<b>Monadenia</b>		Monadeni.				
			<b>Bunnya</b>						
Humboldtian.	Humboldtian.		<b>Humboldtiana</b>		Humboldtian.				
			<b>Lysinoe</b>						
Xanthonych.	Xanthonych.		<b>Leptarionta</b>		Xanthonych.				
			<b>Trionigens</b>						
Xanthonych.	Xanthonych.		<b>Semiconchula</b>		Xanthonych.				
			<b>Xanthonyx</b>						
Xanthonych.	Xanthonych.		<b>Trichodiscina</b>		Xanthonych.				
			<b>Miraverrellina</b>						
Xanthonych.	Xanthonych.		<b>Metostrac.</b>		Xanthonych.				
			<b>Metostracini</b>						
Helminthoglypt.	Helminthoglypt.		<b>Sonorella</b>		Helminthoglypt.				
			<b>Micrarionta</b>						
Helminthoglypt.	Helminthoglypt.		<b>Helminthoglypta</b>		Helminthoglypt.				
			<b>Eremarionta</b>						
Cepol.	Cepol.		<b>Sonorelix</b>		Cepol.				
			<b>Cepolis</b>						
Bradybaen.	Bradybaen.		<b>Bradybaena +2</b>		Bradybaen.				
			<b>Aegista +1</b>						
Bradybaen.	Bradybaen.		<b>Euhadra</b>		Bradybaen.				
			<b>Helicostyl.</b>						
Camaen.	Camaen.		<b>Sinumelont.</b>		Camaen.				
			<b>Xanthomelont.</b>						
Camaen.	Camaen.		<b>Xanthomelon</b>		Camaen.				
			<b>Papuina</b>						
Camaen.	Camaen.		<b>Cristovala</b>		Camaen.				
			<b>Camaena</b>						
Camaen.	Camaen.		<b>Rhagada</b>		Camaen.				
			<b>Ammonitella</b>						
Acav.	Megomphic.		<b>Oreohelic.</b>		Acav.				
			<b>Thysanophor.</b>						
Punct.	Oreohelic.		<b>Thysanophora</b>		Punct.				
			<b>Discolepis</b>						
Pleuront.	Pleuront.		<b>Gonostomopsis</b>		Pleuront.				
			<b>Pleurodonte</b>						
Polygyr.	Polygyr.		<b>Polydonte</b>		Polygyr.				
			<b>Solaropsis</b>						
Polygyr.	Polygyr.		<b>Allogona</b>		Polygyr.				
			<b>Ashmunella</b>						
Polygyr.	Polygyr.		<b>Mesodon</b>		Polygyr.				
			<b>Polygyra</b>						
Polygyr.	Polygyr.		<b>Stenotrema</b>		Polygyr.				
			<b>Vespericola</b>						
Polygyr.	Polygyr.		<b>Triodopsis</b>		Polygyr.				
			<b>Triodopsis</b>						

**Fig. 1.** Comparison between the systems proposed by Hausdorf and Bouchet (in Bouchet and Rocroi, 2005) for Helicoidea (left columns) and Schileyko (2004–2006) for the corresponding taxa (right columns). Both systems are separated by a central column indicating the type genus for each suprageneric taxon. Columns (superfamily, family, subfamily and tribe) appear in the opposite order to Schileyko's classification scheme. Genera in bold type indicate that one or more species were included in this study; asterisk indicates that a non-type genus was analyzed; +n. indicates the number of additional genera included in the analysis for each suprageneric taxon.

Table 1

Taxa used in this study: family, species, locality, voucher, GenBank accession numbers for 16S and 5.8S-ITS2-28S. Superscript numbers refers to the institution of the voucher specimens: 1 – MVHN, Museu Valencià d'Història Natural (Spain), 2 – National Museum Cardiff (UK), 3 – Zoology and Animal Cell Biology department, University of the Basque Country (Spain). Superscript asterisks (\*) mean that the GenBank accession number was published on other study.

Family	Species	Locality	Voucher	GenBank accession number 16S	GenBank accession number 5.8S-ITS2-28S
Bradybaenidae	<i>Acusta despecta</i> (Sowerby, 1839)	(16S): Amami Island, Japan; (5.8-ITS2-28S): Japan		AY137578*	AY841337*
	<i>Aegista vulgivaga</i> (Schmacker and Boettger, 1890)	Osaka City, Japan			AY014139*
	<i>Ainohelix editha</i> (Adams, 1868)	Shimamaki, Hokkaido, Japan			AY841338*
	<i>Bradybaena similaris</i> (Férussac, 1821)	(16S): Brisbaen, Queensland, Australia; (5.8-ITS2-28S): Sri Lanka		GQ851001*	AY014138*
	<i>Chloraea intorta</i> (Sowerby, 1841)	Bohol Island, Philipines			AY841344*
	<i>Euhadra amaliae</i> (Kobelt, 1875)	(16S): Western and middle parts of Japan; (5.8-ITS2-28S): Osaka City, Japan		AF098712*	AY014140*
	<i>Euhadra decorata</i> Pilsbry and Hirase, 1903	(16S): Tamayama, Iwate, Japan; (5.8-ITS2-28S): Japan		AY445012*	AY251834*
	<i>Euhadra sandai</i> (Kobelt, 1878)	(16S): Imajyo, Fukui, Japan; (5.8-ITS2-28S): Osaka City, Japan		AY445021*	AY014141*
	<i>Ezohelix gainesi</i> (Pilsbry, 1900)	Sapporro, Hokkaido, Japan			AY841339*
	<i>Fruticicola fruticum</i> (O.F. Müller, 1774)	Anzù di Feltre (Feltre, Belluno), Italy		AY741450*	
	<i>Nesiohelix bipyramidalis</i> Kuroda and Emura, 1943	Ryukyu, Japan			AY841341*
	<i>Paraegista takahidei</i> Kuroda and Azuma, 1951	Hokkaido, Japan			AY841340*
	<i>Trishoplita hachijoensis</i> Pilsbry, 1902	Nijijima Island, Izu Islands, Japan			AY841345*
Camaenidae	<i>Satsuma (Coniglobus) mercatorius</i> (Pfeiffer, 1845)	(16S): Ryukyu Islands, Japan; (5.8-ITS2-28S): Kikai Island, Ryukyu, Japan		AF098715*	AY841324*
	<i>Satsuma (Coniglobus) nux</i> Möllendorff, 1888	Unknown		EF204786*	EF204880*
	<i>Satsuma (Satsuma) japonica</i> (Pfeiffer, 1847)	(16S): Japan; (5.8-ITS2-28S): Osaka City, Japan		AF098716*	AY014122*
Cepolidae	<i>Cepolis streatorii</i> (Pilsbry, 1889)	Grand Cayman			AY841346*
Cochlicellidae	<i>Cochlicella (Cochlicella) acuta</i> (Da Costa, 1778)	(16S): Lampedusa Island, Valle Imbriacole (Lampedusa e Linosa, Agrigento), Italy; (5.8-ITS2-28S): Porthcurnick, Cornwall, UK		AY741442*	AY014126*
	<i>Cochlicella (Cochlicella) acuta</i> (Da Costa, 1778)	Bakio, Bizkaia, Spain	<sup>3</sup> EHUMC-1003	KJ458503	KJ458599
	<i>Cochlicella (Cochlicella) conoidea</i> (Draparnaud, 1801)	Miramar, Portugal	<sup>3</sup> EHUMC-1004	KJ458504	KJ458600
	<i>Cochlicella (Prietocella) barbara</i> (Linnaeus, 1758)	Antequera, Málaga, Spain	<sup>3</sup> EHUMC-1005	KJ458550	
	<i>Cochlicella (Prietocella) barbara</i> (Linnaeus, 1758)	Gasteiz, Álava, Spain	<sup>3</sup> EHUMC-1006	KJ458551	KJ458633
Elonidae	<i>Elona quimperiana</i> (Férussac, 1821)	Arantzazu: Araotz, Gipuzkoa, Spain		FJ786408*	
	<i>Elona quimperiana</i> (Férussac, 1821)	Arantzazu: Araotz, Gipuzkoa, Spain		FJ786409*	JQ805023*
	<i>Norelona pyrenaica</i> (Draparnaud 1805)	Setcases, Girona, Spain	<sup>3</sup> EHUMC-1007	KJ458543	
	<i>Norelona pyrenaica</i> (Draparnaud 1805)	Querlals: Daió, Girona, Spain	<sup>3</sup> EHUMC-1008	KJ458544	KJ458627
Helicidae	<i>Alabastrina (Alabastrina) alabastrites</i> (Michaud, 1833)	Honaine, Traras Massif, Algeria	<sup>1</sup> MVHN-2169	KJ458484	KJ458582
	<i>Alabastrina (Atlasica) atlasica</i> (Mousson, 1873)	Between Agadir and Essauira, Morocco	<sup>1</sup> MVHN-010211FR03	KJ458490	KJ458588
	<i>Allognathus graellsianus</i> (Pfeiffer, 1848)	Between Caiman and Söller, Mallorca, Spain	<sup>1</sup> MVHN-1789	KJ458485	KJ458583
	<i>Allognathus hispanicus minoricensis</i> (Mittre, 1842)	Alaior, Menorca, Spain	<sup>3</sup> EHUMC-1009	KJ458531	KJ458618
	<i>Arianta arbustorum</i> (Linnaeus, 1758)	Stockholm, Sweden	<sup>1</sup> MVHN-2159	KJ458486	KJ458584
	<i>Arianta xatartii</i> (Farines, 1834)	Núria, Girona, Spain	<sup>1</sup> MVHN-2145	KJ458487	KJ458585
	<i>Cantareus apertus</i> (Born, 1778)	Djelfa, Algeria	<sup>1</sup> MVHN-2013	KJ458491	KJ458589
	<i>Cepaea (Cepaea) hortensis</i> (O.F. Müller, 1774)	Thurso, Highlands, Scotland, UK	<sup>3</sup> EHUMC-1010	KJ458497	KJ458594
	<i>Cepaea (Cepaea) nemoralis</i> (Linnaeus, 1758)	(16S): Pitarque, Teruel, Spain; (5.8-ITS2-28S): Marlborough Downs, Wiltshire, UK	<sup>1</sup> MVHN-2197	KJ458498	AY014130*
	<i>Chilostoma (Cingulifera) cingulatum</i> (Studer, 1820)	Tirol, Halltal, 9 km NE of Innsbruck, Austria		JF717812*	
	<i>Chilostoma (Corneola) desmoulinsii atricha</i> (Bofill, 1915)	Congost de Montrebu, Lleida, Spain	<sup>1</sup> MVHN-2164	KJ458499	KJ458595
	<i>Chilostoma i(Corneola) desmoulinsii bechi</i> (Altimira, 1959)	La Riba, Tarragona, Spain	<sup>1</sup> MVHN-2165	KJ458500	KJ458596
	<i>Chilostoma i(Corneola) desmoulinsii desmoulinsii</i> (Farines, 1834)	Portell, Rambla Celumbres, Castellón, Spain	<sup>1</sup> MVHN-2163	KJ458501	KJ458597
	<i>Chilostoma (Corneola) squamatum</i> (Rossmässler, 1835)	Albanya, near Muga river, Girona, Spain	<sup>1</sup> MVHN-2166	KJ458502	KJ458598
<i>Codringtonia (Codringtonia) codringtonii</i> Gray, 1834	Rodia 1.2 km before, Peloponnese, Greece		JQ240092*		
<i>Cornu aspersum</i> (Müller, 1774)	(16S): Unknown; (5.8-ITS2-28S): Kettering, Northants, UK		AF434797*	AY014128*	
<i>Eobania vermiculata</i> (Müller, 1774)	Girona, Girona, Spain	<sup>1</sup> MVHN-080709DR04	KJ458509		

	<i>Eobania vermiculata</i> (Müller, 1774)	Murchante, Navarre, Spain	<sup>3</sup> EHUMC-1011	KJ458510	KJ458604
	<i>Eobania vermiculata</i> (Müller, 1774)	Calblanque Regional Park, Murcia, Spain	<sup>3</sup> EHUMC-1012	KJ458511	
	<i>Ercetella cephaloeditana</i> (Giannuzzi-Savelli, Oliva and Sparacio, 2012)	Cefalù, La Rocca, Palermo, Sicily, Italy		GQ402397*	
	<i>Ercetella insolida</i> (Monterosato, 1892)	San Vito lo Capo, Cala Mancina, Trapani, Sicily, Italy		GQ402423*	
	<i>Eremina desertorum</i> (Forsskål, 1775)	Unknown			AY841335*
	<i>Helicigona lapicida andorrica</i> (Bourguignat, 1876)	Serrat, Andorra	<sup>3</sup> EHUMC-1013	KJ458523	JQ805027*
	<i>Helicigona lapicida lapicida</i> (Linnaeus, 1758)	(16S): Luxembourg, La Roche-en-Ardenne, chateau, Belgium; (5.8-ITS2-28S): Deepdale, Derbyshire, UK		JF717817*	AY014137*
	<i>Helix (Helix) lucorum</i> Linnaeus 1758	Unknown		AF126144*	AY841334*
	<i>Helix (Helix) melanostoma</i> Draparnaud 1801	Llombai, Algeria	<sup>1</sup> MVHN-783	KJ458524	KJ458612
	<i>Helix (Helix) pomatia</i> Linnaeus, 1758	(16S): Unknown; (5.8-ITS2-28S): Pulpit Down, Buckinghamshire, UK		AF208297*	AY841333*
	<i>Hemicycla (Hemicycla) bidentalis</i> (Lamarck, 1822)	Anaya, Tenerife, Canary Islands, Spain	<sup>1</sup> MVHN-2160	KJ458528	KJ458615
	<i>Hemicycla (Hemicycla) consobrina</i> (Férussac, 1821)	Tenerife, Canary Islands, Spain		HM147230*	
	<i>Hemicycla (Hemicycla) eurythra</i> O. Boettger, 1908	Tenerife, Canary Islands, Spain		HM147226*	
	<i>Hemicycla (Hemicycla) fulgida</i> Alonso and Ibáñez, 2007	Tenerife, Canary Islands, Spain		HM147200*	
	<i>Iberus gualtieranus gualtieranus</i> (Linnaeus, 1758)	Sierra Elvira, Granda, Spain	<sup>3</sup> EHUMC-1014	KJ458530	KJ458617
	<i>Isognomostoma isognomostomos</i> (Schröter, 1784)	Trento-Alto Adige, between Predazzo and Bellamonte, Italy		JF717821*	
	<i>Levantina hierosolyma</i> (Mousson, 1854)	Ksalon, Israel	<sup>1</sup> MVHN-050710FC01	KJ458534	KJ458620
	<i>Marmorana (Ambigua) saxetana</i> (Paulucci, 1886)	Giglio, Il Franco, Tuscany, Italy		GU391400*	
	<i>Marmorana (Ambigua) signata</i> (Férussac, 1821)	Monti Lepini, Latium, Italy		GU391405*	
	<i>Marmorana (Marmorana) serpentina</i> (Férussac, 1821)	Casa Cantoniera, Sardinia, Italy		GU391397*	
	<i>Marmorana (Murella) muralis</i> (Müller, 1774)	(16S): Castle, Fiumedinisi, Sicily; (5.8-ITS2-28S): Pompeya, Nápoles, Italy	<sup>1</sup> MVHN-1276	GU391399*	KJ458621
	<i>Marmorana (Murella) muralis</i> (Müller, 1774)	Caltabellotta, Chiesa di San Pellegrino, Sicily, Italy		EU189886*	
	<i>Marmorana (Murella) scabriuscula</i> (Deshayes, 1830)	Monte Nadore, top, Sicily, Italy		EU189888*	AY014132- AY014133*
	<i>Maurohelix raymondi</i> (Moquin-Tandon, 1848)	Bou-Saad, Algeria	<sup>2</sup> 1984.306.14	KJ458535	KJ458622
	<i>Otala (Dupotetia) sp</i>	Detour to Honaine, between Orán and Tlemcén, Algeria	<sup>1</sup> MVHN-2171	KJ458507	KJ458603
	<i>Otala (Dupotetia) sp</i>	Ghar el Melh, Bizerte, Tunisia	<sup>1</sup> MVHN-260410DR01	KJ458508	
	<i>Otala (Otala) lactea</i> (Müller, 1774)	(16S): Nerja-Frigiliana: 1 Km, Málaga, Spain; (5.8-ITS2-28S): Unknown		AY937264*	AY841336*
	<i>Otala (Otala) punctata</i> (Müller, 1774)	Tlemcen, Algeria	<sup>1</sup> MVHN-2186	KJ458545	KJ458628
	<i>Pseudotachea splendida</i> Draparnaud, 1801	Sierra de Quibas, Murcia, Spain	<sup>1</sup> MVHN-2270	KJ458552	KJ458634
	<i>Pseudotachea splendida</i> Draparnaud, 1801	Náquera, Sierra Calderona, Valencia, Spain	<sup>1</sup> MVHN-080709DR01	KJ458553	
	<i>Pseudotachea splendida</i> Draparnaud, 1801	Sierra Espadán, Castellón, Spain	<sup>1</sup> MVHN-080709DR00	KJ458554	
	<i>Rossmassleria olcese</i> (Pallary, 1898)	Sefliane, Morocco	<sup>2</sup> 1984.384.6	KJ458555	KJ458635
	<i>Theba andalusica</i> Gittenberger and Ripken, 1987	Tarifa, Cádiz, Spain	<sup>1</sup> MVHN-1383	KJ458558	
	<i>Theba geminata</i> (Mousson, 1857)	Teguise, Lanzarote, Las Palmas, Spain	<sup>1</sup> MVHN-241109AZ01	KJ458559	KJ458638
	<i>Theba impugnata</i> Mousson, 1857	Teguise, Lanzarote, Las Palmas, Spain	<sup>1</sup> MVHN-241109AZ02	KJ458560	KJ458639
	<i>Theba pisana</i> (Müller, 1774)	Almería, Almería, Spain	<sup>1</sup> MVHN-1283	KJ458561	
	<i>Theba subdentata</i> Férussac, 1821	El Alquian, Almería, Spain	<sup>1</sup> MVHN-1269	KJ458562	KJ458640
	<i>Tingitana orientalis</i> O. Boettger, 1884	Berkane, Morocco	<sup>1</sup> MVHN-080709DR03	KJ458563	KJ458641
	<i>Tyrrheniberus ridens</i> Von Martens, 1884	Caletta Fuili, Sardinia, Italy		GU391402*	
	<i>Tyrrheniberus villicus</i> (Paulucci, 1882)	Orosei, Sardinia, Italy		GU391410*	
Helicodontidae	<i>Atenia quadrasi</i> (Hidalgo, 1885)	Barranco de los Frailes, Pego, Alicante, Spain		FJ786403*	
	<i>Atenia quadrasi</i> (Hidalgo, 1885)	Celrà, Girona, Spain		FJ786404*	JQ805020*
	<i>Helicodonta obvoluta</i> (Müller, 1774)	Collsacabra, Girona, Spain		FJ786423*	JQ805021*
	<i>Lindholmiola girva</i> (Frivaldszky, 1835)	Igoumenitsa, Greece		AY741448*	
Humboldtianidae	<i>Humboldtiana fasciata</i> Burch and Thompson, 1957	El Chico, Hidalgo, Mexico		DQ324479*	DQ324510*
	<i>Humboldtiana montezuma</i> Pilsbry, 1940	Cumbre Infiernillo, Nuevo León, Mexico		DQ324467*	DQ324508*
	<i>Humboldtiana nuevoleonis</i> Pilsbry, 1927	Arteaga, Coahuila, Mexico		DQ324485*	DQ324524*

(continued on next page)



Table 1 (continued)

Family	Species	Locality	Voucher	GenBank accession number 16S	GenBank accession number 5.8S-ITS2-28S
<i>Hygromiidae</i>	<i>Actinella (Actinella) lentiginosa</i> (Lowe, 1831)	Sao Vicente, Madeira, Portugal	<sup>1</sup> MVHN-2190	KJ458482	KJ458580
	<i>Actinella (Plebecula) giramica</i> (Lowe, 1852)	Pico Serrado, Corral das Freijas, Madeira, Portugal	<sup>1</sup> MVHN-2195	KJ458481	
	<i>Actinella (Plebecula) nitidiuscula</i> (Sowerby, I 1824)	Ponta Sao Lourenço, Madeira, Portugal	<sup>1</sup> MVHN-2193	KJ458483	KJ458581
	<i>Ashfordia granulata</i> (Alder, 1830)	Ordes, A Coruña, Spain	<sup>3</sup> EHUMC-1015	KJ458488	KJ458586
	<i>Ashfordia granulata</i> (Alder, 1830)	Gontan, Ourense, Spain	<sup>3</sup> EHUMC-1016	KJ458489	KJ458587
	<i>Canariella (Canariella) hispidula</i> (Lamarck, 1822)	Las Valladas, Tenerife, Canary Islands, Spain	<sup>1</sup> MVHN-2167	KJ458494	KJ458591
	<i>Candidula corbellai</i> Martínez-Ortí, 2011	Lleida, Lleida, Spain	<sup>1</sup> MVHN-2188	KJ458492	KJ458590
	<i>Candidula gigaxii</i> (L. Pfeiffer, 1847)	Igualeja, Málaga, Spain	<sup>3</sup> EHUMC-1017	KJ458493	
	<i>Candidula intersecta</i> (Poirét, 1801)	Mon Island, Tiornemarke, Denmark		AY741437*	
	<i>Candidula najerensis</i> (Ortiz de Zárate, 1950)	Miño de Medinaceli, Soria, Spain	<sup>3</sup> EHUMC-1018	KJ458495	KJ458592
	<i>Candidula olisippensis</i> (Servain, 1880)	Torro Lamario, Portugal		AY546346*	
	<i>Candidula rugosiuscula</i> (Michaud, 1831)	Carrières-sous-Poissy, France		AY546347*	
	<i>Candidula spadae</i> (Calcara, 1845)	Monte Cucco (Costacciaro, Perugia), Italy		AY741436*	
	<i>Candidula unifasciata</i> (Poirét, 1801)	Parco La Tebaide, Cetinale (Sovicille, Siena), Italy		AY741438*	
	<i>Caseolus compactus</i> (Lowe, 1832)	Ponta Sao Lourenço, Madeira, Portugal	<sup>1</sup> MVHN-2194	KJ458496	KJ45859
	<i>Cernuella (Cernuella) cisalpina</i> (Rossmässler, 1837)	Stazione di Castelnuovo Berardenga (Asciano, Siena), Italy		AY741423*	
	<i>Cernuella (Cernuella) virgata</i> (Da Costa, 1778)	(16S): Stazione di Castelnuovo Berardenga (Asciano, Siena), Italy; (5.8-ITS2-28S): Porthcumick, Cornwall, UK		AY741422*	AY014127*
	<i>Cernuella (Xerocincta) neglecta</i> (Draparnaud, 1805)	Torrente Arbia, Vallina (Castelnuovo Berardenga, Siena), Italy		AY741426*	
	<i>Cernuella (Xerocincta) neglecta</i> (Draparnaud, 1805)	Grasse, France	<sup>3</sup> EHUMC-1019	KJ458571	KJ458648
	<i>Cernuellopsis ghisottii</i> Manganelli and Giusti, 1987	Monte Pollino, Cozzo Vardo (Morano Calabro, Cosenza), Italy		AY741429*	
	<i>Ciliella ciliata</i> (Hartmann, 1821)	Ordesa valley, Torla, Huesca, Spain		FJ786407*	JQ805024*
	<i>Cryptosaccus asturiensis</i> Prieto and Puente, 1994	Somiedo, Asturias, Spain	<sup>3</sup> EHUMC-1020	KJ458505	KJ458601
	<i>Discula (Discula) polymorpha</i> (Lowe, 1831)	Sao Lourenço, Madeira, Portugal	<sup>1</sup> MVHN-2192	KJ458506	KJ458602
	<i>Euomphalia strigella</i> (Draparnaud, 1801)	Pitarque, Teruel, Spain	<sup>1</sup> MVHN-2198	KJ458512	
	<i>Euomphalia strigella</i> (Draparnaud, 1801)	Queralbs: Daió, Girona, Spain	<sup>3</sup> EHUMC-1021	KJ458513	KJ458605
	<i>Ganula gadirana</i> Muñoz, Almodóvar and Arrébola, 1999	Afueras de Algeciras, Cádiz, Spain	<sup>1</sup> MVHN-1382	KJ458514	
	<i>Ganula gadirana</i> Muñoz, Almodóvar and Arrébola, 1999	Valdevaqueros-Punta Paloma, Cádiz, Spain	<sup>3</sup> EHUMC-1022	KJ458515	KJ458606
	<i>Ganula gadirana</i> Muñoz, Almodóvar and Arrébola, 1999	Valdevaqueros-Punta Paloma, Cádiz, Spain	<sup>3</sup> EHUMC-1023	KJ458516	
	<i>Ganula lanuginosa</i> (Boissy, 1835)	Andratx-Sant Elm, Mallorca, Spain	<sup>3</sup> EHUMC-1024	KJ458517	KJ458607
	<i>Ganula lanuginosa</i> (Boissy, 1835)	Coll de Sóller, Mallorca, Spain	<sup>3</sup> EHUMC-1025	KJ458518	
	<i>Ganula sp</i>	Honaine, Trarar Massif, Algeria	<sup>1</sup> MVHN-2179	KJ458519	KJ458608
	<i>Helicella itala</i> (Linnaeus, 1758)	Enol, Asturias, Spain	<sup>1</sup> MVHN-2140	KJ458522	KJ458611
	<i>Helicella orzai</i> Gittenberger and Manga, 1981	Aralar, Navarra, Spain	<sup>3</sup> EHUMC-1026	KJ458525	KJ458613
	<i>Helicella stiparum</i> (Rossmässler, 1854)	Los Alcores, Almería, Spain	<sup>1</sup> MVHN-1285	KJ458526	
	<i>Helicopsis striata</i> (Müller, 1774)	Kyffheuser, Germany		AY546362*	
	<i>Helicopsis turcica</i> (Holten, 1802)	Between Essauira and Agadir, Morocco	<sup>1</sup> MVHN-010211FR04	KJ458527	KJ458614
	<i>Helicotricha carusoi</i> Giusti, Manganelli and Crisci, 1992	Linosa Island, Monte Calcarella (Lampedusa e Linosa, Agrigento), Italy		AY741434*	
	<i>Hygromia (Hygromia) cinctella</i> (Draparnaud, 1801)	Pian di Giuncheto (Cetona, Siena), Italy		AY741421*	
	<i>Hygromia (Riedelia) limbata</i> (Draparnaud, 1805)	Queralbs: Daió, Girona, Spain	<sup>3</sup> EHUMC-1027	KJ458529	KJ458616
	<i>Ichnusomunda sacchii</i> Giusti and Manganelli, 1998	Is Arenas, Cuccuru Pranu (Arbus, Oristano), Italy		AY741424*	
	<i>Leptaxis (Cryptaxis) groviana</i> (A. Ferussac, 1832)	Ponta Sao Lourenço, Madeira, Portugal	<sup>1</sup> MVHN-2189	KJ458533	KJ45861*9
	<i>Leptaxis (Leptaxis) drouetiana</i> (Morelet, 1860)	Faial island, Azores islands, Portugal		AY748301*	
	<i>Leptaxis (Leptaxis) simia</i> (A. Ferussac, 1832)	(16S-ITS2): Sao Vicente, Madeira, Portugal; (28S): Portela and Santaç, Madeira, Portugal	<sup>1</sup> MVHN-2191	KJ458532	KJ458653-AJ550969*
	<i>Mengoana jeschawi</i> (Kobelt, 1878)	Pola de Somiedo, Asturias, Spain	<sup>3</sup> EHUMC-1028	KJ458536	KJ458623
	<i>Mengoana jeschawi</i> (Kobelt, 1878)	Between Cangas and Llanes, Asturias, Spain	<sup>3</sup> EHUMC-1029	KJ458537	
	<i>Monacha (Monacha) cantiana</i> (Montagu, 1803)	(16S): Sopolana, Bizkaia, Spain; (5.8-ITS2-28S): Pulpit Down, Buckinghamshire, UK	<sup>3</sup> EHUMC-1030	KJ458539	AY841332*
	<i>Monacha (Monacha) cartusiana</i> (Müller, 1774)	Cañón del río Dulce, Guadalajara, Spain	<sup>3</sup> EHUMC-1031	KJ458540	KJ458625
<i>Monacha (Monacha) martensiana</i> (Tiberi, 1869)	Piana di Colfiorito (Foligno, Perugia), Italy		AY741420*		
<i>Monacha (Monacha) parumcincta</i> (Menke, 1828)	Medane (Asciano, Siena), Italy		AY741418*		

	<i>Monachoides incarnatus</i> (Müller, 1774)	Enney, Switzerland		AY546371*	
	<i>Montserratina bofilliana</i> (Fagot, 1884)	Bagés, Barcelona, Spain	<sup>1</sup> MVHN-2139	KJ458538	KJ458624
	<i>Montserratina martorelli</i> (Bourguignat, 1870)	Les Planes, Barcelona, Spain	<sup>1</sup> MVHN-2137	KJ458541	KJ458626
	<i>Montserratina martorelli</i> (Bourguignat, 1870)	Collserola, Barcelona, Spain	<sup>1</sup> MVHN-2138	KJ458542	
	<i>Plentuisa vendia Puente and Prieto, 1992</i>	Tielve, Asturias, Spain	<sup>3</sup> EHUMC-1032	KJ458546	KJ458629
	<i>Ponentina revelata</i> (Michaud, 1831)	Valdenoceda, Burgos, Spain	<sup>3</sup> EHUMC-1033	KJ458547	KJ458630
	<i>Ponentina revelata</i> (Michaud, 1831)	Ordes, A Coruña, Spain	<sup>3</sup> EHUMC-1034	KJ458548	KJ458631
	<i>Portugala inchoata</i> (Morelet, 1845)	Conimbriga, Coimbra, Portugal	<sup>3</sup> EHUMC-1035	KJ458549	KJ458632
	<i>Pyrenaeaaria cantabrica</i> (Hidalgo, 1873)	Los Beyos defile, Asturias, Spain		EU310145*	JQ805025*
	<i>Trochoidea (Trochoidea) elegans</i> (Gmelin, 1791)	L'Alcudia, Valencia, Spain	<sup>1</sup> MVHN-1310	KJ458564	KJ458642
	<i>Trochoidea (Trochoidea) pyramidata</i> (Draparnaud, 1805)	Cala de la Mosca, Orihuela, Alicante, Spain	<sup>1</sup> MVHN-120110XT04	KJ458565	KJ458643
	<i>Trochoidea (Trochoidea) trochoides</i> (Poiret, 1789)	Populonia, Italy		AY546379*	
	<i>Trochulus (Trochulus) alpicolus</i> (Eder, 1921)	Bannalppass, Nidwalden, Switzerland		DQ217812*	
	<i>Trochulus (Trochulus) biconicus</i> Eder, 1917	Bannalppass, Nidwalden, Switzerland		DQ217811*	
	<i>Trochulus (Trochulus) caelatus</i> Studer, 1820	Birseschlucht, Bern, Switzerland		DQ217803*	
	<i>Trochulus (Trochulus) hispidus</i> (Linnaeus, 1758)	(16S): Between Lastras and Valle, Cantabria, Spain; (5.8-ITS2-28S): Deepdale, Derbyshire, UK		FJ786447*	AY014125*
	<i>Trochulus (Trochulus) piccardi</i> Pfenninger and Pfenninger, 2005	Chateau d'Oex, Vaud, Switzerland		AY738397*	
	<i>Trochulus (Trochulus) sericeus</i> (Draparnaud, 1801)	Enney, Switzerland		AY546374*	
	<i>Trochulus (Trochulus) striolata</i> (Pfeiffer, 1828)	Deepdale, Derbyshire, UK			AY014124*
	<i>Xerocrassa (Xerocrassa) grabusana</i> Hausdorf and Sauer, 2009	Crete, Greece		JN701847*	
	<i>Xerocrassa (Xerocrassa) mesostena</i> (Westerlund, 1879)	Crete, Greece		JN701876*	
	<i>Xerocrassa barceloi</i> (Hidalgo, 1878)	Calpe, Alicante, Spain	<sup>1</sup> MVHN-1304	KJ458570	
	<i>Xerocrassa grata</i> (F. Haas, 1924)	Benifallet, Tarragona, Spain	<sup>1</sup> MVHN-2196	KJ458575	
	<i>Xerolenta obvia</i> (Menke, 1828)	Fiume Tagliamento, Lago di Cornino (Folgaria nel Friuli, Udine), Italy		AY741431*	
	<i>Xeromunda durieui</i> (L. Pfeiffer, 1848)	Marina di Pescoluse (Salve, Lecce), Italy		AY741432*	
	<i>Xerosecta (Polloneriella) contermina</i> (L. Pfeiffer, 1848)	Lago di Burano (Capalbio, Grosseto), Italy		AY741425*	
	<i>Xerosecta (Xeromagna) adolfi</i> (L. Pfeiffer, 1854)	Castala, Almería, Spain	<sup>1</sup> MVHN-1298	KJ458566	KJ458644
	<i>Xerosecta (Xeromagna) adolfi</i> (L. Pfeiffer, 1854)	Nijar, Almería, Spain	<sup>3</sup> EHUMC-1036	KJ458567	KJ458645
	<i>Xerosecta (Xeromagna) arigonis</i> (A. Schmidt, 1853)	Pitarque, Teruel, Spain	<sup>1</sup> MVHN-2199	KJ458569	KJ458647
	<i>Xerosecta (Xeromagna) promissa</i> (Westerlund, 1893)	Sierra de Benaoján, Málaga, Spain	<sup>1</sup> MVHN-1375	KJ458576	KJ458651
	<i>Xerosecta (Xeromagna) reboudiana</i> (Bourguignat, 1863)	Antequera, Málaga, Spain	<sup>3</sup> EHUMC-1037	KJ458577	KJ458652
	<i>Xerosecta (Xerosecta) cespitum</i> (Draparnaud 1801)	La Garde, France		AY546351*	
	<i>Xerosecta (Xerosecta) explanata</i> (Müller, 1774)	Playa de Daimuz, Valencia, Spain	<sup>1</sup> MVHN-2168	KJ458573	KJ458650
	<i>Xerosecta (Xerosecta) explanata</i> (Müller, 1774)	Cap d'Agde - Sete, Hérault, France	<sup>3</sup> EHUMC-1038	KJ458574	
	<i>Xerotricha apicina</i> (Lamarck, 1822)	Málaga, Málaga, Spain	<sup>1</sup> MVHN-2157	KJ458520	KJ458609
	<i>Xerotricha apicina</i> (Lamarck, 1822)	Between Portimao and Algarve, Portugal	<sup>1</sup> MVHN-2155	KJ458568	KJ458646
	<i>Xerotricha conspurcata</i> (Draparnaud, 1801)	Jadraque, Guadalajara, Spain	<sup>3</sup> EHUMC-1039	KJ458572	KJ458649
	<i>Xerotricha gonzalezi</i> (Azpeitia, 1925)	Pancorbo, Burgos, Spain	<sup>3</sup> EHUMC-1040	KJ458521	KJ458610
	<i>Xerotricha vatonniana</i> (Bourguignat, 1867)	Between Matagañanes and El Cmpillo, Córdoba, Spain	<sup>3</sup> EHUMC-1041	KJ458578	
	<i>Xerotricha vatonniana</i> (Bourguignat, 1867)	Between Matagañanes and El Cmpillo, Córdoba, Spain	<sup>3</sup> EHUMC-1042	KJ458579	
Monadeniidae	<i>Monadenia (Monadenia) fidelis</i> (J.E. Gray, 1834)	Oregon, USA			AY014142*
Pleurodontidae	<i>Dentellaria sinuata</i> (Müller, 1774)	Green Grot Cave, Jamaica			AY841322*
	<i>Thelidomus aspera</i> (Férussac, 1821)	Windsor, Jamaica			AY841321*
Polygyridae	<i>Mesodon (Mesodon) thyroides</i> (Say, 1816)	York Co. Pennsylvania, USA			AY841315*
	<i>Neohelix (Solemborbis) alleni</i> (Wetherby, 1881)	Williams Creek, Iowa, USA			AY841316*
	<i>Vespericola columbiana</i> Henderson, 1928	Eugene, Oregon, USA			AY014120*
Sphincterochilidae	<i>Sphincterochila (Albea) candidissima</i> (Draparnaud, 1801)	Bardenas Reales, Navarra, Spain	<sup>3</sup> EHUMC-1043	KJ458556	KJ458636
	<i>Sphincterochila (Albea) candidissima</i> (Draparnaud, 1801)	Cervera del Maestre, Castellón, Spain	<sup>1</sup> MVHN-280610ZB14	KJ458557	KJ458637
Trissexodontidae	<i>Caracollina (Caracollina) lenticula</i> (Michaud, 1831)	(16S): Tarifa, Cádiz, Spain; (5.8-ITS2-28S): Valverde del Camino, Huelva, Spain		FJ786406*	JQ805002*
	<i>Gasullia gasulli</i> (Ortiz de Zárate and Ortiz de Zárate, 1961)	Nerva, Huelva, Spain		FJ786411*	JQ805000*
	<i>Gasullliella simplicula</i> (Morelet, 1845)	Tharsis, Huelva, Spain		FJ786413*	JQ805001*
	<i>Gittenbergeria turriplana</i> (Morelet, 1845)	(16S): Tavira, Portugal; (5.8-ITS2-28S): Silves, Portugal		FJ786416*	JQ805026*
	<i>Hatumia cobosi</i> (Ortiz de Zárate, 1962)	Enix, Almería, Spain		FJ786420*	JQ804999*

(continued on next page)

Table 1 (continued)

Family	Species	Locality	Voucher	GenBank accession number 16S	GenBank accession number 5.8S-ITS2-28S
	<i>Hatumia riffensis</i> (Ortiz de Zárate, 1962)	Monte Gurugú, Melilla		FJ786422*	JQ804998*
	<i>Hatumia zapateri</i> (Hidalgo, 1870)	Cerro del Olivo, Córdoba, Spain		FJ786421*	JQ804997*
	<i>Mastigophallus rangianus</i> (Michaud, 1831)	Port de la Selva, Girona, Spain		FJ786426*	JQ805019*
	<i>Oestophora barbula</i> (Rossmässler, 1838)	Viches, Jaén, Spain		FJ786437*	JQ805013*
	<i>Oestophora ebría</i> (Corbellá, 2004)	Sierra de las Nieves Natural Park, Málaga, Spain		FJ786430*	JQ805010*
	<i>Oestophora silvae</i> (Ortiz de Zárate, 1962)	Arredondo, Cantabria, Spain		FJ786435*	JQ805011*
	<i>Oestophora tarnieri</i> (Morelet, 1854)	Bobadilla, Los Alcornocales Natural Park, Málaga, Spain		FJ786433*	JQ805009*
	<i>Oestophorella buvinieri</i> (Michaud, 1841)	Matienco, Cantabria, Spain		FJ786440*	JQ805007*
	<i>Suboestophora boscae</i> (Hidalgo, 1869)	Bobadilla, Los Alcornocales Natural Park, Málaga, Spain		FJ786445*	JQ805016*
	<i>Suboestophora jeresae</i> (Ortiz de Zárate, 1962)	Valencia, Spain		FJ786444*	JQ805014*
	<i>Suboestophora tarraconensis</i> (Aguilar-Amat, 1935)	Cunit, Tarragona, Spain		FJ786443*	JQ805015*
	<i>Trissexodon constrictus</i> (Boubée, 1836)	(16S): Kakouetta, Zuberoua, France; (5.8-ITS2-28S): Pagasarri, Bizkaia, Spain		FJ786448*	JQ805017*
	<i>Trissexodon constrictus</i> (Boubée, 1836)	Pagasarri, Bizkaia, Spain		FJ786450*	JQ805018*
	<i>Arion ater</i> (Linnaeus, 1758)	(16S): France; (5.8-ITS2-28S): Kirk Ireton, Derbyshire, UK		HQ659926*	AY014144*
	<i>Discus rotundatus</i> (Müller, 1774)	(16S): Frankfurt, Germany; (5.8-ITS2-28S): Kirkdale, Derbyshire, UK		FJ917265*	AY014097*
	<i>Solatopupa similis</i> (Bruguiere 1792)	(16S): Montpellier, France; (5.8-ITS2-28S): Verdon Gorge, France		DQ305057*	AY014033*
Outgroups					

DNA amplification were 1 min at 96 °C, [30 s at 94 °C, 30 s at 47–55 °C (depending on the annealing temperature of the primer pair used), 1 min at 72 °C] (repeated for 35 cycles) and 10 min at 72 °C. Amplicons were sequenced using the dRhodamine Terminator Cyclor Sequencing Ready reaction Kit (Applied Biosystems, Foster City, CA) run on an ABI PRISM model 3100 Avant Genetic Analyzer and using the same primers as for PCR (see Table 2 for the primers used). The resulting forward and reverse sequences were assembled using SEQUENCHER 4.10.1 (Gene Codes Corporation) and checked for errors/ambiguities. New nucleotide sequences for 16S rRNA and the rRNA gene cluster (5.8S, ITS2 and 28S) were obtained as part of this study. 5.8S fragment is partial and short (–50 bp); therefore we considered 5.8S-ITS2 as a single partition. Consequently, nuclear rRNA gene cluster was divided into two partitions: 5S-ITS2 and 28S. These sequences have been deposited in GenBank under accession numbers KJ458481–KJ458653 (Table 1).

### 2.3. Phylogenetic analyses

Sequences were aligned with Mafft v.7 online version (Katoh et al., 2002) as it has been described to perform better than alternative pairwise alignment methods (Golubchik et al., 2007). We used the Q-INS-i algorithm and default values for the rest of the parameters for the alignment of each gene (Katoh and Toh, 2008).

We aligned our sequences of the 16S rRNA and nuclear rRNA cluster genes with sequences published in GenBank. Several sequences were obtained for all genes considered in this work, but some sequences from GenBank belonging to taxa of interest for this study are provided only for one or two of the genes considered. For this reason, each data set was analyzed separately to cover the maximum information possible and to compare the different topologies obtained. Next, we analyzed three different data sets: the first included the mitochondrial marker 16S rRNA, the second incorporated the nuclear rRNA gene cluster and the third data set was a combined matrix for all genes.

Phylogenetic signal for each gene region analyzed was accessed using the parsimony-based method of Steel et al. (1993) and the entropy-based information method of Xia et al. (2003) and Xia and Lemey (2009), both implemented in DAMBE 5.2.38 (Xia, 2001; Xia and Xie, 2001).

Phylogenetic inference was based on Bayesian (BI), maximum likelihood (ML) and neighbor joining (NJ) inference. We used MrBayes v3.2.2 (Ronquist and Huelsenbeck, 2003) to estimate the topology shown in this work. The evolutionary model considered was GTR + I + G, estimated independently for each of the gene partitions using jModelTest (version 2.1.1; Darriba et al., 2012) applying Akaike weights as selection criterion. MrBayes was run for  $20 \times 10^6$  generations using default values and saving trees each 100 generations. Convergence between runs and the choice of an appropriate burn-in value were assessed by comparing the traces using Tracer v1.5 (Rambaut and Drummond, 2007). Maximum likelihood phylogenies were inferred with RAxML v7.2.8 (Stamatakis, 2006) through the Cipres Science Gateway (Miller et al., 2010) (which includes an estimation of bootstrap node support) using a GTRGAMMA model of evolution and 1000 bootstrapping replicates. Due to the different evolutionary rates of markers considered for this study, in both ML and Bayesian analyses, characters within combined sequence sets were partitioned by gene, allowing different evolution rates for each partition. NJ was performed in PAUP\* version 4.0b10 (Swofford, 2002). For each data set, we used the available substitution and rate heterogeneity model with the closest match to that selected by jModelTest (Darriba et al., 2012). Statistical support for the resulting topologies was assessed by bootstrapping with 5000 pseudoreplicates (Felsenstein, 1985). For the different topologies obtained, we interpreted as significant statistical support values above 70% for



**Table 2**  
List of primers used for amplification and sequencing.

Gene	Primer	Sequence	Reference
16S rRNA	16sar (5')	5' CGCTGTTTATCAAAAACAT 3'	Palumbi et al. (1991)
	16sbr (3')	5' CCGTCTGAACCTCAGATCACGT 3'	Palumbi et al. (1991)
5.8S-ITS2	LSU-1 (5')	5' CTAGCTGCGAGAATTAATGTGA 3'	Wade et al. (2006)
	LSU-3 (3')	5' ACTTCCCTCACGGTACTTG 3'	Wade et al. (2006)
28S	LSU-2 (5')	5' GGGTTGTTTGGGAATGCAGC 3'	Wade et al. (2006)
	LSU-2mod (5')	5' TCTCAGGAGTCGGGTGTTT 3'	This work
	LSU-5 (3')	5' GTTAGACTCCTGTGCCGTG 3'	Wade et al. (2006)

bootstrapping procedures in the ML and NJ analyses and 95% for Posterior Probability (PP) in the BI analysis.

#### 2.4. Divergence time analyses

The use of several calibration nodes has been shown to improve estimates of divergence times and rate estimates (Yang, 2004; Porter et al., 2005; Pérez-Losada et al., 2008). For this study, divergence times were therefore estimated using different genes and multiple fossil calibration points. Time analyses were restricted to the nuclear dataset which allows us to employ more families for the estimation. A relaxed-clock MCMC approach using the uncorrelated lognormal model was implemented in BEAST v1.8 (<http://beast.bio.ed.ac.uk/>), using  $3 \times 10^7$  generations, sampling every 1000th generation and with a burning value of 10%. Independent analyses were performed for the two partitions into which nuclear sequence data were divided. Models of sequence evolution for each nucleotide sequence partition were determined using the corrected Akaike information criterion in jModelTest (Darrriba et al., 2012). The Yule model was chosen as the speciation prior for all three data sets. Information in the set of post-burnin trees was summarized using Tracer v1.5 and TreeAnnotator v1.8 not allowing ESS values <200. The maximum clade credibility tree and clade BPPs were obtained through TreeAnnotator v1.8. Mean values and 95% HPD intervals for the ages of clades and of the stems leading to these clades were calculated using Tracer v1.5.

Calibration was based on fossil evidence and we based divergence time estimates on the age of six fossils attributed to related taxa by Nordsieck (2014) (Table 3). A log-normal prior distribution was assumed for the calibration points (see Ho and Phillips, 2009). Dates of fossils ranged from the Early Eocene (47.8 Ma) to the Late Oligocene (23.03 Ma). Since fossils were ascribed to a geological period, we used the upper limit of each period for divergence time

estimates. The age of a fossil represents the minimum age of the group. Hence, it is more appropriate to present a node within a time interval rather than a fixed time (Norell, 1992). According to Tillier et al. (1996), we considered the origin of the Stylommatophora in the Upper Jurassic (150 Ma) and a normal prior distribution was assumed for the calibration of this point. Calibrations were plotted on the node prior to the basal node of the clade of interest.

### 3. Results

The sequence data obtained are provided in Table 4. Data matrices included 189 sequences for 16S, 136 for the nuclear rRNA gene cluster covering 15 families of the Helicoidea, and 120 for the combined data set covering 11 families. Alignment lengths were 517 base pairs (bp) for 16S, 960 bp for 5.8S-ITS2, and 854 bp for 28S. The length of the mitochondrial/nuclear combined alignment was 2331 bp. The 5.8-ITS2 gene fragment was the most variable, with 67 bp variable sites (46 positions phylogenetically informative PI), the 16S fragment showed 63 bp variable sites (55 positions PI), and the 28S sequence featured 41 bp variable sites (27 positions PI).

Phylogenetic signal analyses based on substitution saturation showed that all three molecular markers should possess enough information to infer phylogenetic relationships among the families considered (Supplementary material S1).

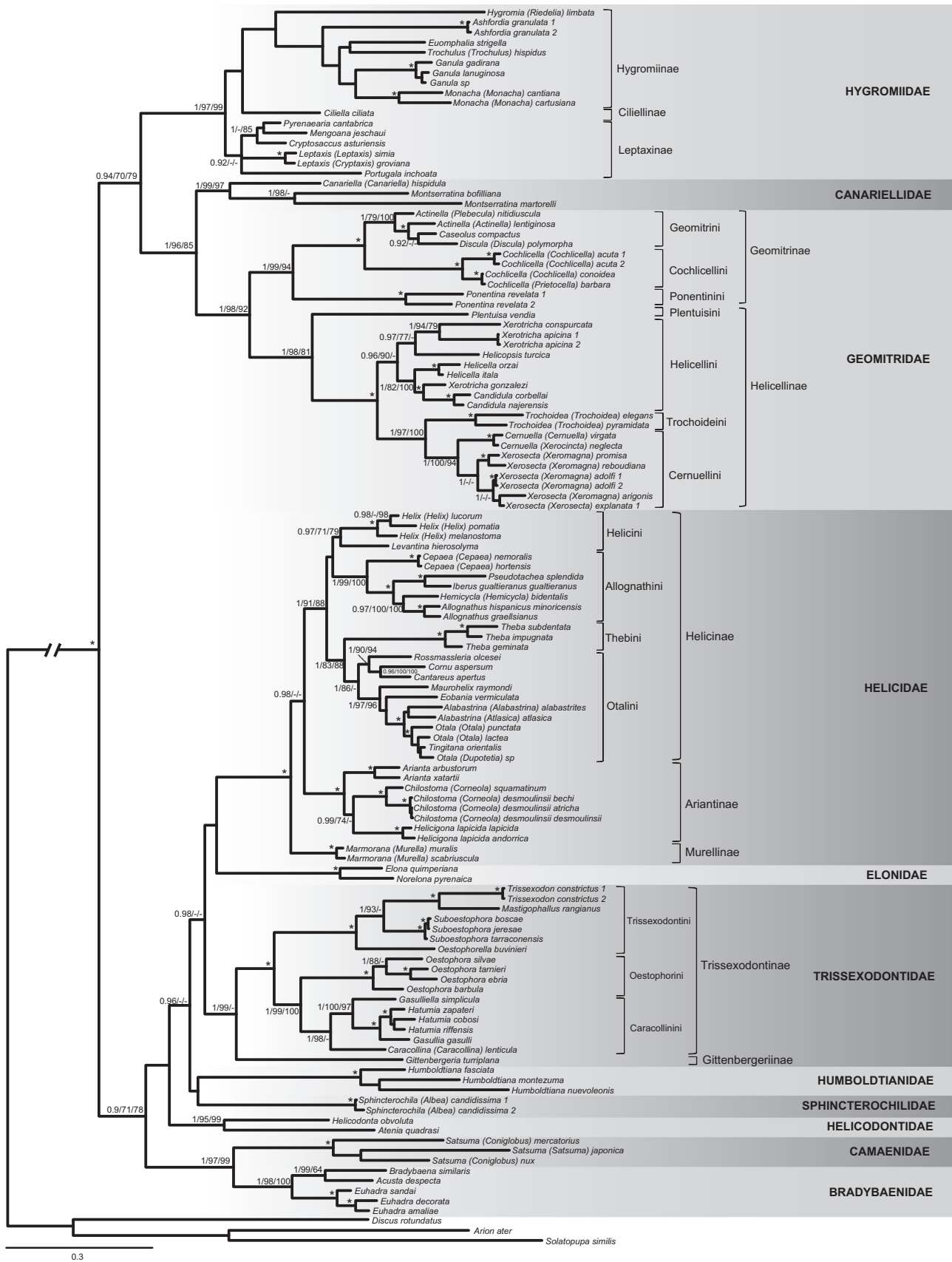
The phylogenetic reconstruction obtained by the concatenated-gene analyses (nuclear rRNA gene cluster + 16S rRNA) is shown in Fig. 2. Unless otherwise indicated, the topology and support of this tree will be referred to in the results section. Other single gene trees (16S rRNA or nuclear rRNA) have been included as supplementary material (Supplementary material S2 and S3). These

**Table 3**  
Summary of node age constraints (minimum ages) used in divergence time estimations based on fossil evidence.

Node	Groups of the crown group	Fossil genus	Fossil age	Upper limit	Source
A	Elonidae	<i>Megalocochlea</i>	Middle Eocene	38.0 Ma	Nordsieck (2014)
B	Helicinae	<i>Parachloraea</i>	Late Eocene	33.9 Ma	Nordsieck (2014)
C	Helicodontidae	<i>Protodrepanostoma</i>	Early Oligocene	28.1 Ma	Nordsieck (2014)
D	Hygromiidae	<i>Loganiopharynx</i>	Early Eocene	47.8 Ma	Nordsieck (2014)
E	Sphincterophilidae	<i>Dentellocaraculus</i>	Middle Eocene	38.0 Ma	Nordsieck (2014)
F	Trissexodontidae	<i>Praeostophorella</i>	Late Oligocene	23.03 Ma	Nordsieck (2014)

**Table 4**  
Length of the sequenced fragments (maximum and minimum) before and after alignment, number of informative sites and evolutionary model selected by Akaike information criteria implemented in jModelTest for the different partitions.

Partition	Maximum length	Minimum length	Aligned length	No. informative sites	Optimal AIC model
16S rRNA	430	211	517	55	GTR + I + G
5.8S-ITS2	607	374	960	46	GTR + I + G
28S	839	315	854	27	GTR + I + G
5.8S-ITS2-28S	1444	834	1814	73	GTR + I + G
Total	1852	1242	2331	185	GTR + I + G



**Fig. 2.** Phylogenetic tree of the Helicoidea based on Bayesian inference (BI), maximum likelihood (ML) and neighbor joining (NJ) analyses of the concatenated data set including 16S rRNA and nuclear 5.8S, ITS2 and 28S sequences. Numbers correspond to BI posterior probabilities, ML bootstrap values and NJ bootstrap, respectively. Asterisks (\*) indicate full support of nodes: BI posterior probabilities = 1.00 ML bootstrap values = 100% and NJ bootstrap = 100%.

topologies include a higher number of taxa because for some species, only sequences of one or two gene fragments were available, especially for taxa represented exclusively by GenBank sequences. Concatenated-gene analyses were better resolved than single-gene analyses, and thus more accurately represent relationships among taxa. Accordingly, the results of single-gene analyses will not be discussed, except when referring to taxa not represented in the concatenated-gene analyses. It should be noted that no single-gene trees featured any well-supported clades in conflict with the concatenated-gene trees discussed.

### 3.1. Family-level classification

All taxa included in our study were grouped into two main clades, with *Cepolis* as sister group in the nuclear rRNA analysis (Supplementary material S3). The first clade (Fig. 2) included Hygromiidae *sensu* Hausdorf and Bouchet (2005) and Cochlicellidae (PP = 0.94; ML = 70%; NJ = 79%). The second clade grouped together all remaining Helicoidea families considered and its monophyly was well supported by ML and NJ in the concatenated-gene tree (PP = 0.9; ML = 71%; NJ = 78%). Clades corresponding to Bradybaenidae, Camaenidae, Cochlicellidae, Elonidae, Helicidae, Helicodontidae, Humboldtianidae, and Sphincterochilidae (represented only by *Sphincterochila candidissima*), were strongly supported (PP = 1.00; ML  $\geq$  95%; NJ  $\geq$  99%) in the concatenated-gene tree. The monophyly of Trissexodontidae was only recovered by BI and ML analyses (PP = 1.00; ML = 99%). The Trissexodontidae also constituted a monophyletic group in the NJ analysis when only nuclear rRNA was considered (NJ = 90%), with *Gittenbergeria* appearing as a separate lineage in the concatenated-gene tree. Nuclear rRNA analysis (Supplementary material S3) also recovered Monadenidae (represented only by *Monadenia fidelis*), Pleurodontidae (PP = 1.00; ML = 100%; NJ = 100%) and Polygyridae (PP = 1.00; ML = 100%; NJ = 100%) with full support.

Relationships among families were not fully resolved even in the concatenated-gene analyses. Nevertheless, the family Cochlicellidae appeared as a derived group within a clade containing all the Hygromiidae *s.l.* genera considered in this study. This relationship among Cochlicellidae and Hygromiidae *s.l.* was recovered with high support in nuclear rRNA and concatenated-gene trees. BI analysis of nuclear rRNA revealed Polygyridae as the sister group (PP = 0.99) of Bradybaenidae (Bradybaeninae + Aegistinae) and Camaenidae, although this sister relationship was not supported by ML and NJ analyses. The nuclear rRNA tree also recovered Monadenidae as the sister group of Humboldtianidae (PP = 0.94; ML = 92%; NJ = 93%), which together with Pleurodontidae form a monophyletic clade supported by BI (PP = 0.99) but not by ML and NJ. Helicodontidae was recovered as the sister group of a clade containing Elonidae, Helicidae, Humboldtianidae, Sphincterochilidae and Trissexodontidae, but this relationship was only supported by the BI analysis in the nuclear rRNA tree (PP = 1.00). Relationships among Elonidae, Helicidae, Humboldtianidae, Sphincterochilidae and Trissexodontidae were not resolved. The sister relationship between Helicidae and Trissexodontidae was recovered only by the nuclear rRNA BI analysis but without significant support (PP = 0.90). *Cepolidae* (represented by *Cepolis streatoris* nuclear rRNA) was recovered as the sister clade of the group joining together all the other families considered in this study.

### 3.2. Genera arrangements within the highly diverse families Helicidae and Hygromiidae

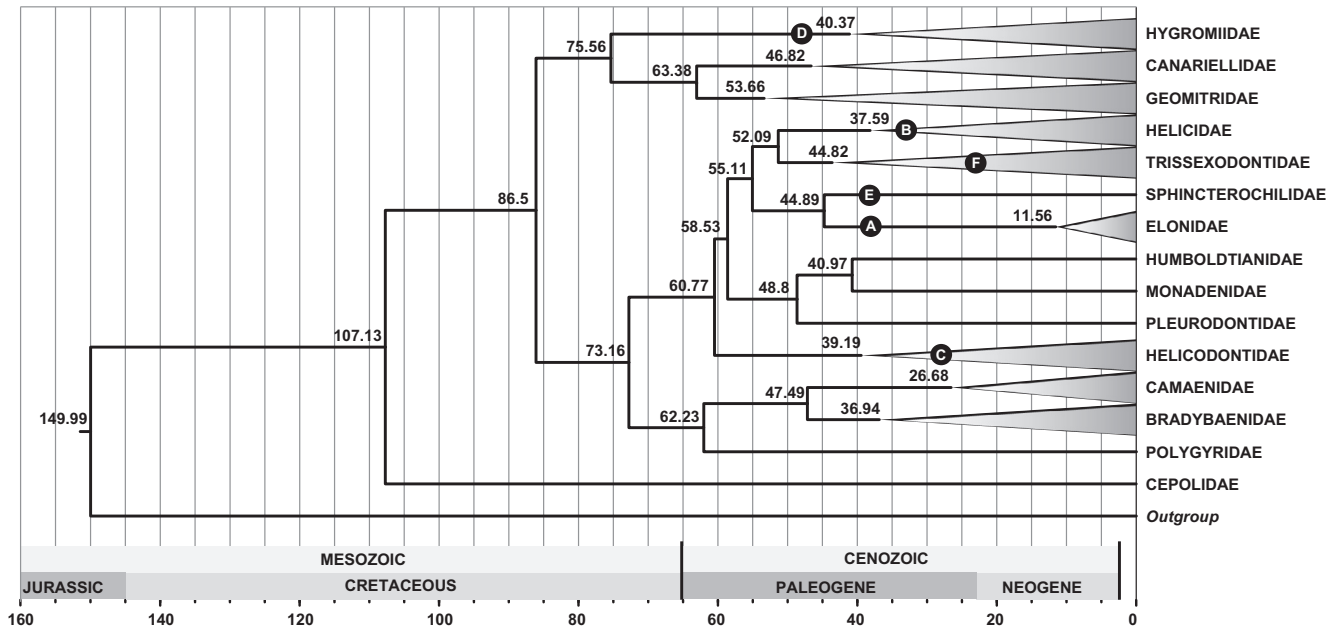
Nuclear rRNA analysis recovered the monophyly of the helioid subfamilies Ariantinae (PP = 1.00; ML = 100%; NJ = 100%) and Helicinae (containing representatives of the three tribes: Helicini, Murellini and Thebini) although the monophyly of the Helicinae

was weakly supported (PP = 0.78; NJ = 78%). In contrast, the concatenated-gene tree recovered Murellini as the sister group of Ariantinae and Helicini + Thebini with strong BI support (PP = 0.98). Nevertheless, ML and NJ analyses recovered Ariantinae, Murellini and Helicini as three different lineages in the concatenated-gene tree. Thebini emerged as a different lineage in the BI and ML analysis of the 16S gene fragment, being the sister group of the other helioids. In contrast, Thebini was included with strong support within the Helicini in the nuclear rRNA (PP = 1.00; ML = 97%; NJ = 79%) and concatenated-gene trees (PP = 1.00; ML = 91%; NJ = 88%), as the sister clade of a group containing the genera *Alabastrina*, *Cantareus*, *Cornu*, *Eobania*, *Maurohelix*, *Otala*, *Rossmassleria* and *Tingitana* (PP = 1.00; ML = 83%; NJ = 88% in the concatenated-gene tree; PP = 1.00; ML = 96%; NJ = 96% in the nuclear rRNA tree). This group formed a polytomy with another two lineages of Helicini in the BI analysis. One of these lineages grouped *Allognathus*, *Hemicycla*, *Iberus* and *Pseudotachea* as the sister group of *Cepaea* (PP = 1.00; ML = 99%; NJ = 100%). The other clade grouped *Helix* and *Levantina* genera (and *Eremina* in nuclear rRNA analysis) (PP = 0.97%; ML = 71%; NJ = 79%).

None of the polytypic subfamilies of the Hygromiidae *s.l.* (Ciliellinae, Geomitrinae, Hygromiinae and Monachainae) were recovered as monophyletic lineages. Three main clades were obtained within the Hygromiidae *s.l.* The first main clade was highly resolved (PP = 1.00; ML = 97%; NJ = 99%) and grouped Euomphalini, Monachaini, Hygromiini (without *Cernuella*), Leptaxini and Trochulini, although relationships among these taxa were not fully resolved. *Ciliella*, *Cryptosaccus*, *Ganula* and *Pyrenaearia* also clustered within this clade. The second hygromiid clade grouped *Canariella* and *Montserratina* (PP = 1.00; ML = 99%; NJ = 97%). The third hygromiid clade grouped Cochlicellidae with Geomitriini and Trochoideini (Geomitrinae), Helicellini (Hygromiinae) and Pontentiniinae (PP = 1.00; ML = 98%; NJ = 92%). Within this third clade, the genus *Plentuisa* was recovered as the sister group of Helicellini + Trochoideini + *Cernuella* (Hygromiini) (PP = 1.00; ML = 98%; NJ = 81%) and grouped with a clade formed by Pontentiniinae and Geomitriini + Cochlicellidae (PP = 1.0; ML = 99%; NJ = 94%). The third hygromiid clade was recovered as the sister group of the second hygromiid clade (PP = 1.00; ML = 96%; NJ = 85%). The sister relationship of the first hygromiid clade with the second + third hygromiid clades was supported in the concatenated-gene tree (PP = 0.94; ML = 70%; NJ = 79%) and by BI and ML phylogenetic analyses in the nuclear rRNA tree (PP = 1.00; ML = 79%; NJ = 68%).

### 3.3. Timing of diversification and biogeographic patterns

Divergence time estimates using BEAST rendered a well-resolved maximum clade credibility tree (see Fig. 3). This tree represents the estimated divergence time chronogram using the maximum clade credibility consensus BMCMC tree based on the nuclear rRNA gene cluster matrix, and six fossil calibrations. Divergence times and 95% highest posterior density (HPD) intervals are given in Table 5. Multiple independent Bayesian runs produced large effective sample sizes and convergence statistics in Tracer that indicated the convergence of all analyses. Variation in the rate of evolution across lineages was evident. According to our divergence time analyses (Table 5), the origin of Helicoidea dates back to 107.13 Ma, in the Early Cretaceous. The two main clades obtained share a similarly aged MRCA, 75.56 Ma for Hygromiidae *s.l.* (Hygromiidae *s.str.* Canariellidae, Geomitridae) and 73.16 Ma for the clade grouping the rest of the families considered (Bradybaenidae, Camaenidae, Elonidae, Helicidae, Helicodontidae, Humboldtianidae, Monadenidae, Pleurodontidae, Polygyridae, Sphincterochilidae and Trissexodontidae). According to our reconstruction of the Hygromiidae *s.l.* clade, the three European families considered started their diversification in the Eocene (33.9–56.0 Ma), with ages



**Fig. 3.** Maximum clade credibility tree generated by the BEAST analysis for the 5.8S-ITS2–28S concatenated data set. Node numbers indicate estimated mean ages provided by the BEAST analysis. The bar below provides ages in millions of years (My).

**Table 5**

Posterior characteristics of nodes of Helicoidea and all families: estimated mean ages and 95% highest probability density (HPD) intervals of the MRCA and the posterior probability.

Group	Mean age MRCA in Ma	95% HPD interval in Ma	Posterior prob.
Helicoidea	107.13	82.65–137.99	0.95
Bradybaenidae	36.94	25.13–51.29	1.00
Camaenidae	26.68	15.73–38.97	1.00
Canariellidae	46.82	28.83–65.73	1.00
Elonidae	11.56	4.27–21.61	1.00
Geomitridae	53.66	40.51–67.98	1.00
Helicidae	37.59	34.44–42.53	1.00
Helicodontidae	39.19	20.72–57.53	1.00
Hygromiidae	40.37	26.78–59.32	1.00
Trissexodontidae	44.82	34.98–55.06	1.00

ranging between 40.37 Ma (Hygromiidae *s.str.*) and 53.66 Ma (Geomitridae). The second main clade diverged into two groups. The first group started its diversification 62.23 Ma ago and includes families distributed outside the western Palaeartic region: Bradybaenidae (36.94 Ma) living in Asia, Camaenidae (26.68 Ma) in SE Asia and Australia and Polygyridae in North America. The second cluster dates back to 60.77 Ma and joins families of the western Palaeartic region [Elonidae (11.56 Ma), Helicidae (37.59 Ma), Helicodontidae (39.19 Ma), Sphincterochilidae and Trissexodontidae (44.82 Ma)] with the Humboldtianidae, Monadenidae and Pleurodontidae distributed across North and South America.

#### 4. Discussion

Recent molecular studies are gradually improving our knowledge of relationships among the Helicoidea (Manganelli et al., 2005; Wade et al., 2006; Groenenberg et al., 2011). The present study mainly focuses on the helicoidean families that exist in the western Palaeartic region, although we also included representatives of other phylogenetically closely related helicoidean families (after Wade et al., 2006, 2007). Compared to prior studies, the increased number of taxa sampled and the use of mitochondrial

and nuclear genes yielded some new insights into relationships, and allowed for direct comparisons with earlier investigations examining families of land snails.

##### 4.1. Congruence of phylogenetic clades with current classification

All the families considered in our study were grouped into two main clades. Thus, Bradybaenidae, Camaenidae, Elonidae, Helicidae, Helicodontidae, Humboldtianidae, Monadenidae, Pleurodontidae, Polygyridae, Sphincterochilidae and Trissexodontidae clustered together as the sister group of Hygromiidae *s.l.* This basal dichotomy between Hygromiidae *s.l.* and the other helicoidean families included in our study is consistent with the classification by Koene and Schulenburg (2005), who grouped Helicidae, Helminthoglyptidae (including Humboldtianidae and Monadenidae) and Bradybaenidae (including Camaenidae) as the sister group of Hygromiidae *s.l.* In contrast, Wade et al. (2007) clustered, although without support, the Hygromiidae *s.l.* with the pleurodontids *Pleurodonte sinuata* and *Theliodomus asper*, as the sister group of a large clade including the other helicoidean taxa, excluding the pleurodontids *Polydotes* and *Zachrysia*. Here, we included the nuclear sequences for *Pleurodonte sinuata* and *Theliodomus asper* of Wade et al. (2007), but both taxa grouped with Monadenidae and Humboldtianidae, confirming the monophyly of the non-Hygromiidae *s.l.* basal clade. Manganelli et al. (2005) clustered the Helicodontidae within the Hygromiidae *s.l.* However, our results located this family outside the Hygromiidae.

Eleven helicoidean families were identified in the concatenated-gene analysis and four additional families appeared in the nuclear rRNA tree, with Cepolidae as the sister group of the other Helicoidea. The family status was confirmed for Bradybaenidae, Camaenidae, Elonidae, Helicidae, Helicodontidae, Humboldtianidae, Monadenidae, Pleurodontidae, Polygyridae, Sphincterochilidae and Trissexodontidae. All these families, defined on the basis of morphological characters (reviewed in Nordsieck, 2010), were also considered in the classification of Hausdorf and Bouchet (2005). In the helicoidean classification by Schileyko (2004, 2006a, 2006b), Cepolidae was ascribed to a subfamily of Helminthoglyptidae (a family not included in our study), while Trissexodontidae were



distributed among four subfamilies within the Helicodontidae. Gómez-Moliner et al. (2013) also recovered the families Trissexodontidae and Helicodontidae as separated lineages. The family Hygromiidae *sensu* Hausdorf and Bouchet (2005) was divided into three clades which are here given familial rank. Hygromiidae *s.str.*, Canariellidae and Geomitridae are here shown to be distinct clades with high support. Nevertheless, we should mention that we did not examine some suprageneric hygromiid taxa restricted to the eastern Mediterranean and Caucasus regions (Hesseolinae Schileyko, 1991 and Metafruticicolinae Schileyko, 1972), Tien-Shan mountains in Central Asia (Archaicinae Schileyko, 1978 and Paedhoplitinae Schileyko, 1978), and Tropical Africa (Halolimnolhelicinae Nordsieck, 1986 and Vicariihelicinae Schileyko, 1991). The monophyly of all above mentioned 14 families examined was highly supported by the different phylogenetic analyses. However, the family Cochlicellidae *sensu* Hausdorf and Bouchet (2005) and Schileyko (2004) was not recovered as a distinct clade of familial rank, but was included within the Geomitridae. Sister relationships between Helicidae and Trissexodontidae recovered by some but not all the analyses performed require confirmation. The monophyly of the Hygromiidae *s.l.* (Hygromiidae *s.str.* + Canariellidae + Geomitridae, present work) was not observed by Manganelli et al. (2005). Koene and Schulenburg (2005) did recover the monophyly of the Hygromiidae *s.l.* but without statistical support. Wade et al. (2007) also recovered this monophyly, but only supported by NJ analysis. In the present study, the monophyly of the clade grouping Hygromiidae *s.str.*, Canariellidae and Geomitridae was supported by ML and NJ analyses in the concatenated-gene tree and by BI, ML and NJ in the nuclear rRNA tree. Besides, the sister relationship between Canariellidae and Geomitridae was highly supported by all phylogenetic analyses.

Relationships among families outside the western Palaearctic region were congruent with the data of Wade et al. (2006, 2007) clustering Brabybaenidae, Camaenidae and Polygyridae with high support. Nuclear rRNA analysis also grouped together Humboldtianidae, Monadenidae and Pleurodontidae with strong support. *Satsuma* and *Coniglobus* classified by Schileyko (2004) within the Aegystinae (Bradybaenidae) were ascribed to the Camaenidae according to Vaught (1989) and Wade et al. (2007).

#### 4.1.1. Family Hygromiidae *s.str.*

Twelve genera found to cluster within this clade appeared within three main groups here considered subfamilies: Ciliellinae, Leptaxinae and Hygromiinae. The genus *Hygromia* was grouped with *Ashfordia*, *Euomphalia* and *Monacha*, (Monachinae), *Trochulus* (Trochulinae: Trochulini) and *Ganula* (Hygromiinae: Hygromiini). This indicates that neither Trochulinae Lindholm, 1927 *sensu* Schileyko nor Hygromiinae *sensu auctores* are monophyletic groups. All the Monachinae genera included in the present study were assigned to this group, consistent with the data of Koene and Schulenburg (2005) relating *Monacha* to the mesophilic Hygromiidae *s.l.*, but not with the scheme of Steinke et al. (2004), who clustered *Monacha* with the xerophilic Hygromiidae *s.l.* (Geomitridae in the present study).

The phylogenetic relationships of the genera included within this family were not fully resolved. Besides, some clusters were not statistically supported. Thus, before suggesting any further subdivisions, more work is needed on this family including the study of more taxa and/or more gene fragments. *Cryptosaccus* and *Pyrenaearia*, considered Hygromiini by Schileyko (2006b), and *Mengoana* (Monachinae *sensu* Schileyko) were grouped with high support values by BI and NJ analyses in the concatenated-gene tree. The relationship of *Trochulus* with the Monachinae, although not supported, was also reported by Koene and Schulenburg (2005) and Wade et al. (2006, 2007). According to Koene and Schulenburg (2005), *Monachoides*, *Perforatella* and

*Pseudotrachia* also belong to this family; *Pseudotrachia* being grouped with *Trochulus* and the other two genera being closely related to *Hygromia* and *Ashfordia* based only on the 28S gene fragment (data not shown).

Ciliellinae, only represented by *Ciliella ciliata*, was diagnosed by its simplified genital system with a long free oviduct, a thick bursa copulatrix duct and a short flagellum, and no signs of stimulatory organs (Fig. 4). Schileyko (2006b) also included *Schileykiella*, *Cilliellopsis* and *Tyrrenhiellina* (all from Tyrrhenian Islands) within the Ciliellinae.

Leptaxinae, considered a tribe by Hausdorf and Bouchet (2005) and represented in our study by five genera (*Cryptosaccus*, *Leptaxis*, *Mengoana*, *Portugala* and *Pyrenaearia*), shows no neat diagnostic differences with respect to the Hygromiinae with its single stimulatory apparatus (Fig. 4), as may be seen by examining the drawings of Schileyko (2006b). Leptaxinae shows a reduction trend and even loss of the accessory sac (*Cryptosaccus*, *Leptaxis* and *Portugala*), or dart sac (*Mengoana*). Although our study was centred on western European taxa and we lacked representatives from Central and Eastern Europe, we suggest this clade could have originated in the Iberian Peninsula: four genera are endemic to the northwestern and western Iberian Peninsula, *Leptaxis* being endemic to Macaronesia.

The subfamily Hygromiinae includes six genera: *Hygromia*, *Ashfordia*, *Euomphalia*, *Trochulus*, *Ganula* and *Monacha*. This group is anatomically very diverse and specimens have both a double or single stimulatory system consisting of dart-sac, accessory sac and mucous glands, or these are transformed to vaginal appendiculata (Fig. 4): *Monacha* is the only hygromiid genus with a free right ommatophoral retractor (r.o.r.) (but see Hausdorf (2000) for some exceptions), *Euomphalia* and *Trochulus* have a double stimulatory apparatus, while *Ashfordia* has no stimulatory organ. Only *Hygromia* and *Ganula* have the single stimulatory system comprising dart-sac, accessory sac and mucous glands.

#### 4.1.2. Family Canariellidae (Schileyko, 1991)

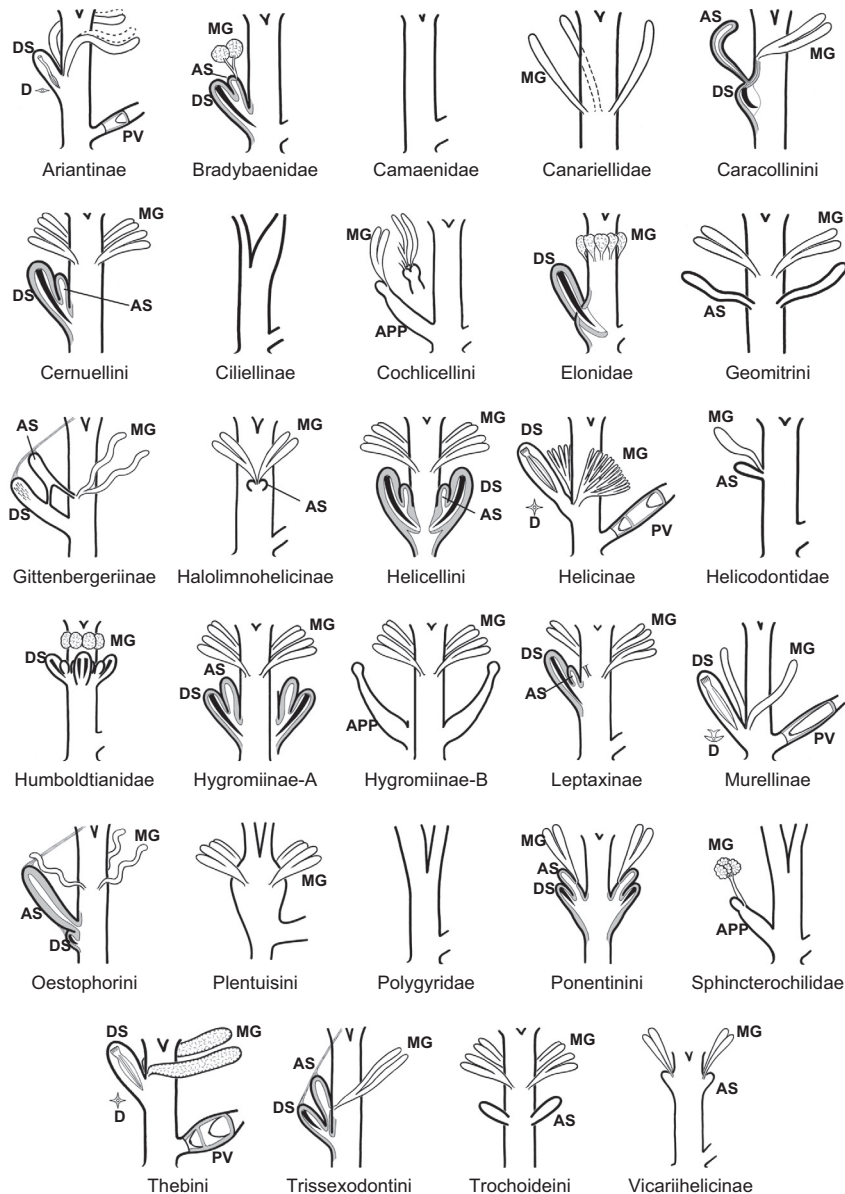
Our data recovered a close relationship between *Canariella* and *Montserratina*, which were grouped within a separated clade that was highly supported by nuclear rRNA and all-gene concatenated analyses. Schileyko (1991) created the monogeneric subfamily Canariellinae within the Hygromiidae. Hausdorf and Bouchet (2005) and Bank et al. (2001) included *Canariella* within the Ciliellinae. However, Schileyko (2006a) assigned *Montserratina* to the Monachinae Wenz, 1930. Our analyses revealed that both genera were neither related to *Ciliella* nor *Monacha*. On the basis of our results, the subfamily Canariellinae (including *Montserratina*) should be elevated to family rank. The close relationship between *Canariella* and *Montserratina* was indicated by Ibáñez et al. (1995) based on shell microsculpture and anatomical similarities. Anatomically, this family is characterized by having a short flagellum and a stimulatory apparatus (Fig. 4) comprised only of one to three single mucous glands (lacking dart or accessory sacs).

#### 4.1.3. Family Geomitridae Boettger, 1909

Both classification systems (Fig. 1) ascribed Geomitriini and Trochoideini Nordsieck, 1987 to the Geomitridae. Besides, Hausdorf and Bouchet (2005) included the Asian Paedhoplitini Schileyko, 1978 within Geomitridae (considered a separate subfamily by Schileyko, 2006b). We had no Paedhoplitini representatives available for our study.

Two main clades were recovered within the Geomitridae, here considered of subfamily rank: Geomitridae and Helicellinae. Geomitridae *sensu* Schileyko (2006b) and Hausdorf and Bouchet (2005) including Geomitriini and Trochoideini emerged here as polyphyletic and both tribes were assigned to different subfamilies: Geomitriini to the Geomitridae, and Trochoideini to the





**Fig. 4.** Diagram illustrating the diagnostic features of the stimulatory apparatus of the genital system for the families, subfamilies and tribes of the superfamily Helicoidea defined in this study. The inner structure of the dart and accessory sacs are indicated when necessary; the inner structure of the penis and cross section of the dart are included only for the subfamilies of Helicinae. Shortness of the vagina is indicated by a lengthened oviducal/spermathecal fork. Oligotypical taxa are represented by their type or better-known genera; polytypical taxa are represented in general terms (defective genital systems are not indicated). The great variation within Hygromiinae is represented by two schemes, each showing a single/double stimulatory apparatus (AS: accessory sac; APP: appendicula; D: cross-section of dart; DS: dart sac; MG: mucous glands; PP: penial verge).

Helicellinae. The Macaronesian genera *Actinella*, *Caseolus* and *Discula* (Geomitriini) were grouped with *Cochlicella* and its allies (Cochlicellini in our classification), with *Ponentina* (Ponentinini in our proposal) as their sister group.

The Helicellinae grouped together another four clades, that are here ranked as tribes. One clade contained *Cernuella* (Hygromiinae: Cernuellini *sensu* Schileyko, 2006b) as the sister group of *Xerosecta* (Hygromiinae: Hygromiini). Because *Hygromia* was clustered within a different group in our analyses, the name Cernuellini Schileyko, 1991 should be applied here. The second clade, Trochoideini Nordsieck, 1987, was recovered as the sister group of Cernuellini. The other two clades were grouped together and designated Helicellini Ihering, 1909 (grouping *Candidula*, *Helicella* and *Xerotricha*) and Plentuisini new tribe, monotypic for *Plentuisa vendia*. In agreement with Manganelli et al. (2005) *Ichnusomunda sacchii* and *Polloneriella*

*contermina* grouped with Cernuellini in our 16S tree (Supplementary material S2).

The most characteristic feature of the anatomy of geomitrids is that they have a free r.o.r. (passing outside the peni-oviducal angle) and a double stimulatory apparatus. However, there are some exceptions and one subclade has a crossing r.o.r. (*Ponentina*) and four subclades have a single stimulatory apparatus. A free r.o.r. has been linked to adaptation to xeric habitats (Schileyko, 1978, 1991; Giusti and Manganelli 1987; Nordsieck, 1987). Nordsieck (1993) suggested that this feature arose independently several times, allowing hygromiids *s.l.* to have independently colonized xeric habitats on several occasions. Our results indicate that all helicoidean genera with a free r.o.r. (except *Monacha*) belong to this clade, suggesting that the free r.o.r. arose only once within the Geomitridae, since this family thrives in prevailing xeric habitats.

Geomitrinae. Although Schileyko (2006b) stated that the r.o.r. passes through the peni-oviducal angle in Geomitrinae, it is certainly free in this subfamily as in other geomitrids (Mandahl, 1950). The stimulatory apparatus of the Geomitridini (Fig. 4) consists of one accessory sac and one bifurcate mucous gland, and may be single (in *Discula* and in other genera not included in our study) or double (in *Actinella* and *Caseolus*). Geomitridini is endemic to Macaronesia (Azores and Madeira). The stimulatory apparatus of the monotypic Pontinini is double (Fig. 4); it is composed of two small dart sacs with two accessory sacs attached to these, and two bifurcate mucous glands joined to the accessory sacs, although this apparatus may be somewhat reduced (Holyoak and Holyoak, 2012).

Helicellinae. We cannot define the Helicellinae based on genital structure because this subfamily fulfils the general criterion for the r.o.r. and stimulatory apparatus common to geomitrids. Nevertheless, our phylogenetic tree reveals its uniqueness and its division into four clades. The tribe Helicellini is characterized by having a double stimulatory apparatus (secondarily single in *Candidula*) with each unit consisting of one accessory sac opening into a large dart sac and two bifurcate mucous glands connected to the vagina (Fig. 4). The close relationship of *Candidula* and *Helicella* was already been recognized by Hausdorf (1988) based on morphological characters. The tribe Trochoideini has two small accessory sacs (without dart sacs) and four bifurcate mucous glands (Fig. 4). Unlike other Helicellinae, the tribe Cernuellini has a single stimulatory apparatus very similar to each unit of the Helicellini (Fig. 4). Other genera ascribed by Schileyko (2006b) to the Cernuellini have a similar stimulatory system, and probably belong to this phylogroup. Nevertheless, Schileyko (2006b) also included *Candidula* within the Cernuellini, a genus that according to our results belongs to the Helicellini. Plentuisini new tribe is characterized by a defective double stimulatory apparatus, with neither a dart sac nor accessory sac, but with four bifurcate mucous glands (Fig. 4). Although *Plentuisa* has a free r.o.r., Puente and Prieto (1992) claimed it showed a close relationship with Trochulinae and Schileyko (2006b) ascribed it to the Monachinae. Our data allocate *Plentuisa* to the Geomitridae, being neither related to *Trochulus* nor to *Monacha*.

Plentuisini new tribe. Shell minute, depressed, umbilicate, hairy. Right ommatophore retractor independent of genital system. Penis short, with rudimentary flagellum. Vagina short and wide, with inner lumen occupied by four double longitudinal folds. Stimulatory apparatus lacking dart and accessory sacs, having only two pairs of forked mucous glands.

**4.1.3.1. Tribe Cochlicellini Schileyko, 1972.** One of the most surprising results of our study was the phylogenetic position recovered for the cochlicellids *Cochlicella* and *Prietocella*. These two subgenera, together with *Monilearia* and *Obelus* (not included in this study) were considered a separate family in the classifications of Hausdorf and Bouchet (2005) and Schileyko (2004).

The structure and position of the stimulatory apparatus in cochlicellids (Fig. 4) is so peculiar that interrelations between this taxon and other Helicoidea families have been largely controversial. The apparatus, inserted in the atrium, consists of one long appendage with one or several bifurcate mucous gland(s) opening at the base of a small apical thickening (Schileyko and Menkhorst, 1997). The apical thickening of the stimulatory apparatus can be single (subgen. *Cochlicella*, *Obelus*) or multiple (*Monilearia*, subgen. *Prietocella*) (Schileyko, 2004). Schileyko (1991) and Schileyko and Menkhorst (1997) suggested that cochlicellids should be assigned to a separate family (Cochlicellidae) and believed that they could have originated from the Aegistinae (Bradybaenidae), including Cochlicellidae within the Xanthonychoidea (Schileyko and Menkhorst, 1997; Schileyko, 2004). In contrast, Ibáñez et al.

(2006) mentioned the similarity of the stimulatory apparatus of cochlicellids to the penis appendage of the Orthurethra.

According to partial 16S rRNA gene sequences, Manganelli et al. (2005) recovered *Cochlicella* as the sister group of *Sphincterochila* within the Helicellinae (Hygromiidae s.l.). Steinke et al. (2004) also recovered the sister relationship of these two genera, closely related to the Helicellinae using sequences of two mitochondrial and two nuclear DNA gene fragments. Groenenberg et al. (2011), using the 16S sequences published by Manganelli et al. (2005), also recovered the sister relationship between *Cochlicella* and *Sphincterochila* but grouped them with Monachinae and Bradybaenidae. The close relationship between *Cochlicella* and *Sphincterochila* was nevertheless not statistically supported in any of these works. *Cochlicella* did form a derived group within the Helicoidea when nuclear rRNA sequences were included. This genus was recovered as the sister group of the geomitrid genera *Actinella*, *Caseolus* and *Discula* with full support (BI = 1.00; ML = 100%; NJ = 100%) in both nuclear rRNA and all-gene concatenated analyses. Consequently, our data strongly suggest that *Cochlicella* should be assigned to the Geomitridae. This interpretation is consistent with the findings of Wade et al. (2006, 2007) who recovered *Cochlicella acuta* within the Hygromiidae s.l., but as the sister group of *Cernuella virgata*, and of Steinke et al. (2004) and Manganelli et al. (2005), who recovered a close relationship between *Cochlicella* and the Helicellinae. The phylogenetic relationships observed between cochlicellids and geomitrids suggest that cochlicellids might be given the rank of tribe (Cochlicellini) being recovered as an ingroup of Geomitrinae. Accordingly, the peculiar stimulatory apparatus probably arose from the accessory sac with its attached mucous glands that elongated and shifted down to the atrium.

#### 4.1.4. Family Sphincterochilidae

Forcart (1972) recognized the morphological singularity of *Sphincterochila*, proposing a superfamily (Sphincterochiloidea) for this genus, which Schileyko (1991) considered related to the infra-order Zonitina rather than Helixinia. This interpretation was justified by the oxygnathous mandible and the tripartite sole of the foot, both of which are absent in other Helicoidea taxa. Schileyko (2004) and Hausdorf and Bouchet (2005) assigned family rank to this taxon, although the former author ascribed it to the Xanthonychoidea. Recent molecular studies by Steinke et al. (2004), Manganelli et al. (2005) and Groenenberg et al. (2011) recovered a sister relationship between *Sphincterochila* and *Cochlicella*, grouping them within the Hygromiidae s.l. (Canariellidae, Geomitridae and Hygromiidae, present work) although without statistical support (see discussion on the Cochlicellini). This relationship was highly inconsistent with classifications based on morphology (Nordsieck, 1987; Schileyko, 1991, 2004). In the present study, *Sphincterochila* was recovered in nuclear rRNA and concatenated-gene trees as a separate lineage within a clade grouping Elonidae, Helicidae, Humboldtianidae and Trissexodontidae, not related to *Cochlicella*.

#### 4.1.5. Family Trissexodontidae

Schileyko (1991, 2006b) grouped the Helicodontidae and Trissexodontidae within a single family Helicodontidae comprising seven subfamilies. According to Gómez-Moliner et al. (2013) and the present results, however, they should be treated as two separate families as in the classification of Hausdorf and Bouchet (2005). The monophyly of the Trissexodontidae was not supported in the study by Gómez-Moliner et al. (2013) using the same DNA gene fragments as in the present study, together with the COI gene fragment. This monophyly was strongly supported by our BI and ML analyses in the concatenated-gene tree as well as by BI, ML and NJ analyses in the nuclear rRNA gene tree, suggesting that the mitochondrial COI gene fragment has no phylogenetic signal

at the family level in this group. Consequently, the present data strongly confirm the inclusion of *Gittenbergeria* in the family Trissexodontidae. A further two differences from the results obtained by Gómez-Moliner et al. (2013) should be highlighted. The genus *Oestophorella* was here recovered with high support as the sister group of the other genera included in the Trissexodontini (*Trissexodon*, *Mastigophallus* and *Suboestophora*). However, Gómez-Moliner et al. (2013) did not resolve the position of *Oestophorella* within this tribe, and assigned it as the sister group of *Suboestophora*, but without statistical support. On the other hand, our results confirmed the monophyly of *Hatumia*, which was recovered as a paraphyletic group by Gómez-Moliner et al. (2013).

#### 4.1.6. Family Helicidae Rafinesque, 1815

We included 23 Helicidae taxa of generic rank in our concatenated-gene analysis, but neither *Lampadia* (Lampadiini Schileyko, 2006) nor *Cylindrus* (Cylindruini Schileyko, 2006) were included in our study. Groenenberg et al. (2011) and Cadahía et al. (2014) showed that *Cylindrus* is the sister group of *Arianta*, and thus, Cylindruini should be included in the synonymy of Ariantinae. All helicid taxa clustered within three main groups that were here considered of subfamily rank: Ariantinae Mörch, 1864, Helicinae and Murellinae Hesse, 1918. The basal relationships of these three subfamilies were not resolved in the concatenated-gene tree. BI and ML analysis recovered *Theba* (Thebini sensu Hausdorf and Bouchet, 2005 and Schileyko, 2006a) as the sister group of the other Helicidae genera in the 16S tree, but it appeared as a derived group within the Helicinae when nuclear rRNA was included in the analysis. This is consistent with the results of Wade et al. (2007) who recovered *Theba pisana* within the Helicinae. *Theba* also appeared close to several Helicinae genera (*Cantareus*, *Cornu*, *Eobania*, *Otala*) by Koene and Schulenburg (2005), but these authors also recovered Ariantinae and Murellinae within the Helicinae. The consideration of Thebini as a tribe within the Helicinae implies the consideration of a further three taxa with the same rank: Helicini, and the revalidated Allognathini Westerlund, 1902 and Otalini Pfeffer, 1930. The monophyly of these four tribes was supported by BI and ML analyses in the nuclear rRNA and the all-gene concatenated analyses. Only Otalini in the concatenated-gene tree and Helicini in the nuclear rRNA tree were not supported by NJ analysis. The monophyly of the Ariantinae was confirmed, although only three genera were here included, and *Helicigona* and *Chilostoma* (subgen. *Corneola*) were recovered as sister groups, closely related to *Arianta*. These results are in agreement with the phylogeny obtained by Groenenberg et al. (2011) in their extensive work on the Ariantinae.

The classification of *Murella* is controversial. It was considered a tribe of Helicinae by Nordsieck (1987) and Hausdorf and Bouchet (2005), while Schileyko (2006a) placed it within the Ariantinae. Koene and Schulenburg (2005) and Manganelli et al. (2005) described *Murella* as the sister group of the Ariantinae within the Helicinae. However, we recovered *Murella* as a separate lineage in our nuclear rRNA and concatenated-gene analyses. This indicates that *Murella* should be classified within a separate subfamily, the Murellinae. *Marmorana* and *Tyrrheniberus* (no nuclear rRNA sequences available) also belong to the Murellinae since they clustered with *Murella* in a clade highly supported by all the 16S phylogenetic analyses.

Chromosome number seems to be relevant for the diagnosis of subfamilies and tribes within the Helicidae (reviewed in Aparicio, 1981). The ancestral number for Helicidae seems to be  $n = 30$  and this number also exists in the Ariantinae (ranging from 29 to 31), Murellinae and Thebini (Helicinae). Chromosome number in the remaining Helicinae tribes varies from 22 to 27, suggesting their derived nature, although Rainer (1967) reported  $n = 30$  for the helicine *Caucasotachea leucoranea* (Mousson, 1863).

The presence of two verges inside the penis (Fig. 4) is a synapomorphy of Helicinae (Schileyko, 2004). This subfamily is also characterized by a calcareous dart with four blades along its axis (Fig. 4), forming a cross in transverse section (Koene and Schulenburg, 2005), a feature also present in the Murellinae suggesting a closer phylogenetic relationship between both subfamilies. Allognathini could be characterized by a reproductive apparatus with a long vagina, a dart sac near the atrium, mucous glands with 2–4 branches, and a flagellum of medium size; chromosome number,  $n = 22–25$ . Helicini have a long vagina, a dart sac positioned near the atrium, two mucous glands from bifurcate to multiramous, and a very long flagellum; chromosome number,  $n = 27$ . Otalini have a long vagina, one dart sac near the atrium, two multiramous mucous glands, and a flagellum of medium size; chromosome number,  $n = 25–27$ . Thebini have a short vagina, a dart sac in the upper vagina and broadly joined to the vagina wall, two simple, inflated and alveolar mucous glands, and a rudimentary flagellum (Fig. 4); chromosome number,  $n = 30$ .

Based on their current distribution, the three subfamilies (Ariantinae, Helicinae and Murellinae) probably originated and diversified within Europe. Subsequently, the Helicinae would have colonized North Africa, giving rise to the North African tribes Thebini and Otalini.

#### 4.2. Divergence time

The data obtained by BEAST analysis should be considered a first approach to deciphering the diversification time of the Helicoidea. Fossil records are critical to interpret the history of a group and it is important to accurately establish the age of each lineage. However, many taxa representing the families under study are lacking in the fossil record or there is no accurate information about their age, so it is difficult to compare inferred results with the palaeontological record. The main problem is that the fossil record for land snails in the Cretaceous is very scarce, and it is completely absent for European helicoideans (Nordsieck, 2014). Since the appearance of some uncertain Stylommatophoran families assigned to the Carboniferous (300 Ma) (Solem and Yochelson, 1979; Benton, 1993), there is a gap of over 180 My before land snails started to be represented in the fossils of the Cretaceous, probably due to a very low probability of fossilization (Naggs and Raheem, 2005). For this study, we used six fossil calibrations at the family level. The use of the uncorrelated relaxed molecular clock (see Fig. 3; Table 5) dated the origin of Helicoidea (107 Ma) to the end of the Early Cretaceous. Thus, these data supported the general hypothesis that considered the Helicoidea to be essentially of Laurasian origin (Nordsieck, 1986a; Tillier, 1989).

Our molecular tree showed a main division of Helicoidea into two principal clades diverging around 86 Ma ago that started their radiation nearly simultaneously in the Late Cretaceous.

The group radiating 75.56 Ma ago includes only elements belonging to the Hygromiidae s.l. (Hygromiidae s.str., Canariellidae, Geomitridae). Currently, the Hygromiidae s.l. are mainly distributed throughout the western Palearctic region (Schileyko, 2006b) with some suprageneric taxa extending towards Central Asia (Archaicinae and Paedhoplitinae) and Tropical Africa (Halolimnohelicinae). Our results are consistent with the hypothesis that the Hygromiidae s.l. originated in the western Palearctic. Although the Central Atlantic Ocean started to open at around 195 Ma ago (Smith et al., 1994; Torsvik et al., 2012) breaking up Pangaea into Laurasia and Gondwana, and the South Atlantic opened at around 130 Ma, Europe and North America were still connected to each other 100 Ma ago (Torsvik et al., 2012). According to Sanmartín et al. (2001), the opening of the North Atlantic occurred during the Late Cretaceous (90 Ma). Thus, the vicariant event prompting the origin of the Hygromiidae s.l. could have been the opening of the North



Atlantic Ocean, and they were confined to the landmasses that were to subsequently give rise to current Eurasia.

The second basal clade split around 73 Ma ago into two lineages: one American–Asian–Australian lineage (Polygyridae, Bradybaenidae and Camaenidae) that could have colonized Asia via the Bering land bridge and given rise to the Bradybaenidae–Camaenidae complex (see Wade et al., 2007 for the polyphyly of both families) that subsequently extended to Australia, according to the scenario proposed by Solem (1997) and Hugall and Stanisic (2011); and an American–European lineage joining the rest of the Helicoidea families distributed in the western Palaearctic (excluding the Hygromiidae *s.l.*), together with the American Humboldtianidae, Monadenidae and Pleurodontidae. It is difficult to propose a biogeographic scenario for this American–European group. A plausible explanation, supported by the presence of North American representatives in both lineages, is that this second basal clade could have started its diversification in North America, and spread to the East to give rise to families confined to the western Palaearctic (Elonidae, Helicidae, Helicodontidae, Sphincterochilidae and Trissexodontidae). The presence of the Caribbean family Cepolidae as the basal taxon of the families considered in our study supports a Nearctic origin of this lineage. The history of the Holarctic realm is complicated (Sanmartín et al., 2001) by the repeated connection/disconnection by land bridges between Europe and North America that persisted at least until the Early Eocene (50 Ma) (McKenna, 1983; Tiffney, 1985). The Thulean bridge, that connected southern Europe to Greenland through the British Isles, is considered to have been the most important bridge for exchange of temperate taxa during the earliest part of the Early Eocene (55 Ma), when the climate became markedly warmer (McKenna, 1983). It could also have been the route for the colonization of Europe by the ancestors of the European representatives of the American–European lineage. The split between Nearctic and Palaearctic helicoidean lineages of this second clade is dated around 54–60 Ma ago, but their basal relationships were not reliably resolved. It does not allow to state if the origin of the European families occurred once or several times by dispersive events from North America or by a vicariant process as a result of the breakdown of the Thulean bridge. Nevertheless, other large-scale passive dispersal events (winds, drifting floats, birds, etc.) cannot be discarded as it has been confirmed for other taxa (Rees, 1965; Vagvolgyi, 1975; Gittenberger, 1984; Kirchner et al., 1997).

At the family level, divergence time analysis showed that major diversification processes within the Hygromiidae *s.l.* clade started after the Cretaceous–Paleogene boundary (53.66 Ma for Geomitridae, 46.82 Ma for Canariellidae and 40.37 Ma for Hygromiidae *s.str.*). It is widely known that the Cretaceous–Tertiary boundary had a major impact on terrestrial biotas, including several terrestrial gastropod families thought to have disappeared during this period (Benton, 1993; Vajda et al., 2001; McCleod, 2004). However, this event opened a new opportunity for posterior radiation events during the early Cenozoic as is also well documented for many taxa, including different groups of Stylommatophoran land snails (Wade et al., 2006; Rowson et al., 2010; Uit de Weerd and Gittenberger, 2013). The current presence of some Hygromiidae *s.l.* taxa in Central Asia and Tropical Africa can be explained by posterior dispersal events as described for other invertebrate taxa. As an example, the subfamily Phalangiinae (Phalangiidae, Opiliones, Arachnida), with a Holarctic range (Giribet and Kury, 2007), is represented also in the Ethiopian region by 9 endemic genera out of 26 genera that could be the result of dispersive expansion via a land bridge across the ‘Mediterranean’ sea (Staręga, 1984). With regard to the endemic subfamilies of Hygromiidae (Paedhoplitinae and Archaicinae) from the Tien Shan mountains, Schileyko (1978: Figs. 33, 35) suggested a quite recent origin based on morphological characteristics, and that they could

be Pleistocene derivatives of the Trochulinae subfamily. Diversification processes in Trissexodontidae (44.82 Ma) slightly predated that of the Helicodontidae (39.19 Ma) and Helicidae (37.59 Ma). Divergence times obtained for the Sphincterochilidae (represented only by *Sphincterochila candidissima*) and Elonidae (represented only by the two extant species) cannot be considered in terms of their diversification origin.

The role of the Iberian plate in the evolution of the western Palaearctic Helicoidea has not been considered previously. Nevertheless, the Iberian plate which became isolated from Laurasia at the beginning of the Cretaceous and remained isolated during large periods in the Cenozoic (see Smith et al., 1994) could explain the presence of many ancient taxa endemic to the Iberian Peninsula. Besides, the Thulean bridge directly connected North America with the British Isles and the Iberian Peninsula, suggesting that the latter could have played an important role in the colonization processes of Europe. Hence, some family level taxa of helicoideans, like Trissexodontidae, Elonidae, Plentuisinae, Ponentininae, Monserratininae and Leptaxinae share the Iberian peninsula as their unique distribution area (with Neogene colonization of Macaronesia by *Leptaxis* and related genera in Leptaxinae and of the Rifian region by several species of *Hatumia* and *Oestophora* in Trissexodontidae as the only exceptions).

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2014.11.014>.

## References

- Aparicio, M.T., 1981. Cytotaxonomic studies of the family Helicidae (Gastropoda, Pulmonata). *Genética Ibérica* 33, 211–224.
- Bank, R.A., 2011. Fauna Europea Project. Checklist of the Land and Freshwater Gastropoda of the Iberian Peninsula (Spain, Portugal, Andorra, Gibraltar).
- Bank, R.A., Bouchet, P., Falkner, G., Gittenberger, E., Hausdorf, B., von Proschwitz, T., Ripken, T.E.J., 2001. Supraspecific classification of European non-marine Mollusca (CLECOM Sections I + II). *Heldia* 4, 77–128.
- Benton, M.J., 1993. *The Fossil Record* 2, London.
- Bouchet, P., Rocroi, J.P., 2005. Classification and nomenclator of gastropod families. *Malacologia* 47, 1–397.
- Cadahía, L., Harl, J., Duda, M., Sattmann, H., Kruckenhausner, L., Fehér, Z., Zopp, L., Haring, E., 2014. New data on the phylogeny of Ariantinae (Pulmonata, Helicidae) and the systematic position of *Cylindrus obtusus* based on nuclear and mitochondrial DNA marker sequences. *J. Zool. Syst. Evol. Res.* 52, 163–169.
- Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. JModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* 9, 772.
- Davison, A., Wade, C.M., Mordan, P.B., Chiba, S., 2005. Sex and darts in slugs and snails (Mollusca: Gastropoda: Stylommatophora). *J. Zool.* 267, 329.
- Davison, A., Barton, N.H., Clarke, B., 2009. The effect of coil phenotypes and genotypes on the fecundity and viability of *Partula suturalis* and *Lymnaea stagnalis*: implications for the evolution of sinistral snails. *J. Evolution. Biol.* 22, 1624–1635.
- Dayrat, B., Conrad, M., Balayan, S., White, T.R., Albrecht, C., Golding, R., Gomes, S.R., Harasewych, M.G., Martins, A.M.D.F., 2011. Phylogenetic relationships and evolution of pulmonate gastropods (Mollusca): new insights from increased taxon sampling. *Mol. Phylogenet. Evol.* 59, 425–437.

- Elejalde, M.A., Madeira, M.J., Arrébola, J.R., Muñóz, B., Gómez-Moliner, B.J., 2008. Molecular phylogeny, taxonomy and evolution of the land snail genus *Iberus* (Pulmonata: Helicidae). *J. Zool. Syst. Evol. Res.* 46, 193–202.
- Elejalde, M.A., Madeira, M.J., Prieto, C.E., Backeljau, T., Gómez-Moliner, B.J., 2009. Molecular phylogeny, taxonomy and evolution of the land snail genus *Pyrenaearia* (Gastropoda: Helicoidea). *Am. Malacol. Bull.* 27, 69–81.
- Falkner, G., Bank, R.A., von Proschwitz, T., 2001. CLECOM-PROJECT: check-list of the non-marine molluscan species-group taxa of the states of northern, Atlantic and central Europe (CLECOM I). *Heldia* 4, 1–76.
- Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Florentino, V., Salomone, N., Manganelli, G., Giusti, F., 2010. Historical biogeography of Tyrrhenian land snails: the Marmorana–Tyrrheniberus radiation (Pulmonata, Helicidae). *Mol. Phylogenet. Evol.* 55, 26–37.
- Forcart, L., 1972. Systematische stellung und Unterteilung der Gattung *Sphincterochila* Ancey. *Arch. Moll.* 102 (4–6), 147–164.
- Giribet, G., Kury, A.B., 2007. Phylogeny and biogeography. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 62–87.
- Gittenberger, E., 1984. Vicariantists and dispersalists among the Chondrinidae. In: Solem, A., van Bruggen, A.C. (Eds.), *World-Wide Snails*. Brill and Backhuys, Leiden, pp. 56–69.
- Giusti, F., Manganelli, G., 1987. Notulae malacologicae, XXXVI. On some Hygromiidae (Gastropoda: Helicoidea) living in Sardinia and in Corsica. (Studies on the Sardinian and Corsican malacofauna VI). *Syst. Biol.* 23, 123–205.
- Golubchik, T., Wise, M.J., Easteal, S., Jeremiin, L.S., 2007. Mind the gaps: evidence of bias in estimates of multiple sequence alignments. *Mol. Biol. Evol.* 24, 2433–2442.
- Gómez-Moliner, B.J., Elejalde, A.M., Arrébola, J.R., Puente, A.I., Martínez-Ortí, A., Madeira, M.J., 2013. Molecular phylogeny of the Helicodontidae and Trissexodontidae (Gastropoda). *Zool. Scr.* 42, 170–181.
- Greve, C., Hutterer, R., Groh, K., Haase, M., Misof, B., 2010. Evolutionary diversification of the genus *Theba* (Gastropoda: Helicidae) in space and time: a land snail conquering islands and continents. *Mol. Phylogenet. Evol.* 57, 572–584.
- Groeneweg, D.S.J., Neubert, E., Gittenberger, E., 2011. Reappraisal of the “Molecular phylogeny of Western Palaearctic Helicidae s.l. (Gastropoda: Stylommatophora)”: when poor science meets GenBank. *Mol. Phylogenet. Evol.* 61, 914–923.
- Hausdorf, F.B., 1988. Zur kenntnis der systematischen Beziehungen einiger Taxa der Helicellinae Ihering 1909 (Gastropoda: Hygromiidae). *Arch. Moll.* 119 (1/3), 9–37.
- Hausdorf, B., 2000. The genus *Monacha* in the Western Caucasus (Gastropoda: Hygromiidae). *J. Nat. Hist.* 2000 (34), 1575–1594.
- Hausdorf, B., Bouchet, P., 2005. Pulmonata: 263–270. In: Bouchet, P., Rocroi, J.P. (Eds.), *Classification and Nomenclator of Gastropod Families*. Malacologia 47, 1–397.
- Hesse, P., 1931. Zur Anatomie und Systematik paläarktischer Stylommatophoren. *Zoologica* 31 (81), 1–118.
- Hesse, P., 1934. Zur Anatomie und Systematik paläarktischer Stylommatophoren. *Zoologica* 34 (85), 1–57.
- Hirano, T., Kameda, Y., Kimura, K., Chiba, S., 2014. Substantial incongruence among the morphology, taxonomy, and molecular phylogeny of the land snails *Aegista*, *Landouria*, *Trishoplita*, and *Pseudobuliminus* (Pulmonata: Bradybaenidae) occurring in East Asia. *Mol. Phylogenet. Evol.* 70, 171–181.
- Ho, S.Y.W., Phillips, M.J., 2009. Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. *Syst. Biol.* 58, 367–380.
- Holyoak, D.T., Holyoak, G.A., 2012. A review of the genus *Ponentina* Hesse 1921 with description of seven new species from Portugal and Spain (Gastropoda, Pulmonata: Hygromiidae). *J. Conchol.* 41 (2), 173–238.
- Holzner, W.E., Colgan, D.J., Lydeard, C., 2010. Pulmonate phylogeny based on 28S rRNA gene sequences: a framework for discussing habitat transitions and character transformation. *Mol. Phylogenet. Evol.* 57, 1017–1025.
- Hugall, A.F., Stanistic, J., 2011. Beyond the prolegomenon: a molecular phylogeny of the Australian camaenid land snail radiation. *Zool. J. Linn. Soc.-Lond.* 161, 531–572.
- Ibáñez, M., Alonso, M.R., Ponte-Lira, C.E., 1995. El género *Canariella* Hesse, 1918, y su posición en la familia Hygromiidae (Gastropoda, Pulmonata, Helicoidea). *Boll. Malacol.* 36 (1–2), 111–137.
- Ibáñez, M., Groh, K., Alonso, M.R., Castillo, C., Yanes, Y., 2006. The subgenus *Monilearia* (Lyrula) Wollaston, 1878 (Gastropoda: Helicoidea: Cochlicellidae) from Lanzarote and Fuerteventura (Canary Islands), with the description of *Monilearia* (*Lyrula*) *tubaeformis* sp. nov. *Zootaxa* 1320, 29–41.
- Katoh, K., Toh, H., 2008. Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. *BMC Bioinformatics* 9, 212.
- Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucl. Acids Res.* 30, 3059–3066.
- Kirchner, C., Krätzner, R., Welter-Schultes, F.W., 1997. Flying snails—how far can *Truncatellina* (Pulmonata: Vertiginidae) be blown over the sea? *J. Mollus. Stud.* 63, 479–487.
- Koene, J.M., Schulenburg, H., 2005. Shooting darts: co-evolution and counter-adaptation in hermaphroditic snails. *BMC Evol. Biol.* 5, 25.
- Kotsakiozi, P., Parmakelis, A., Giokas, S., Papanikolaou, I., Valakos, E.D., 2012. Mitochondrial phylogeny and biogeographic history of the Greek endemic land-snail genus *Codringtonia* Kobelt 1898 (Gastropoda, Pulmonata, Helicidae). *Mol. Phylogenet. Evol.* 62, 681–692.
- Madeira, M.J., Elejalde, A., Chueca, L.J., Gómez-Moliner, B.J., 2010. Phylogenetic position of the genus *Cryptazeca* and the family Azecidae within the system of the Stylommatophora. *Malacologia* 52, 163–168.
- Mandahl, B., 1950. Systematische Untersuchungen über die Helicidenfauna von Madeira. *Abh. Senckenberg Naturf. Ges.* 469, 1–93.
- Manganelli, G., Salomone, N., Giusti, F., 2005. A molecular approach to the phylogenetic relationships of the western palaearctic Helicoidea (Gastropoda: Stylommatophora). *Biol. J. Linn. Soc.* 85, 501–512.
- McCleod, N., 2004. End-cretaceous extinctions. In: Selley, R.C., Cooks, L.R.M., Plimer, I.R. (Eds.), *Encyclopedia of Geology*. Academic Press, London, pp. 372–386.
- McKenna, M.C., 1983. Cenozoic paleogeography of North Atlantic land bridges. In: Bott, M.H.P., Saxov, S., Talwani, M., Thiede, J. (Eds.), *Structure and Development of the Greenland-Scotland Bridge: New Concepts and Methods*. Plenum, New York, pp. 51–395.
- Mejia, O., Zúñiga, G., 2007. Phylogeny of the three brown banded land snail genus *Humboldtiana* (Pulmonata: Humboldtianidae). *Mol. Phylogenet. Evol.* 45, 587–595.
- Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*. New Orleans, pp. 1–8.
- Moquin-Tandon, A., 1855. Histoire naturelle des mollusques terrestres et fluviatiles de France. In: Baillièvre, J.B. (Ed.), vol. I, Paris.
- Naggs, F., Raheem, D., 2005. Sri Lankan snail diversity: faunal origins and future prospects. *Rec. Western Aust. Mus.* 68, 11–29.
- Nordsieck, H., 1985. The system of the Stylommatophora (Gastropoda), with special regard to the systematic position of the Clausiliidae. I. Importance of the excretory and genital systems. *Arch. Moll.* 116, 1–24.
- Nordsieck, H., 1986a. The system of the Stylommatophora (Gastropoda), with special regard to the systematic position of the Clausiliidae. II. Importance of the shell and distribution. *Arch. Moll.* 117 (1/3), 93–116.
- Nordsieck, H., 1986b. Das System der tertiären Helicoidea Mittel- und Westeuropas (Gastropoda: Stylommatophora). *Heldia* 4 (1), 109–120.
- Nordsieck, H., 1987a. Revision des Systems der Helicoidea (Gastropoda: Stylommatophora). *Arch. Moll.* 118 (1/3), 9–50.
- Nordsieck, H., 1987b. Revision des Systems der Helicoidea (Gastropoda: Stylommatophora). *Arch. Moll.* 118, 9–50.
- Nordsieck, H., 1993. Das system der paläarktischen Hygromiidae (Gastropoda: Stylommatophora: Helicoidea). *Arch. Moll.* 122, 1–23.
- Nordsieck, H., 2010. Higher classification of Helicoidea (Gastropoda: Stylommatophora) and the molecular analyses of their phylogeny. <http://hdl.handle.net/10061/117602/535642a24c0d8aa06/index.html#535642a24c0d9260c>.
- Nordsieck, H., 2014. Annotated Check-List of the Genera of Fossil Land Snails (Stylommatophora) of Western and Central Europe (Cretaceous – Pliocene) <<http://www.hnords.de/5356429d6b117f602/535642a24c0d78e01/>>.
- Norell, M.A., 1992. Taxic origin and temporal diversity: the effect of phylogeny. In: Novacek, M.J., Wheeler, Q.D. (Eds.), *Extinction and Phylogeny*. Columbia University Press, New York, pp. 89–118.
- Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G., 1991. The Simple Fool's Guide to PCR, Version 2.0. Department of Zoology, University of Hawaii, Honolulu, HI.
- Pérez-Losada, M., Harp, M., Høeg, J.T., Achituv, Y., Jones, D., Watanabe, H., Crandall, K.A., 2008. The tempo and mode of barnacle evolution. *Mol. Phylogenet. Evol.* 46, 328–346.
- Pilsbry, H.A., 1893–95. *Manual of Conchology*, 9(2). In: *Guide to the Study of Helices*. Academy of Natural Sciences of Philadelphia.
- Porter, M.L., Pérez-Losada, M., Crandall, K.A., 2005. Model-based multi-locus estimation of decapod phylogeny and divergence times. *Mol. Phylogenet. Evol.* 37, 355–369.
- Puente, A.I., 1994. Estudio taxonómico y biogeográfico de la superfamilia Helicoidea Rafinesque, 1815 (Gastropoda: Pulmonata: Stylommatophora) de la Península Ibérica e Islas Baleares. Thesis Dissertation. University of the Basque Country, Spain.
- Puente, A.I., Prieto, C.E., 1992. *Plentuisa vendia*, a new genus and species from the Picos de Europa (North of the Iberian Peninsula) (Gastropoda: Helicoidea: Hygromiidae). *J. Conchol.* 34, 159–168.
- Rainer, M., 1967. Chromosomenuntersuchungen an Gastropoden (Stylommatophora). *Malacologia* 5, 341–373.
- Rambaut, A., Drummond, A.J., 2007. *Molecular Evolution, Phylogenetics and Epidemiology*, Tracer v.1.5 <<http://tree.bio.ed.ac.uk/software/tracer/>> (accessed 01.01.13).
- Rees, W.J., 1965. The aerial dispersal of Mollusca. *Proc. Malacol. Soc. Lond.* 36, 269–282.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.
- Rowson, B., Tattersfield, P., Symondson, W.O.C., 2010. Phylogeny and biogeography of tropical carnivorous land-snails (Pulmonata: Streptaxoidea) with particular reference to East Africa and the Indian Ocean. *Zool. Scripta* 40, 85–98.
- Sanmartín, I., Engloff, H., Ronquist, F., 2001. Patterns of animal dispersal, vicariance and diversification in the Holarctic. *Biol. J. Linn. Soc.* 73, 345–390.
- Schileyko, A.A., 1978. Land molluscs of the superfamily Helicoidea. In: *Fauna of USSR Molluscs*, vol. 3(6), 117, pp. 1–384.



- Schileyko, A.A., 1979. The system of the order Geophila (=Helicida) (Gastropoda Pulmonata). In: Boss, K.J., Jacobson, M.K. (Eds.), *Special Occasional Publication*, vol. 6(80), pp. 44–69.
- Schileyko, A.A., 1989. Taxonomic status, phylogenetic relations and system of the Helicoidea sensu lato (Pulmonata). *Arch. Moll.* 120, 187–236.
- Schileyko, A.A., 1991. Taxonomic status, phylogenetic relations and system of the Helicoidea sensu lato (Pulmonata). *Arch. Moll.* 126, 187–236.
- Schileyko, A.A., 2003. Treatise on Recent Terrestrial Pulmonate Molluscs. Part 11. Trigonochlamyidae, Papillodermidae, Vitrinidae, Limacidae, Bielziidae, Agriolimacidae, Boettgeriidae, Camaenidae. *Ruthenica* 2, 1467–1626.
- Schileyko, A.A., 2004. Treatise on recent terrestrial pulmonate molluscs. Part 12. Bradybaenidae, Monadeniidae, Xanthonychidae, Epiphragmophoridae, Helminthoglyptidae, Eloniidae, Humboldtianidae, Sphincterochilidae, Cochlicellidae. *Ruthenica* 2, 1627–1763.
- Schileyko, A.A., 2006a. Treatise on recent terrestrial pulmonate molluscs. Part 13. Helicidae, Pleurodontidae, Polygyridae, Ammonitellidae, Oreohelicidae, Thysanophoridae. *Ruthenica* 2, 1765–1906.
- Schileyko, A.A., 2006b. Treatise on recent terrestrial pulmonate molluscs. Part 14. Helicodontidae, Ciliellidae, Hygromiidae. *Ruthenica* 2, 1907–2047.
- Schileyko, A.A., Menkhorst, H.P.M.G., 1997. Composition and phylogenetic relations of the Cochlicellidae (Gastropoda, Pulmonata). *Ruthenica* 7, 51–60.
- Scott, B., 1997. Biogeography of the Helicoidea (Mollusca: Gastropoda: Pulmonata): land snails with a Pangean distribution. *J. Biogeogr.* 24, 399–407.
- Smith, A.G., Smith, D.G., Funnell, B.M., 1994. *Atlas of Mesozoic and Cenozoic Coastlines*. Cambridge University Press, Cambridge.
- Solem, A., 1984. A world model of land snail diversity and abundance. In: Solem, A., van Bruggen, A.C. (Eds.), *World-Wide Snails: Biogeographical Studies on Non-Marine Mollusca*, Leiden, pp. 6–22.
- Solem, A., 1997. Camaenid land snails from Western and central Australia (Mollusca: Pulmonata: Camaenidae). VII. Records of the Western Australian Museum 50(1), 461–1906.
- Solem, A., Yochelson, E.L., 1979. North American Paleozoic Land Snails, With a Summary of Other Paleozoic Nonmarine Snails. Geological Survey Professional Paper.
- Stamatakis, A., 2006. RAXML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22 (21), 2688–2690.
- Starega, W., 1984. Revision der Phalangiidae (Opiliones), III. Die afrikanischen Gattungen der Phalangiinae, nebst Katalog aller afrikanischen Arten der Familie. *Ann. Zool. (Polska Akademia Nauk)* 38 (1), 1–79.
- Steel, M.A., Lockhart, P.J., Penny, D., 1993. Confidence in evolutionary trees from biological sequence data. *Nature* 364, 440–442.
- Steinke, D., Albrecht, C., Pfenninger, M., 2004. Molecular phylogeny and character evolution in the Western Palaearctic Helicidae s.l. (Gastropoda: Stylommatophora). *Mol. Phylogenet. Evol.* 32, 724–734.
- Swofford, D.L., 2002. PAUP. Phylogenetic Analysis Using Parsimony (and Other Methods). Version 4.0b10 win 32. Sinauer Associates, Sunderland.
- Tiffney, B.H., 1985. The Eocene North Atlantic land bridge its importance in Tertiary and modern phytogeography of the Northern Hemisphere. *J. Arnold Arboretum* 66, 243–273.
- Tillier, S., 1989. Comparative morphology, phylogeny and classification of land snails and slugs (Gastropoda: Pulmonata: Stylommatophora). *Malacologia* 30, 1–303.
- Tillier, S., Masselot, M., Tillier, A., 1996. Phylogenetic relationships of the pulmonate gastropods from rRNA sequences, and tempo and age of the stylommatophoran radiation. In: Taylor, J.D. (Ed.), *Origin and evolutionary Radiation of the Mollusca*. Oxford University Press, Oxford, pp. 267–284.
- Torsvik, T.H., Van der Voo, R., Preeden, U., Mac Niocaill, C., Steinberger, B., Doubrovine, P.V., van Hinsbergen, D.J.J., Domeier, M., Gaina, C., Tohver, E., Meert, J.G., McCausland, P.J.A., Cocks, L.R.M., 2012. Phanerozoic polar wander, palaeogeography and dynamics. *Earth-Sci. Rev.* 114, 325–368.
- Uit de Weerd, D.R., Gittenberger, E., 2013. Phylogeny of the land snail family Clausiliidae (Gastropoda: Pulmonata). *Mol. Phylogenet. Evol.* 67, 201–216.
- Vagvolgyi, J., 1975. Body size, aerial dispersal, and origin of the pacific land snail fauna. *Syst. Biol.* 24, 465–488.
- Vajda, V., Raine, J.L., Hollis, C.J., 2001. Indication of global deforestation at the Cretaceous-Tertiary boundary by New Zealand fern spike. *Science (New York, N.Y.)* 294, 1700–1702.
- Vaught, K.C., 1989. A Classification of the Living Mollusca. American Malacologists Inc., Melbourne, USA, 195 pp.
- Wade, C.M., Mordan, P.B., Clarke, B., 2001. A phylogeny of the land snails (Gastropoda: Pulmonata). *Proc. Roy. Soc. Lond. B Bio.* 268, 413–422.
- Wade, Christopher M., Mordan, Peter B., Naggs, F., 2006. Evolutionary relationships among the Pulmonate land snails and slugs (Pulmonata, Stylommatophora). *Biol. J. Linn. Soc.* 87, 593–610.
- Wade, Christopher M., Hudelot, C., Davison, A., Naggs, F., Mordan, Peter B., 2007. Molecular phylogeny of the helicoid land snails (Pulmonata: Stylommatophora: Helicoidea), with special emphasis on the Camaenidae. *J. Mollus. Stud.*, 411–415.
- Xia, X., 2001. DAMBE: Data Analysis in Molecular Biology and Evolution 5.2.5. Kluwer Academic Publishers.
- Xia, X., Lemey, P., 2009. Assessing substitution saturation with DAMBE. In: Lemey, Philippe, Salemi, Marco, Vandamme, Anne-Mieke (Eds.), *The Phylogenetic Handbook: A Practical Approach to DNA and Protein Phylogeny*, second ed. Cambridge University Press, pp. 615–630.
- Xia, X., Xie, Z., 2001. DAMBE: software package for data analysis in molecular biology and evolution. *J. Hered.* 92, 371–373.
- Xia, Xuhua, Xie, Zheng, Salemi, M., Chen, L., Wang, Y., 2003. An index of substitution saturation and its application. *Mol. Phylogenet. Evol.* 26, 1–7.
- Yang, Z., 2004. A heuristic rate smoothing procedure for maximum likelihood estimation of species divergence times. *Acta Zool. Sinica* 50, 645–656.
- Zhang, Z.-Q., 2013. Animal biodiversity: an update of classification and diversity in 2013. *Zootaxa* 3703, 5–11.
- Zilch, A., 1960. Gastropoda Teil 2. Euthyneura. *Handbuch Paläozool.* 6, 520–543.