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ISIMEROPE, A NEW GENUS OF HYDROBIIDAE (CAENOGASTROPODA: RISSOOIDEA) FROM GREECE

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ABSTRACT

Isimerope semele n. gen. and n. sp., a valvatiform hydrobiid from southern continental Greece, is described based on morphological and molecular data. *Isimerope* is distinguished from other European and circum-Mediterranean valvatiform hydrobiid genera by a unique combination of morphological characters, including distinctive male and female genitalia. *Isimerope* is differentiated from morphologically similar *Graecoarganiella*, which is also endemic to Greece, by a 10.15% mean COI sequence divergence. *Isimerope semele* is composed of three small populations living in disturbed habitats, including springs and a river.

INTRODUCTION

The hydrobiid fauna of Europe is particularly rich in valvatiform species (Bodon, Manganelli & Giusti, 2001), but their anatomical study is challenging because of their minute size (Arconada & Ramos, 2006). During the last few decades many new valvatiform taxa have been described from the Mediterranean region, including France (e.g. Bodon *et al.*, 2001), Italy (e.g. Bodon & Giusti, 1986; Bodon *et al.*, 1995; Manganelli *et al.*, 1998), Spain (e.g. Ramos *et al.*, 2000; Arconada & Ramos, 2001, 2002, 2006, 2007; Arconada *et al.*, 2007), Israel (e.g. Schütt, 1991), Turkey (e.g. Schütt & Şeşen, 1989) and the Balkan Peninsula (e.g. Radoman, 1966, 1983).

Seven valvatiform or valvatiform-planispiral genera (*Daphniola* Radoman, 1973, *Fissuria* Boeters, 1981, *Graecoarganiella* Falniowski & Szarowska, 2011a, *Hauffenia* Pollonera, 1898, *Islamia* Radoman, 1973, *Prespolitorea* Radoman, 1983 and *Pseudoislamia* Radoman, 1979) and thirteen species have been recorded so far from Greece (Reischütz, 1988; Reischütz & Reischütz, 2004; Bank, 2006; Radea, 2011; Falniowski & Szarowska, 2011a). Most of these taxa are crenobionts and a high percentage (42.8% of the genera, 92.3% of the species) is endemic to this country (Gittenberger, 1982; Radoman, 1983; Reischütz, 1988; Falniowski & Szarowska, 2000, 2011a, 2011b; Reischütz & Reischütz, 2004; Radea, 2011).

In 2012, we surveyed 25 localities across Greece for hydrobiids, within the framework of the research project '*Species on the brink of extinction*', which was designed to assess the conservation status of hydrobiids that were classified as threatened in Greece (IUCN, 2012). Here we describe a new genus and a new species of a valvatiform hydrobiid gastropod that was collected from three localities in south continental Greece during the course of this survey.

MATERIAL AND METHODS

Specimens of the new taxon were collected from Megali Vrysi (stream), 'Second Spring' (spring) and Piana (river) in Peloponnisos (Fig. 1). Snails were collected by hand from stones, gravel and dead leaves and transported alive to the laboratory.

Two specimens from each locality were stored in −20°C for molecular analysis and the rest were preserved unrelaxed in 70% ethanol for morphological and anatomical studies. Shell characters (shell height and width, apertural height and width) were measured using the micrometer of a stereomicroscope.

For each population two specimens of each sex were dissected and studied anatomically. Before dissection, the shell of each specimen was removed by soaking in Perenny solution. External morphology was photographed using a Canon EOS 1000D camera attached to a stereomicroscope (Stemi 2000-C, Zeiss, Germany). Radulae and opercula were cleaned with KOH solution (5 g/l) at room temperature, rinsed in distilled water and air-dried before being mounted on stubs and spray-coated in gold–palladium. The protoconch, operculum and radula were studied using scanning electron microscopy (SEM; Jeol JSM–35 operating at 25 kV). Morphological terminology follows that of Hershler & Ponder (1998).

Only a few specimens were collected from each locality (i.e. 9 specimens from Megali Vrysi, 12 from 'Second Spring' and 10 from Piana) owing to the small size of the populations. The

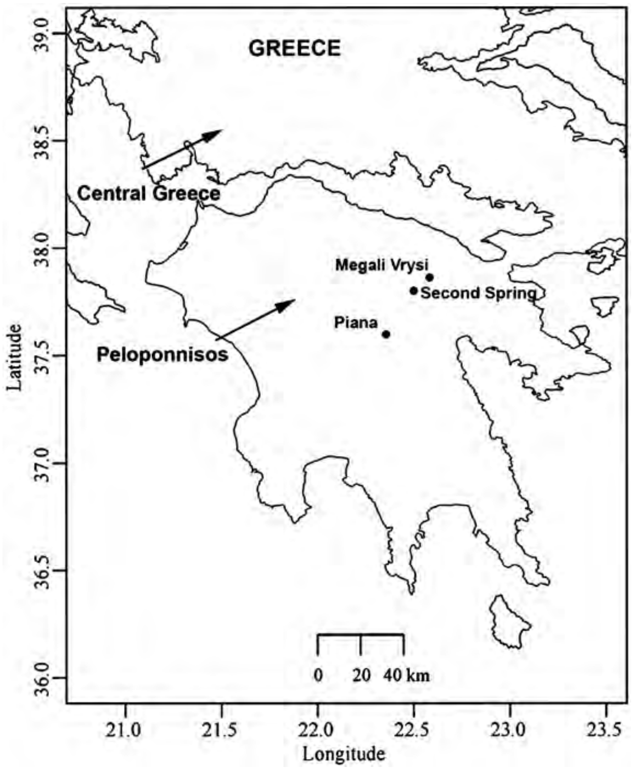

Figure 1. Map showing the collection localities of *Isimerope semele*.

collected material was deposited in the Zoological Museum of the University of Athens (ZMUA) and the personal collection of C. Radea.

DNA extraction, amplification and sequencing

The entire animal was used for genomic DNA isolation. Details of the specimens used in the molecular analyses are provided in Table 1. DNA was extracted using the Purelink Genomic DNA mini kit (Invitrogen) following the manufacturers' protocol. A fragment of the mitochondrial gene cytochrome oxidase subunit I (COI) was amplified from each specimen using the universal primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). PCR was performed in a 25 µl volume, in which 1–2 µl of template DNA was mixed with 0.2 mM dNTPs, 0.4 mM of each primer and 0.5 units of Taq polymerase. The concentration of the $MgCl_2$ was 3.5 mM. Thermocycling was performed in a MyCycler (Biorad) thermocycler. The cycle program comprised an initial denaturation step at 95°C for 3 min, followed by 40 cycles of 15 s at 95°C, 1 min at 42°C, and 1.5 min at 72°C. The cycling was ended with 10 min sequence extension at 72°C. Automated sequencing of both strands of the PCR amplicons was performed in a PE-ABI3740 automated sequencer (using Big-Dye terminator chemistry). The primers in the sequencing reactions were the same as in the PCR amplifications. Sequences generated for this study were deposited in GenBank under the accession numbers (Table 1).

Sequence alignment and genetic data analysis

The newly generated sequences were viewed and edited using CodonCode Aligner v. 2.06 (Genecodes Corporation). The authenticity of the mtDNA sequences and the homology with the targeted mitochondrial gene were evaluated by a BLAST search in the NCBI genetic database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The genetic distances separating individual or grouped sequences were calculated using MEGA5 (Tamura *et al.*, 2011) implementing the Kimura 2-parameter (K2P) model (Kimura, 1980) of nucleotide substitution. We chose this model because it is one of the most frequently used in pairwise comparisons of taxa. Variable and parsimony-informative sites were estimated with MEGA5. We also included in our analyses COI sequences from other valvatiform genera. Sequences were aligned with CodonCode Aligner v. 2.06. Bayesian phylogenetic analysis was performed using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003). *Bythinella austriaca* was used as the outgroup. Modeltest v. 3.7 (Posada & Crandall, 1998) and the Akaike Information Criterion (Akaike, 1974) were used to select the appropriate substitution model. During the MrBayes analysis model, parameter values were treated as unknown and were estimated during the run. The number of generations was set to 2×10^6 and two independent runs were performed simultaneously. In each run four chains were involved. The average standard deviation of split frequencies of the two simultaneous and independent runs was used to determine the stationarity point of likelihoods (see MrBayes v. 3.1.2 manual). According to this index, stationarity in the analyses was achieved well before 0.25×10^6 generations. A tree was sampled every 100th generation and, consequently, the summaries of the Bayesian analysis relied on 4×10^5 samples (sum of two runs). From each run 15001 samples were used, while 4999 were discarded as burn-in. From the remaining 30002 trees (sum of two runs), a 50% majority-rule consensus tree was constructed. Support for the nodes was assessed by posterior probabilities.

We also compared the COI sequences of the new taxon with those of hydrobiid species deposited in GenBank that exhibited high levels of similarity based on a blast homology search.

SYSTEMATIC DESCRIPTION

Family HYDROBIIDAE Troschel, 1857

Isimerope Radea & Parmakelis, new genus

Type species: *Isimerope semele* new species, by original designation.

Etymology: The generic name was derived from Greek mythology: the first element Isi (Ίση in Greek), is an adjective meaning 'equivalent', and the second, Merope (Μερόπη in Greek), is the name of one of the seven Pleiades, daughters of Atlas and Pleione. Gender feminine.

Diagnosis: Shell minute (maximum height 1.55 mm, maximum width 2.00 mm), dextral, valvatiform; operculum without peg; central tooth trapezoidal with one basal cusp on each side; ctenidium absent; penis with narrow lobe along inner edge (at 2/3 of its length), distal section swollen, pigmented black; bursa copulatrix pyriform, positioned posterior to albumen gland; renal oviduct well-developed and coiled in an M shape; seminal receptacle absent.

Referred species: *Isimerope semele* n. sp., described below.

Isimerope semele Radea & Parmakelis, new species

(Figs 2–7)

Types: Holotype: ZMUA 4092; Megali Vrisi, Pharmakas Mt., Argolida, Peloponnisos, Greece, 37.77011°N, 22.52231°E, 1150 m a.s.l., leg. C. Radea and Th. Constantinidis, 3 April 2012. Paratype (from same lot): ZMUA 4093.

Table 1. Species, families (according to Wilke *et al.*, 2013), locality details, GenBank accession numbers and publication references for COI sequences used in the phylogenetic analysis of this study.

Species	Family	Country	Sampling locality	GenBank accession number	Reference
Bythinella austriaca (Frauenfeld, 1857)	Bythinellidae	Poland	Młynnik spring, Ojców National Park	FJ545132	Falniowski, Szarowska & Sirbu (2009)
Daphniola exigua (A. Schmidt, 1856)	Hydrobiidae	Greece	Two springs close to the railway station Ag. Paraskevi	EU047765	Falniowski, Szarowska & Grzmil (2007)
Daphniola graeca Radoman, 1973	Hydrobiidae	Greece	Daphne spring about 30 km north of Larissa	EU047763	Falniowski *et al.* (2007)
Daphniola louisi Falniowski & Szarowska, 2000	Hydrobiidae	Greece	Spring at the monastery of Kessariani, Athens, Attica	EF070618	Szarowska (2006)
Graecoarganiella parnassiana Falniowski & Szarowska, 2011	Hydrobiidae	Greece	Parnassos Mt., S of Eptalophos, N of Kalania, cistern and spring	JN202348-52	Falniowski & Szarowska (2011a)
'*Graecoarganiella* sp.'	Hydrobiidae	Greece	Mainalo Mt., WSW of Piana, WNW of Tripolis, Peloponnisos, cistern and spring	JN202353-54	Falniowski & Szarowska (2011a)
Hauffenia sp.	Hydrobiidae	Slovakia	Pätřočnica spring, Gemerska Hôrka	EF070614	Szarowska (2006)
Hauffenia sp. 1	Hydrobiidae	Slovakia	Kunova Teplica	JF313940	Šteffek *et al.* (2011)
Hauffenia tellinii Pollonera, 1898)	Hydrobiidae	Italy	Friuli, Venetia Julia, Gorizia, Isonzo River, near Sagrado, spring	AF367640	Wilke *et al.* (2001)
Horatia strumi	Hydrobiidae	Spain	Cadiz province, Zahara, Puente de Los Palominos Creek (1487)	AF213345	Wilke, Rolán & Davis (2000)
Hydrobia acuta acuta (Draparnaud, 1805)	Hydrobiidae	France	Hérault, Etang du Prévost	AF278808	Wilke *et al.* (2000)
Hydrobia ventrosa (Montagu, 1803)	Hydrobiidae	United Kingdom	North Sea, Snettisham lagoon	AF118335	Wilke & Davis (2000)
Isimerope semele n. sp.	Hydrobiidae	Greece	Megali Vrisi, Pharmakas Mt., Argolida	KC841149	Present study
Isimerope semele n. sp.	Hydrobiidae	Greece	Second Spring, Pharmakas Mt., Argolida	KC841150	Present study
Isimerope semele n. sp.	Hydrobiidae	Greece	Elissonas River, Mainalon Mt., Piana, Arkadia	KC841151	Present study
Pseudamnicola lucensis (Issel, 1866)	Hydrobiidae	Italy	Tuscany, Lucca, Bagni di Luca, Bagni Caldi, thermal spring	AF367651	Wilke *et al.* (2001)

Referred material: ZMUA 4094; a small spring, just a few kilometres away from the type locality, 'Second Spring', Pharmakas Mt., Argolida, Peloponnisos, 37.76389°N, 22.5125°E, 1230 m a.s.l., leg. C. Radea and Th. Constantinidis, 3 April 2012. ZMUA 4095; Piana, Elissonas river, Mainalo Mt., Arkadia, Peloponnisos, 37.57611°N, 22.23917°E, 1010 m a.s.l., leg. C. Radea and A. Marmari, 23 June 2012.

Etymology: The specific name (in apposition) was derived from Greek mythology: Semele, (Σεμέλη in Greek), was the mortal mother, by Zeus, of Dionysus, god of wine, vineyards, pleasure, ritual madness and ecstasy.

Diagnosis: As for genus.

Shell (Figs 2A–E, 3A): Colourless, valvatiform, with up to 3.5 whorls, thin, transparent when fresh; spire depressed, blunt; whorls rounded, with shallow sutures. Measurements: Table 2. Periostracum pale corneous; columella thick (Fig. 2A–E); umbilicus open, deep, not very wide, in some specimens partially covered by reflected columellar margin (Fig. 2B); aperture subcircular to ovate, inner lip adnate, outer lip prosocline (Fig. 2C–D); peristome continuous, thickened at columellar margin, reflected at columellar and slightly at lower margin, outer margin simple. Protoconch sculptured with dense net of irregularly shaped depressions (Fig. 3A–C).

Operculum (Fig. 3D): Ovate, thin, corneous, paucispiral; dark orange, darker at nucleus; inner side weakly convex; nucleus subcentral; muscle attachment area ovate.

Radula (Fig. 4A): Central tooth trapezoidal, dorsal edge strongly concave (Fig. 4B, C), one pair of medium-sized basal cusps (bc2), basal tongue broadly V-shaped and about equal to lateral margin; median cusp blunt, broader and longer than laterals, five lateral cusps on each side of median cusp, the outer three not well defined; at the edges of radula, median cusp short and square, lateral cusps poorly delineated. Lateral tooth face taller than wide (Fig. 4C), basal tongue well developed; outer wing moderately flexed; cutting edge much shorter than outer wing; central cusp longer than lateral cusps, 6 lateral cusps on outer side, 4–5 on inner side usually shorter than outer ones or not well defined. Inner marginal teeth with *ca.* 22–24 long equal-sized cusps (Fig. 4D). Outer marginal teeth with *ca.* 12–15 cusps (Fig. 4E).

Non-genital anatomy: In living specimens, mantle grey-black with white border, the colour being visible under the transparent shell; snout grey-black, longer than wide, parallel-sided with strong distal lobation; eye spots present; tentacles rather wide with a median longitudinal black stripe up to the half of their length; soft body pigmentation variable. Ctenidium absent. U-shaped rectum containing ovate, orange-yellow, longitudinally packed faecal pellets. Nervous system (Fig. 5):

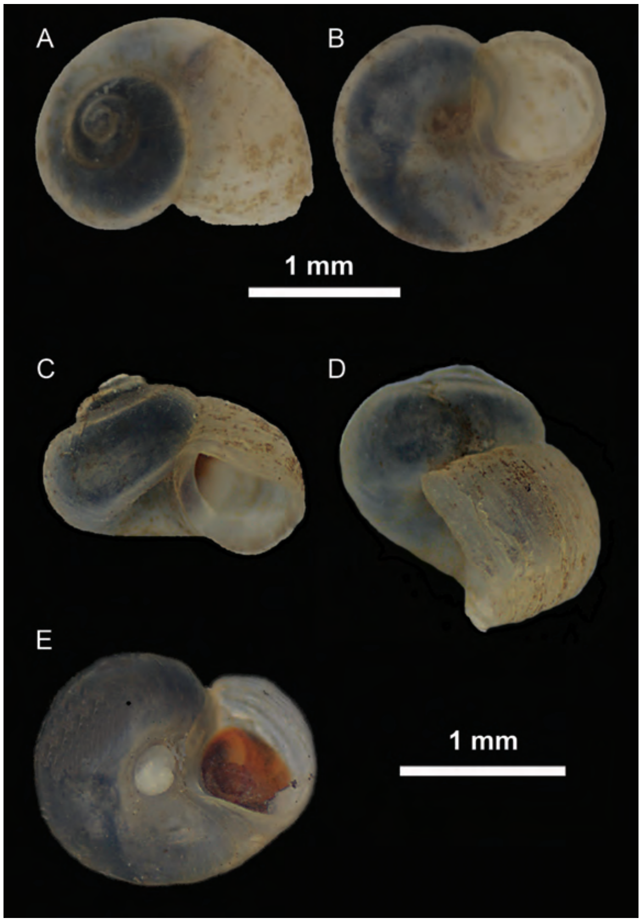

Figure 2. Shell of *Isimerope semele*. **A.** Apical view. **B.** Basal view. **C**, **D.** Lateral view. **E.** Basal view showing egg capsule in the umbilicus.

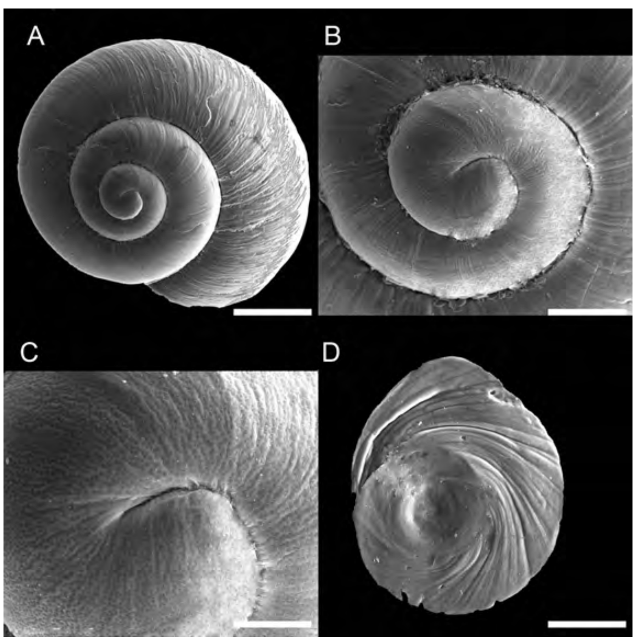

Figure 3. Scanning electron micrographs of shell and operculum of *Isimerope semele*. **A.** Apical view of shell. **B**, **C.** Close-up views of shell apex showing protoconch sculpture. **D.** Operculum, outer side. Scale bars: **A** = 200 μm; **B, D** = 100 μm; **C** = 50 μm.

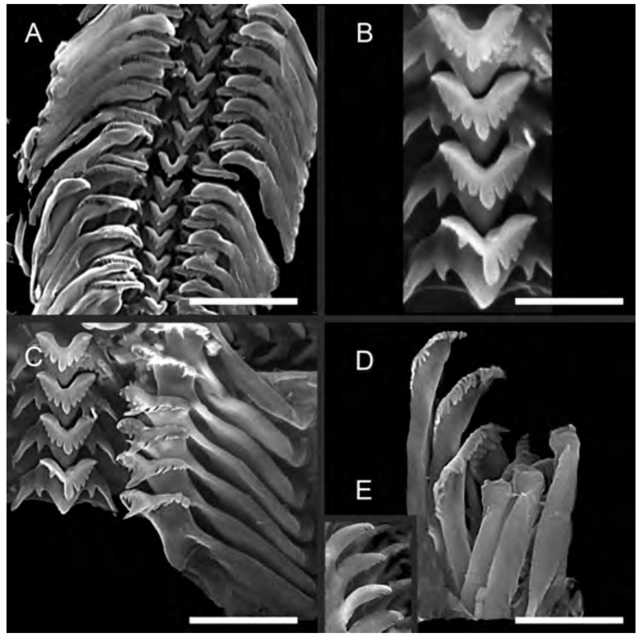

Figure 4. Scanning electron micrographs of radula of *Isimerope semele*. **A**. Portion of radular ribbon. **B.** Central radular teeth. **C.** Central and lateral radular teeth. **D.** Inner marginal teeth. **E.** Outer marginal teeth. Scale bars **A, E** = 20 μm; **B** = 5 μm; **C, D** = 10 μm.

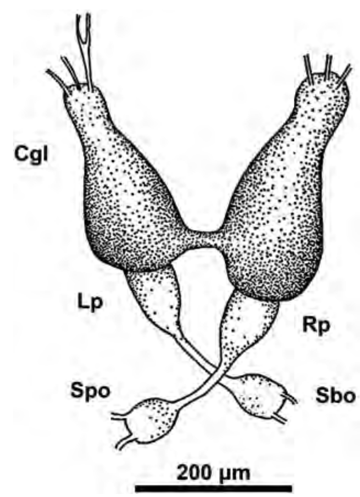

Figure 5. Nervous system of *Isimerope semele*. Abbreviations: Cgl, cerebral ganglia; Lp, left pleural ganglion; Rp, right pleural ganglion; Sbo, suboesophageal ganglion; Spo, supraoesophageal ganglion.

cerebral ganglia similarly sized, light grey; supraoesophageal and suboesophageal ganglia white; supraoesophageal connective longer than suboesophageal connective.

Female reproductive system (Fig. 6A): Pallial oviduct glands with straight border, capsule gland comprising about 2/3 of pallial oviduct; bursa copulatrix medium-sized, pyriform, positioned posterior to albumen gland; renal oviduct unpigmented, well-developed, coiled tightly in an M shape; seminal receptacle absent; coiled oviduct somewhat widened near bursa copulatrix, partly overlapping bursa copulatrix, having a pink pearly sheen.

Egg capsule (Fig. 2E): An egg capsule (containing a single egg) was found inside the umbilicus of several specimens.

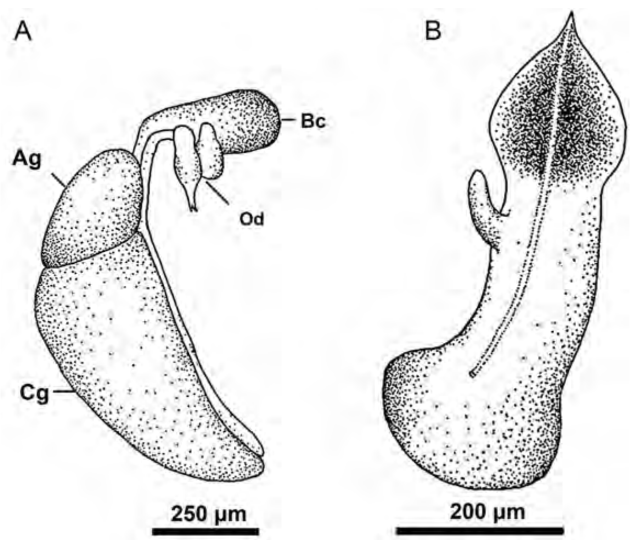

Figure 6. Reproductive anatomy of *Isimerope semele*. **A.** Female genitalia (viewed from left side). **B.** Penis, dorsal surface. Abbreviations: Ag, albumen gland; Bc, bursa copulatrix; Cg, capsule gland; Od, oviduct.

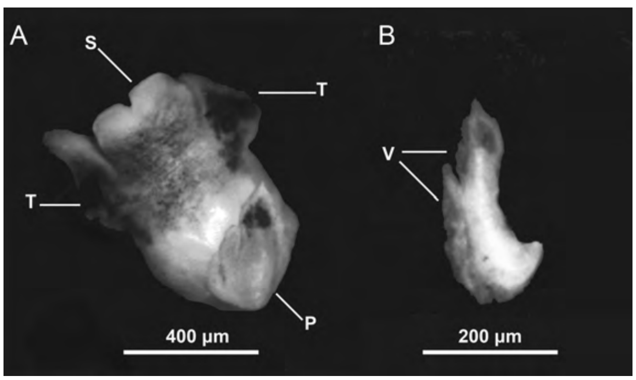

Figure 7. Intraspecific variability of the penis in *Isimerope semele*. **A.** Penis without 'veil'. **B.** Penis with a 'veil' on its left side. Abbreviations: P, penis; T, cephalic tentacles; S, snout; V, 'veil'.

Male reproductive system: Penis (Figs 6B, 7) whitish, long, wide and flat, bent back upon itself and wrinkled near base; distal section black, broadly conic and pointed at tip; inner edge bearing a narrow, unpigmented lobe at 2/3 of penis length; penis usually having a thin, completely transparent extension (resembling a veil) along the length of the inner edge; penial duct almost straight, centrally positioned; penial base expanded, usually black pigmented ventrally, attachment area well behind right eye. Prostate elongate, bean-shaped. Vas deferens narrow, straight, containing sperm of iridescent pink colour.

Distribution and habitat: *Isimerope semele* appears to be restricted to Peloponnisos. At the type locality it was found on stones, gravel and dead leaves of *Juglans regia* in a spring brook together with numerous *Bythinella* sp. In the 'Second Spring', specimens were found on stones in a spring with *Juncus* sp. In Piana, the snails were found on stones along the bank of the Elissonas River.

Remarks: The shells of the specimens from Megali Vrisi and 'Second Spring' were heavily encrusted with epibionts, the diatom *Cocconeis* sp. being the most numerous, whereas the specimens from Piana did not bear any epibionts.

Table 2. Shell morphometry of *Isimerope semele* n. sp. Measurements are in mm.

Locality		sh	sw	ah	aw	sh/sw	ah/aw	sh/ah	sw/aw
Megali Vrisi	Min	0.80	1.55	0.75	0.65	0.80	1.12	0.94	2.00
n = 9	Max	1.10	1.80	0.95	0.85	1.10	1.46	1.29	2.62
	x	0.91	1.66	0.87	0.73	0.91	1.19	1.04	2.26
	SD	0.10	0.08	0.06	0.07	0.10	0.11	0.11	0.19
	CV*	0.11	0.05	0.07	0.09	0.11	0.09	0.11	0.09
Second Spring	Min	0.95	1.30	0.60	0.70	0.58	1.00	1.10	1.86
n = 12	Max	1.15	1.80	1.00	0.90	0.81	1.42	1.75	2.57
	x	1.05	1.65	0.87	0.76	0.64	1.14	1.21	2.18
	SD	0.06	0.16	0.13	0.06	0.36	0.17	0.43	0.22
	CV*	0.05	0.10	0.16	0.09	0.57	0.15	0.36	0.10
Piana	Min	1.10	1.60	0.80	0.70	0.67	1.00	1.29	2.00
n = 10	Max	1.55	2.00	0.95	1.00	0.84	1.14	1.69	2.38
	x	1.26	1.72	0.85	0.81	0.73	0.97	1.48	2.14
	SD	0.13	0.14	0.05	0.08	0.06	0.08	0.13	0.12
	CV*	0.10	0.08	0.06	0.10	0.08	0.08	0.09	0.06

Abbreviations: sh, shell height; sw, shell width; ah, aperture height; aw, aperture width; CV* = (1 + 1/4n)*SD/*x*, (coefficient of variation corrected for sample size, Sokal & Rohlf, 1995); Max, maximum; Min, minimum; *n*, number of specimens; SD, standard deviation; *x*, mean.

Sequence data and phylogenetic analysis

The lengths of the COI fragments obtained from the three specimens were 615, 618 and 643 bp (Table 2). After alignment with sequences retrieved from GenBank the dataset consisted of 664 bp, of which 247 sites were variable and 201 were parsimony informative. Of the newly sequenced specimens, 20 sites were variable and none were parsimony informative. The mean sequence divergence (K2P distance) among the newly sequenced specimens was 2.3%. The Bayesian tree (Fig. 8) is only moderately well resolved, as expected since only a single mtDNA gene was used. Nonetheless, the placement of the new taxon within the clade composed of *Graecorganiella* specimens is well supported.

The specimen from Piana was clustered (with strong support) with two specimens identified as '*Graecoarganiella* sp.' by Falniowski & Szarowska (2011a) from nearby localities, while the two specimens from Argolida (Megali Vrisi and 'Second Spring') grouped together. All five of these individuals formed a well-supported clade. The sequence divergence between the three newly-sequenced individuals and other hydrobiid genera, apart from *Graecoarganiella*, was greater than 15%.

DISCUSSION

Following Bodon *et al*. (2001) and Arconada & Ramos (2001, 2006, 2007), the genera of Hydrobiidae are distinguished by the combination of features from both male and female genitalia. *Isimerope* is differentiated from *Tarraconia* Ramos & Arconada, 2000 (type species *Hauffenia (Neohoratia) gasulli* Boeters, in Gasull, 1981), the only known valvatiform genus having a bursa copulatrix and no seminal receptacles, by the broad, medium-sized, medially positioned penial lobe of the latter. *Tarraconia* is further differentiated from *Isimerope* in having a ctenidium, lacking a connection between the left pleural and suboesophageal ganglia, having a centrally located opercular nucleus, a very wide umbilicus, and a varix behind

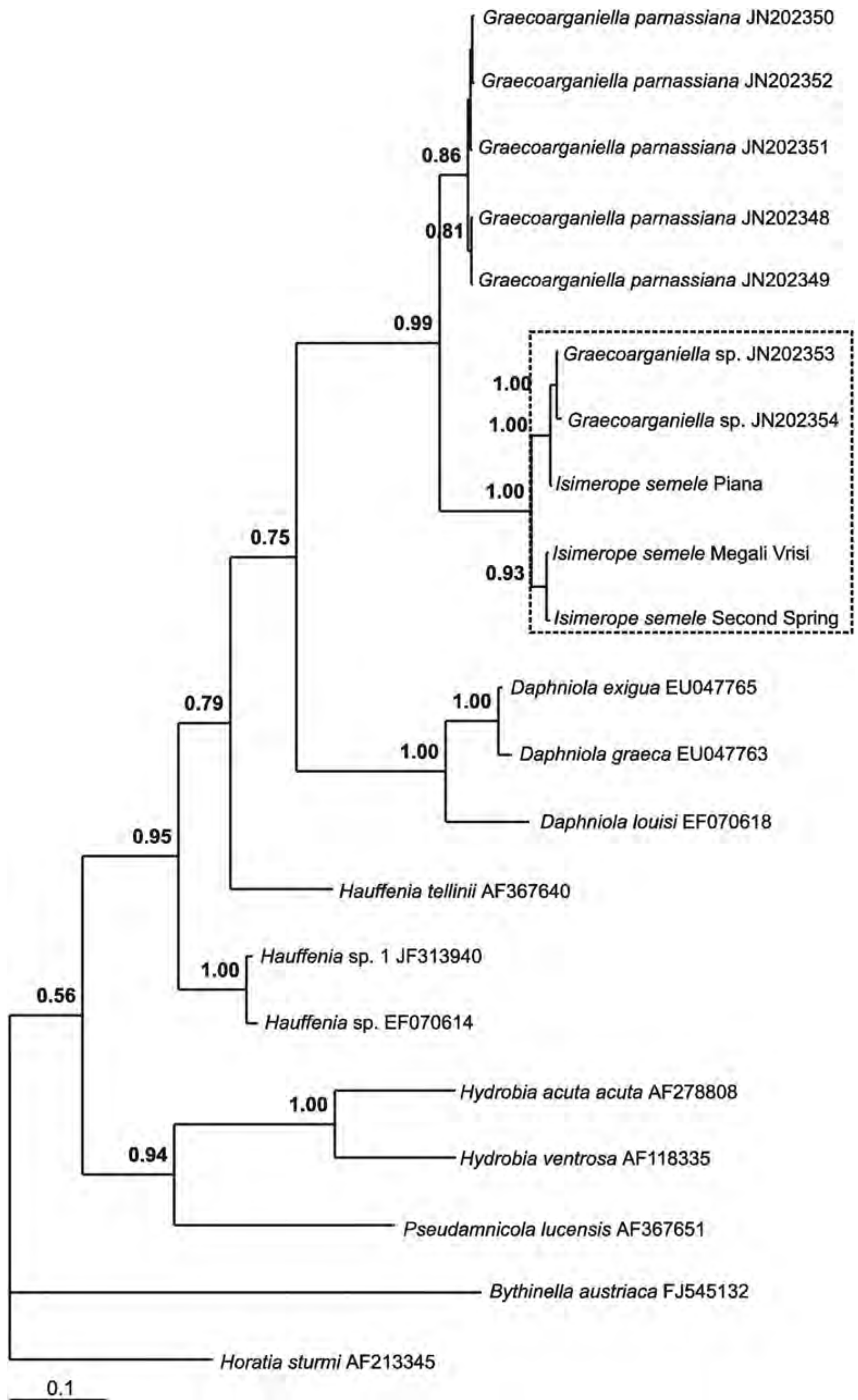

Figure 8. Bayesian phylogeny of selected Hydrobiidae. The dashed outline encompasses the three investigated *Isimerope semele* specimens and the similar '*Graecoarganiella* sp.' sequences of Falniowski & Szarowska (2011a). Numbers on nodes are posterior probabilities (only values above 0.5 are shown). The species/subspecies names are given as registered in GenBank and followed by their GenBank accession numbers (see Table 1). According to Arconada & Ramos (2001), *Horatia sturmi* is a composite of two species, *Boetersiella sturmi* and *Chondrobasis levantina*. Unfortunately we cannot determine to which of these two species the sequence with accession number AF213345 belongs.

the outer margin of the peristome (Ramos *et al*., 2000). Besides the similarity in the female genitalia, *Isimerope* resembles *Tarraconia* in carrying an egg capsule inside the umbilicus and having eye spots, a U-shaped rectum and an operculum without a peg.

Other valvatiform genera having a distally positioned penial lobe, i.e. *Horatia* Bourguignat, 1887, *Karevia* Hadžišče, 1959, *Kerkia* Radoman, 1978, *Ohridohauffenia* Hadžišče, 1959, *Ohrigocea* Hadžišče, 1959, *Prespolitorea* Radoman 1983, *Pseudohoratia* Radoman, 1967, *Strugia* Radoman, 1973 (Radoman, 1973, 1978, 1983; Bodon *et al*., 2001), are distinguished from *Isimerope* by the different overall shape and length of the penis and its lobe and by other characters detailed in Table 3.

The new genus is also anatomically differentiated from another valvatiform hydrobiid genus that was recently described from Greece, *Graecoarganiella* Falniowski & Szarowska, 2011 (type species *G. parnasianna* by original designation) (Falniowski & Szarowska, 2011a). *Graecoarganiella* has two seminal receptacles, an elongated black-pigmented penis with a simple or double lobe near its basal area and 5–6 well-defined lateral cusps on each side of the central tooth of radula (Falniowski & Szarowska, 2011a: 195, Fig. 8C). The type species of *Graecoarganiella* was collected on Parnassos Mountain and, according to the authors, another species of the same genus, '*Graecoarganiella* sp.', was collected from Piana on Mainalo Mountain, in a locality very close to that of our third *Isimerope semele* population. Falniowski & Szarowska (2011a) suggested that the populations from Parnassos and Piana are two congeneric species based on genetic data alone, without anatomical evidence.

Isimerope is well differentiated from non-valvatiform hydrobiids both morphologically and genetically (COI sequence divergence >15%). The new genus, apart from anatomical features, is further differentiated from the morphologically similar *Graecoarganiella parnassiana* by mean COI sequence divergence of 10.15%. This level of genetic divergence falls within the range of values recorded for closely related hydrobiid genera (e.g. *Andriohydrobia*, *Hydrobia*, *Peringia*, *Ventrosia vs Salenthydrobia* 10.38–14.83%, Wilke, 2003; *Floridobia vs Nymphophilus* 10.0–10.9%, *Floridobia vs Pyrgulopsis* 8.1–12.9%, Liu & Hershler, 2005; *Grossuana vs Daphniola* 10.2–12.3%, Falniowski *et al.*, 2007) and other risooidean genera (e.g. *Chorrobius* and *Minckleyella vs* morphologically similar genera 8.6–13.6% and 5.2–13.5% respectively, Hershler, Liu & Landye, 2011).

Based on the phylogenetic tree (Fig. 8), it is evident that *Graecoarganiella parnassiana* forms a clade well separated from the clade of '*Graecorganiella* sp.' and *I. semele*. '*Graecorganiella* sp.' is almost identical (0.7% sequence divergence) in its COI sequence with our specimen from Piana, which has a quite different anatomy from *G. parnassiana*. The similarity in external morphology between *I. semele* and '*Graecoarganiella* sp.' (Falniowski & Szarowska 2011a: 192, fig. 4G–I), the closely proximal collection sites, and the results of our molecular analysis suggest that '*Graecoarganiella* sp.' from Piana should be assigned to *I. semele*.

The phylogenetic clade comprising the three investigated specimens of the present study and the sequences of '*Graecoarganiella* sp.' from Piana exhibits some internal topological structure, with the two subclades corresponding to the

Table 3. *Isimerope* compared morphologically with 31 other valvatiform genera distributed in the Balkan Peninsula and Mediterranean areas.

	Bursa copulatrix	Seminal receptacle(s)	Penis	Penial lobe(s)	Ctenidium	Eyes	Operculum	Umbilicus	Rectum
Isimerope new genus	1	0	1	3	0	1	0	1	(U)
Arganiella	1	1	0	0	1	0	0	2	(U) or (S)
Boetersiella	1	1	0	0	0	1	0	2	(U)
Bracenica	1	3	1	2	–	0	1	3	–
Chondrobasis	1	1	1	1	0	1	0	2	(U)
Dabriana	1	1	0	0	1	0	–	1	–
Daphniola	1	3	1	2	1	1	0	0	–
Fissuria	1	3	3	1+3	1	0	0	0,1,2,3	(S)
Gocea	1	3	1*	4*	–	1	1	2	–
Graecoarganiella	1	3	1	1	0	1	–	2	(S)
Hauffenia	1	2	0,1	0,4	1, 0	0	1	2	(Z) or (?)
Heraultiella	1	1	0	0	1	0	0	2	(U) or (V)
Horatia	1	3	1,2	3	1	1	0	1	(0)
Iberhoratia	1	3	1	2	1	1	0	2	(U) or (S)
Islamia	0	3	1	4	1	1	0	0	(U)
Josefus	0	3	1	4	0	1	0	1	(U)
Karevia	1	3	1	3	–	1	0*	3	–
Kerkia	1	1	1	3	1	0	1	2	(S)
Lyhnidia	1	2	1*	4*	–	1	0	0	–
Milesiana	0	3	1	2	1	1	0	2	(U)
Ohridohauffenia	1	3	1	3	–	1	0	1	–
Ohrigocea	1	3	1	3	–	1	0	2	–
Pezzolia	0,1	3	0	0	0	0	0	2	(S)
Prespolitorea	1	3	1	3	–	1	0*	1	–
Pseudohoratia	1	2	1	3	1	1	1	0,1,2	(0)
Pseudoislamia	1	3	1	4	–	1	0	2	–
Sardohoratia	1	3	0	0	0	0	0	0	(S)
Sheitanok	1	1	0	0	1**	1	0*	3	–
Spathogyna	1	3	1	2	1	1	0*	2	(V)
Strugia	1	2	1	3	–	1	0	2	–
Tarraconia	1	0	1	2	1	1	0	3	(U)
Zaumia	1	2	1*	4*	–	0	0	1	–

Character states: bursa copulatrix: absent (0), present (1); seminal receptacle: absent (0), distal seminal receptacle (1), proximal seminal receptacle (2), distal and proximal seminal receptacle (3); penis: simple (0), with one lobe (1), with two lobes (2), with more than two lobes (3); penial lobe(s): absent (0), basal lobe (1), medial lobe (2), lobe at 2/3 of penis length (3), apical lobe (4); ctenidium: absent (0), present (1); eyes: absent (0), present (1); operculum: simple (0), peg-bearing (1); umbilicus: narrow (0), medium (1), wide (2), very wide (3); rectum: without or almost without bend (0), Z-like (Z), U-like (U), S-like (S), V-like (V), ?-like (?); *: deduced by Bodon *et al.* (2001); **: Schütt & Şessen, 1989: 117, fig. 2B; – : no data. Sources: Arconada & Ramos, 2001, 2006, 2007; Bodon & Giusti, 1986; Bodon *et al.*, 1995, 2001; Manganelli *et al.*, 1998; Radoman, 1966, 1983; Ramos *et al.*, 2000; Schütt, 1991; Schütt & Şessen, 1989.

specimens from Arkadia and Argolida, respectively. The mean sequence divergence separating these two subclades is approximately 3%, lying within the range of values reported for conspecific populations of hydrobiid (e.g. *Pyrgulopsis* 0–3.44%, Hurt, 2004; *Grossuana* 3.4%, Szarowska *et al*., 2007) and other rissooidean genera (e.g. *Austropyrgus* 3–5%, Perez *et al*., 2005).

The new hydrobiid genus described here is indicative of the high diversity and local endemism of aquatic snails in Greece (see Bank, 2006). The new species seems to have a small distribution range and is restricted to local hydrographic systems as are many other Balkan freshwater gastropods (Regnier, Fontaine, & Bouchet, 2009). This species may be vulnerable to extirpation owing to the small size of its populations and local habitat perturbations, including pollution due to livestock.

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REFERENCES

AKAIKE, H. 1974. New look at statistical-model identification. *IEEE Transactions on Automatic Control*, **Ac19**: 716–723.

ARCONADA, B., DELICADO, D. & RAMOS, M.-A. 2007. A new genus and two new species of Hydrobiidae (Mollusca, Caenogastropoda) from the Iberian Peninsula. *Journal of Natural History*, **41**: 2007–2035.

ARCONADA, B. & RAMOS, M.-A. 2001. New data on Hydrobiidae systematics: two new genera from Iberian Peninsula. *Journal of Natural History*, **35**: 949–984.

ARCONADA, B. & RAMOS, M.-A. 2002. *Spathogyna*, a new genus for *Valvata* (?*Tropidina*) *fezi*, Altimira, 1960 from eastern Spain: another case of pseudohermaphroditism in the Hydrobiidae (Gastropoda). *Journal of Molluscan Studies*, **68**: 319–327.

ARCONADA, B. & RAMOS, M.-A. 2006. Revision of the genus *Islamia* Radoman 1973 (Gastropoda, Caenogastropoda, Hydrobiidae), on the Iberian Peninsula and description of two new genera and three new species. *Malacologia*, **48**: 77–132.

ARCONADA, B. & RAMOS, M.-A. 2007. Description of a new species of the genus *Arganiella* Giusti & Pezzoli 1980 (Mollusca, Gastropoda, Hydrobiidae). *Graellsia*, **63**: 61–70.

BANK, R.A. 2006. Towards a catalogue and bibliography of the freshwater Mollusca of Greece. *Heldia*, **6**: 51–86.

BODON, M. & GIUSTI, F. 1986. A new valvatoid shelled hydrobiid from Liguria (Italy) (Gastropoda: Prosobranchia). *Archiv für Molluskenkunde*, **117**: 61–71.

BODON, M., MANGANELLI, G. & GIUSTI, F. 2001. A survey of the European valvatiform hydrobiid genera, with special reference to *Hauffenia* Pollonera, 1898 (Gastropoda: Hydrobiidae). *Malacologia*, **43**: 103–215.

BODON, M., MANGANELLI, G., SPARACIO, I. & GIUSTI, F. 1995. Two new species of the genus *Islamia* Radoman 1973 from Italian islands (Prosobranchia, Hydrobiidae). *Journal of Molluscan Studies*, **61**: 43–54.

FALNIOWSKI, A. & SZAROWSKA, M. 2000. A new species of *Daphniola* Radoman 1973 (Gastropoda: Hydrobiidae) from Greece. *Folia Malacologica*, **8**: 181–188.

FALNIOWSKI, A. & SZAROWSKA, M. 2011a. A new genus and new species of valvatiform hydrobiid (Rissooidea; Caenogastropoda) from Greece. *Molluscan Research*, **31**: 189–199.

FALNIOWSKI, A. & SZAROWSKA, M. 2011b. The genus *Daphniola* Radoman, 1973 (Caenogastropoda: Hydrobiidae) in the Peloponnese, Greece. *Folia Malacologica*, **19**: 131–137.

FALNIOWSKI, A., SZAROWSKA, M. & GRZMIL, P. 2007. *Daphniola* Radoman, 1973 (Gastropoda: Hydrobiidae): shell biometry, mtDNA, and the Pliocene flooding. *Journal of Natural History*, **41**: 2301–2311.

FALNIOWSKI, A., SZAROWSKA, M. & SIRBU, I. 2009. *Bythinella* Moquin-Tandon, 1856 (Gastropoda: Rissooidea: Bythinellidae) in Romania: species richness in a glacial refugium. *Journal of Natural History*, **43**: 2955–2973.

FOLMER, O., BLACK, M., HOEH, W., LUTZ, R. & VRIJENHOEK, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**: 294–299.

GITTENBERGER, E. 1982. Eine neue *Horatia* Art aus Griechenland (Prosobranchia, Hydrobiidae). *Basteria*, **46**: 68.

HERSHLER, R., LIU, H.-P. & LANDYE, J.J. 2011. Two new genera and four new species of freshwater cochliopid gastropods (Rissooidea) from Northeastern Mexico. *Journal of Molluscan Studies*, **77**: 8–23.

HERSHLER, R. & PONDER, W.F. 1998. A review of morphological characters of hydrobioid snails. *Smithsonian Contributions to Zoology*, **600**: 1–55.

HURT, C.R. 2004. Genetic divergence, population structure and historical demography of rare springsnails (*Pyrgulopsis*) in the lower Colorado River basin. *Molecular Ecology*, **13**: 1173–1187.

IUCN. 2012. *IUCN Red List of Threatened Species*. Version 2012.1. <www.iucnredlist.org>. (accessed on July 7, 2012).

KIMURA, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide-sequences. *Journal of Molecular Evolution*, **16**: 111–120.

LIU, H.-P. & HERSHLER, R. 2005. Molecular systematics and radiation of western North American nymphophiline gastropods. *Molecular Phylogenetics and Evolution*, **34**: 284–298.

MANGANELLI, G., BODON, M., CIANFANELLI, S., TALENTI, E. & GIUSTI, F. 1998. New hydrobiids from subterranean waters of Eastern Sardinia, Italy (Gastropoda Prosobranchia: Hydrobiidae). *Basteria*, **62**: 43–67.

PEREZ, K.E., PONDER, W.F., COLGAN, D.J., CLARK, S.A. & LYDEARD, C. 2005. Molecular phylogeny and biogeography of spring-associated hydrobiid snails of the Great Artesian Basin, Australia. *Molecular Phylogenetics and Evolution*, **34**: 545–556.

POSADA, D. & CRANDALL, K.A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**: 817–818.

RADEA, C. 2011. A new species of hydrobiid snails (Mollusca, Gastropoda, Hydrobiidae) from central Greece. *Zookeys*, **138**: 53–64.

RADOMAN, P. 1966. Die Gattungen *Pseudamnicola* und *Horatia*. *Archiv für Molluskenkunde*, **95**: 243–253.

RADOMAN, P. 1973. New classification of fresh and brackish water Prosobranchia from the Balkans and Asia Minor. *Prirodnjacki Mujei u Beogradu, Posebna Izdanja*, **32**: 1–30.

RADOMAN, P. 1978. Neue Vertreter der Gruppe Hydrobioidea von der Balkanhalbinsel. *Archiv für Molluskenkunde*, **109**: 27–44.

RADOMAN, P. 1983. *Hydrobioidea a superfamily of Prosobranchia (Gastropoda). I. Sistematics* (sic). Serbian Academy of Sciences of Arts and Monographs, Department of Sciences, Beograd.

RAMOS, M.A., ARCONADA, B., ROLAN, E. & MORENO, D. 2000. A new genus and a new species of hydrobiid snail (Mollusca: Gastropoda: Hydrobiidae) from eastern Spain. *Malacologia*, **42**: 75–101.

REGNIER, C., FONTAINE, B. & BOUCHET, P. 2009. Not knowing, not recording, not listing: numerous unrecognized mollusc extinctions. *Conservation Biology*, **23**: 1214–1221.

REISCHÜTZ, A. & REISCHÜTZ, P.L. 2004. Helleniká pantoia 7. *Hauffenia edlingeri* nov. spec. (Gastropoda: Prosobranchia: Hydrobiidae) aus Arkadien (Peloponnes, Griechenland). *Nachrichtenblatt der Ersten Vorarlberger Malakologischen Gesellschaft*, **12**: 1–2.

REISCHÜTZ, P.L. 1988. Beiträge zur Molluskenfauna Thrakiens und Ostmakedoniens, II. *Annalen des Naturhistorischen Museums in Wien*, **90B**: 341–356.

RONQUIST, F. & HUELSENBECK, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**: 1572–1574.

SCHÜTT, H. 1991. A contribution to the knowledge of some inland water hydrobiid snails in Israel (Gastropoda, Prosobranchia). *Basteria*, **55**: 129–137.

SCHÜTT, H. & ŞEŞEN, R. 1989. Eine besondere Quellschnecke aus Ostanatolien (Prosobranchia: Hydrobiidae). *Archiv für Molluskenkunde*, **120**: 175–178.

SOKAL, R.R. & ROHLF, F.J. 1995. *Biometry. The principles and practice of statistics in biological research*, Edn 3. Freeman, New York.

ŠTEFFEK, J., FALNIOWSKI, A., SZAROWSKA, M. & GREGO, J. 2011. '*Hauffenia*' Pollonera, 1898 (Caenogastropoda: Hydrobiidae) in Slovakia: a preliminary report. *Folia Malacologica*, **19**: 1–7.

SZAROWSKA, M. 2006. Molecular phylogeny, systematics and morphological character evolution in the Balkan Rissooidea (Caenogastropoda). *Folia Malacologica*, **14**: 99–168.

SZAROWSKA, M., GRZMIL, P., FALNIOWSKI, A. & SIRBU, I. 2007. *Grossuana codreanui* (Grossu, 1946) and the phylogenetic relationships of the East Balkan genus *Grossuana* (Radoman, 1973) (Gastropoda: Rissooidea). *Hydrobiologia*, **579**: 379–391.

TAMURA, K., PETERSON, D., PETERSON, N., STECHER, G., NEI, M. & KUMAR, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**: 2731–2739.c.

WILKE, T. 2003. *Salenthydrobia* gen. nov. (Rissooidea:z Hydrobiidae): a potential relict of the Messinian salinity crisis. *Zoological Journal of the Linnean Society*, **137**: 319–336.

WILKE, T. & DAVIS, G.M. 2000. Infraspecific mitochondrial sequence diversity in *Hydrobia ulvae* and *Hydrobia ventrosa* (Hydrobiidae: Rissoacea: Gastropoda): Do their different life histories affect biogeographic patterns and gene flow? *Biological Journal of the Linnean Society*, **70**: 89–105.

WILKE, T., DAVIS, G.M., FALNIOWSKI, A., GIUSTI, F., BODON, M. & SZAROWSKA, M. 2001. Molecular systematics of Hydrobiidae (Gastropoda: Rissooidea): testing monophyly and phylogenetic relationships. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **151**: 1–21.

WILKE, T., HAASE, M., HERSHLER, R., LIU, H.-P., MISOF, B. & PONDER, W. 2013. Pushing short DNA fragments to the limit: Phylogenetical relationships of hydrobioid gastropods (Caenogastropoda: Rissooidea). *Molecular Phylogenetics and Evolution*, **66**: 715–736.

WILKE, T., ROLÁN, E. & DAVIS, G.M. 2000. The mudsnail genus *Hydrobia* s.s. in the northern Atlantic and western Mediterranean: a phylogenetic hypothesis. *Marine Biology*, **137**: 827–833.