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Unraveling a new lineage of Hydrobiidae genera (Caenogastropoda: Truncatelloidea) from the Ponto-Caspian region

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Abstract. Phylogenetic analyses of the highly diverse (non-marine aquatic) gastropod family Hydrobiidae Stimpson, 1865 have revealed seven main lineages, most of which represent subfamilies. The subfamily Pseudamnicolinae Radoman, 1977, and specifically the genus *Pseudamnicola* Paulucci, 1878 (mainly inhabiting western and central Mediterranean regions), contributes substantially to this hydrobiid richness. Most of its congeners have been described in terms of their shell and penis features, which are of limited diagnostic value. Hence, the taxonomic status of some *Pseudamnicola* species needs to be revised, particularly of those inhabiting marginal regions, such as the Ponto-Caspian domain, largely occupied by the subfamily Pyrgulinae Brusina, 1882. Here we present a molecular phylogeny including species of both subfamilies along with extended morphological descriptions to confirm assignments of the Iranian species *Pseudamnicola zagrosensis* Glöer & Pešić, 2009; *Sarkia kermanshahensis* Glöer & Pešić, 2009 (originally within *Pseudamnicola*) and *P. saboori* Glöer & Pešić, 2009. Our COI-based tree rejects these assignments suggesting a new potential lineage, sister to the pyrgulinid species, and comprising three genera: *Shadinia* Akramowski, 1976, *Intermaria* gen. nov. and *Persipyrgula* gen. nov. These genera differ molecularly by 3.6%–8.5%, and are diagnosable by penis, female genitalia and radula features. Our findings evidence the high morphological variability of pyrgulinid species and provide insight into the origins and evolution of the freshwater Ponto-Caspian fauna.

Keywords. Freshwater snails, *Pseudamnicola*, Cryptic taxa, Anatomy, mtDNA.

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Introduction

The family Hydrobiidae Stimpson, 1865 (*sensu* Wilke *et al.* 2013) is thought to be the most diverse family of non-marine aquatic gastropods, with ca 70 genus-level and 550 species-level taxa. Phylogenetic analyses have recovered seven main lineages within this family, most of which represent subfamilies (see Wilke *et al.* 2013). Among the genera contributing most to this remarkable richness is the genus *Pseudamnicola* Paulucci, 1878 (subfamily Pseudamnicolinae Radoman, 1977). Despite the promotion of its former congeneric subgenus *Corrosella* Boeters, 1970 to the genus level (see Delicado *et al.* 2015), *Pseudamnicola* still comprises around 70 nominal species distributed mainly across the western and central Mediterranean region (Glöer *et al.* 2015). However, this richness may be overestimated due to erroneous assignments to the genus since most of its species have been diagnosed according only to a few shell features or at most to both shell and penis descriptions. *Pseudamnicola* species, like most hydrobiids, are characterized by a minute, unsculpted shell and simple penis, such that only comprehensive studies including the description of several anatomical structures and molecular data have been able to distinguish among congeners (Hershler & Ponder 1998; Wilke *et al.* 2001; Arconada & Ramos 2003; Strong *et al.* 2008). Morphological and molecular data may offer varying resolution in cases of cryptic species (Wilke *et al.* 2002; Liu *et al.* 2003; Delicado & Ramos 2012) or when high morphological disparity exists among close related taxa (Wilke *et al.* 2007; Delicado *et al.* 2014). Thus, unraveling the systematics of the hydrobiids family calls for an integrative approach.

Although a systematic revision of *Pseudamnicola* is still pending, Delicado *et al.* (2015) revealed the monophyly of *Pseudamnicola* s. str. and proposed a series of diagnostic characters including a simple ovate-conic shell shape, single elongated seminal receptacle and broadly triangular penis with several folds across its surface. Hence, we propose the revision of the taxonomic status of some *Pseudamnicola* species originally described according to shell features only. These include species inhabiting Ponto-Caspian regions, marginal areas of the family's main distribution range, such as Iran (Glöer & Pešić 2009, 2012), Turkey (Yıldırım *et al.* 2006; Glöer & Georgiev 2012; Glöer *et al.* 2014, 2015) and Georgia (Badzoshvili 1979). One such species *Sarkhia kermanshahensis* (Glöer & Pešić, 2009) has been recently assigned by Glöer & Pešić (2012) to an independent genus of unclear phylogenetic relationship. Freshwater systems in these areas are mainly occupied by members of the subfamily Pyrgulinae (see Radoman 1983; Wilke *et al.* 2007). These share some morphological and anatomical similarities with *Pseudamnicola* (e.g., complete ctenidium, yellowish, oval operculum, pigmented renal oviduct and nervous system), yet they also differ in terms of other features (e.g., shell shape and sculpture, penis shape, number of seminal receptacles, number of basal cusps in the radular central tooth, etc.) and also appear relatively distant from *Pseudamnicola* in the Hydrobiidae phylogeny (Wilke *et al.* 2013).

Through a molecular/anatomical approach, the present study revises the taxonomic status of the *Pseudamnicola* species from Iran described by Glöer & Pešić (2009), i.e., *P. zagrosensis* Glöer & Pešić, 2009, *Sarkhia kermanshahensis* (Glöer & Pešić, 2009) (originally described as *Pseudamnicola kermanshahensis*) and *P. saboori* Glöer & Pešić, 2009. We also examined whether the springsnail genus *Shadinia* Akramowski, 1976, cited only from a few localities of Iran's neighbour Armenia, anatomically and molecularly resembles the pyrgulinid taxa. So far, three species of this genus have been described based both on shell characteristics (Shadin 1952; Egorov 2006) and some anatomical structures (Akramovski 1976: figs 29, 30; Glöer *et al*. 2016). Their phylogenetic relationships within the family Hydrobiidae, nevertheless, remain unclear. Accordingly, we here examine several anatomical structures of the type species of *Shadinia*, *S. terpoghassiani* (Shadin, 1952), and obtained partial gene sequences in this and the recently described species *S. bjniensis* Glöer *et al*., 2016.

Our results indicate that the *Pseudamnicola* species described by Glöer & Pešić (2009) and the genus *Shadinia* are likely pyrgulinid taxa. This information provides clues about the actual biodiversity and biogeographic patterns of the hydrobiid subfamilies Pseudamnicolinae and Pyrgulinae, with possible implications for on-going and future research efforts targeted at understanding the origins and evolution of freshwater biodiversity, such as the projects PRIDE (Ponto-Caspian biodiversity Rise and Demise) or SCOPSCO (Scientific Collaboration On Past Speciation Conditions in Ohrid).

Material and methods

To assess the taxonomic status of the three *Pseudamnicola* species from Iran described in Glöer & Pešić (2009), we examined molecular and anatomical data of the original material collected by these authors, together with a sample of *Shadinia terpoghassiani* (type species) collected from the type locality (Lake Aiger-Lich, Metsamor, Armenia, 40.14288° N 44.17117° E) in 2008 as well as a molecular sequence of *Shadinia bjniensis*. The *Shadinia terpoghassiani* sample is preserved in 80% ethanol and deposited in the biodiversity collection at Justus Liebig University Giessen (UGSB). The third described species, *Shadinia akramowskii* (Shadin, 1952), could not be relocated yet.

Total DNA obtained here *de novo* was isolated from one individual per species following the CTAB protocol of Wilke *et al.* (2006). A partial 658-bp of the mitochondrial fragment cytochrome *c* oxidase subunit I (COI) was PCR amplified with the primers LCO1490 and HCO2198 (Folmer *et al.* 1994). Thermal cycling conditions were as described in Delicado *et al.* (2012) and were conducted with an annealing temperature of 48º C. Final PCR products were sequenced in an ABI 3730 XL sequencer (Life Technologies, Carlsbad, CA, USA) using a Big Dye Terminator kit v. 3.1 (Life Technologies). The new sequences were deposited in GenBank under the accession numbers indicated in Table 1.

DNA sequences were edited in Sequencher 4.6 (Gene Codes, Ann Arbor, MI) and aligned manually in PAUP* 4.0a123 (Swofford 2002) together with the sequences of other related hydrobiid species obtained from GenBank (Table 1). Phylogenetic reconstruction was conducted using maximum likelihood (ML) and Bayesian-inference (BI). ML analysis was performed in PHYML v3.0 (Guindon & Gascuel 2003) using the evolutionary model selected in jModelTest v. 2.1.4 (Darriba *et al*. 2012) under corrected Akaike's information criterion (Akaike 1974; Sugiura 1978; Hurvich & Tsai 1989). The BI was run through two independent runs of four Metropolis-coupled chains in MrBayes 3.1.2 (Huelsenbeck 2000; Huelsenbeck & Ronquist 2001), with 5 million generations each and a sample frequency of 1000. The analysis was terminated when the standard deviation of split frequencies reached values of < 0.01 in MrBayes 3.1.2. Convergence between runs was in addition monitored by reviewing that each posterior parameter reached values of effective sample size, estimated in Tracer 1.5 (Rambaut & Drummond 2009), greater than 200. The first 10% sampled trees were discarded as burn-in. The robustness of the inferred topologies was assessed by bootstrapping (Felsenstein 1985) with 1000 pseudoreplicates in ML, and by posterior probabilities (BPPs) of Bayesian trees.

The number of the dissected specimens and their respective localities are indicated in Tables 2 and 3. Dissections and measurements were made with a Keyence VHX-2000E digital microscope in combination with the program VHX-2000 Communication software version 2.3.5.0 (Keyence Corporation, 2009–2012). Radulae were extracted from buccal mass by applying the first step of the established Proteinase K protocol for DNA isolation (Wilke *et al.* 2006). After mounting on stubs and drying, radulae were sputter coated with gold (Balter Sputter Coater SCD004) for 50 sec. in order to photograph them with a field emission scanning electron microscope (FESEM) DSM982 Gemini (Carl Zeiss GmbH, Germany). Morphological character states are based on the terminology of Hershler & Ponder (1998). Whorls were counted according to the method of Ramos *et al.* (2000). The concentration of the nervous system was calculated as the RPG ratio (Davis *et al.* 1976) and also characterized using the categories of Davis *et al.* (1984, 1986, 1992) as follows: dorsal nerve ring concentrated (≤ 0.29); moderately concentrated (0.30–0.49); elongated (0.50–0.67); extremely elongated (≥ 0.68). Analysis of variance (ANOVA) was applied to test for statistical significance among morphological dimensions of the Ponto-Caspian species studied here. These calculations have been done using the package MBESS (Kelley & Lai 2011) for the

Table 1. Locality name, GenBank accession numbers and original references for the species employed in the molecular study.

Taxon	Locality	GenBank # COI	Original reference
Outgroup			
Mercuria similis	Italy, Friuli-Venetia-Julia, Udine, Aquileia, Canale Panigai	AF367646	Wilke *et al.* 2001
Hydrobiinae			
Hydrobia acuta	France, Hérault, Etang du Prévost	AF278808	Wilke *et al.* 2000
Peringia ulvae	Russia, Lagoon 'Levin navolok'	AF118302	Wilke & Davis 2000
Salenthydrobia ferrerii	Italy, Lecce, Porto Cesareo, Torre Lapillo, Bambinello Spring	AF449205	Wilke 2003
Ecrobia ventrosa	Great Britain, Snettisham Lagoon	AF118335	Wilke & Davis 2000
Pseudamnicolinae			
Pseudamnicola lucensis	Italy, Tuscany, Bagni di Lucca, Bagni Caldi, thermal spring	AF367651	Wilke *et al.* 2001
Corrosella falkneri	Spain, Granada, Orce, La Armada spring	JF312224	Delicado *et al.* 2012
Diegus gasulli	Spain, Almería, Rambla de Retamar	KF060743	Delicado *et al.* 2014
Pyrgulinae			
Chilopyrgula sturanyi	Macedonia, Lake Ohrid, S of Sveti Zaum	EF379284	Wilke *et al.* 2007
Dianella thiesseana	Greece, Lake Trichonis at Loutres Mirtias	AY676127	Wilke *et al.* 2007
Euxinipyrgula milachevitchi	Russia, Sea of Azov, Miusski Liman	EF379290	Wilke *et al.* 2007
Falsipyrgula pfeiferi	Turkey, Isparta, Lake Eĝirdir	EF379296	Wilke *et al.* 2007
Ginaia munda munda	Macedonia, Lake Ohrid	JN398637	Schreiber *et al.* 2012
Ginaia munda sublitoralis	Macedonia, Lake Ohrid	JN398630	Schreiber *et al.* 2012
Intermaria kermanshahensis	Iran, Kermanshah Province, spring between Sarab and Sahneh city	KT896670	Present study
Intermaria zagrosensis	Iran, Kermanshah Province, Sar Pol Kangarar village, Sar Pol Kangarar stream	KT896669	Present study

Macedopyrgula pavlovici	Macedonia, Lake Ohrid	JN398635	Schreiber *et al.* 2012
Macedopyrgula wagneri	Macedonia, Lake Ohrid	JN398617	Schreiber *et al.* 2012
"*Micromelania*" *lincta*	Romania, Lake Razim, Sarichioi	EF379292	Wilke *et al.* 2007
Ohridopyrgula macedonica	Macedonia, Lake Ohrid, Otesevo	EF379286	Wilke *et al.* 2007
Persipyrgula saboori	Iran, Khorrasan Province, Zou Eram village, Zou Eram spring	KT896671	Present study
Pyrgula annulata	Italy, Brescia, Lake Garda, Desenzano del Garda	AY341258	Wilke *et al.* 2007
Shadinia terpoghassiani	Armenia, south of Metzamor, lake Aiger-Lich outflow	KT896672	Present study
Shadinia bjniensis	Armenia, Kotyak province, Bjni, Hrazdan river	KT896673	Present study
Turricaspia sp.	Ukraine, Kherson, lower Dnieper, near hydrobiological station	EF379294	Wilke *et al.* 2007
Xestopyrgula dybowskii	Macedonia, Lake Ohrid, S of Sveti, Zaum	EF379288	Wilke *et al.* 2007

R statistical environment (R Development Core Team 2011). Resulting parameter values are shown in Tables 2 and 3.

The localities are listed according to the code: spring or lake, city, province, country, co-ordinates, altitude (when measured) and date of collection.

Abbreviations

Shell characters

AH = aperture height
AL = aperture length
AW = aperture width
LBW = length of body whorl
NSW = number of shell whorls
SL = shell length
SW = shell width
WAW = width of the antepenultimate whorl
WBW = width of the body whorl
WPW = width of the penultimate whorl

Anatomical characters

Ag = albumen gland
Bc = bursa copulatrix
Cg = capsule gland
Ct = ctenidium
dBc = duct of the bursa copulatrix

L = length
Os = osphradium
P = penis
Po = pallial oviduct
Pr = prostate gland
Ro = renal oviduct
SR = seminal receptacle
W = width

Collections

UGSB = Biodiversity collection at Justus Liebig University Giessen
ZMH = Zoologisches Museum Hamburg

Results

Phylogenetic inferences were based on 658 bp of the COI gene under the nucleotide substitution model TrN (Tamura & Nei 1993) + I (invariable sites) + G (rate variation among sites). Average base frequencies for the data set were 27.4% A, 14.1% C, 15% G and 43.5% T. Both tree topologies (ML and BI) indicated that the *Pseudamnicola* species from the Ponto-Caspian region formed a monophyletic group independent of the subfamily Pseudamnicolinae (Fig. 1). However, this monophyly was well supported by ML (bootstrap value = 77%) but less supported by BI (BPP = 0.84). This newly discovered lineage appeared as sister to the pyrgulinid clade with high support (85% of bootstrap in ML and 1.00 of PP in BI). Species of *Shadinia* also grouped within this newly discovered lineage as sister to the species *P. zagrosensis* and *Sarkhia kermanshahensis*. The close relationship between the latter taxa suggests they could both belong to the same genus. Additionally, *Shadinia* and *P. saboori* may constitute two different genera. Sequence differences between species of this lineage (uncorrected pairwise distance, p-distance) ranged from 0.1% to 8.5% for COI (Table 4), and mean divergences between this lineage and the Pseudamnicolinae and Pyrgulinae species were 13.6% and 13%, respectively.

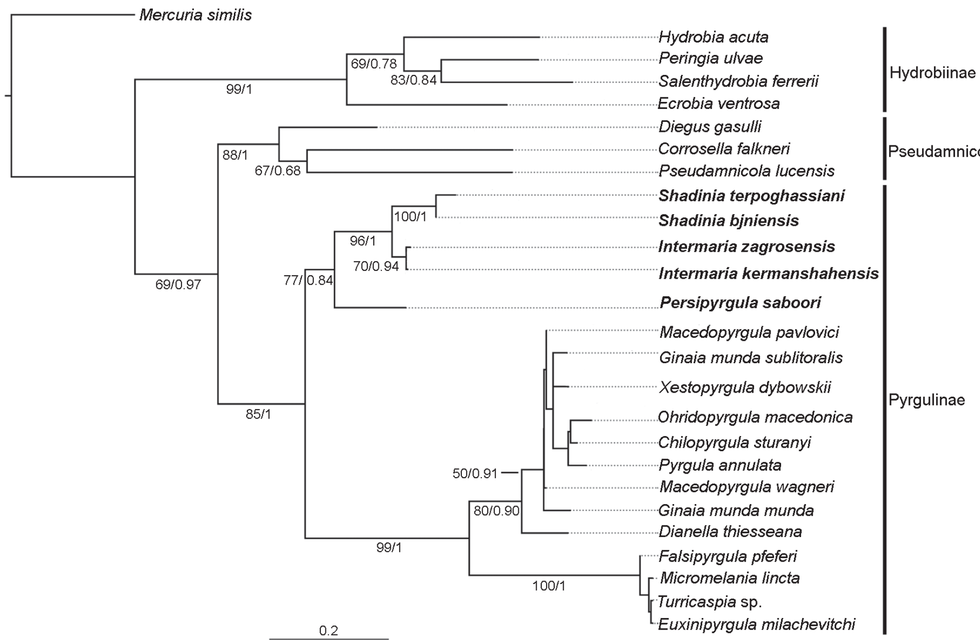

Fig. 1. Bayesian inference of hydrobiid species based on COI sequences. Values below branches indicate bootstrap supports for maximum likelihood and BPPs for Bayesian inference. Black bars on the right denote subfamily assignments. Scale bar: expected change per site.

Morphological data also supported the existence of three different groups, which are hereafter treated as independent genera within the newly recovered hydrobiid lineage. The inclusion of this group within one or other subfamily is discussed in the following section.

Class Gastropoda (Cuvier, 1797)
Superorder Caenogastropoda Cox, 1960
Superfamily Truncatelloidea Gray, 1840
Family Hydrobiidae Stimpson, 1865

Genus ***Intermaria*** gen. nov.
urn:lsid:zoobank.org:act:1CDF6CD1-5D3C-447F-BAAE-7197FBEB2614

Diagnosis

Shell ovate-conic, 3 to 4.5 mm high; large and convex body whorl; rest of whorls small and slightly convex; aperture pyriform, angled on top and often fused to the body whorl. Operculum corneous, yellowish, thin, pliable, ellipsoidal, paucispiral with submarginal nucleus. Two pairs of basal cusps on radular central tooth. Ctenidium occupying nearly the entire length of pallial cavity bearing well-developed gill filaments. Osphradium opposite approximate middle of ctenidium. Bursa copulatrix lying against the middle section of the albumen gland; pigmented renal oviduct; one elongate seminal receptacle. Prostate gland bean-shaped, about twice as long as wide; exit of the pallial vas deferens from the posterior-most section of the prostate gland and seminal vesicle entering the prostate gland in its middle section; penis simple, gradually tapering, with its distal end tapered and often with a small distal lobe on the inner edge. Nervous system with black pigmentation typically elongated.

Etymology

From Latin *inter*- (between) -*maria* (seas), referring to the occurrence of the genus in the continental area between the Mediterranean and the Caspian seas.

Type species

Pseudamnicola zagrosensis Glöer & Pešić, 2009.

Remarks

Intermaria species, like those of *Pseudamnicola*, have an ovate-conic simple shell, one elongated seminal receptacle and a pigmented renal oviduct and nervous system. However, *Intermaria* differs in having smaller shell dimensions (e.g., when compared with the 5 mm height of *P. granjaensis* Glöer & Zettler, 2007 see Delicado *et al.* 2014), a more conic-shaped shell, two basal cusps on the central radular tooth (one in *Pseudamnicola*), a shorter prostate gland, and a gradually tapering penis with a small distal lobe on the inner edge (penis is triangular with many surface folds and has a blunt end in *Pseudamnicola* see Delicado *et al.* 2015).

Intermaria zagrosensis (Glöer & Pešić, 2009) comb. nov.
Figs 2–3, Tables 2–3

Pseudamnicola zagrosensis Glöer & Pešić, 2009: 37, pl. 6, figs 4–6.

New diagnosis

Shell ovate-conic, yellowish, with pyriform aperture; protoconch microsculpture wrinkled; central radular tooth formula (5)4–C–4(5)/2–2; pyriform bursa copulatrix; one seminal receptacle elongate with a short duct; penis gradually tapering, with a small distal lobe on inner edge, end tapered and grayish pigmented; nervous system elongated (mean RPG ratio = 0.60) and slightly black pigmented.

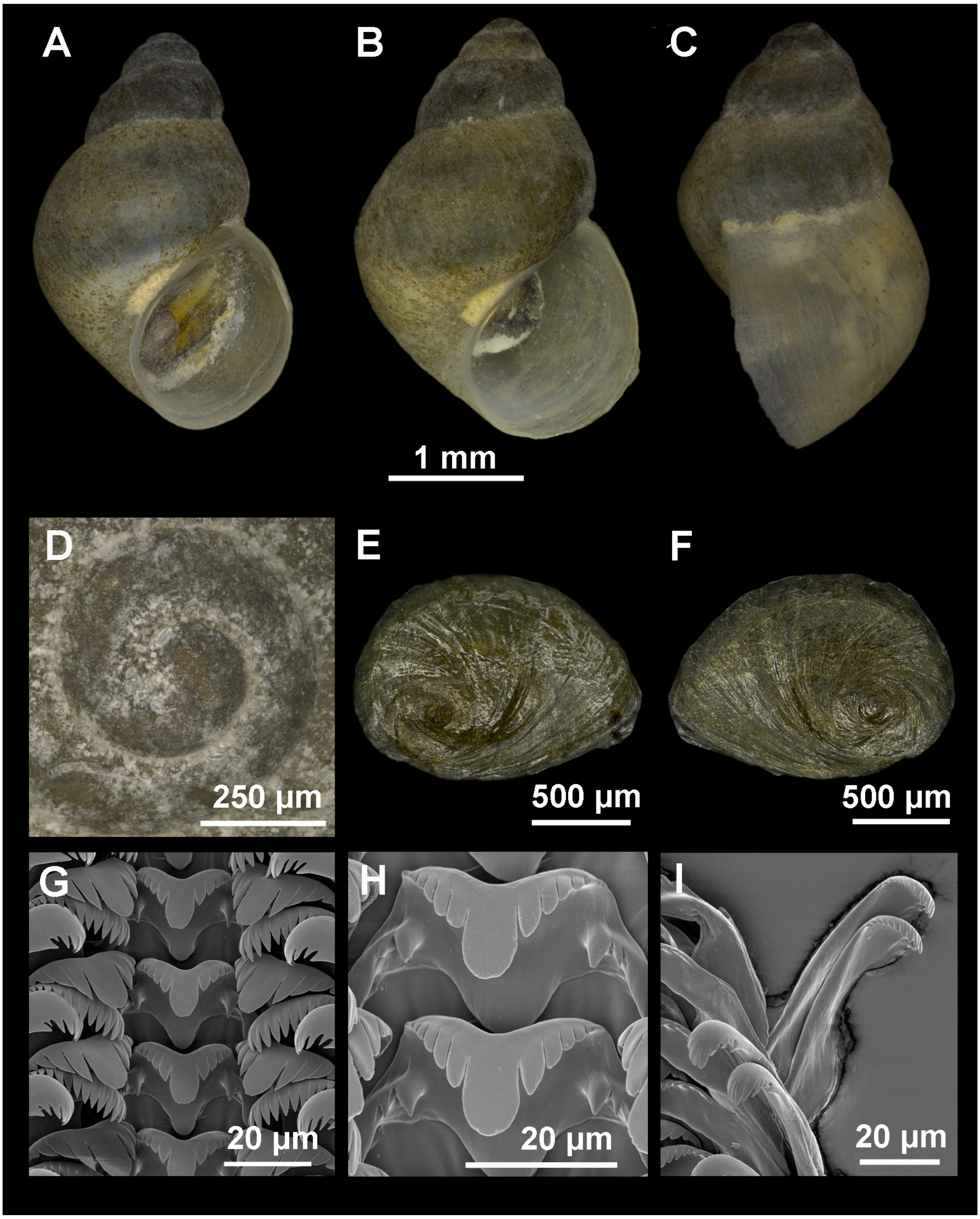

Fig. 2. Shell, operculum and radula of *Intermaria zagrosensis* (Glöer & Pešić, 2009) gen. et comb. nov. from Sar Pol Kangarar stream, Kermanshah Province, Iran. **A–B**. Shell in front view. **C**. Shell in lateral view. **D**. Protoconch and microsculpture. **E–F**. Internal and external side of the operculum. **G**. Rows of teeth of the radula. **H**. Central teeth. **I**. Detail of outer marginal teeth.

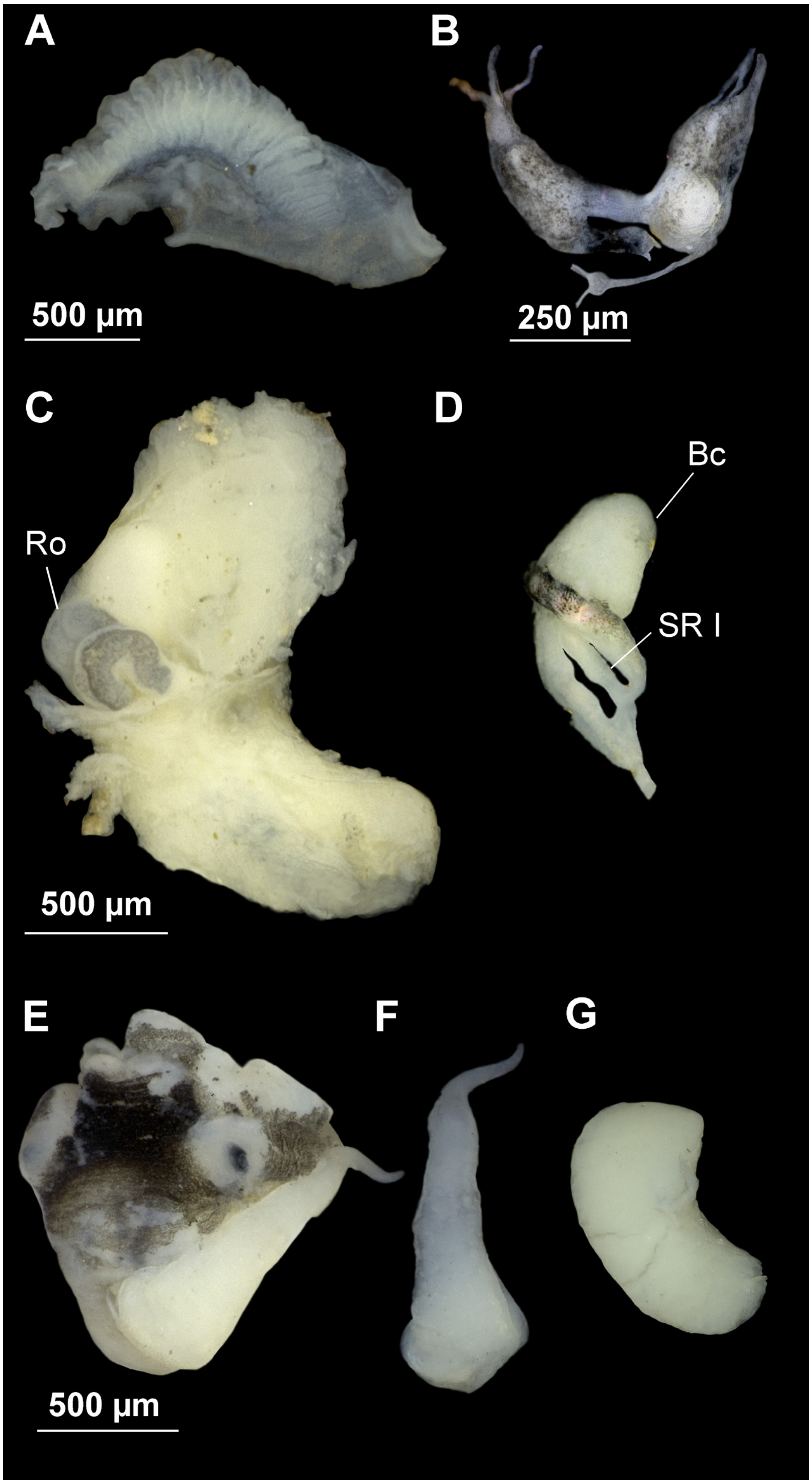

Fig. 3. Anatomy of *Intermaria zagrosensis* (Glöer & Pešić, 2009) gen. et comb. nov. from Pol Kangarar stream, Kermanshah Province, Iran. **A**. Ctenidium and osphradium. **B**. Partial nervous system. **C**. Female genitalia. **D**. Bursa copulatrix and seminal receptacle. **E–F**. Head of a male and penis. **G**. Prostate gland. Anatomical abbreviations given in the Material and methods section.

Table 2. Shell measurements (in mm) of the species: **1**. *Intermaria zagrosensis* (Glöer & Pešić, 2009). **2**. *Intermaria kermanshahensis* (Glöer & Pešić, 2009). **3**. *Persipyrgula saboori* (Glöer & Pešić, 2009). **4**. *Shadinia terpoghassiani* (Shadin, 1952). ANOVA results are shown through effect size F, degrees of freedom (df), residuals (r) and the resulting significance (p–value: *** when 0; ** when 0.001; * when 0.01; NS = no significant).

	1	**2**	**3**	**4**	
	Mean ± SD; CV (Max–Min) (n = 11)	Mean ± SD; CV (Max–Min) (n = 7)	Mean ± SD; CV (Max–Min) (n = 7)	Mean ± SD; CV (Max–Min) (n = 20)	ANOVA ($F_{df,r}$)
SL	3.58 ± 0.40; 0.11 (4.50–2.98)	3.30 ± 0.36; 0.11 (4.00–2.80)	2.90 ± 0.67; 0.23 (4.40–2.48)	3.02 ± 0.25; 0.08 (3.61–2.60)	$F_{3,40}$ = 6.1**
SW	2.28 ± 0.33; 0.15 (3.00–1.74)	2.00 ± 0.13; 0.07 (2.70–1.77)	1.84 ± 0.52; 0.28 (3.00–1.48)	1.68 ± 0.14; 0.09 (1.96–1.41)	$F_{3,40}$ = 10.4***
SL/SW	1.58 ± 0.10; 0.07 (1.71–1.45)	1.58 ± 0.05; 0.03 (1.66–1.50)	1.59 ± 0.08; 0.05 (1.70–1.48)	1.80 ± 0.10; 0.06 (2.05–1.63)	$F_{3,40}$ = 19.4***
AH	1.82 ± 0.22; 0.12 (2.34–1.58)	1.62 ± 0.13; 0.08 (1.71–1.37)	1.31 ± 0.15; 0.11 (1.50–1.09)	1.45 ± 0.11; 0.08 (1.68–1.25)	$F_{3,37}$ = 18.3***
SL–LBW	0.83 ± 0.08; 0.10 (0.97–0.67)	0.75 ± 0.08; 0.10 (0.83–0.61)	0.66 ± 0.05; 0.07 (0.75–0.62)	0.84 ± 0.11; 0.14 (1.08–0.66)	$F_{3,37}$ = 5.9**
WBW	1.98 ± 0.19; 0.09 (2.26–1.58)	1.92 ± 0.12; 0.06 (2.07–1.76)	1.51 ± 0.07; 0.04 (1.62–1.44)	1.58 ± 0.13; 0.08 (1.88–1.37)	$F_{3,37}$ = 26.3***
AL	1.77 ± 0.16; 0.09 (2.11–1.53)	1.67 ± 0.11; 0.07 (1.79–1.54)	1.27 ± 0.12; 0.09 (1.39–1.06)	1.41 ± 0.12; 0.08 (1.70–1.24)	$F_{3,37}$ = 28.2***
AW	1.36 ± 0.09; 0.07 (1.50–1.18)	1.23 ± 0.10; 0.08 (1.35–1.06)	0.97 ± 0.05; 0.07 (1.06–0.90)	1.03 ± 0.08; 0.08 (1.13–0.86)	$F_{3,37}$ = 41.9***
WPW	1.20 ± 0.12; 0.10 (1.41–0.98)	1.17 ± 0.05; 0.04 (1.24–1.10)	0.96 ± 0.05; 0.05 (1.02–0.90)	1.03 ± 0.09; 0.08 (1.24–0.9)	$F_{3,37}$ = 13.9***
WAW	0.13 ± 0.02; 0.18 (0.19–0.11)	0.13 ± 0.02; 0.19 (0.18–0.11)	0.10 ± 0.02; 0.20 (0.13–0.07)	0.13 ± 0.03; 0.24 (0.23–0.08)	$F_{3,37} = 1.4^{NS}$
NSW	4.15 ± 0.21; 0.05 (4.50–4.00)	4.37 ± 0.14; 0.03 (4.50–4.25)	4.20 ± 0.25; 0.06 (4.50–4.00)	4.13 ± 0.17; 0.04 (4.50–4.00)	$F_{3,37} = 2.6^{NS}$

Material examined

Holotype

IRAN: ZMH 51406: 4.5 mm height, 3.0 mm width.

Paratypes

IRAN: ZMH 51407 (5 ex.) and P. Glöer's (68 ex.) collection.

Type locality

IRAN: Sar Pol Kangavar stream, Kangavar city, Kermanshah Province, 34º30' N, 47º55' E, 27 Jun. 2005.

Table 3. Ctenidium, osphradium. Female and male genitalia and nervous system measurements (in mm) of the species: **1**. *Intermaria zagrosensis* (Glöer & Pešić, 2009). **2**. *Intermaria kermanshahensis* (Glöer & Pešić, 2009). **3**. *Persipyrgula saboori* (Glöer & Pešić, 2009). **4**. *Shadinia terpoghassiani* (Shadin, 1952). For ANOVA results effect size F, degrees of freedom (df), residuals (r) and the resulting significance (p–value: *** when 0; ** when 0.001; * when 0.01; NS = not significant) are shown.

	1	2	3	4	
	Mean ± SD; CV (Max–Min) 3 ♀ – 2 ♂	Mean ± SD; CV (Max–Min) 1 ♀ – 3 ♂	Mean ± SD; CV (Max–Min) 2 ♀ – 2 ♂	Mean ± SD; CV (Max–Min) 5 ♀ – 3 ♂	ANOVA ($F_{df,r}$)
CL	1.43 ± 0.14; 0.09 (1.56–1.28)	0.93 ± 0.08; 0.08 (1.00–0.84)	0.79 ± 0.05; 0.07 (0.85–0.72)	1.12 ± 0.08; 0.08 (1.25–1.00)	$F_{3,15}$ = 32.9***
Os L	0.41 ± 0.09; 0.23 (0.40–0.27)	0.32 ± 0.02; 0.05 (0.34–0.30)	0.24 ± 0.06; 0.24 (0.33–0.20)	0.32 ± 0.06; 0.17 (0.40–0.27)	$F_{3,15}$ = 4.8*
Os W	0.20 ± 0.00; 0.00 (0.20–0.20)	0.10 ± 0.00; 0.00 (0.10–0.10)	0.12 ± 0.03; 0.26 (0.16–0.09)	0.13 ± 0.03; 0.21 (0.15–0.08)	$F_{3,15}$ = 11.8***
PoL	2.29 ± 0.20; 0.09 (2.44–2.06)	1.71	1.20 ± 0.11; 0.09 (1.28–1.13)	1.66 ± 0.22; 0.13 (2.00–1.40)	$F_{3,7}$ = 12.6**
PoW	0.71 ± 0.08; 0.12 (0.80–0.63)	0.5	0.35 ± 0.03; 0.10 (0.38–0.33)	0.53 ± 0.06; 0.11 (0.57–0.43)	$F_{3,7}$ = 12.6**
AgL	1.11 ± 0.13; 0.09 (1.23–0.98)	0.98	0.60 ± 0.01; 0.01 (0.61–0.60)	0.75 ± 0.04; 0.06 (0.80–0.68)	$F_{3,7}$ = 22.9***
CgL	0.13 ± 0.05; 0.05 (1.17–1.07)	0.69	0.60 ± 0.14; 0.23 (0.70–0.50)	0.93 ± 0.09; 0.10 (1.00–0.77)	$F_{3,7}$ = 14.7**
BC L	0.71 ± 0.11; 0.16 (0.84–0.63)	0.4	0.38 ± 0.11; 0.27 (0.46–0.31)	0.35 ± 0.07; 0.21 (0.42–0.25)	$F_{3,7}$ = 10.7**
BC W	0.39 ± 0.04; 0.10 (0.43–0.36)	0.25	0.21 ± 0.06; 0.27 (0.25–0.17)	0.21 ± 0.05; 0.23 (0.27–0.15)	$F_{3,7}$ = 10.3**
dBC L	0.40 ± 0.05; 0.11 (0.43–0.35)	0.45	0.38 ± 0.03; 0.07 (0.40–0.36)	0.46 ± 0.04; 0.10 (0.50–0.40)	$F_{3,7} = 2^{NS}$
SR I L	0.28 ± 0.03; 0.11 (0.31–0.25)	0.24	0.08 ± 0.01; 0.18 (0.09–0.07)	0.26 ± 0.10; 0.41 (0.42–0.15)	$F_{3,7} = 2.8^{NS}$
SR II L	absent	absent	absent	0.16 ± 0.04; 0.26 (0.22–0.12)	–
P L	1.37 ± 0.11; 0.08 (1.45–1.30)	1.00 ± 0.00; 0.00 (1.00–1.00)	0.78 ± 0.12; 0.15 (0.87–0.70)	1.09 ± 0.23; 0.21 (1.27–0.83)	$F_{3,6}$ = 5.5*
P W	0.30 ± 0.00; 0.00 (0.30–0.30)	0.30 ± 0.02; 0.07 (0.32–0.28)	0.17 ± 0.03; 0.20 (0.20–0.15)	0.42 ± 0.06; 0.13 (0.47–0.36)	$F_{3,6}$ = 17.7**
P L/Head	1.18 ± 0.14; 0.11 (1.28–1.08)	0.90 ± 0.14; 0.16 (1.06–0.80)	0.92 ± 0.11; 0.12 (1.00–0.84)	0.98 ± 0.15; 0.15 (1.10–0.81)	$F_{3,5} = 1.8^{NS}$
ProL	1.10	0.84 ± 0.11; 0.13 (0.94–0.72)	0.68 ± 0.01; 0.01 (0.69–0.68)	0.76 ± 0.03; 0.04 (0.78–0.72)	$F_{3,5}$ = 7.7*
ProW	0.55	0.38 ± 0.04; 0.10 (0.42–0.35)	0.34 ± 0.01; 0.04 (0.35–0.33)	0.37 ± 0.03; 0.08 (0.40–0.34)	$F_{3,5}$ = 10.8*
RPG	0.61 ± 0.03; 0.05 (0.64–0.59)	0.50 ± 0.07; 0.13 (0.55–0.41)	0.52 ± 0.02; 0.05 (0.55–0.49)	0.45 ± 0.03; 0.06 (0.46–0.42)	$F_{3,10}$ = 11***

Other localities

IRAN: Darband stream in Darband village (Azna to Dorud road, ca 16 km to Azna city), Lorestan Province, 33º25' N, 49º17' E, ca 1800 m a.s.l., 23 Jun. 2005.

Description

Shell ovate-conic with 4–4.5 whorls, height 3–4.5 mm (Fig. 2A–C, Table 2); periostracum yellowish; protoconch approximately 400 µm wide with 1.3 whorls and nucleus around 125 µm long; protoconch microsculpture wrinkled (Fig. 2D); body whorl about ⅔ of total length; rest of whorls slightly convex with deep sutures; aperture complete, pyriform, often attached to body whorl on the top; thin inner peristome but thicker than outer lip; peristome margin slightly sinuate (Fig. 2C).

Operculum with ca 2 whorls (Fig. 2E–F) and muscle attachment area oval and located near the nucleus.

Radula intermediate length (25% total shell length) bearing about 45 rows of teeth; central tooth formula (5)4–C–4(5)/2–2 (Fig. 2G–H); lateral teeth formula 4–C–4; inner marginal teeth having 18–20 sharp cusps; outer marginal teeth having 15–19 sharp cusps (Fig. 2I).

Pigmentation and anatomy

Head dark brown pigmented from snout to neck; tentacles brown pigmented except on ocular lobes; snout as long as wide, with medial lobation. Ctenidium extended across most of pallial cavity with 18–21 gill filaments; osphradium two times longer than wide and opposite middle of ctenidium (Fig. 3A, Table 3).

Nervous system with black pigmentation and elongate (mean RPG ratio 0.60, Table 3); cerebral ganglia equal in size (Fig. 3B).

Female pallial oviduct with a capsule gland longer than albumen gland (Fig. 3C, Table 3); pyriform bursa copulatrix with a duct longer than its length, and lying against the middle section of the albumen gland; renal oviduct white from the insertion point of bursal duct to the seminal receptacle and hereafter black making one or two loops; one elongate seminal receptacle with short duct (Fig. 3D).

Male genitalia with penis gradually tapering with a small distal lobe on inner edge; distal end tapered, and grayish pigmented on the distal section (Fig. 3E–F); prostate gland twice as long as wide (Fig. 3G, Table 3).

Remarks

This species anatomically resembles *I. kermanshahensis*, snails of *I. zagrosensis*, however, are larger and present more cusps in lateral and marginal radular teeth, nervous system with higher RPG ratio, and relatively larger bursa copulatrix and penis.

Ecology and distribution

Recorded in streams and springs from the Kermanshah and Lorestan provinces (Iran).

Intermaria kermanshahensis (Glöer & Pešić, 2009) comb. nov.
Figs 4–5, Tables 2–3

Pseudamnicola kermanshahensis Glöer & Pešić, 2009: 38, pl. 6, figs 7–10.

Sarkhia kermanshahensis – Glöer & Pešić, 2012: 33, fig. 12h comb. nov.

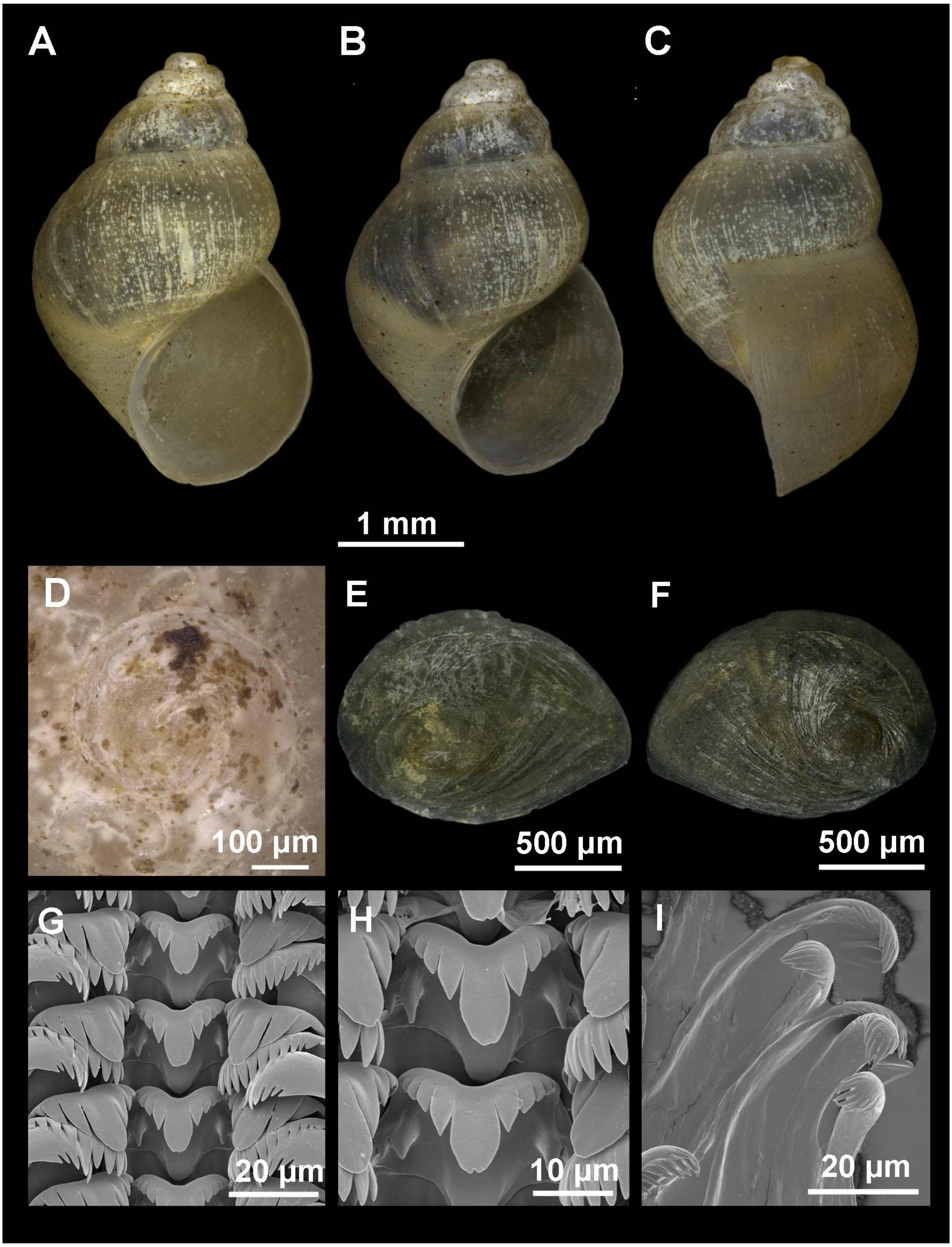

Fig. 4. Shell, operculum and radula of *Intermaria kermanshahensis* (Glöer & Pešić, 2009) gen. et comb. nov. from a spring near Sarabe - Sahneh city, Kermanshah Province, Iran. **A–B**. Shell in front view. **C**. Shell in lateral view. **D**. Protoconch and microsculpture. **E–F**. Internal and external side of the operculum. **G**. Rows of teeth of the radula. **H**. Central teeth. **I**. Detail of outer marginal teeth.

New diagnosis

Shell ovate-conic, yellowish, with pyriform aperture; protoconch microsculpture slightly wrinkled; central radular tooth formula 4–C–4/2–2; pyriform bursa copulatrix; one seminal receptacle elongate with short duct; penis gradually tapering with small distal lobe on inner edge, end tapered; nervous system elongated (mean RPG ratio = 0.50) with slight black pigmentation.

Material examined

Holotype

IRAN: ZMH 51404: 4 mm height, 2.7 mm width.

Paratypes

IRAN: ZMH 51405 (5 ex.) and P. Glöer's (66 ex.) collection.

Type locality

IRAN: spring between Sarab and Sahneh cities, Kermanshah Province, 34º27' N 47º44' E, 27 Jun. 2005.

Description

Shell ovate-conic with 4.25–4.5 whorls, height 3–4 mm (Fig. 4A–C, Table 2); periostracum yellowish; protoconch approximately 500 µm wide with 1.4 whorls and nucleus around 135 µm long; protoconch microsculpture slightly wrinkled (Fig. 4D); body whorl about ⅔ total length; rest of whorls slightly convex with deep sutures; aperture complete, pyriform, inner lip thicker than outer lip; peristome margin straight (Fig. 4C).

Operculum with 2 whorls approximately (Fig. 4E–F) and muscle attachment area oval and located near the nucleus.

Radula intermediate length (25% total shell length) bearing about 55 rows of teeth; central tooth formula 4–C–4/2–2 (Fig. 4G, H); lateral teeth formula 3–C–3; inner marginal teeth having 15–18 sharp cusps; outer marginal teeth having 12–14 sharp cusps (Fig. 4I).

Pigmentation and anatomy

Head light brown pigmented from snout to neck; tentacles also brown pigmented except on ocular lobes; snout as long as wide, with medial lobation. Ctenidium extended across most of pallial cavity with 18–21 narrow gill filaments; osphradium three times longer than wide and opposite middle of ctenidium (Fig. 5A, Table 3).

Nervous system with black pigmentation and elongate (mean RPG ratio 0.50); cerebral ganglia equal in size (Fig. 5B, Table 3).

Female pallial oviduct with a capsule gland slightly shorter than albumen gland (Fig. 5C, Table 3); pyriform bursa copulatrix with a duct as long as bursa length, and lying against the middle section of the albumen gland; renal oviduct white from the insertion point of bursal duct to the seminal receptacle and hereafter black making one or two loops; one elongate seminal receptacle with short duct (Fig. 5D).

Male genitalia with penis gradually tapering bearing a small distal lobe in the inner edge; slightly grayish pigmented on the distal section in some specimens (Fig. 5E, F); prostate gland about two times longer than wide (Fig. 5G, Table 3).

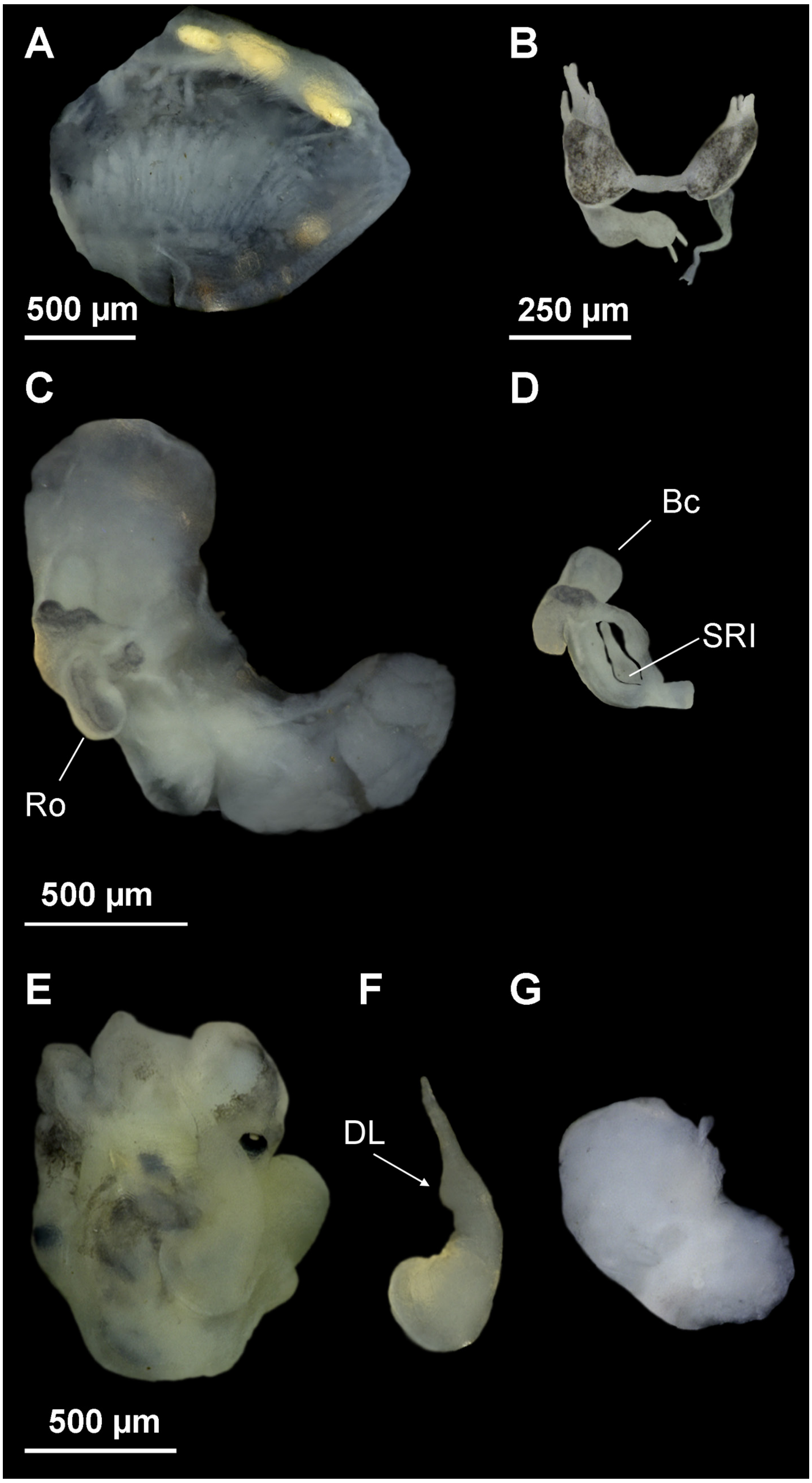

Fig. 5. Anatomy of *Intermaria kermanshahensis* (Glöer & Pešić, 2009) gen. et comb. nov. from a spring near Sarabe - Sahneh city, Kermanshah Province, Iran. **A**. Ctenidium and osphradium. **B**. Partial nervous system. **C**. Female genitalia. **D**. Bursa copulatrix and seminal receptacle. **E–F**. Head of a male and penis. **G**. Prostate gland. Anatomical abbreviations given in the Material and methods section, except DL (distal lobe).

Remarks

Apart from differences in body dimensions and in certain anatomical features (explained above), the penial distal lobe is more prominent in this species than in *I. zagrosensis*. Uncorrected genetic distances are, on the contrary, low between these two species (0.2% for COI fragment, Table 4), though sister taxa in the subfamily Pyrgulinae are often genetically very close (e.g., COI p-distances of 0.3% between species of the genus *Macedopyrgula* Radoman, 1973). Given this minor genetic variation between congeners, these observed anatomical differences could potentially reflect intraspecific variation.

Ecology and distribution

Known only from a spring in the Kermanshah Province, Iran.

Genus ***Persipyrgula*** gen. nov.
urn:lsid:zoobank.org:act:6E9922EE-56CD-4BBC-BF4E-9224C75859C0

Type species

Pseudamnicola saboori Glöer & Pešić, 2009.

Diagnosis

Shell ovate-conic, 2.5 to 4.5 mm high; large and convex body whorl; rest of the whorls small and slightly convex; aperture complete, pyriform, slightly angled on the top and separated from body whorl. Operculum corneous, yellowish, thin, pliable, ellipsoidal, paucispiral with submarginal nucleus. From one to two pairs of basal cusps in radular central tooth. Ctenidium occupying nearly the entire length of pallial cavity and bearing well-developed gill filaments. Osphradium opposite approximate middle of ctenidium. Bursa copulatrix posterior positioned relative to albumen gland; pigmented renal oviduct; one small pyriform seminal receptacle without duct. Prostate gland bean-shaped, about twice as long as wide; exit of the pallial vas deferens from the posterior-most section of the prostate gland and seminal vesicle entering the prostate gland in its middle section; penis simple, gradually tapering, with a distal end tapered. Nervous system with black pigmentation typically elongated.

Etymology

Referring to Persia, the historic name of the region where the genus was found, and to *Pyrgula*, type genus of the subfamily Pyrgulinae.

Remarks

Though similar in shell features, this genus differs from *Pseudamnicola* mainly due to its small and pyriform seminal receptacle in the female genitalia (long and elongate in *Pseudamnicola*), shorter prostate gland, tapered and simple penis (triangular and folded in the latter), and because of the occasional possession of two pairs of basal cusps in the central radular tooth. Differences between *Persipyrgula* gen. nov. and *Intermaria* gen. nov. are: shell dimensions (larger in the latter), aperture not fused to body whorl in *Persipyrgula* gen. nov., one (occasionally two) vs two pairs of basal cusps in the central radular tooth, small pyriform vs elongate seminal receptacle, and absence vs presence of a small distal lobe on the inner edge of the penis, respectively.

Persipyrgula saboori (Glöer & Pešić, 2009) comb. nov.
Figs 6–7, Tables 2–3

Pseudamnicola saboori Glöer & Pešić, 2009: 36, pl. 6, figs 1–3.

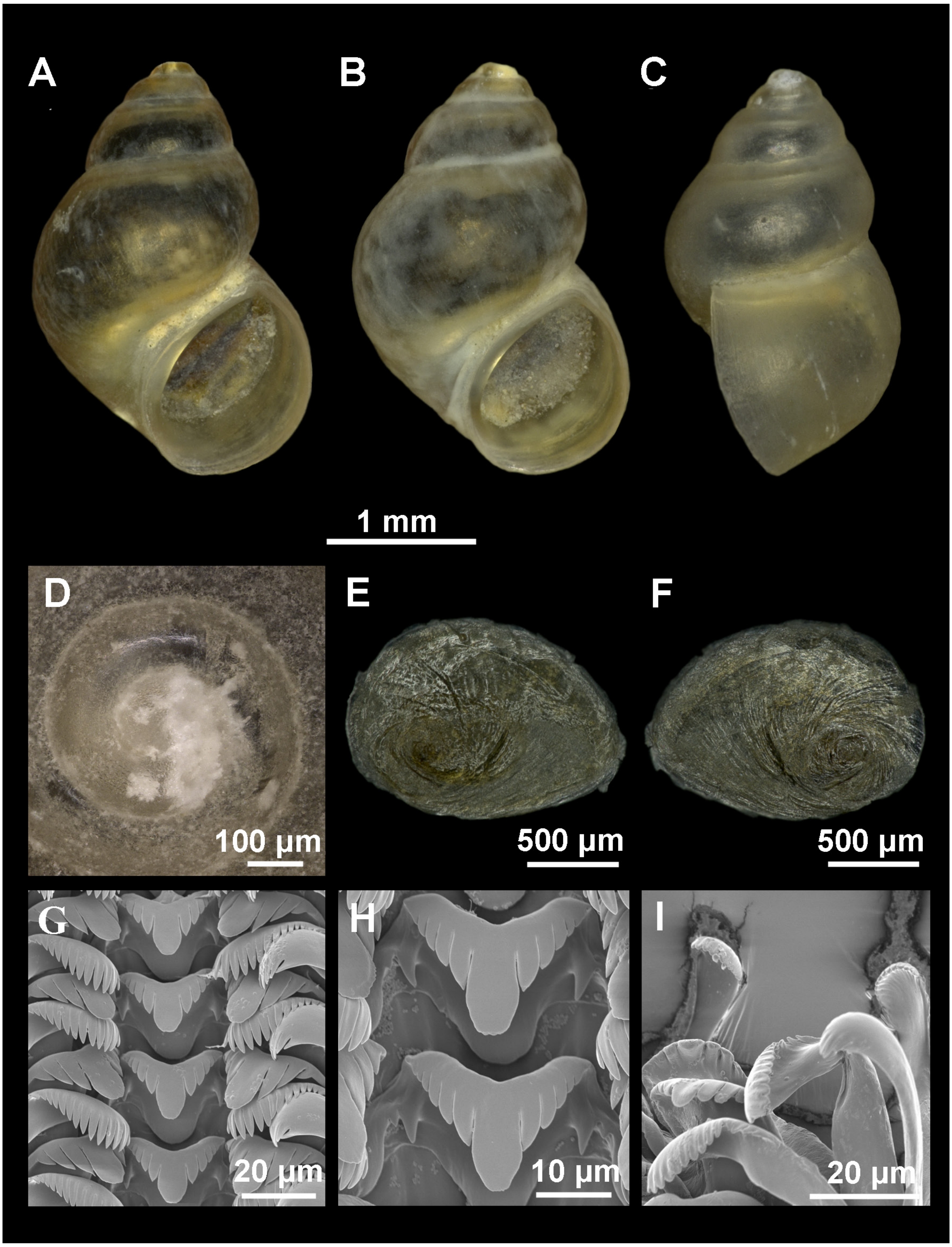

Fig. 6. Shell, operculum and radula of *Persipyrgula saboori* (Glöer & Pešić, 2009) gen. et comb. nov. from Zou Eram spring in Zou Eram village, Khorrasan Province, Iran. **A–B**. Shell in front view. **C**. Shell in lateral view. **D**. Protoconch and microsculpture. **E–F**. Internal and external side of the operculum. **G**. Rows of teeth of the radula. **H**. Central teeth. **I**. Detail of outer marginal teeth.

New diagnosis

Shell ovate-conic, yellowish, with pyriform aperture; protoconch microsculpture wrinkled; central radular tooth formula 5–C–5/(2)1–1(2); pyriform bursa copulatrix, with a duct longer than bursa; one small pyriform seminal receptacle with no distinct duct; penis gradually tapering and simple, with distal end tapered and grayish pigmented; nervous system elongated (mean RPG ratio = 0.52) with slight black pigmentation.

Material examined

Holotype

IRAN: ZMH 51402: 4.4 mm height, 3.0 mm width.

Paratypes

IRAN: ZMH 51403 (5 ex.) and P. Glöer's (46 ex.) collection.

Type locality

IRAN: Zou Eram spring in Zou Eram village, Shirvan city, Khorasan Province, 37°20' N, 57°40' E, ca 1600 m, 11 Jun. 2005.

Description

Shell ovate-conic with 4–4.5 whorls, height 2.5–4.4 mm (Fig. 6A–C, Table 2); periostracum yellowish; protoconch approximately 350 µm wide with 1.25 whorls and nucleus around 120 µm long; protoconch microsculpture wrinkled, more intense on apex (Fig. 6D); body whorl about ⅔ total length; rest of whorls slightly convex with a deep suture; aperture complete and pyriform; inner lip thicker than outer lip; peristome margin straight (Fig. 6C).

Operculum with 2.5 whorls approximately (Fig. 6E–F) and muscle attachment area oval located near the nucleus.

Radula intermediate length (30% total shell length) bearing about 50 rows of teeth; central tooth formula 5–C–5/(2)1–1(2) (Fig. 6G–H); lateral teeth formula 3–C–3; inner marginal teeth having 15–18 cusps; outer marginal teeth having 14–16 cusps (Fig. 6I).

Pigmentation and anatomy

Head dark brown pigmented from snout to the penial base; tentacles also brown pigmented except on ocular lobes; snout as long as wide, with medial lobation. Ctenidium in middle region of pallial cavity with 18–20 narrow gill filaments; osphradium two times longer than wide and opposite middle of ctenidium (Fig. 7A, Table 3).

Nervous system with black pigmentation and elongate (mean RPG ratio 0.52, Table 3); cerebral ganglia equal in size (Fig. 7B).

Female pallial oviduct with a capsule gland and albumen gland similar in size (Fig. 7C, Table 3); pyriform bursa copulatrix with a duct about 50–100% of bursa length; renal oviduct white straight from the insertion point of bursal duct to where it begins to fold; hereafter black pigmented making a simple loop; one small pyriform seminal receptacle with no distinct duct (Fig. 7D).

Male genitalia with penis simple, gradually tapering, with distal end tapered grayish pigmented (Fig. 7E–F); prostate gland bean-shaped, about two times longer than wide (Fig. 7G, Table 3).

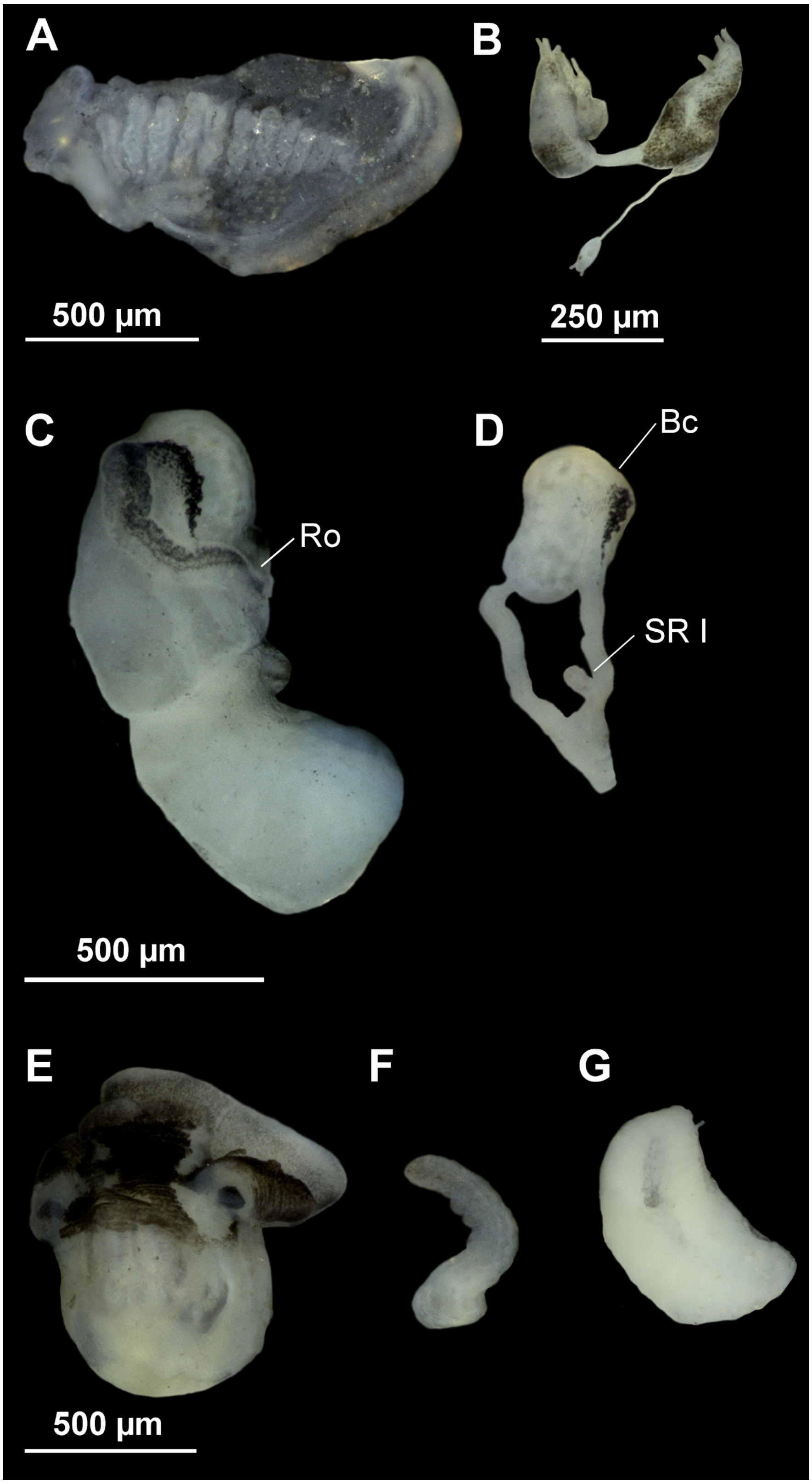

Fig. 7. Anatomy of *Persipyrgula saboori* (Glöer & Pešić, 2009) gen. et comb. nov. from Zou Eram spring in Zou Eram village, Khorrasan Province, Iran. **A**. Ctenidium and osphradium. **B**. Partial nervous system. **C**. Female genitalia. **D**. Bursa copulatrix and seminal receptacle. **E–F**. Head of a male and penis. **G**. Prostate gland. Anatomical abbreviations given in the Material and methods section.

Remarks

The small pyriform seminal receptacle and the posterior position of bursa copulatrix relative to albumen gland are the exceptional features of this species related to the species comprising the new pyrgulinid lineage found in this study. Moreover, *P. saboori* is the most distantly related taxa of this newly recovered lineage (COI p-distances from 6.5 to 8.5%, Table 4).

Ecology and distribution

Only recorded in Khorasan and Markazi provinces (Iran).

Genus ***Shadinia*** Akramowski, 1976

Type species

Pyrgula terpoghassiani Shadin, 1952.

New diagnosis

Shell ovate-conic, 3 to 5 mm high; large and convex body whorl; rest of the whorls tall and convex; aperture complete, pyriform, angled on the top and fused to the body whorl. Operculum corneous, yellowish, thin, pliable, ellipsoidal, paucispiral with submarginal nucleus. From two to three pairs of basal cusps in radular central tooth. Ctenidium occupying nearly the entire length of pallial cavity and bearing well-developed gill filaments. Osphradium opposite approximate middle of ctenidium. Bursa copulatrix lying against the middle section of the albumen gland; pigmented renal oviduct; two opposite seminal receptacles, SRII smaller than SRI. Prostate gland bean-shaped, about twice as long as wide; exit of the pallial vas deferens from the posterior-most section of the prostate gland and seminal vesicle entering the prostate gland in its middle section; penis simple, gradually tapering, with a distal end tapered dark pigmented. Nervous system with black pigmentation and moderately concentrated.

Shadinia terpoghassiani (Shadin, 1952)

Figs 8–9; Tables 2–3

Pyrgula terpoghassiani Shadin, 1952: 227

Pyrgula terpoghassiani – Akramowski 1952, nom. nud.

New diagnosis

Shell ovate-conic, yellowish, with two weak parallel keels along the body whorl; pyriform aperture; protoconch microsculpture wrinkled; central radular tooth formula 3–C–3/(2)3–3(2); pyriform bursa copulatrix; SRI elongate with a short duct and SRII smaller, globular and without duct; penis gradually tapering and simple, with a distal end tapered and black pigmented; nervous system moderately concentrated (mean RPG ratio = 0.45) and with slight black pigmentation.

Material examined

Type material not specified.

Type locality

ARMENIA: Lake Aiger-Lich, south of Metzamor, Armavia province, 40° 08.573' N, 44°10.270' E, 18 May 2008.

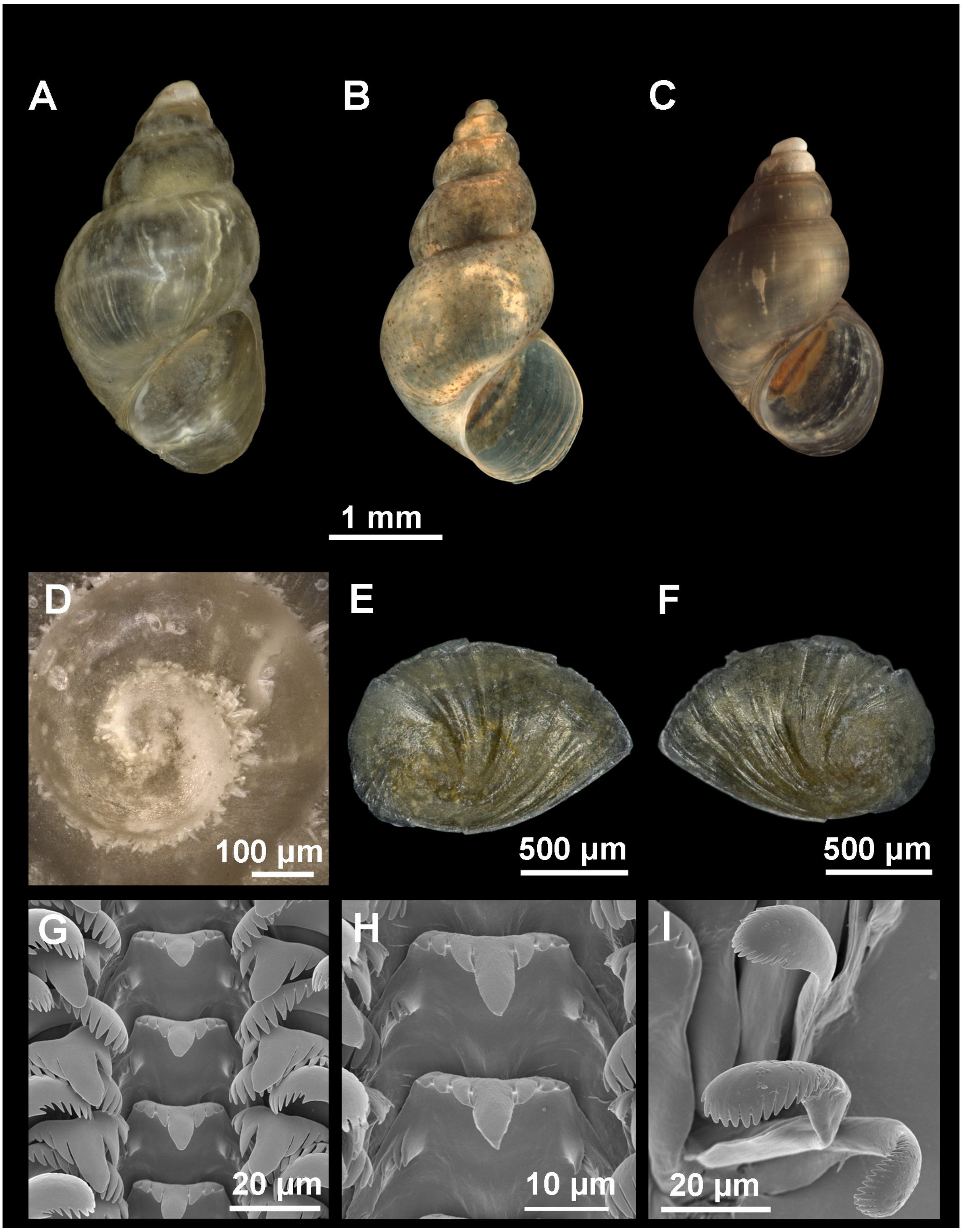

Fig. 8. Shell, operculum and radula of *Shadinia terpoghassiani* (Shadin, 1952) from Lake Aiger-Lich, Armenia. **A–C**. Shell in front view. **D**. Protoconch and microsculpture. **E–F**. Internal and external side of the operculum. **G**. Rows of teeth of the radula. **H**. Central teeth. **I**. Detail of outer marginal teeth.

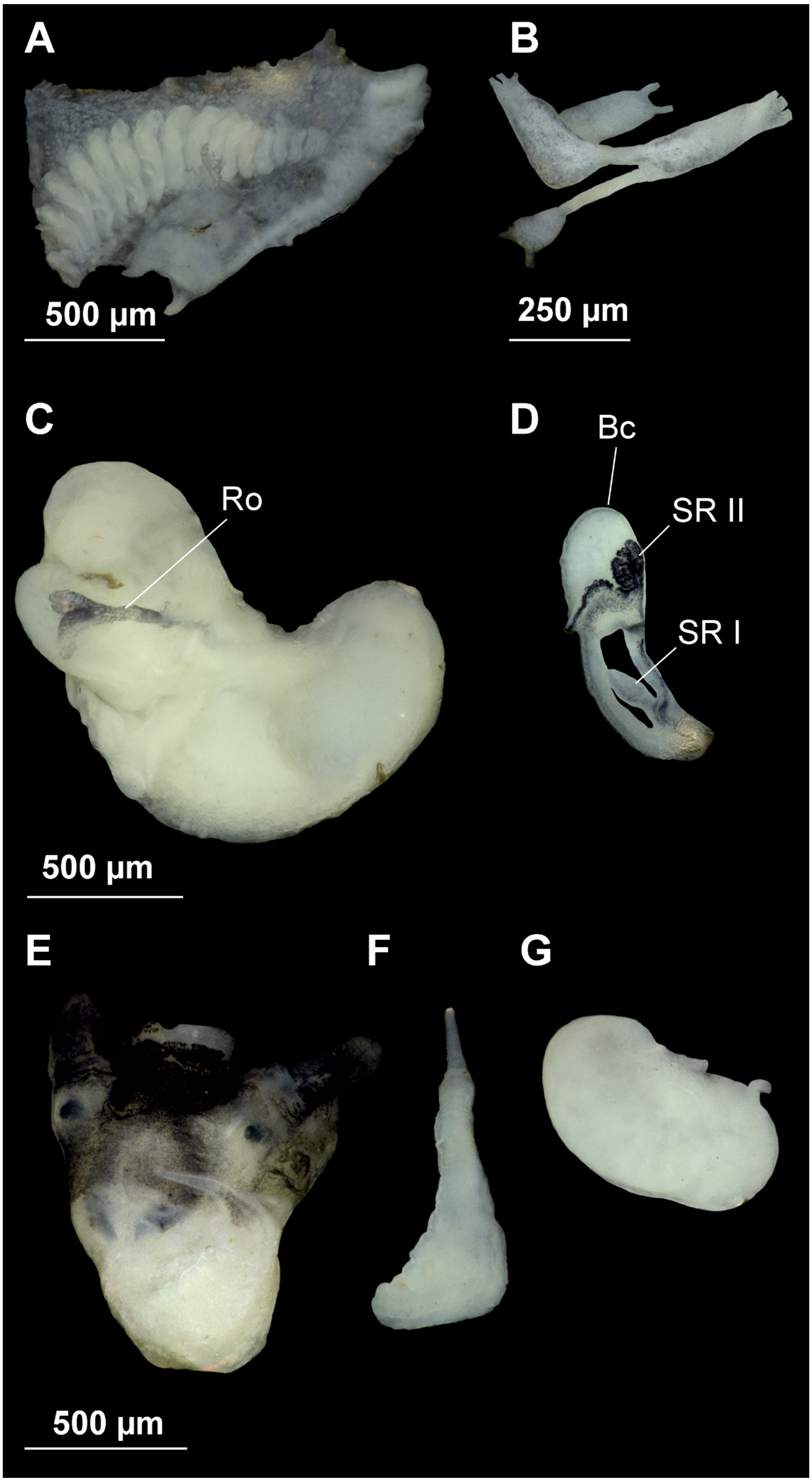

Fig. 9. Anatomy of *Shadinia terpoghassiani* (Shadin, 1952) from Lake Aiger-Lich, Armenia. **A**. Ctenidium and osphradium. **B**. Partial nervous system. **C**. Female genitalia. **D**. Bursa copulatrix and seminal receptacle. **E–F**. Head of a male and penis. **G**. Prostate gland. Anatomical abbreviations given in the Material and methods section.

Description

Shell ovate-conic with 4–4.5 whorls, height 2.6–3.6 mm (Fig. 8A–C, Table 2); periostracum yellowish; protoconch approximately 385 µm wide with 1.3 whorls and nucleus around 125 µm long; protoconch microsculpture wrinkled (Fig. 8D); body whorl about ⅔ total length and bearing two weak spiral keels; rest of whorls tall and convex with a deep suture; aperture complete, pyriform, with an inner lip thicker than outer lip; peristome margin slightly sinuate.

Operculum with 2.5 whorls approximately (Fig. 8E–F) and muscle attachment area oval and located near the nucleus.

Radula intermediate length (25% total shell length) bearing around 45 rows of teeth; central tooth formula 3–C–3/(2)3–3(2) (Fig. 8G–H); lateral teeth formula 3–C–3; inner and outer marginal teeth bearing 17–21 and 19–25 cusps, respectively (Fig. 8I).

Pigmentation and anatomy

Head dark brown pigmented from snout to penial base; pigmentation clearer on neck; tentacles also brown pigmented except on ocular lobes; snout as long as wide, with medial lobation. Ctenidium in middle region of pallial cavity with 18–20 gill filaments; osphradium two to three times longer than wide and opposite middle of ctenidium (Fig. 9A, Table 3).

Nervous system with black pigmentation and moderately concentrated (mean RPG ratio 0.45, Table 3); cerebral ganglia equal in size (Fig. 9B).

Female genitalia with a capsule gland longer than albumen gland (Fig. 9C, Table 3); pyriform bursa copulatrix lying against the middle section of the albumen gland; bursal duct longer than bursa length; renal oviduct straight and white from the insertion point of bursal duct to SRII; hereafter black pigmented making one or two loops; two opposite seminal receptacles; SRI pyriform with short duct and SRII smaller, black pigmented, globular and sessile (Fig. 9D).

Male genitalia bearing a penis simple, gradually tapering, with a distal end tapered, and black pigmented on the distal section (Fig. 9E–F); bean-shaped prostate gland about two times longer than wide (Fig. 9G, Table 3).

Remarks

Specimens from the type locality varied in shell dimensions and whorl convexity (Fig. 8A–C and Shadin 1952: fig. 51), though they were similar in their anatomical features. So far, *Shadinia* is the only pyrgulinid genus bearing two seminal receptacles (for a morphological review of the Pyrgulinae subfamily see Radoman (1983); the anatomy of *Pyrgula* Cristofori & Jan, 1832 and *Dianella* Gude, 1913 is described in Szarowska 2006). Size of seminal receptacles varies slightly between the three anatomically known species, being smaller in *S. terpoghassiani* than in *S. bjniensis*. Moreover, some specimens of *S. terpoghassiani* are larger (shell height *S. bjniensis* 3.6–4.0 mm, *S. terpoghassiani* 5.2 mm, see Shadin 1952) and often two weak spiral keels are present in the shell body whorl. Three basal cusps in the central radular tooth are also present in *S. akramowskii* (Shadin 1952). The species of *Shadinia* here analyzed differ from each other by 0.4% COI p-distances (Table 4).

Ecology and distribution

Armenia and Nakhchivan province of Azerbaijan (Akramowski 1976).

Table 4. Uncorrected COI p-distances between species of the subfamilies Pyrgulinae and Pseudamnicolinae.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. *Pseudamnicola lucensis*	-																			
2. *Corrosella falkneri*	13.7	-																		
3. *Diegus gasulli*	12.9	11.2	-																	
4. ***Intermaria zagrosensis***	15.2	13.7	10.8	-																
5. ***Persipyrgula saboori***	14.3	12.6	10.6	**6.7**	-															
6. ***Intermaria kermanshahensis***	15.1	13.5	10.6	**0.1**	**6.5**	-														
7. ***Shadinia bjniensis***	15.6	14.1	11.7	**3.5**	**8.3**	**3.6**	-													
8. ***Shadinia terpoghassiani***	15.9	14.5	12.5	**3.7**	**8.5**	**3.9**	**0.4**	-												
9. *Pyrgula annulata*	16.8	17.8	14.6	12.2	12.5	12.4	11.6	11.8	-											
10. *Chilopyrgula sturanyi*	17.2	17.1	14.6	13.2	12.8	13.3	12.4	12.6	1.9	-										
11. *Ohridopyrgula macedónica*	16.9	16.5	13.8	12.2	12.9	12.4	11.4	11.6	2.2	1.9	-									
12. *Xestopyrgula dybowskii*	17.4	16.9	14.3	13.2	13.0	13.3	12.2	12.4	3.8	2.8	4.1	-								
13. *Dianella thiesseana*	16.9	16.8	13.4	12.1	13.1	12.2	11.7	11.9	5.8	5.3	5.5	5.5	-							
14. *Euxinipyrgula milachevitchi*	18.5	18.2	13.7	14.1	13.8	14.3	14.0	14.2	11.1	11.3	11.6	10.8	11.4	-						
15. *Micromelania lincta*	18.3	18.4	14.1	14.4	13.9	14.6	14.0	14.2	11.6	11.8	11.8	11.3	11.9	0.6	-					
16. *Turricaspia sp.*	18.9	18.5	14.0	14.3	14.1	14.4	13.8	14.0	11.4	11.6	11.9	11.1	11.8	0.3	0.6	-				
17. *Falsipyrgula pfeiferi*	18.7	18.2	13.5	14.1	13.8	14.3	14.0	14.2	11.0	11.1	11.4	11.0	11.3	0.9	0.9	0.9	-			
18. *Ginaia munda munda*	17.6	16.4	12.9	11.3	11.9	11.4	10.9	11.1	3.3	3.4	4.1	3.3	4.5	10.7	11.1	11.0	10.5	-		
19. *Ginaia munda sublitoralis*	17.8	16.1	13.5	11.9	12.3	12.1	11.7	11.9	3.1	2.6	3.4	2.2	5.0	10.3	10.8	10.7	10.2	2.6	-	
20. *Macedopyrgula pavlovici*	16.3	16.0	13.2	11.7	11.9	11.9	11.1	11.3	2.4	1.9	2.7	2.1	4.0	10.3	10.7	10.6	10.1	1.9	1.4	-
21. *Macedopyrgula wagneri*	16.9	16.7	13.5	12.4	12.5	12.5	11.7	11.9	2.8	2.2	3.1	2.4	4.1	10.5	11.0	10.8	10.3	1.9	1.7	0.3

Discussion

Our results were consistent with our proposed hypothesis of the incorrect assignment of the *Pseudamnicola* species from Iran described by Glöer & Pešić (2009). Thus, molecular and morphological analyses revealed that shell shape and penis morphology alone are not sufficiently informative for genus assignment. Our phylogenetic data recovered those Iranian species and the genus *Shadinia* as a potential monophyletic group appearing outside *Pseudamnicola* and the subfamily Pseudamnicolinae (Fig. 1). A sister relationship between this new lineage and other pyrgulinid species was identified (despite the limited resolution of COI for inferring phylogenetic relationships on the subfamily level). However, p-distances between the Ponto-Caspian genera and the molecularly analyzed Pseudamnicolinae and Pyrgulinae taxa proved similar (13.6% and 13%, respectively). In effect, our phylogenetic reconstruction suggests these new taxa may comprise a different subfamily intermediate between the other two. Notwithstanding, this would need confirmation through more comprehensive sampling of the area and examining other potential new representatives of this lineage. As more shared synapomorphies with the pyrgulinid group were detected (such as a tapered penis, zero to two seminal receptacles, zero to three pairs of basal cusps in the central radular tooth and spiral keels on the shell, see Radoman 1955, 1983) than with *Pseudamnicola* (diagnosed by, one elongate seminal receptacle, one pair of basal cusps in the central radular tooth, triangular penis with folds and simple shells slightly longer than wide, of which the latter two are not present in Pyrgulinae, see Boeters 1988; Szarowska *et al.* 2009; Delicado *et al.* 2014), we tentatively consider these taxa as belonging to the subfamily Pyrgulinae.

Despite the low p-distances characterizing these Ponto-Caspian species, morphological evidence led us to consider them as different genera, here described as *Intermaria* gen. nov. and *Persipyrgula* gen. nov. This pattern of high morphological variability between closely related genera has been also observed in other pyrgulinid groups (see Wilke *et al.* 2007). In addition, the discovery of these genera suggests that other species from the Ponto-Caspian region assigned to *Pseudamnicola* according to shell shape and penis morphology could effectively be members of this newly discovered lineage or even constitute other clades. For instance, *P. kayseriensis* Glöer, Yıldırım & Kebapçi, 2015, *P. gullei* Glöer, Yıldırım & Kebapçi, 2015 and *P. vinarskii* Glöer & Georgiev, 2012 from Turkey bear similar shell and penis morphologies as the genera here described, and despite a more conical shell, the species

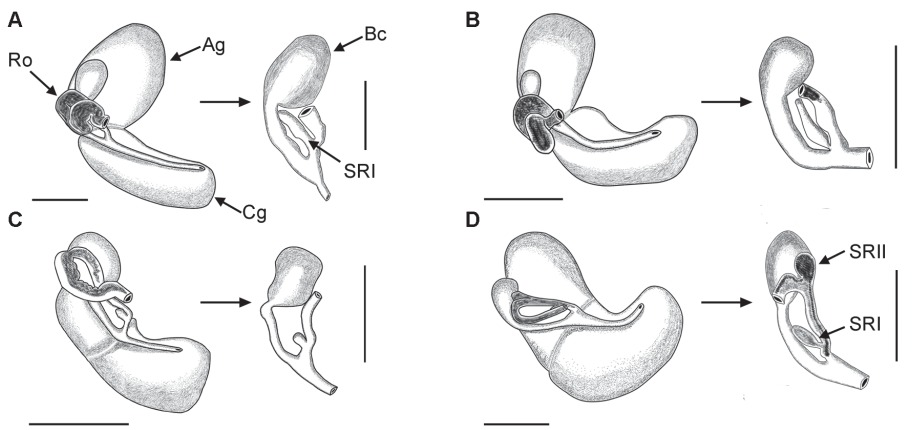

Fig. 10. Distal female genitalia and associated structures of the Ponto-Caspian species. **A.** *Intermaria zagrosensis* (Glöer & Pešić, 2009) gen. et comb. nov. **B**. *Intermaria kermanshahensis* (Glöer & Pešić, 2009) gen. et comb. nov. **C**. *Persipyrgula saboori* (Glöer & Pešić, 2009) gen. et comb. nov. **D**. *Shadinia terpoghassiani* (Shadin, 1952). Anatomical abbreviations given in the Material and method section. Scale bars: 500 µm.

Sarkhia sarabensis Glöer & Pešić, 2012 from Iran also shows similar penis characteristics. Additional anatomical descriptions and phylogenetic data might soon clarify this issue.

In conclusion, our morphological and molecular data question the species richness of the genus *Pseudamnicola*, and indicate that the morphological characters traditionally used for the recognition of Ponto-Caspian hydrobiid species (i.e., conchyliologic) need to be complemented with other features mainly related to their genital and trophic systems. The high morphological disparity observed among closely related pyrgulinid genera calls for an intensive study of the reason why some hydrobiids are so similar and others so diverse. Our phylogeny identified a new potential lineage that could represent a new subfamily between Pseudamnicolinae and Pyrgulinidae, thus contributing to the knowledge of evolutionary patterns in the family Hydrobiidae. Our findings also provide future direction for research on the biodiversity, systematics and biogeography of hydrobiid gastropods, particularly those from the Ponto-Caspian region.

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