

# TWO NEW GENERA AND FOUR NEW SPECIES OF FRESHWATER COCHLIOPID GASTROPODS (RISSOOIDEA) FROM NORTHEASTERN MEXICO

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## ABSTRACT

We describe four new species of cochliopid snails from thermal springs in northeastern Mexico (Chihuahua, Coahuila and Durango States). Two of these are placed in *Pseudotryonia* and represent the first Mexican records for this genus. They are diagnosed by unique or unique combinations of shell and anatomical characters, are well differentiated genetically from both each other and other congeners (mtCOI sequence divergence  $\geq 2.0\%$ ) and were resolved as sister species within the *Pseudotryonia* clade in our molecular phylogenetic analyses. The other two species resemble *Ipnobius*, *Pseudotryonia* and *Tryonia* in having a thin, narrow shell, penis ornamented with a small number of glandular papillae and ovoviviparous reproductive mode, but are well differentiated (from these and each other) by other morphological/anatomical characters and mtCOI sequences (8.6–12.5% and 5.2–12.1% divergence, respectively) and consequently are placed in new monotypic genera (*Chorrobius* and *Minckleyella*). *Chorrobius* and *Minckleyella* formed a clade with *Ipnobius*, *Mexipyrgus* and *Tryonia* in all of our molecular phylogenetic analyses. *Chorrobius* was consistently delineated as a divergent lineage within this clade, but its relationships were otherwise unresolved. *Minckleyella* was nested within *Tryonia* in most of the trees and was sister to this genus in the others; despite this finding we erected a new genus for this snail because of its highly distinctive anatomical features and large genetic divergence relative to *Tryonia* ( $5.2 \pm 0.6\%$ ). Each of the four new species is endemic to highly disturbed single springs or local spring systems and may require protection.

## INTRODUCTION

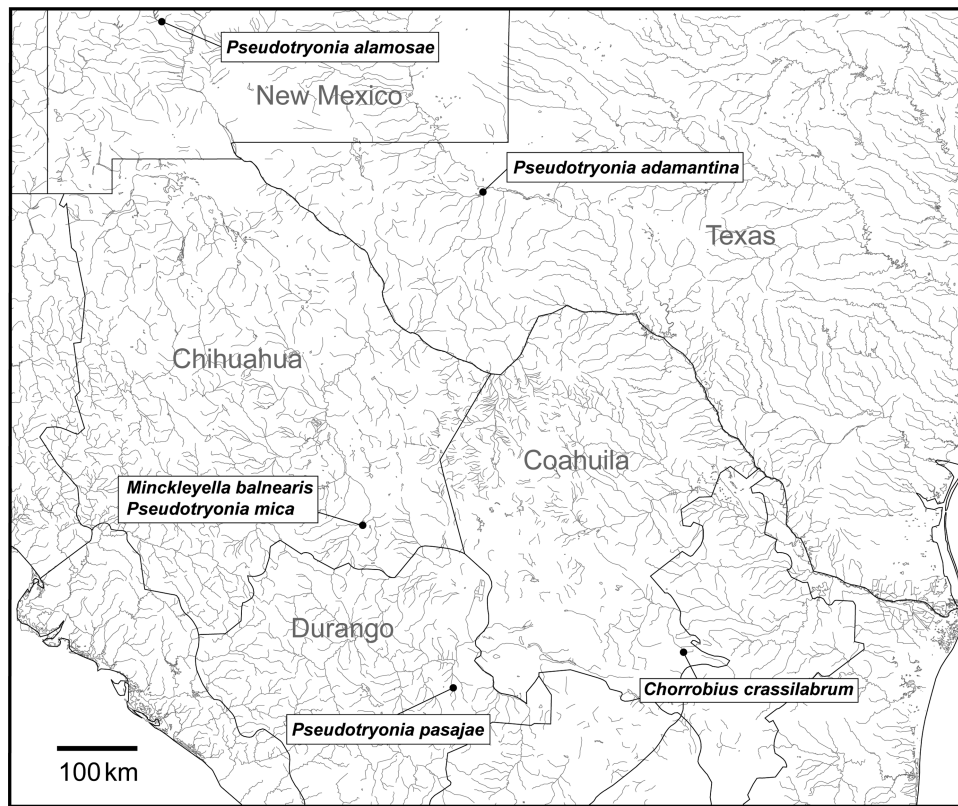
The large radiation of fresh- and brackish-water cochliopid gastropods in temperate and tropical America (30 genera, 240 species; Hershler & Thompson, 1992) is poorly known and only beginning to be studied using modern systematic-phylogenetic methods. One of the focal points of this recent research has been a large group of species that share a thin, narrow shell, penis ornamented with a small number of glandular papillae and ovoviviparous reproductive mode; most of these species live in thermal springs and most are distributed in the American Southwest. This morphologically distinctive assemblage was previously classified as a single genus, *Tryonia* (Taylor, 1987; Hershler & Thompson, 1987, 1992), but has recently been partitioned into three genera (*Tryonia sensu stricto* and newly erected *Ipnobius* and *Pseudotryonia*) that are diagnosed by unique or unique combinations of reproductive anatomical characters (Hershler, 2001) and form evolutionarily distinct lineages based on mitochondrial DNA sequences (Hershler, Liu & Mulvey, 1999a; Liu, Hershler & Thompson, 2001). The phylogenetic relationships of these three genera have not been well resolved, although molecular evidence (Hershler *et al.*, 1999a; Liu *et al.*, 2001) suggests that they are not closely related and that *Tryonia* may be sister to *Mexipyrgus*, a monotypic genus that is locally endemic in northeastern Mexico (Hershler, 1985). *Tryonia* currently consists of 17 species that are scattered throughout the American Southwest, and single species in both Florida and Guatemala (Hershler, 2001; Hershler, Liu & Stockwell, 2002a); *Ipnobius* is monotypic and endemic to the lower Amargosa River basin (Death

Valley, CA, USA) (Hershler, 2001); *Pseudotryonia* contains two species in the Pecos River basin and single species in both Alabama and Florida (Hershler, 2001; Thompson, 2001).

The current study was prompted by the discovery in northeastern Mexico of four undescribed species of *Tryonia*-like snails that do not conform to the genus as currently diagnosed. Herein we provide taxonomic descriptions of these novelties, which consist of two species of *Pseudotryonia* that represent the first Mexican records for the genus, and two highly distinctive snails that cannot be accommodated in any cochliopid genus and consequently are placed in new (monotypic) genera. We also analyse mitochondrial DNA sequence variation to assess the divergence and phylogenetic relationships of these new taxa. This paper may also provide the impetus for protection of these snails, each of which is endemic to heavily impacted single springs or local spring systems in freshwater ecoregions (Guzmán, Río Conchos, Río Salado) that have been assigned to the highest (critical or endangered) North American conservation priority categories (Abell *et al.*, 2000). This paper is the second in a series that treats the large undescribed cochliopid fauna of northeastern Mexico (Hershler, Liu & Landye, 2002b).

## MATERIAL AND METHODS

The geographic locations of the four new species described herein are shown in Figure 1. Three of these species were first collected by J.J.L. during 1971 when he participated in biotic surveys of aquatic systems in northeastern Mexico while with



**Figure 1.** Map showing the collecting localities of the new species described in this paper. The distributions of the two previously described species of *Pseudotryonia* from the American Southwest are also shown.

W.L. Minckley (Arizona State University). Description of these and other new molluscs that were discovered during that survey was delayed. The fourth new species (from Durango State) was discovered during the course of more recent fieldwork that was largely focused on collecting fresh material from previously known cochliopid populations for molecular studies.

Anatomical study was based on specimens that were relaxed with menthol crystals and fixed in dilute formalin prior to preservation in 70% ethanol. Snails used for mtDNA sequencing were preserved in 90% ethanol in the field. Types and other voucher material from this study were deposited in the National Museum of Natural History (USNM) collection. Relevant material from the Bell Museum of Natural History (BellMNH) was also examined during the course of this study. Variation in the number of cusps on the radular teeth ( $n = 5$  for each species) was assessed using the method of Hershler *et al.* (2007a). Gill filament counts were obtained from five adult females for each sample. Two to five specimens of each sex were dissected for each species for other anatomical details. Other methods of morphological study and descriptive terminology are those of Hershler (2001) and Hershler *et al.* (2002b). Shell data were analysed using Systat for Windows 11.00.01 (SSI, 2004).

The molecular phylogenetic analyses included the four species described herein, eight species of *Tryonia* (including four undescribed species from northeastern Mexico that we are currently studying), each of the previously described species of *Ipnobius* and *Pseudotryonia*, eight species belonging to six other (North or Central American) cochliopid genera, and *Phrantela marginata*, which was used as the outgroup (see Hershler *et al.*, 1999a). Genomic DNA was extracted from entire snails using a CTAB protocol (Bucklin, 1992). A 658-bp segment of cytochrome *c* oxidase subunit I (COI) was amplified and sequenced with primers LCO1490 and HCOI2198 (Folmer

*et al.*, 1994) following the protocols of Liu, Hershler & Clift (2003). Sequences were determined for both strands and then edited and aligned using Sequencher™ version 4.8. We sequenced two to five specimens from each sample of the four new species treated herein to assess variation. Sample information and GenBank accession numbers for the sequenced specimens utilized in this study are in Table 1.

Sequence divergences (uncorrected *p*-distance) within and between phylogenetic lineages were calculated using MEGA4 (Tamura *et al.*, 2007), with standard errors estimated by 1,000 bootstrap replications with pairwise deletion of missing data. In order to provide a readable tree and reduce computation time, only one example of each haplotype per sample was used in the phylogenetic analyses. Base compositional differences were first evaluated using the  $\chi^2$  test. MrModeltest 2.3 (Nylander, 2004) was used to obtain an appropriate substitution model (using the Akaike Information Criterion) and parameter values for the analyses. Phylogenetic relationships were inferred using four different methods of phylogenetic reconstruction: genetic distance, maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference. The distance, MP and ML analyses were performed using PAUP\*4.0b10 (Swofford, 2002) and the Bayesian analyses were performed using MrBayes 3.04 (Huelsenbeck & Ronquist, 2001). For the distance analysis, an appropriate genetic distance was used to generate a neighbour-joining (NJ) tree (Saitou & Nei, 1987). The MP analysis was conducted with equal weighting, using the heuristic search option with tree bisection–reconnection branch swapping and 100 random additions. The appropriate model was applied for the ML analysis. An NJ tree with appropriate genetic distance was used as the initial topology for branch swapping. Node support was evaluated by 10,000 bootstrap pseudo-replicates except for

**Table 1.** Specimen codes, locality details, GenBank accession numbers and publication references for COI sequences analysed in this study.

Species	Code	Country	State or Province	GenBank accession #	Reference
<i>Aphaostracon</i> sp.	AS	USA	Florida	AF129319	Hershler <i>et al.</i> (1999a)
<i>Aphaostracon</i> sp.	LP	USA	Florida	AF129320	Hershler <i>et al.</i> (1999a)
<i>Chorrobilus crassilabrum</i>	A ( <i>n</i> = 3)	Mexico	Coahuila	HM149777	
<i>Chorrobilus crassilabrum</i>	B	Mexico	Coahuila	HM149778	
<i>Chorrobilus crassilabrum</i>	C	Mexico	Coahuila	HM149779	
<i>Eremopyrgus eganensis</i>		USA	Nevada	AF129317	Hershler <i>et al.</i> (1999a)
<i>Eremopyrgus elegans</i>	A	Mexico	Chihuahua	AF388167	Hershler <i>et al.</i> (2002b)
<i>Eremopyrgus elegans</i>	B	Mexico	Chihuahua	AF388168	Hershler <i>et al.</i> (2002b)
<i>Ipnobius robustus</i>		USA	California	AF061774	Hershler, Mulvey & Liu (1999b)
<i>Juturnia coahuilae</i>		Mexico	Coahuila	AF354761	Liu <i>et al.</i> (2001)
<i>Juturnia kosteri</i>		USA	New Mexico	AF129310	Hershler <i>et al.</i> (1999a)
<i>Juturnia tularosae</i>		USA	New Mexico	AF474371	Hershler <i>et al.</i> (2002a)
<i>Littoridinops monroensis</i>	CA	USA	California	EF490565	Hershler <i>et al.</i> (2007b)
<i>Littoridinops monroensis</i>	SJ	USA	Florida	AF129323	Hershler <i>et al.</i> (1999a)
<i>Littoridinops monroensis</i>	MD	USA	Maryland	AF367644	Wilke <i>et al.</i> (2001)
<i>Littoridinops tenuipes</i>	ME	USA	Maine	EF490566	Hershler <i>et al.</i> (2007b)
<i>Mexipyrgus carranzae</i>		Mexico	Coahuila	AF129325	Hershler <i>et al.</i> (1999a)
<i>Minckleyella balnearis</i>	A	Mexico	Chihuahua	HM149773	
<i>Minckleyella balnearis</i>	B	Mexico	Chihuahua	HM149774	
<i>Phrantela marginata</i>		Australia	Tasmania	AF129331	Hershler <i>et al.</i> (1999a)
<i>Pseudotryonia adamantina</i>		USA	Texas	AF129300	Hershler <i>et al.</i> (1999a)
<i>Pseudotryonia alamosae</i>		USA	New Mexico	AF129303	Hershler <i>et al.</i> (1999a)
<i>Pseudotryonia brevissima</i>		USA	Florida	AF061765	Hershler <i>et al.</i> (1999a)
<i>Pseudotryonia grahamae</i>		USA	Alabama	AF129318	Hershler <i>et al.</i> (1999a)
<i>Pseudotryonia mica</i>	A	Mexico	Chihuahua	HM149772	
<i>Pseudotryonia mica</i>	B	Mexico	Chihuahua	HM149775	
<i>Pseudotryonia pasajae</i>	( <i>n</i> = 4)	Mexico	Durango	HM149776	
<i>Spurwinkia salsa</i>	ME	USA	Maine	AF354765	Liu <i>et al.</i> (2001)
<i>Spurwinkia salsa</i>	MD	USA	Maryland	AF367633	Wilke <i>et al.</i> (2001)
<i>Tryonia aequicostata</i>	LE	USA	Florida	AF129301	Hershler <i>et al.</i> (1999a)
<i>Tryonia aequicostata</i>	AS	USA	Florida	AF129302	Hershler <i>et al.</i> (1999a)
<i>Tryonia clathrata</i>		USA	Nevada	AF061767	Hershler <i>et al.</i> (1999a)
<i>Tryonia imitator</i>	PE	USA	California	AF061769	Hershler <i>et al.</i> (1999a)
<i>Tryonia imitator</i>	MO	USA	California	AF061770	Hershler <i>et al.</i> (1999a)
<i>Tryonia porrecta</i>	CA	USA	California	AY803025	Hershler, Mulvey & Liu (2005)
<i>Tryonia porrecta</i>	NV	USA	Nevada	AY803033	Hershler <i>et al.</i> (2005)
<i>Tryonia salina</i>		USA	California	AF061776	Hershler <i>et al.</i> (1999a)
<i>Tryonia</i> n. sp. 1		Mexico	Chihuahua	AY803035	Hershler <i>et al.</i> (2005)
<i>Tryonia</i> n. sp. 2		Mexico	Chihuahua	AY803038	Hershler <i>et al.</i> (2005)
<i>Tryonia</i> n. sp. 3		Mexico	Chihuahua	AY803037	Hershler <i>et al.</i> (2005)
<i>Tryonia</i> n. sp. 4		Mexico	Chihuahua	AY803039	Hershler <i>et al.</i> (2005)
<i>Zetekina</i> sp. 1		Panama	Veraguas	AF354766	Liu <i>et al.</i> (2001)
<i>Zetekina</i> sp. 2		Panama	Chiriquí	AF354767	Liu <i>et al.</i> (2001)

the ML analysis, in which support values were based on 100 replications. For the Bayesian analysis, three short runs were first conducted using the default random tree option to determine when the log-likelihood sum reached a stable value (by plotting the log-likelihood scores of sample points against generation time). Metropolis-coupled Markov chain Monte Carlo simulations were then run with four chains using the model selected through Modeltest 3.7 for 1,000,000 generations, and Markov chains were sampled at intervals of 10 generations to obtain 100,000 sample points. At the end of the analysis, the average standard deviation of split frequencies was around 0.01, indicating that the runs had reached convergence. The sampled trees with branch lengths were used to generate a 50% majority rule consensus tree with the first 5,000 trees, equal to 50,000 generations, removed to ensure that the chain sampled on a stationary portion.

## SYSTEMATIC DESCRIPTIONS

### Genus *Pseudotryonia* Hershler, 2001

*Type species:* *Tryonia alamosae* Taylor, 1987, by original designation.

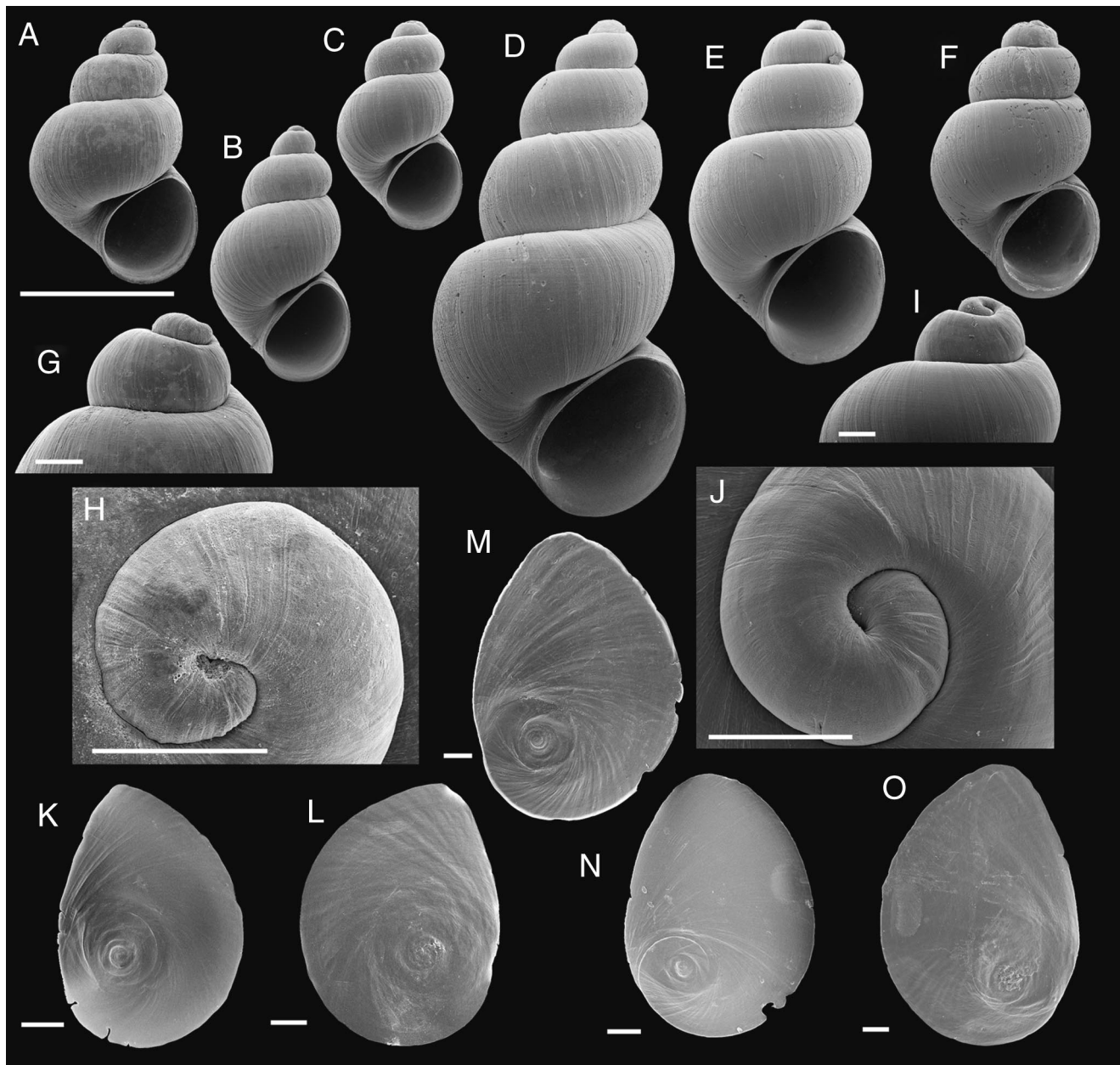
*Diagnosis:* Hershler, 2001: 15–16.

### *Pseudotryonia mica* new species

(Figs 2A–C, G–H, K–L, 3A–E, 4A–C)

*Types:* Holotype, USNM 1001930, Ojo de Dolores, main spring pool, south-southwest of Ciudad Jimenez, Chihuahua, Mexico, 27°1'53.5"N, 104°57'12.0"W, leg. by R.H. and J.J.L., 5/12/2002. Paratypes (from same lot), USNM 1138441.





**Figure 2.** Scanning electron micrographs of shells and opercula of *Pseudotryonia mica* and *P. pasajae*. **A.** Holotype, *P. mica* (USNM 1001930). **B, C.** Paratypes, *P. mica* (USNM 1138441). **D.** Holotype, *P. pasajae* (USNM 1133662). **E, F.** Paratypes, *P. pasajae* (USNM 1138442). **G.** Lateral view of shell apex, *P. mica* (USNM 1138441). **H.** Protoconch, *P. mica* (USNM 1138441). **I.** Lateral view of shell apex, *P. pasajae* (USNM 1138442). **J.** Protoconch, *P. pasajae* (USNM 113844). **K, L.** Opercula (outer, inner sides), *P. mica* (USNM 1138441). **M–O.** Opercula (outer, outer, inner sides), *P. pasajae* (USNM 1138442). Scale bars **A–F,** 1.0 mm; **G–O,** 100  $\mu$ m.

**Etymology:** The specific epithet is from the Latin *mica*, meaning bit or grain (and treated as a substantive noun in apposition), and refers to the small size of this snail.

**Referred material:** Chihuahua, Mexico: USNM 905266, USNM 905270, Ojo de Delores, outflow at northeast end of parking lot, 27°1'55"N, 104°57'10.0"W (estimated), leg. J.J.L. *et al.*, 30/8/1971. USNM 874126, *ibid.*, leg. J.J.L. and D. Wong. USNM 854939, *ibid.*, 27°1'54.6"N, 104°57'10.2"W, leg. R.H. and J.J.L., 10/12/1998.

**Diagnosis:** Shell small (maximum height, 2.2 mm), ovate-conic; penis having a small papilla on both the inner and outer edges distally. Differentiated from congeners having a similar pattern

of penial ornament (*P. adamantina* [Taylor, 1987]; *P. pasajae*, described next) by its less incised teleoconch whorls, absence of a posterior caecum of stomach, shorter pallial section of prostate gland, smaller penial stylet, proximally kinked sperm duct, longer sperm tube and mtCOI sequences (see Remarks below). *Pseudotryonia mica* can be further distinguished from its Mexican congener by its smaller size, broader shell, shorter outer wing of the lateral radular teeth and pigmented seminal receptacle.

**Description:** Shell (Fig. 2A–C) ovate-conic, up to 2.2 mm tall, whorls 3.75–4.50. Male shells (Fig. 2B, C) are smaller and narrower and have relatively smaller apertures than those of females (Fig. 2A; Table 2). Periostracum tan or olive.

**Table 2.** Shell parameters for new species described herein and results of *t*-tests (separate variances) for sexual dimorphism in the two species of *Pseudotryonia*.

	WH	SH	SW	HBW	WBW	AH	AW	SW/SH	HBW/SH	AH/SH
<i>Pseudotryonia mica</i> , holotype, USNM 1001930										
	4.75	1.90	1.20	1.31	1.06	0.80	0.65	0.63	0.69	0.42
<i>P. mica</i> , paratypes, USNM 1138441 (females, <i>n</i> = 13)										
Mean	4.29	1.92	1.17	1.35	1.04	0.81	0.67	0.61	0.71	0.43
SD	0.17	0.13	0.08	0.08	0.06	0.03	0.03	0.03	0.01	0.02
Range	4.0–4.25	1.77–2.20	1.05–1.32	1.25–1.54	0.95–1.15	0.75–0.87	0.64–0.75	0.58–0.66	0.69–0.74	0.40–0.45
<i>ibid.</i> (males, <i>n</i> = 15)										
Mean	4.07	1.54	0.90	1.09	0.82	0.62	0.54	0.58	0.71	0.40
SD	0.20	0.13	0.06	0.08	0.05	0.04	0.03	0.03	0.02	0.02
Range	3.75–4.25	1.32–1.77	0.77–0.98	0.91–1.21	0.72–0.90	0.53–0.67	0.47–0.60	0.55–0.64	0.68–0.75	0.38–0.43
<i>T</i>	3.156	7.612	10.614	8.651	10.455	14.676	10.245	2.664	0.259	3.585
df	26.0	25.8	22.1	25.4	23.8	25.8	25.8	24.6	22.5	25.9
<i>P</i>	0.004*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.013*	0.798	0.001*
<i>P. pasajae</i> , holotype, USNM 1133662										
	5.75	3.57	1.84	2.08	1.62	1.22	1.02	0.52	0.58	0.34
<i>P. pasajae</i> , paratypes, USNM 1138442 ( <i>n</i> = 30)										
Mean	5.89	3.67	1.88	2.14	1.70	1.25	1.05	0.51	0.59	0.34
SD	0.35	0.32	0.12	0.12	0.09	0.07	0.07	0.02	0.03	0.02
Range	5.25–6.25	3.25–4.25	1.71–2.16	1.98–2.39	1.57–1.87	1.16–1.40	0.96–1.20	0.48–0.55	0.53–0.63	0.30–0.37
<i>ibid.</i> (males, <i>n</i> = 15)										
Mean	5.32	2.95	1.53	1.82	1.36	1.07	0.88	0.52	0.62	0.36
SD	0.22	0.24	0.08	0.11	0.07	0.06	0.06	0.02	0.02	0.01
Range	5.00–5.75	2.61–3.36	1.41–1.68	1.69–2.00	1.26–1.47	0.93–1.16	0.78–0.98	0.50–0.55	0.60–0.65	0.34–0.38
<i>T</i>	6.695	8.437	11.257	9.043	14.183	8.970	8.037	–1.025	–4.954	–4.411
df	40.5	36.2	39.2	30.9	35.0	33.8	31.7	25.7	39.9	35.3
<i>P</i>	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.314	0.000*	0.000*
<i>Chorrobrius crassilabrum</i> , holotype, USNM 873243										
	4.25	2.24	1.49	1.66	1.36	1.00	0.87	0.67	0.74	0.45
<i>C. crassilabrum</i> , paratypes, USNM 1138443 ( <i>n</i> = 30)										
Mean	4.15	1.93	1.28	1.46	1.15	0.87	0.74	0.67	0.76	0.45
SD	0.18	0.13	0.06	0.07	0.06	0.04	0.03	0.04	0.03	0.03
Range	3.75–4.5	1.72–2.18	1.19–1.44	1.33–1.64	1.04–1.31	0.81–0.95	0.68–0.78	0.59–0.76	0.70–0.79	0.40–0.51
<i>Minckleyella balnearis</i> , holotype, USNM 874037										
	4.25	2.10	1.39	1.52	1.11	1.01	0.80	0.66	0.73	0.48
<i>M. balnearis</i> , paratypes, USNM 1138444 ( <i>n</i> = 15)										
Mean	3.87	1.66	1.13	1.28	0.93	0.83	0.71	0.68	0.77	0.50
SD	0.30	0.13	0.06	0.08	0.07	0.05	0.05	0.02	0.02	0.03
Range	3.0–4.25	1.47–1.93	1.05–1.23	1.15–1.47	0.85–1.11	0.76–0.92	0.63–0.77	0.64–0.73	0.74–0.80	0.45–0.55

Abbreviations: WH, total number of whorls; SH, shell height; SW, shell width; HBW, height of body whorl; WBW, width of body whorl; AH, aperture height; AW, aperture width; *T*, *t*-value; df, degrees of freedom.

\**P* ≤ 0.05.

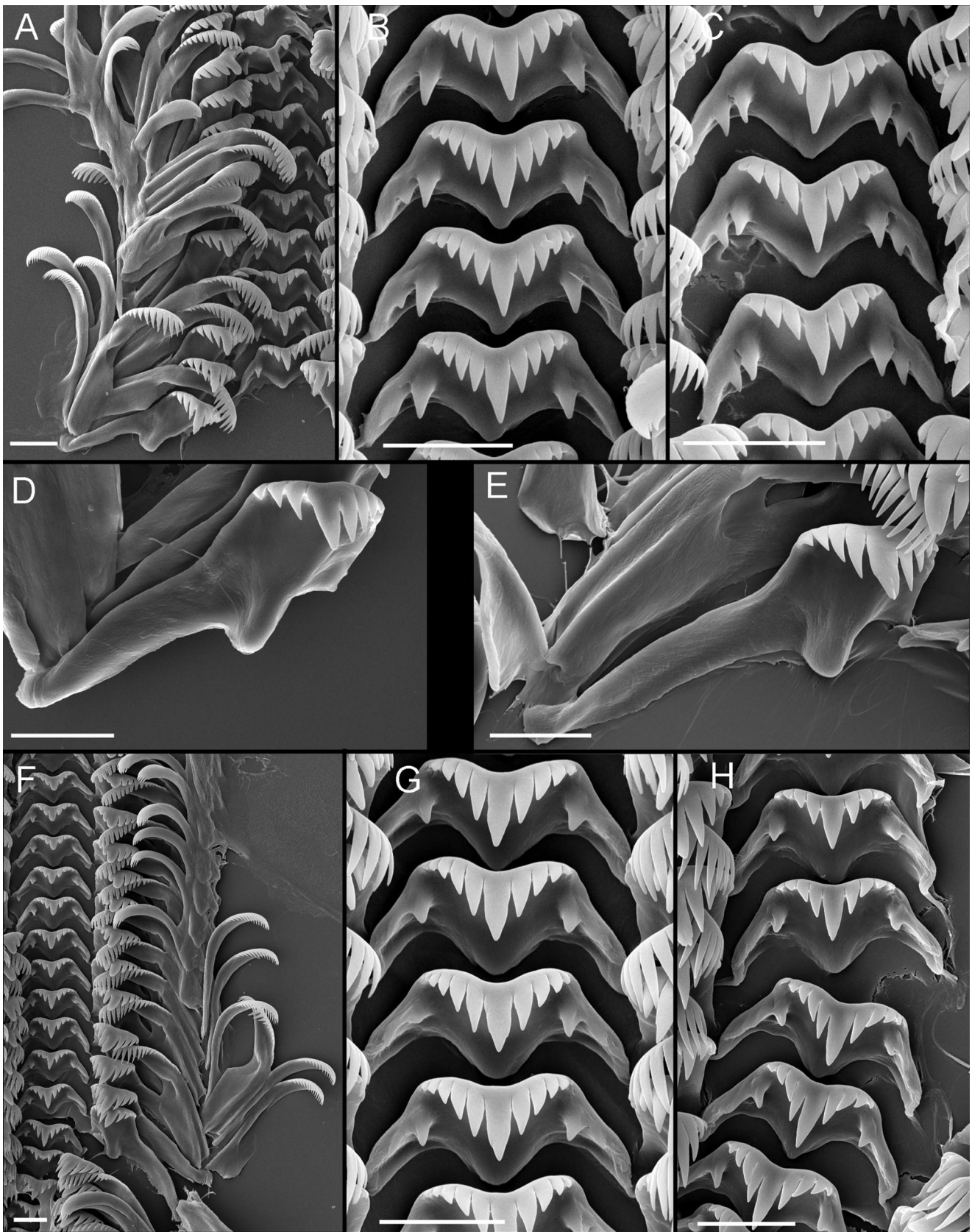
Protoconch and/or initial 1.5 teleoconch whorls often markedly tilted (Fig. 2G). Protoconch surface having a few colabral folds (Fig. 2H). Teleoconch whorls medium to highly convex, evenly rounded, often shouldered, last two whorls often sculptured with numerous spiral striae. Aperture ovate, slightly narrowed adapically, apertural lip usually thin, parietal lip usually adnate, rarely slightly or considerably separated from body whorl, columellar lip sometimes narrowly reflected toward umbilical region. Outer lip orthocline or slightly prosocline, sometimes weakly sinuate. Umbilicus narrow or otherwise small, rarely absent.

Inner (Fig. 2K) and outer (Fig. 2L) sides of operculum smooth. Animal lightly pigmented aside from black coating on dorsal surface of testis. Ctenidial filaments 21–25. Osphradium small, ovate, positioned slightly posterior to middle of ctenidial axis. Posterior caecum of stomach absent. Portion of radular ribbon shown in Figure 3A. Dorsal edge of central radular

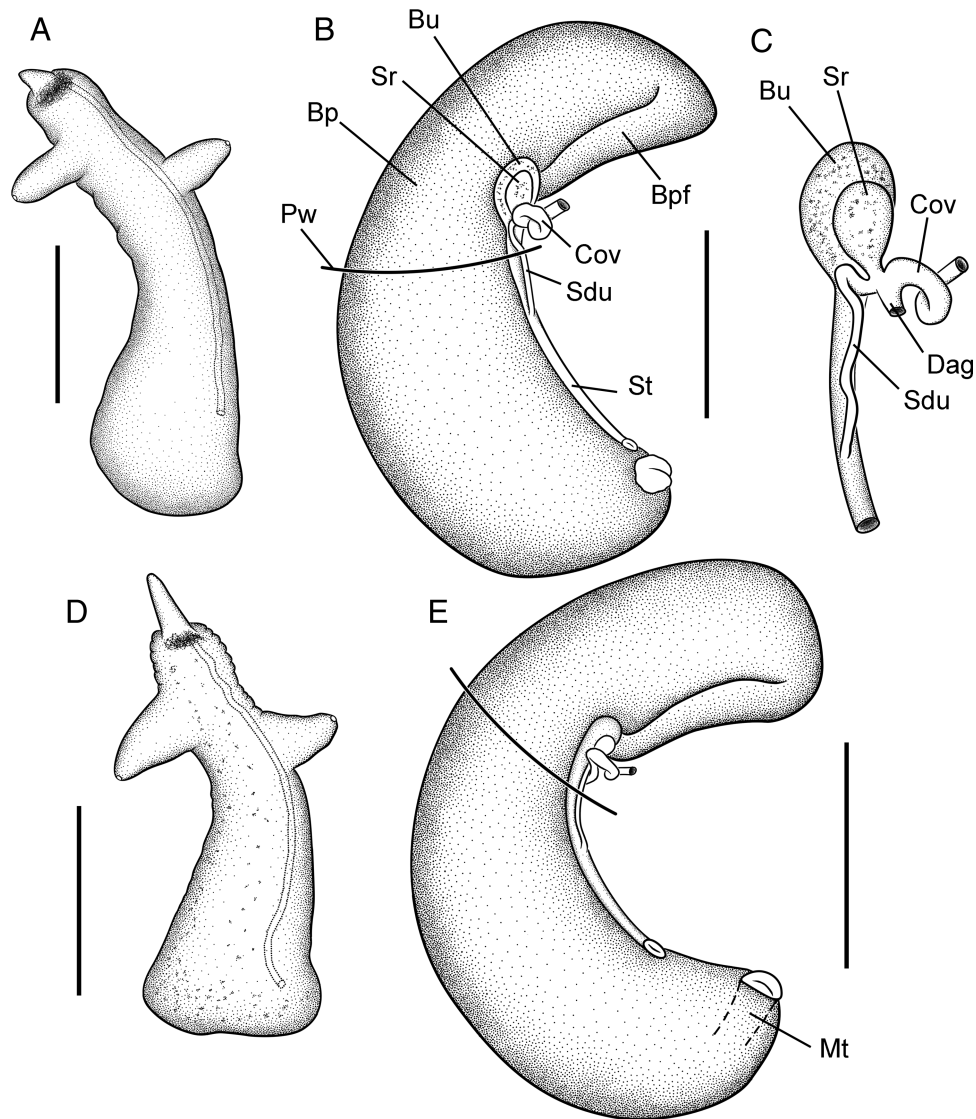
teeth concave, basal tongue V- or U-shaped, median cusps elongate, distally pointed, basally parallel-sided, lateral cusps 3–5, basal cusps 1–2, outer most cusp smaller, sometimes weakly developed (Fig. 3B, C). Lateral teeth (Fig. 3D) having 3–4 cusps on both inner and outer sides, outer wing moderately flexed, length 170% width of tooth face, central cusp distally weakly pointed or rounded. Inner marginal teeth with 14–20 cusps, outer marginal teeth with 23–30 cusps.

Prostate gland small, about 25% in pallial roof, bean-shaped. Penis (Fig. 4A) medium-sized, narrow, slightly tapering distally. Penial duct narrow, nearly straight, near outer edge, stylet small. Penis having a small, narrow papilla on both the inner and outer edges distally (28/29 specimens examined); one specimen differed in lacking a papilla on inner edge. Penis having dark (internal) pigment patch near distal tip, otherwise pigmented with scattered black granules. Brood pouch and associated structures shown in Figure 4B, C. Coiled oviduct a vertical or





**Figure 3.** Scanning electron micrographs of radula of *Pseudotryonia mica* (USNM 1138441) and *P. pasajae* (USNM 1138442). **A.** Portion of radular ribbon, *P. mica*. **B, C.** Central teeth, *P. mica*. **D.** Lateral tooth, *P. mica*. **E.** Lateral and inner marginal teeth, *P. pasajae*. **F.** Portion of radular ribbon, *P. pasajae*. **G, H.** Central teeth, *P. pasajae*. Scale bars = 10  $\mu\text{m}$ .



**Figure 4.** Reproductive anatomy of *Pseudotryonia mica* (USNM 874126) and *P. pasajae* (USNM 1138442). **A.** Penis (dorsal surface), *P. mica*. **B, C.** Female brood pouch and associated structures (**C**, viewed from left side, embryos not shown; close-up of sperm pouches and associated ducts), *P. mica*. **D.** Penis (dorsal surface), *P. pasajae*. **E.** Female brood pouch and associated structures (viewed from left side, embryos not shown), *P. pasajae*. Scale bars **A, B**, 250  $\mu\text{m}$ ; **D, E**, 500  $\mu\text{m}$ . Abbreviations: Bp, brood pouch; Bpf, posteriorly folded section of brood pouch; Bu, bursa copulatrix; Cov, coiled oviduct; Dag, connection between coiled oviduct and albumen gland; Mt, muscular tube at distal end of brood pouch; Pw, posterior wall of pallial cavity; Sdu, sperm duct; Sr, seminal receptacle; St, sperm tube.

posterior-oblique loop. Brood pouch containing about seven embryos, opening terminal, sphincter-like. Albumen gland positioned on right side of bursa copulatrix. Bursa copulatrix ovate, lightly pigmented. Seminal receptacle about 50–66% size of bursa copulatrix, ovate or globular, lightly pigmented. Sperm duct having small, tight coil proximally, distal section straight or weakly undulating. Sperm tube opening a little behind distal edge of brood pouch, slightly muscularized.

*Distribution and habitat:* This species is endemic to the type locality (Ojo de Dolores), which is in the headwaters of the Río Conchos (Río Grande basin). Ojo de Dolores, also referred to in the literature as El Ojo de la Hacienda Dolores, is a large, thermal (*c.* 30°C) spring pool that has been developed into a heavily utilized public bathing area. *Pseudotryonia mica* was collected from the main pool (Fig. 5A) and the uppermost section of the outflow ditch (Fig. 5B); it was most abundant in silt and on dead plant matter. In both the spring pool and

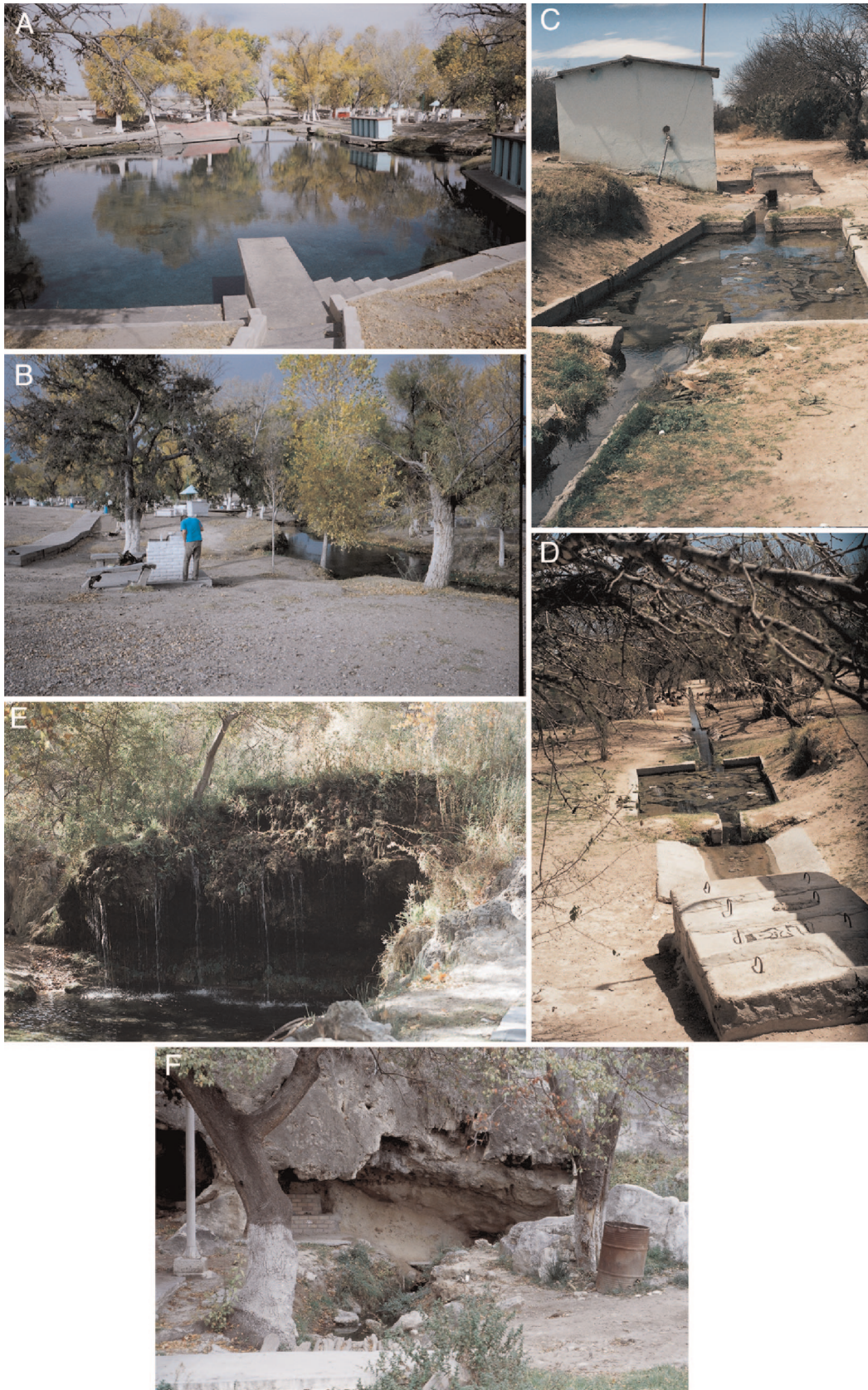
outflow *P. mica* lives in sympatry with *Minckleyella balnearis* (described below). Two species of minnows are also endemic to this spring (Hubbs & Springer, 1957; Miller, 1976), one of which (*Cyprinodon macrolepis*) is listed by the International Union for Conservation of Nature (IUCN) as endangered (Contreras-Balderas & Almada-Villela, 1996a).

*Remarks:* This species and the one described next are placed in *Pseudotryonia* based on the combination of a small number of glandular papilla on the edges of the penis and the nearly straight female sperm duct that opens into the sperm tube in the posterior portion of the pallial cavity (Hershler, 2001). *Pseudotryonia mica* differs from morphologically similar *P. adamantina* and *P. pasajae* by 2.3% and 2.0%, respectively, in its COI sequences and from other members of the genus by 2.5–8.2%.

#### *Pseudotryonia pasajae* new species

(Figs 2E, F, I, J, M–O, 3E–H, 4D, E)





**Figure 5.** Photographs of habitats occupied by species described in this paper. **A.** Ojo de Dolores, Chihuahua, main spring pool, type locality of *Pseudotryonia mica* (photograph taken on 5/12/2001). **B.** Ojo de Dolores, outflow (on upper right), type locality of *Minckleyella balnearis* (5/12/2001). **C, D.** Unnamed spring at El Tanque, Chihuahua, type locality of *P. pasajae*; snails were collected in the large pool in the center of photograph **C** (3/3/2010); photograph **D** was taken at spring source looking downflow (3/3/2010). **E.** Unnamed spring on road to Los Liros (south-southeast of El Chorro), Coahuila, type locality of *Chorrobius crassilabrum* (11/11/2009). **F.** Unnamed spring at roadside park adjacent to Mexico Highway 57 containing *C. crassilabrum* (12/12/1998). This figure appears in colour in the online version of *Journal of Molluscan Studies*.



*Types:* Holotype, USNM 1133662, unnamed spring at El Tanque (second pool below spring source), west-northwest of Cuencamé de Ceniceros, Durango, Mexico, 24°54'32"N, 103°47'7"W (estimated), leg. J.J.L., 12/11/2009. Paratypes (from same lot), USNM 1138442.

*Etymology:* The species name is a noun in the genitive case that commemorates the historic El Camino Real village of Pasaje (see Moorhead, 1995), which is about 2 km northwest of the type locality.

*Diagnosis:* Shell small to medium-sized (maximum height, 4.3 mm), ovate-conic or turritiform; penis having a small papilla on both the inner and outer edges distally. Differs from all other congeners in having a muscular, tube-like distal section of the (female) brood pouch. Further distinguished from closely similar *P. adamantina* by its larger size, smaller shell umbilicus, shorter pallial section of the prostate gland, unpigmented seminal receptacle, slightly muscularized opening of the sperm tube and mtCOI sequences (see Remarks below).

*Description:* Shell (Fig. 2D–F) ovate-conic to turritiform, up to 4.3 mm tall, whorls 5.00–6.50. Male shells (Fig. 2E, F) are smaller and have lower spires and relatively smaller apertures than those of females (Fig. 2D) (also see Table 2). Periostracum tan. Protoconch (Fig. 2I) sometimes tilted, surface sculptured with several folds (Fig. 2J). Teleoconch whorls medium convex, often deeply incised, strongly shouldered, often having distinct subsutural angulation, last 1.5 whorls usually sculptured with numerous fine spiral striae. Aperture ovate, rounded apically, apertural lip thin or slightly thickened, parietal lip usually adnate, rarely slightly separated from body whorl, columellar lip sometimes narrowly reflected towards umbilical region. Outer lip orthocone or slightly prosocline. Umbilicus narrow or otherwise small; less developed in male than in female shells.

Outer side of operculum smooth (Fig. 2M) or with weakly frilled whorl edges (Fig. 2N), inner side smooth (Fig. 2O), sometimes having weak rim. Animal rather darkly pigmented, especially on snout and dorsal surfaces of stomach and testis. Ctenidial filaments 27–30. Osphradium small, narrow, positioned at or slightly posterior to centre of ctenidial axis. Stomach having small posterior caecum. Portion of radular ribbon shown in Figure 3F. Dorsal edge of central radular teeth weakly concave, basal tongue V- or U-shaped, median cusps elongate, distally pointed, basally parallel-sided, lateral cusps 4–5, basal cusps usually 1, rarely 2 (Fig. 3G, H). Lateral teeth (Fig. 3E) having 4–6 cusps on inner and 3–4 cusps on outer side, outer wing moderately flexed, length about 200% width of tooth face, central cusp often curved, distally pointed, sometimes bifurcate. Inner marginal teeth with 18–22 cusps, outer marginal teeth with 18–25 cusps.

Prostate gland small, about 33% in pallial roof, bean-shaped. Penis (Fig. 4D) medium-sized, narrow-rectangular, base and medial section tapering. Penial duct narrow, nearly straight, opening through large, elongate stylet. Penis having a small narrow or conical papilla both on inner edge near distal terminus and on outer edge slightly proximal to the above (16/17 specimens); one specimen differed in lacking a papilla on inner edge. Penis having dark (internal) pigment patch distally, otherwise pigmented with scattered black granules. Brood pouch and associated structures shown in Figure 4E. Coiled oviduct a vertical loop. Brood pouch containing about 10–12 embryos, distal-most section consisting of a short, narrow, muscularized tube (overlapped on the left side by the adjacent thin walled section), terminal opening

sphincter-like. Albumen gland positioned on right side of bursa copulatrix. Bursa copulatrix ovate or globular, lightly pigmented. Seminal receptacle about 50% the size of bursa copulatrix, ovate or globular, unpigmented. Sperm tube opening about 66% into pallial cavity, slightly muscularized.

*Distribution and habitat:* The single locality for this species is a highly modified, mildly thermal (23°C) spring in the upper Rio Nazas drainage (Guzmán basin) that flows into a series of two concrete-lined pools before entering a ditch (Fig. 5C, D). *Pseudotryonia pasajae* was abundant in fine mud at the mouth of the lower pool. The head of the spring has been capped and much of its flow diverted to a pump. The upper pool is used as a washing area by the local human population and the lower spring run is utilized by goats.

*Remarks:* *Pseudotryonia pasajae* differs from other congeners by 2.0–8.2% in its COI sequence.

### ***Chorrobium* new genus**

*Type species:* *Chorrobium crassilabrum* new species, by original designation.

*Etymology:* Refers to the occurrence of this genus in the Cañon el Chorro (canyon of running waters); gender masculine.

*Diagnosis:* Shell small, ovate-conic; penis having a small papilla on both the inner and outer edges. Females ovoviviparous. Bursa copulatrix present, seminal receptacle present, coiled oviduct pigmented, sperm duct opening to bursa copulatrix, sperm tube short. *Chorrobium* is distinguished from all other cochliopid genera by the combination of penis ornamented with a small number of glandular papillae, sperm duct opening into the bursa copulatrix, and short sperm tube. The genus is further differentiated from morphologically similar genera by the broad columellar shelf of the (shell) apertural lip, distally tapered penis and absence of a terminal stylet on the penis. *Chorrobium* also differs from *Ipnobius* by having a bursa copulatrix and by the horizontal shape of the posterior brood pouch coil; from *Pseudotryonia* by its pigmented coiled oviduct and larger bursa copulatrix; from *Tryonia* by the opening of the visceral vas deferens into the ventral edge of the prostate gland; and from the genus described next by the larger number of lateral cusps on the central radular teeth, more tightly coiled renal oviduct and more posteriorly positioned bursa copulatrix.

*Referred species:* *Chorrobium crassilabrum*, described below.

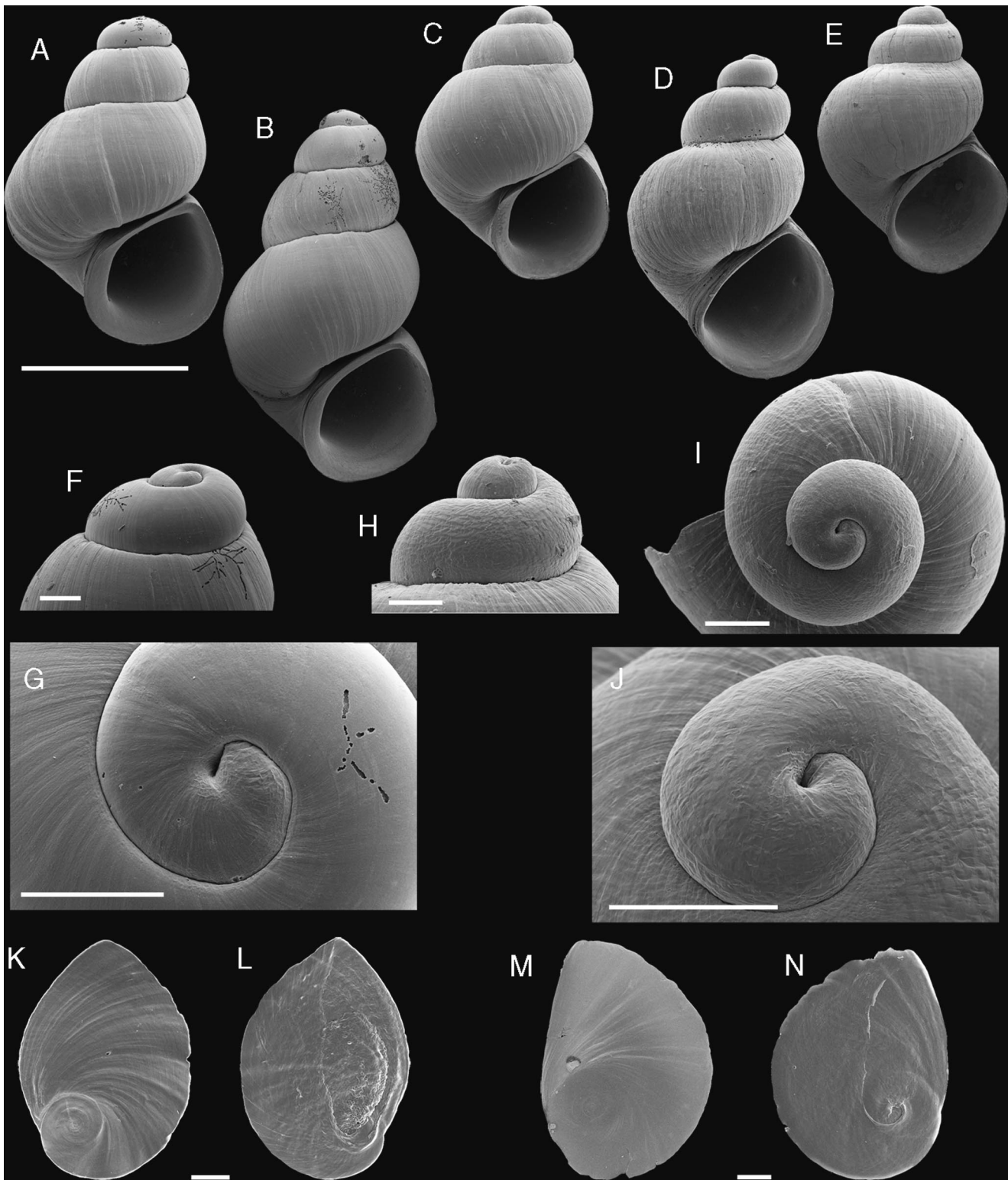
*Remarks:* *Chorrobium* differs from the morphologically similar genera listed above by 8.6–13.6% mtCOI sequence divergence.

### ***Chorrobium crassilabrum* new species**

(Figs 6A–C, F, G, K, L, 7A–D, 8A–C)

*Types:* Holotype, USNM 873243, unnamed spring in private recreation area on road to Los Liros (south-southeast of El Chorro), east-southeast of Saltillo, Coahuila, Mexico, 25°22'49"N, 100°47'20"W (estimated), leg. J.J.L., 5/9/1971. Paratypes (from same lot), USNM 1138443.

*Referred material:* Coahuila, Mexico: USNM 1133662, unnamed spring in private recreation area on road to Los Liros, 25°22'49"N, 100°47'20"W (estimated), leg. J.J.L., 11/11/2009. USNM 873249, unnamed spring beneath Mexico Highway 57 at Los Liros turnoff, 25°22'34"N, 100°47'45"W (estimated), leg. J.J.L., 18/4/1978. USNM 873244, unnamed spring at roadside park adjacent to Mexico Highway 57, 25°22'49"N, 100°47'34"W (estimated), leg. J.J.L., 18/4/1978. USNM

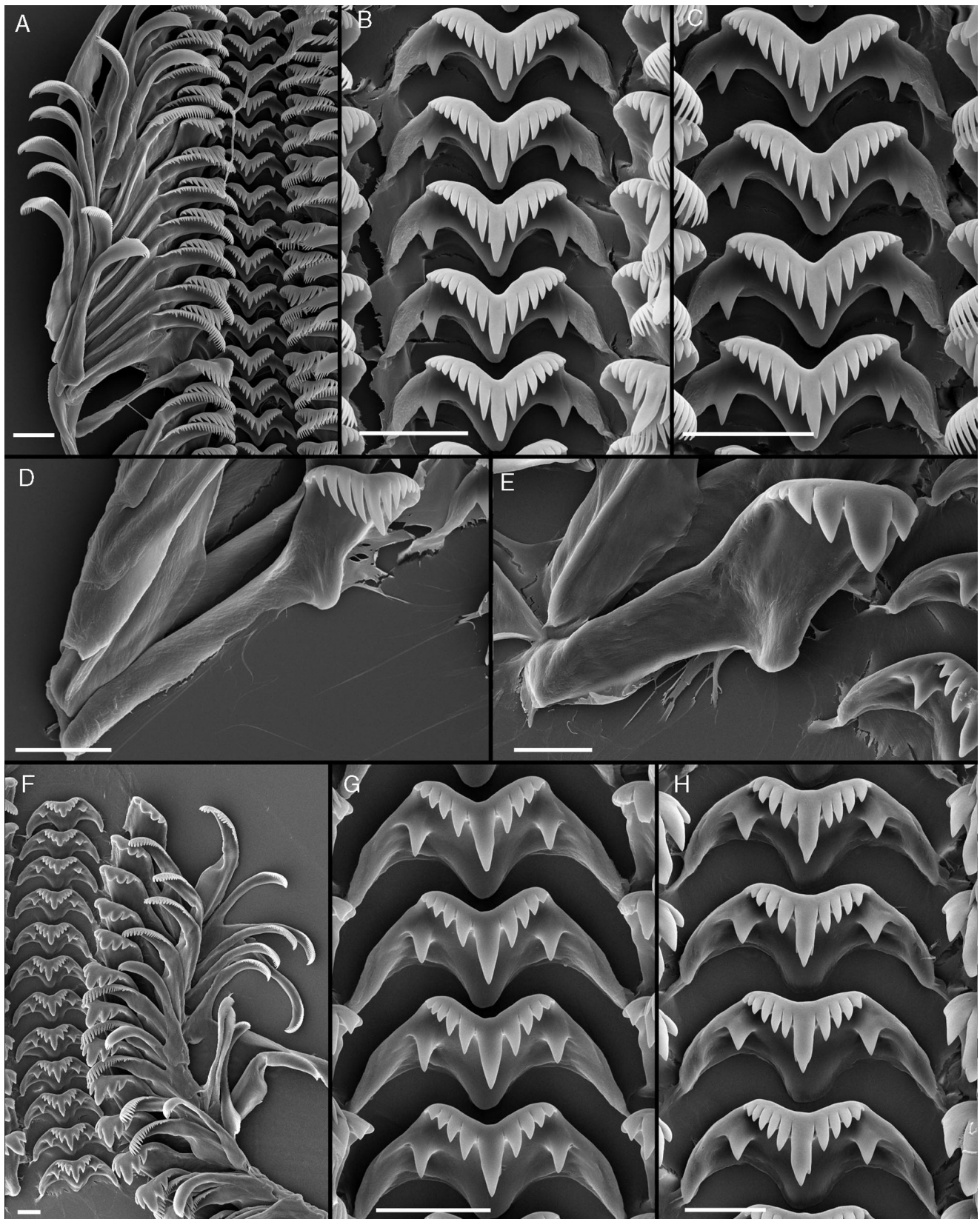


**Figure 6.** Scanning electron micrographs of shells and opercula of *Chorrobilus crassilabrum* and *Minckleyella balnearis*. **A.** Holotype, *C. crassilabrum* (USNM 873243). **B.** Paratype, *C. crassilabrum* (USNM 1138443). **C.** *C. crassilabrum* (USNM 873249). **D.** Holotype, *M. balnearis* (USNM 874037). **E.** Paratype, *M. balnearis* (USNM 1138444). **F.** Shell apex, *C. crassilabrum* (USNM 1138443). **G.** Protoconch, *C. crassilabrum* (USNM 1138443). **H.** **I.** Shell apex, *M. balnearis* (USNM 1138444). **J.** Protoconch, *M. balnearis* (USNM 1138444). **K, L.** Opercula (outer, inner sides), *C. crassilabrum* (USNM 1138443). **M, N.** Opercula (outer, inner sides), *M. balnearis* (USNM 1138444). Scale bars **A–E**, 1.0 mm; **F–N**, 100  $\mu$ m.

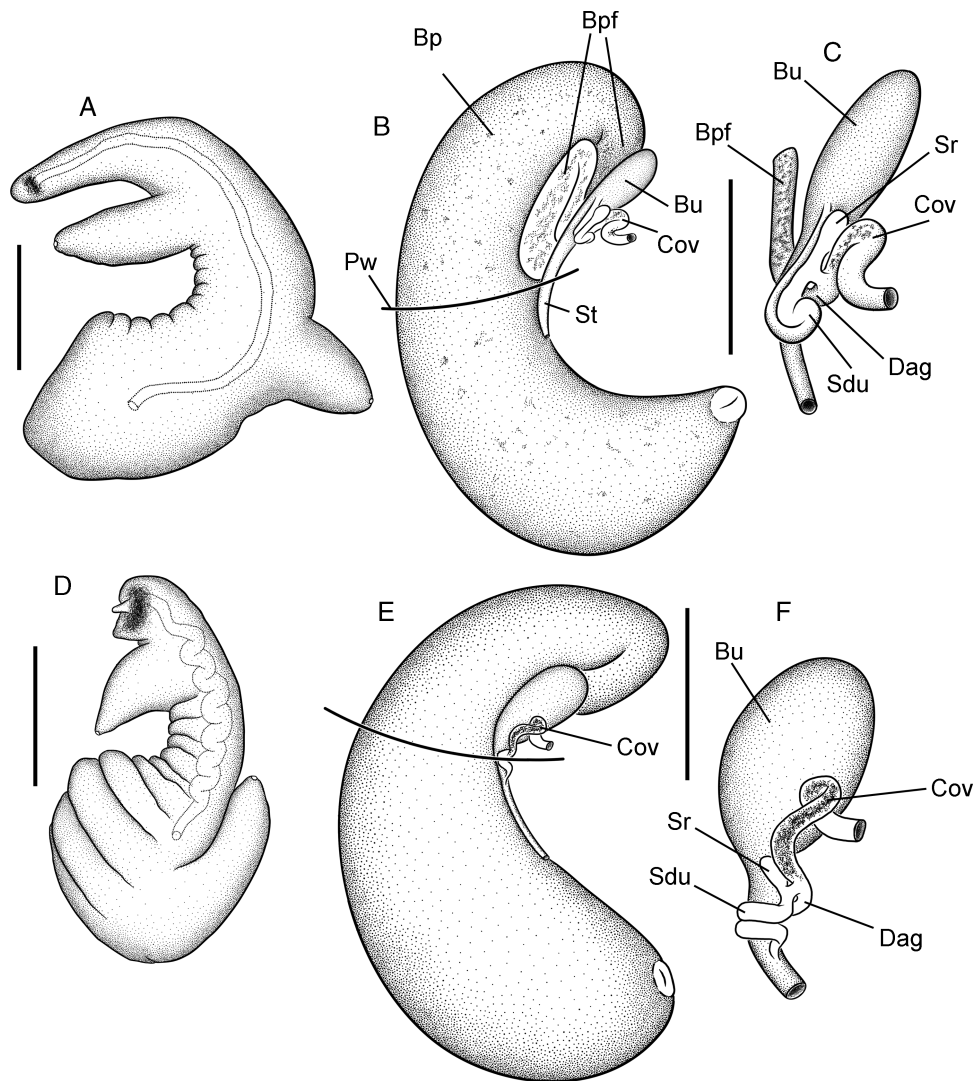
874129, USNM 883178, *ibid.*, leg. J.J.L. and D. Wong, 10/4/1991. BellMNH 21292, Parque El Chorro, head of Cañon El Chorro, leg. D.W. Taylor, 8/10/1968.

*Etymology:* A nominative singular noun, derived from the Latin *crassus* (thick, fat) and *labrum* (lip), that refers to the markedly thickened shell lip in this species.





**Figure 7.** Scanning electron micrographs of radula of *Chorobius crassilabrum* (USNM 1138443) and *Minckleyella balnearis* (USNM 1138444). **A.** Portion of radular ribbon, *C. crassilabrum*. **B, C.** Central teeth, *C. crassilabrum*. **D.** Lateral tooth, *C. crassilabrum*. **E.** Lateral tooth, *M. balnearis*. **F.** Portion of radular ribbon, *M. balnearis*. **G, H.** Central teeth, *M. balnearis*. Scale bars = 10  $\mu\text{m}$ .



**Figure 8.** Reproductive anatomy of *Chorrobrius crassilabrum* (USNM 1138443) and *Minckleyella balnearis* (USNM 1138444). **A.** Penis (dorsal surface), *C. crassilabrum*. **B, C.** Female brood pouch and associated structures (**B**, viewed from left side, embryos not shown; **C**, close-up of sperm pouches and associated ducts). **D.** Penis (dorsal surface), *M. balnearis*. **E, F.** Female brood pouch and associated structures (**E**, viewed from left side, embryos not shown; **F**, close-up of sperm pouches and associated ducts). Scale bars **A**, 125  $\mu\text{m}$ ; **B, D, E**, 250  $\mu\text{m}$ . Abbreviations: Bp, brood pouch; Bpf, posteriorly folded section of brood pouch; Bu, bursa copulatrix; Cov, coiled oviduct; Dag, connection between coiled oviduct and albumen gland; Pw, posterior wall of pallial cavity; Sdu, sperm duct; Sr, seminal receptacle; St, sperm tube.

*Diagnosis:* As for genus.

*Description:* Shell (Fig. 6A–C) ovate-conic, up to 2.2 mm tall, whorls 3.75–4.5, sexual dimorphism not readily apparent. Periostracum thin, tan. Apex (Fig. 6F) blunt, protoconch (Fig. 6G) small (about 0.5 whorl), coarsely wrinkled. Teleoconch whorls weakly convex, evenly rounded, sometimes narrowly shouldered, sometimes sculptured with a few evenly spaced spiral lines. Aperture medium-sized, pyriform, apertural lip thickened; parietal lip complete, adnate or slightly separated from body whorl, narrowly reflected; columellar lip broadly reflected onto umbilical region. Outer lip prosocline, sinuate. Umbilicus narrow or absent.

Operculum thin, ovate, multispiral, nucleus highly eccentric, outer side smooth (Fig. 6K), inner side slightly thickened along attachment scar margin near nucleus (Fig. 6L). Head-foot almost pale to near uniform dark brown, pallial roof and visceral coil dark brown dorsally. Ctenidium well developed, connected to pericardium by short efferent vein, ctenidial filaments 10–12, finger-like, lateral surfaces smooth. Osphradium

short, narrow, positioned slightly posterior to centre of ctenidial axis. Kidney having rather large pallial portion. Style sac about as long as remainder of stomach, posterior caecum of stomach absent. Portion of radular ribbon shown in Figure 7A. Central teeth (Fig. 7B, C) trapezoidal, dorsal edge of teeth strongly concave, lateral side of teeth convex, basal tongue V- or U-shaped, median cusps elongate, distally pointed and sometimes bi- or trifurcate, basally parallel-sided, lateral cusps 7–10, basal cusps 1–2, outer most cusp weakly developed. Lateral teeth (Fig. 7D) having 4–6 cusps on inner side and 5–7 cusps on outer side, outer wing narrow, strongly flexed, length 250% width of tooth face, central cusp curved, distally pointed, basal tongue weakly developed. Inner marginal teeth with 26–32 cusps, outer marginal teeth with 24–31 cusps, inner edges of both marginal teeth having rectangular wings.

Testis abutting posterior edge of stomach, overlapping most of digestive gland, composed of compound lobes; seminal vesicle exiting from near anterior end of testis. Prostate gland medium-



sized, bean- or banana-shaped, about 50% in pallial roof. Visceral vas deferens opening to prostate gland ventrally near posterior edge. Penis (Fig. 8A) medium-sized, narrow, inner edge from base to edge of papilla having small folds, distal section tapering, often coiled, stylet absent. Penial duct narrow, positioned near centrally, weakly undulating along most of length. Penis having a small, narrow or conical papilla both on the inner edge distally and on the outer edge at or slightly proximal to mid-length (28/30 specimens); one specimen differed in lacking a papilla on inner edge and another had two (very small) papillae on both the inner and outer edges. Penis entirely pale or having a small brown (internal) pigment patch near distal tip. Females ovoviviparous, brood pouch containing about 15–20 embryos, most of which are very small. Ovary a small, simple sac posterior to stomach. Brood pouch and associated structures shown in Figure 8B, C. Brood pouch thin-walled, posterior section having two horizontal, lightly pigmented folds, anterior opening terminal, slightly muscularized. Albumen gland very small, positioned on right side of sperm tube. Coiled oviduct a single, tight, posterior-oblique loop, lightly pigmented, opens to distal end of albumen gland. Bursa copulatrix small, usually narrow, sometimes ovate, posterior edge about even with posterior edge of brood pouch, duct exiting from anterior edge. Seminal receptacle much smaller than bursa copulatrix, pouch-like, positioned on anteroventral portion of bursa copulatrix, duct medium length. Sperm duct elongate, proximal section having single tight coil, distal section looping posteriorly along left side of sperm tube, opening to anterior half of bursa copulatrix. Sperm tube narrow, opening in posterior 33% of pallial cavity.

*Distribution and habitat:* *Chorrobium crassilabrum* is distributed in a series of small, mildly thermal (18–19°C) springs closely proximal to Mexico Highway 57 at or just south of the head of Cañon el Chorro, Río Salado (Río Grande) basin. Snails were found on rocks and decaying plant matter in three springs (two of which are shown in Fig. 5E, F) and may be present in others within this small, but well watered area, which is sometimes referred to as El Chorro de Arteaga (Dinerstein *et al.*, 2001). An undescribed species of *Pyrgulopsis* (Hydrobiidae) also lives in these waters (unpubl.) as does one of the few remaining populations (Contreras-Balderas, 1969; Miller, Minckley & Norris, 2005) of the endangered (Contreras-Balderas & Almada-Villela, 1996b) Saltillo chub (*Gila modesta*). This spring complex has been impacted by the introduction of exotic fish and by recreational activities (Dinerstein *et al.*, 2001); it has been designated as a national park and is listed by the World Wildlife Fund as an important site for conservation of freshwater biodiversity in the Chihuahuan Desert (Dinerstein *et al.*, 2001) and in North America generally (Abell *et al.*, 2000).

*Remarks:* Dwight W. Taylor may have been the first to collect this species (in 1968); he identified it as a *Tryonia* and used an unpublished manuscript name for it on his original field label (BellMNH 21292). *Chorrobium crassilabrum* or the undescribed species of *Pyrgulopsis* which also lives in the Cañon el Chorro springs may be the ‘hydroboid [sic] snail’ reported from this area by Dinerstein *et al.* (2001: 110).

### ***Minckleyella* new genus**

*Type species:* *Minckleyella balnearis* new species, by original designation.

*Etymology:* Named after Wendell L. Minckley, a renowned ichthyologist, ecologist and conservationist (Collins *et al.*, 2002) who led the pioneering surveys of aquatic ecosystems in

northern Mexico during the late 1960s and early 1970s that resulted in the discovery of this snail and many other previously undescribed species. Gender feminine.

*Diagnosis:* Shell small, ovate-conic; penis having a large papilla on both the inner and outer edges. Females ovoviviparous. Bursa copulatrix present, seminal receptacle present, coiled oviduct pigmented, sperm duct opening to sperm tube, sperm tube short. *Minckleyella* is distinguished from all other cochliopid genera by having the combination of a penis ornamented with a small number of glandular papillae and a coiled sperm duct that opens into the pallial section of the sperm tube. The genus is further differentiated from other morphologically similar genera by having a distally elongate section of the renal oviduct. *Minckleyella* also differs from *Ipnobius* by having a bursa copulatrix and having a papilla on the inner edge of penis; from *Pseudotryonia* by its pigmented coiled oviduct and larger bursa copulatrix; and from *Tryonia* by the opening of the visceral vas deferens into the ventral edge of the prostate gland.

*Referred species:* *Minckleyella balnearis*, described below.

*Remarks:* *Minckleyella* differs from morphologically similar genera (*Chorrobium*, *Ipnobius*, *Minckleyella*, *Tryonia*) by 5.2–13.5% mtCOI sequence divergence.

### ***Minckleyella balnearis* new species**

(Figs 6D, E, H–J, M, N, 7E–H, 8D–F)

*Types:* Holotype, USNM 874037, Ojo de Dolores (outflow at northeast end of parking lot), south-southwest of Ciudad Jimenez, Chihuahua, Mexico, 27°1'55"N, 104°57'10"W (estimated), leg. J.J.L. 30/8/1971. Paratypes (from same lot), USNM 1138444, USNM 873253.

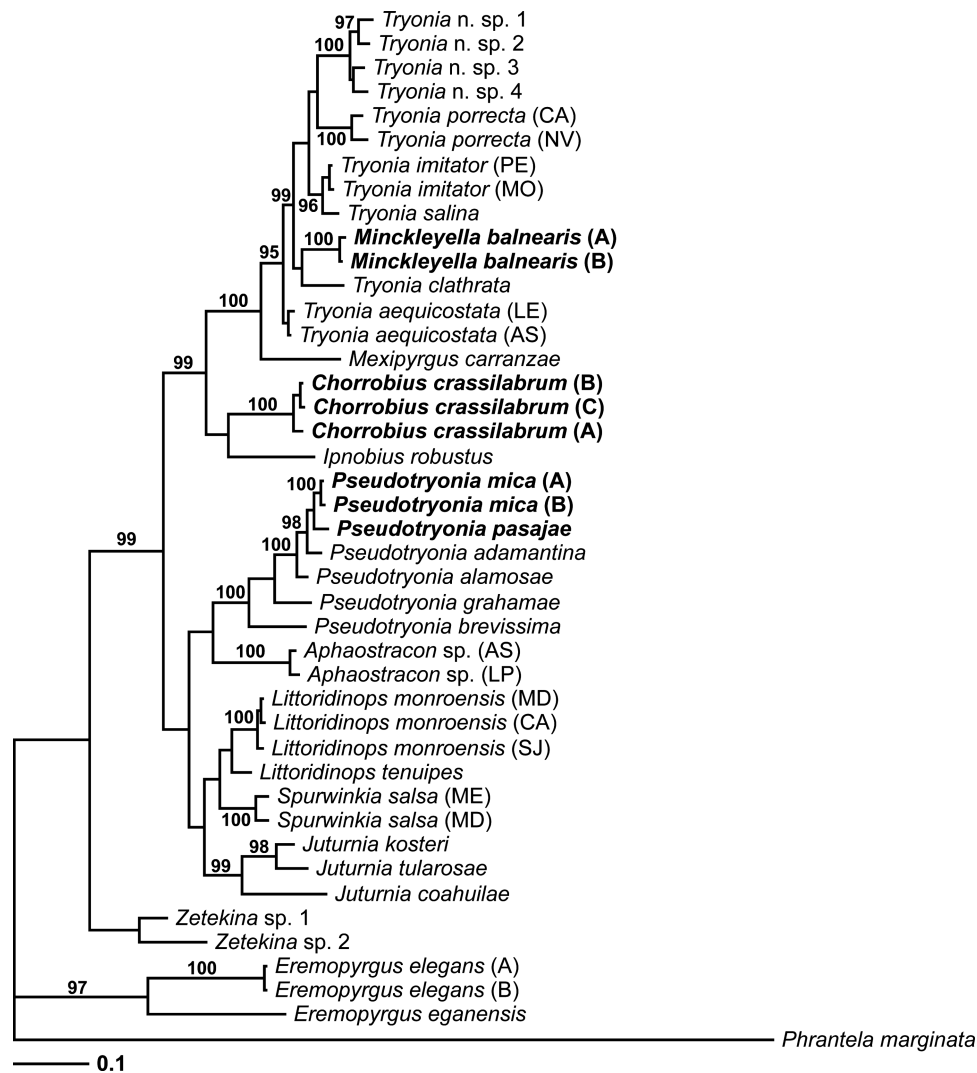
*Referred material:* Chihuahua, Mexico: USNM 905271, Ojo de Dolores (outflow at northeast end of parking lot), 27°1'54.6"N, 104°57'10.2"W, leg. J.J.L. and D. Wong, 5/5/1991. USNM 1001928, Ojo de Dolores (main spring pool), 27°1'56.5"N, 104°57'9.7"W, leg. R.H. and J.J.L., 12/5/2002. USNM 1001929, Ojo de Dolores (outflow from main spring pool), 27°1'53.5"N, 104°57'12.0"W, leg. R.H. and J.J.L., 12/5/2002.

*Etymology:* From the Latin adjective *balnearis*, meaning of a bath, and referring to the occurrence of this snail in a spring that has been modified into a public bathing area.

*Diagnosis:* As for genus.

*Description:* Shell (Fig. 6D, E) ovate-conic, up to 2.2 mm tall, whorls 3.00–4.50, sexual dimorphism not readily apparent. Periostracum thin, light tan. Apex (Fig. 6H) slightly tilted, often eroded, protoconch extent not clearly delineated; initial 2.0 whorls (Fig. 6I, J) weakly wrinkled, sculpture sometimes spirally arranged or cancellate. Teleoconch whorls medium to highly convex, often shouldered and distinctly angled below the suture, later portion having a variable number of evenly spaced spiral lines, some of which may be prominent. Aperture large, D-shaped, slightly angled above, parietal lip complete, slightly thickened, adnate or slightly separated from body whorl; columellar lip slightly thickened, often narrowly reflected onto umbilical region. Outer lip thin, prosocline, weakly sinuate. Umbilicus small or absent.

Operculum thin, ovate, multispiral, nucleus eccentric, outer side (Fig. 6M) smooth, inner side (Fig. 6N) smooth or slightly thickened along inner edge and/or near nucleus. Head–foot lightly pigmented apart from brown snout, pallial roof and visceral coil pale aside from dark pigment on dorsal surfaces of stomach and testis. Ctenidium well developed, abutting



**Figure 9.** Bayesian tree based on the COI data set. Posterior probabilities are provided when >95%. Sequences for the species described herein are in bold face. Terminals and specimen codes are labeled as in Table 1.

pericardium, ctenidial filaments 17–19, broadly triangular, lateral surfaces pleated. Osphradium small, narrow, centrally positioned along ctenidial axis. Kidney having short pallial portion. Style sac about as long as remainder of stomach, posterior caecum of stomach small. Portion of radular ribbon shown in Figure 7F. Central teeth (Fig. 7G, H) trapezoidal, dorsal edge of teeth weakly to moderately concave, lateral sides of teeth convex, basal tongue U-shaped, median cusps elongate, distally pointed, sometimes basally parallel-sided, lateral cusps 4–6, basal cusps 1. Lateral teeth (Fig. 7E) having 2–3 cusps on inner side and 3–4 cusps on outer side, outer wing broad, moderately flexed, length 130% width of tooth face, central cusp broad, distally pointed, basal tongue well developed. Inner marginal teeth with 20–31 cusps, outer marginal teeth with 27–35 cusps, inner edges of both marginal teeth having rectangular wings.

Testis overlapping posterior section of stomach, overlapping most of digestive gland, composed of compound lobes; seminal vesicle opening from well behind anterior end of testis. Prostate gland medium-sized, bean-shaped, about 33% in pallial roof. Visceral vas deferens opening to prostate gland ventrally near posterior edge. Penis (Fig. 8D) medium-sized, basally expanded, medial and distal sections slightly tapering, inner edge from base to edge of papilla having deep folds, distal edge

weakly rounded, slightly expanded on inner side, stylet small. Penial duct rather broad, positioned near centrally, strongly undulating along most of length. Penis having a large papilla on the inner edge distally and a somewhat larger papilla originating from outer edge and adjacent dorsal surface of base (29/30 specimens); one specimen differed in having two papillae on the inner edge, the distal of which was very small. Penis darkly pigmented near distal tip, medial and basal sections pale or brown. Females ovoviparous, brood pouch containing about 5–7 embryos. Ovary a small, simple sac posterior to stomach. Brood pouch and associated structures shown in Figure 8E, F. Brood pouch thin-walled, posterior section folded, anterior opening terminal, slightly muscularized. Albumen gland very small, positioned on right side of bursa copulatrix. Coiled oviduct consisting of a small, posterior-oblique loop proximally and an elongate, weakly arched distal section, pigmented, opens to distal end of albumen gland. Bursa copulatrix small, ovate, positioned well anterior to posterior edge of brood pouch, duct exiting from anterior edge. Seminal receptacle much smaller than bursa copulatrix, sac-like, positioned along anteroventral portion of bursa copulatrix, duct short. Sperm duct having a single vertical coil, opens to proximal portion of sperm tube a little in front of posterior wall of pallial cavity. Sperm tube narrow, opening in posterior 33% of pallial cavity.



*Distribution and habitat:* *Minckleyella balnearis* is endemic to Ojo de Dolores (described above in the treatment of *P. mica*) where it has been collected in the main spring pool and upper section of the outflow. Specimens were mostly commonly found on submerged vegetation and decaying plant matter near the edges of these water bodies.

## MOLECULAR ANALYSES

Thirteen specimens were newly sequenced for this study and are deposited in GenBank under accession numbers HM149772–149779. A total of 658 bp of COI was analysed, of which 263 (40.0%) were variable and 205 (31.2%) were parsimony-informative. Overall nucleotide composition was biased towards thymine (T) (39.3%) and adenine (A) (24.9%), followed by guanine (G) (18.8%) and cytosine (C) (17.1%), as typically observed in gastropod mitochondrial genes (e.g. Hershler, Liu & Thompson, 2003). There was no significant frequency bias among species ( $\chi^2 = 71.47$ ,  $df = 126$ ,  $P = 0.99$ ).

The MP analysis yielded sixteen equally parsimonious trees of 823 steps (CI = 0.46, RI = 0.70). MrModeltest selected the HKY model, with some sites assumed to be invariable and with variable sites assumed to follow a discrete gamma distribution (e.g. HKY + I + G), as the best fit for the combined dataset using the Akaike Information Criterion. The HKY + I + G model was used for the Bayesian analysis. In the Bayesian analysis, the ln likelihood scores started at around -8,700 and quickly converged upon a stable value of about -4,950 after c. 5,000 generations. HKY distance was used to generate an NJ tree based on the clustering method of Saitou & Nei (1987). The ML analysis was based on the HKY + I + G model.

The NJ, ML, MP and Bayesian analyses were generally concordant in relevant details and thus we illustrated only the Bayesian topology (Fig. 9). *Eremopyrgus* and *Zetekina* were basal to two variably supported clades that contained the rest of the taxa. *Pseudotryonia* was consistently resolved as a well-supported monophyletic group (in one of these clades) within which *P. mica* and *P. pasajae* formed a variably supported terminal subunit sister to *P. adamantina*. The other clade contained *Tryonia* and the rest of the *Tryonia*-like genera. *Chorrobius* and *Ipobius* were depicted as either a weakly supported subclade (Bayesian, ML, NJ, 10/16 MP trees) or as successive basal lineages (6/16 MP trees). *Mexipyrgus*, *Minckleyella* and *Tryonia* consistently formed a subclade, although this relationship was strongly supported only in the Bayesian analysis (100%). *Mexipyrgus* was basally positioned within this subclade; *Minckleyella* was either nested within (ML, NJ, Bayesian, 2/16 MP trees) or sister to *Tryonia* (12/16 MP trees).

## DISCUSSION

*Tryonia sensu lato* was previously shown to contain three anatomically distinctive and genetically divergent lineages (Hershler et al., 1999a; Liu et al., 2001) that are currently recognized as separate genera (Hershler, 2001). The present study has delineated two additional such lineages of *Tryonia*-like snails in northern Mexico. One of these (*Chorrobius*) was depicted (with weak support) as closely related to *Ipobius* in some of our molecular phylogenetic analyses, but we have not found any morphologic evidence supporting this relationship. *Chorrobius* and *Ipobius* are well differentiated from other *Tryonia*-like snails both morphologically and genetically (COI sequence divergence 8.6–12.5% and 8.4–11.8%, respectively) and probably represent either old or rapidly evolved lineages. The other divergent lineage detected in our study was resolved as a distinct, monophyletic group in only a small portion of the

molecular phylogenetic analyses, while in the others it was nested within the genus *Tryonia*. Despite this finding, we have nonetheless described this snail as a new (monotypic) genus (*Minckleyella*) because of its large divergence ( $5.2 \pm 0.6\%$ ) relative to *Tryonia* (sequence variation among species of the latter is  $4.5 \pm 0.5\%$ ) and because it has a unique combination of anatomical features that precludes its assignment to any currently recognized cochliopid genus. We also consider the alternative option of maintaining monophyly by treating the entire *Minckleyella* + *Mexipyrgus* + *Tryonia* clade as a single genus to be inappropriate because it lumps highly divergent lineages together and the resulting assemblage cannot be diagnosed morphologically. Regardless of how the unusual species that we have described as *Minckleyella balnearis* is treated taxonomically, its apparently close molecular phylogenetic relationship with morphologically divergent *Tryonia* raises interesting questions about the process of evolutionary diversification in this component of the cochliopid radiation that merit additional study. The use of additional genetic markers will also enable a more robust test of whether *M. balnearis* is phylogenetically distinct from the *Tryonia* clade.

The small number of new species that have been described subsequent to Hershler & Thompson's (1992) review of the cochliopids (12 including those described herein; Hershler & Velkovrh, 1993; Hershler, 1999; Thompson, 2001; Hershler et al., 2002a, b; Thompson & Hershler, 2002; Pons da Silva & Veitenheimer-Mendes, 2004) does not appear to be consistent with their hypothesis that the diversity of the temperate and tropical American fauna is poorly known and will eventually be greatly increased, but this is surely a reflection of paucity of recent field surveys rather than an actual slowing of discovery rate. Note that in addition to the novelties erected herein, three other new genera have been described from (widely separated parts of) the region since 1992 (*Andesipyrgus*, northern South America; *Eremopyrgus*, Great Basin; *Tepalcatia*, central Mexico), which suggests that more divergent cochliopid lineages will likely be discovered with further exploration of poorly known areas. Our studies of *Tryonia sensu lato* also suggest that anatomical and molecular investigations of the other large cochliopid genera of the region requiring revision (*Aroapyrgus*, *Cochliopina*, *Heleobia* and *Pyrgophorus*; Hershler & Thompson, 1992) may lead to the detection of additional clades hidden within the confines of traditional taxonomy.

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## REFERENCES

- ABELL, R.A., OLSON, D.M., DINERSTEIN, E., HURLEY, P.T., DIGGS, J.T., EICHBAUM, W., WALTERS, S., WETTENGEL, W., ALLNUTT, T., LOUCKS, C.J. & HEDAO, P. 2000. *Freshwater*

- ecoregions of North America – a conservation assessment*. Island Press, Washington, DC and Covelo, CA.
- BUCKLIN, A. 1992. Use of formalin-preserved samples for molecular analysis. *Newsletter of Crustacean Molecular Techniques*, **2**: 3.
- COLLINS, J.P., DEACON, J., DOWLING, T. & MARSH, P. 2002. Wendell Lee Minckley 1935–2001. *Copeia*, **2002**: 258–262.
- CONTRERAS-BALDERAS, S. 1969. Perspectivas de la ictiofauna en las zonas áridas del Norte de México. In: [*Proceedings*] *International symposium on increasing food production in arid lands; 22–25 April 1968; Monterrey, Mexico* (T.W. Box & P. Rojas-Mendoza, eds), pp. 293–304. International Center for Arid and Semi-arid Land Studies, Texas Technological College, Lubbock, ICASALS Publication 3.
- CONTRERAS-BALDERAS, S. & ALMADA-VILLELA, P. 1996a. *Cyprinodon macrolepis*. In: *IUCN 2010. IUCN Red List of Threatened Species. Version 2010.1*. Available from <http://www.iucnredlist.org/apps/redlist/details/6156/0> (accessed 1 April 2010).
- CONTRERAS-BALDERAS, S. & ALMADA-VILLELA, P. 1996b. *Gila modesta*. In: *IUCN 2010. IUCN Red List of Threatened Species. Version 2010.1*. Available from <http://www.iucnredlist.org/apps/redlist/details/9188/0> (accessed 1 April 2010).
- DINERSTEIN, E., OLSON, D., ATCHLEY, J., LOUCKS, C., CONTRERAS-BALDERAS, S., ABELL, R., IÑIGO, E., ENKERLIN, E., WILLIAMS, C. & CASTILLEJA, G. 2001. *Ecoregion-based conservation in the Chihuahuan Desert – a biological assessment*. 2nd printing with corrections. World Wildlife Fund, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), The Nature Conservancy, PRONATURA Noreste, and the Instituto Tecnológico y de Estudios Superiores de Monterrey (ITESM). Available from <http://www.worldwildlife.org/what/wherewework/chihuahuandesert/WWFBinaryitem2757.pdf> (accessed 1 April 2010).
- 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.
- HERSHLER, R. 1985. Systematic revision of the Hydrobiidae (Gastropoda: Rissoacea) of the Cuatro Ciénegas basin, Coahuila, Mexico. *Malacologia*, **26**: 31–123.
- HERSHLER, R. 1999. A systematic review of the hydrobiid snails (Gastropoda: Rissooidea) of the Great Basin, western United States. Part II. Genera *Colligyrus*, *Eremopyrgus*, *Fluminicola*, *Pristinicola*, and *Tryonia*. *Veliger*, **42**: 306–337.
- HERSHLER, R. 2001. Systematics of the North and Central American aquatic snail genus *Tryonia* (Rissooidea: Hydrobiidae). *Smithsonian Contributions to Zoology*, **612**: 1–53.
- HERSHLER, R., DAVIS, C.L., KITTING, C.L. & LIU, H.-P. 2007b. Discovery of introduced and cryptogenic cochliopid gastropods in the San Francisco Estuary, California. *Journal of Molluscan Studies*, **73**: 323–332.
- HERSHLER, R., LIU, H.-P., FREST, T.J. & JOHANNES, E.J. 2007a. Extensive diversification of pebblesnails (Lithoglyphidae: *Fluminicola*) in the upper Sacramento River basin, northwestern United States. *Zoological Journal of the Linnean Society*, **149**: 371–422.
- HERSHLER, R., LIU, H.-P. & LANDYE, J.J. 2002b. A new species of *Eremopyrgus* (Hydrobiidae: Cochliopinae) from the Chihuahuan Desert, Mexico: phylogenetic relationships and biogeography. *Journal of Molluscan Studies*, **68**: 7–15.
- HERSHLER, R., LIU, H.-P. & MULVEY, M. 1999a. Phylogenetic relationships within the aquatic snail genus *Tryonia*: implications for biogeography of the North American Southwest. *Molecular Phylogenetics and Evolution*, **13**: 377–391.
- HERSHLER, R., LIU, H.-P. & STOCKWELL, C.A. 2002a. A new genus and species of aquatic gastropods (Rissooidea: Hydrobiidae) from the North American Southwest: phylogenetic relationships and biogeography. *Proceedings of the Biological Society of Washington*, **115**: 171–188.
- HERSHLER, R., LIU, H.-P. & THOMPSON, F.G. 2003. Phylogenetic relationships of North American nymphophiline gastropods based on mitochondrial DNA sequences. *Zoologica Scripta*, **32**: 357–366.
- HERSHLER, R., MULVEY, M. & LIU, H.-P. 1999b. Biogeography in the Death Valley region: evidence from springsnails (Hydrobiidae: *Tryonia*). *Zoological Journal of the Linnean Society*, **126**: 335–354.
- HERSHLER, R., MULVEY, M. & LIU, H.-P. 2005. Genetic variation in the Desert springsnail (*Tryonia porrecta*): implications for reproductive mode and dispersal. *Molecular Ecology*, **14**: 1755–1765.
- HERSHLER, R. & THOMPSON, F.G. 1987. North American Hydrobiidae (Gastropoda: Rissoacea): redescription and systematic relationships of *Tryonia* Stimpson, 1865 and *Pyrgulopsis* Call and Pilsbry, 1886. *Nautilus*, **101**: 25–32.
- HERSHLER, R. & THOMPSON, F.G. 1992. A review of the aquatic gastropod subfamily Cochliopinae (Prosobranchia: Hydrobiidae). *Malacological Review Supplement*, **5**: 1–140.
- HERSHLER, R. & VELKOVHR, F. 1993. A new genus of hydrobiid snails (Mollusca: Gastropoda: Prosobranchia: Rissooidea) from northern South America. *Proceedings of the Biological Society of Washington*, **106**: 182–189.
- HUBBS, C. & SPRINGER, V.G. 1957. A revision of the *Gambusia nobilis* species group, with descriptions of three new species, and notes on their variation, ecology, and evolution. *Texas Journal of Science*, **9**: 279–327.
- HUELSENBECK, J.P. & RONQUIST, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**: 754–755.
- LIU, H.-P., HERSHLER, R. & CLIFT, K. 2003. Mitochondrial DNA sequences reveal extensive cryptic diversity within a western American springsnail. *Molecular Ecology*, **12**: 2771–2782.
- LIU, H.-P., HERSHLER, R. & THOMPSON, F.G. 2001. Phylogenetic relationships of the Cochliopinae (Rissooidea: Hydrobiidae): an enigmatic group of aquatic gastropods. *Molecular Phylogenetics and Evolution*, **21**: 17–25.
- MILLER, R.R. 1976. Four new pupfishes of the genus *Cyprinodon* from Mexico, with a key to the *C. eximius* complex. *Bulletin of the Southern California Academy of Sciences*, **75**: 68–75.
- MILLER, R.R., MINCKLEY, W.L. & NORRIS, S.M. 2005. *Freshwater fishes of Mexico*. University of Chicago Press, Chicago, IL.
- MOORHEAD, M.L. 1995. *New Mexico's Royal Road. Trade and travel on the Chihuahua Trail*. University of Oklahoma Press, Norman, OK.
- NYLANDER, J.A.A. 2004. *MrAIC*. Program distributed by the author (available from <http://www.abc.se/~nylander>). Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.
- PONS DA SILVA, M.C. & VEITENHEIMER-MENDES, I.L. 2004. Nova espécie de *Heleobia* (Rissooidea, Hydrobiidae) da planície costeira do sul do Brasil. *Iheringia*, **94**: 89–94.
- SAITOU, N. & NEI, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**: 406–425.
- SWOFFORD, D.L. 2002. *PAUP\*: Phylogenetic analysis using parsimony (and other methods)*. Version 4.0b10. Sinauer Associates, Sunderland, MA.
- SYSTAT SOFTWARE, INC. (SSI). 2004. *Systat® for Windows®*. Richmond, California.
- TAMURA, K., DUDLEY, J., NEI, M. & KUMAR, S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**: 1596–1599.
- Taylor, D.W. 1987. Fresh-water molluscs from New Mexico and vicinity. *New Mexico Bureau of Mines and Mineral Resources Bulletin*, **116**: 1–50.
- THOMPSON, F.G. 2001. A new hydrobiid snail from a saline spring in southern Alabama (Gastropoda, Prosobranchia, Rissooidea). *American Malacological Bulletin*, **16**: 41–46.
- THOMPSON, F.G. & HERSHLER, R. 2002. *Tepalcattia*, a new genus of hydrobiid snails (Prosobranchia: Rissooidea) from the Rio Balsas basin, central Mexico. *Proceedings of the Biological Society of Washington*, **115**: 189–204.
- WILKE, T., DAVIS, G.M., FALNIOWSKI, A., GIUSTI, F., BODON, M. & SZAROWSKA, M. 2001. Molecular systematics of Hydrobiidae (Mollusca: Gastropoda: Rissooidea): testing monophyly and phylogenetic relationships. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **151**: 1–21.