

NEW SPECIES AND RECORDS OF CHIHUAHUAN DESERT SPRINGSNAILS, WITH A NEW COMBINATION FOR *TRYONIA BRUNEI*

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ABSTRACT.—This is the last in a series of papers clarifying the taxonomy of a critically imperiled assemblage of cochliopid gastropods (*Tryonia* sensu lato) that inhibit thermal springs in the Chihuahuan Desert (Mexico and United States). We describe 2 new narrowly endemic species of *Tryonia* from Chihuahua, both of which appear to have been recently extirpated, and we provide new records for 4 congeners (also from Chihuahua) and for a species of *Pseudotryonia* (from Durango). The 2 new species of *Tryonia* differ from closely similar regional congeners in shell and penial characters. On the basis of new anatomical data, we also transfer *T. brunei* Taylor, 1987 to the genus *Juturnia* and provide evidence that this species, which was endemic to the Phantom Lake spring complex in west Texas, became extinct sometime after 1984. Our findings provide additional insight into the complex biogeographic history of the Chihuahuan Desert cochliopids and further document the recent decline of regional spring-dwelling biota as a result of groundwater mining.

RESUMEN.—Este es el último de una serie de artículos que clarifican la taxonomía del grupo *cochliopid gastropods* (*Tryonia* sensu lato), que se encuentra en peligro de extinción, que habita los manantiales de aguas termales en el Desierto de Chihuahua, en México y los Estados Unidos. Describimos dos nuevas especies endémicas de *Tryonia* de Chihuahua, las cuales parecen haber sido eliminadas recientemente, y proveemos nuevos registros de cuatro congéneres (también de Chihuahua) y de una especie de *Pseudotryonia* (de Durango). Las dos nuevas especies de *Tryonia* son diferentes de los congéneres regionales similares en el caparazón y en la estructura genital. Además transferimos *T. brunei*, Taylor, 1987, al género *Juturnia* basados en la nueva información anatómica y aportamos evidencia de que esta especie, que era endémica de los manantiales del lago Phantom en el oeste de Texas, se extinguió después del año 1984. Nuestros hallazgos suministran información adicional sobre la compleja historia biogeográfica de este grupo de gasterópodos del Desierto de Chihuahua y documentan la reciente disminución de la biota regional de los manantiales como resultado de la minería de aguas subterráneas.

This is the last in a series of papers (Hershler et al. 2002a, 2002b, 2011a, 2011b) clarifying the taxonomy of a critically imperiled fauna of cochliopid gastropods (*Tryonia* sensu lato) that inhibit thermal springs in the Chihuahuan Desert (Mexico and United States). In a previous contribution to this series, 13 new species of *Tryonia* were described from the region (Hershler et al. 2011b). Here we describe 2 new congeners from Chihuahua, both of which appear to have been recently extirpated as a result of groundwater pumping, and we provide new records (also from Chihuahua) for 4 congeners. In another paper in this series, 2 new species of *Pseudotryonia* were described from northeastern Mexico (Hershler et al. 2011a); herein we detail a new record for one of these species from Durango. On the basis of new anatomical data, we also

transfer *T. brunei* Taylor, 1987 to the genus *Juturnia* and provide evidence that this species, which was endemic to the Phantom Lake spring complex in west Texas, became extinct sometime after 1984. Our findings further clarify the diversity and distributions of this morphologically similar, but evolutionarily diverse, species assemblage and provide additional documentation of the devastating effect of anthropogenic activities on spring-dwelling biota of the Chihuahuan Desert.

METHODS

The geographic locations of the populations and species treated herein are shown in Fig. 1. Types and other voucher material from this study were deposited in the National Museum of Natural History (USNM) collection. Relevant

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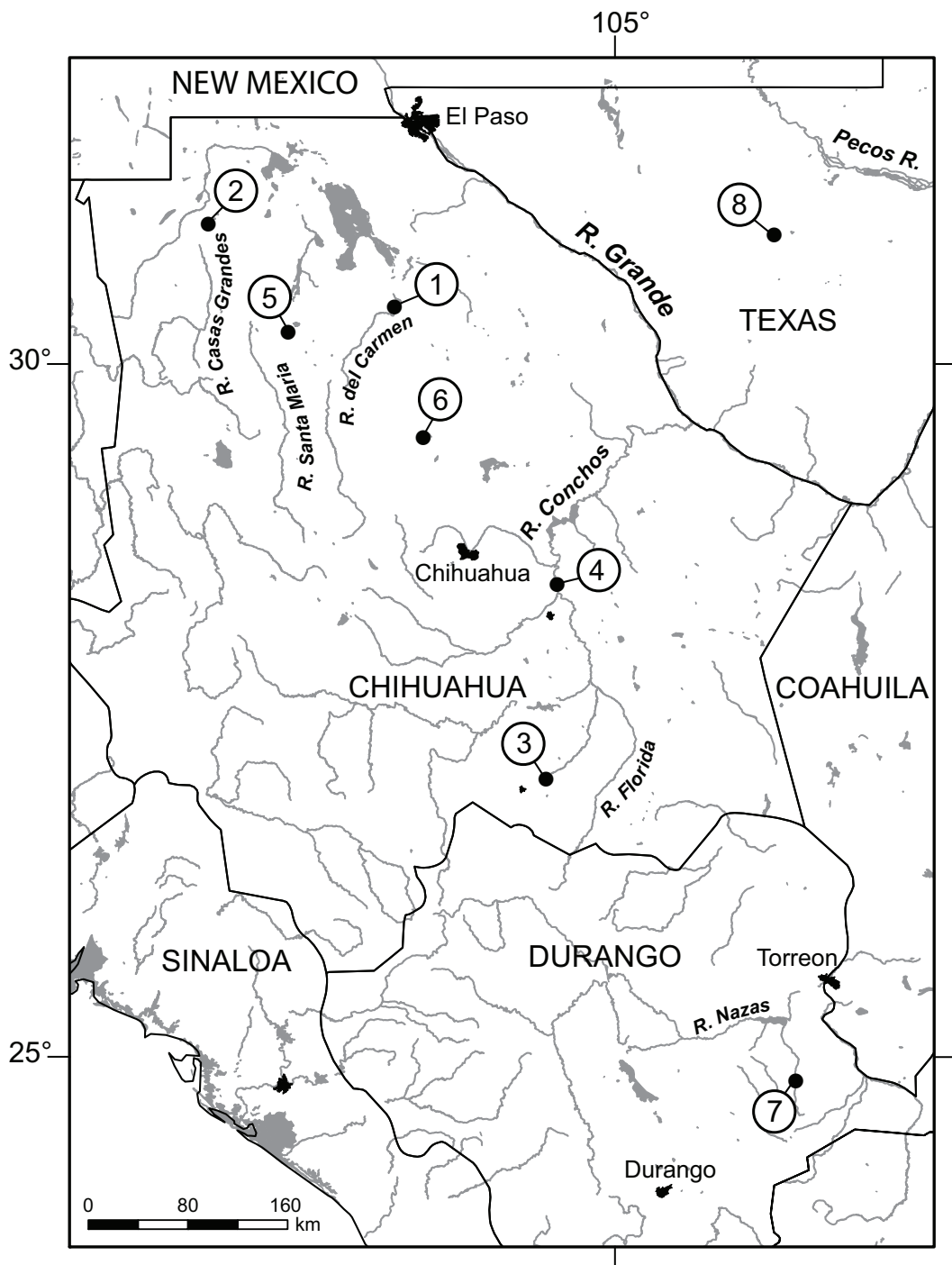


Fig. 1. Map of the Chihuahuan Desert region showing collecting localities: 1, Ojo Caliente de Santa Rosa, Chihuahua (*Tryonia santarosae*); 2, springs southeast of Ascensión, Chihuahua (*T. shikueii*); 3, spring at San Gregorio, Chihuahua (*T. allendae*); 4, springs southeast of Julimes, Chihuahua (*T. julimesensis*, *T. minckleyi*); 5, springs at La Nariz, Chihuahua (*T. peregrina*); 6, spring north of La Laguna, Chihuahua (*Tryonia* sp.); 7, spring at San Pedro de Ocuila, Durango (*Pseudotryonia pasajae*); 8, Phantom Lake spring, Texas (*Juturnia brunei*).

materials from the Bell Museum of Natural History (BellMNH) and the University of Texas at El Paso Centennial Museum (UTEP) also were examined.

Snails used for mtDNA sequencing were preserved in 90% ethanol in the field. Methods of molecular study are those of Hershler et al. (2011b). Genomic DNA was extracted from entire snails (2–4 specimens per sample) using a CTAB protocol (Bucklin 1992); each specimen was analyzed separately for mtDNA. LCO1490 and HCO2198 (Folmer et al. 1994) were used to amplify a 658–base pair (bp) fragment of COI. Sequences were determined for both strands and then edited and aligned using Sequencher™ version 5.1. New sequences were deposited in GenBank under accession numbers KF972420–KF972424. The molecular phylogenetic analysis included the sequences reported herein and previously published haplotypes of other regional species of *Pseudotryonia* and *Tryonia* (Hershler et al. 2011a, 2011b). *Phrantela marginata* was used as the root. Sample information and GenBank accession numbers for sequences used in the molecular phylogenetic analysis are in Table 1. Sequence divergences (uncorrected p distance) within and between species were calculated using MEGA5 (Tamura et al. 2011), with standard errors estimated by 1000 bootstrap replications with pairwise deletion of missing data. 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 by Bayesian analysis (MrBayes 3.1.2; Huelsenbeck and Ronquist 2001). Metropolis-coupled Markov chain Monte Carlo simulations were run with 4 chains using the model selected through MrModeltest 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 0.004, 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 25% of trees removed to ensure that the chain sampled on a stationary portion.

Large adult females were used for shell measurements and illustrations, unless indicated otherwise. The total number of shell whorls was counted (WH) for each specimen.

The height and width of the entire shell (SH, SW), body whorl (HBW, WBW), and aperture (AH, AW) were measured from camera lucida outline drawings, using a digitizing pad linked to a personal computer (see Hershler 1989). Shell data were analyzed and *t* tests were conducted using Systat for Windows 11.00.01 (SSI 2004). Sexual dimorphism in shells, which is commonly observed in species of *Tryonia* sensu lato (e.g., females are larger; Taylor 1987, Hershler et al. 2011a), could not be quantified owing to the generally small sample sizes. Variation in the number of cusps on the radular teeth ($n = 5$ for each sample) was assessed using the method of Hershler et al. (2007). Penial variation was described from series of adult specimens ($n = 30$ when sufficient material was available) that were relaxed with menthol crystals and fixed in dilute formalin prior to preservation in 70% ethanol. Other methods of morphological study and descriptive terminology are those of Taylor (1987), Hershler (2001), and Hershler et al. (2011b).

In the previous papers in this series, we used an evolutionary lineage concept in describing new species for those snails that are both morphologically diagnosable as well as phylogenetically independent and substantially divergent genetically (per Hershler et al. 2007). Unfortunately, molecular data could not be obtained for most of the populations treated herein (the majority of which became extinct prior to this study). In these cases, we describe new species for those snails that are morphologically well differentiated from geographically proximal congeners. Our brief taxonomic descriptions focus on those aspects of morphology that have proven most useful in discriminating species of *Tryonia* sensu lato (Taylor 1987, Hershler 2001, Hershler et al. 2011a). Internal (reproductive) anatomy was studied only to determine generic placement (per Hershler 2001).

SYSTEMATICS

Family Cochliopidae Tryon, 1866

Genus *Tryonia* Stimpson, 1865

Type species: *Tryonia clathrata* Stimpson, 1865, by original designation

Diagnosis: Hershler (2001)

Tryonia santarosae, new species

TYPES.—Holotype, USNM 873280, Ojo Caliente de Santa Rosa, 21.0 km south of Villa

TABLE 1. Specimen codes, locality details (museum voucher numbers for new sequences are in parentheses), GenBank accession numbers, and publications references for mtCOI sequences analyzed in this study.

Species	Specimen code	Locality	Accession number
<i>Pseudotrygonia adamantina</i>		Diamond Y Spring, Pecos Co., Texas	AF129300 ^a
<i>Pseudotrygonia alamosae</i>		Spring west of Ojo Caliente, Socorro Co., New Mexico	AF129303 ^a
<i>Pseudotrygonia brevissima</i>		Lake Panasoffkee, Sumter Co., Florida	AF061765 ^a
<i>Pseudotrygonia grahamae</i>		Salt Spring, 22 km south-southeast of Jackson, Clarke Co., Alabama	AF129318 ^a
<i>Pseudotrygonia mica</i>	mica1	Ojo de Dolores, south-southwest of Ciudad Jiminez, Chihuahua, Mexico	HMI49772 ^b
	mica2	Ojo de Dolores, south-southwest of Ciudad Jiminez, Chihuahua, Mexico	HMI49775 ^b
<i>Pseudotrygonia pasajae</i>	pasajae1	Spring at El Tanque, west-northwest of Cuencamé de Centeeros, Durango, Mexico	HMI49776 ^b
	pasajae51A (n = 2)	Spring, San Pedro de Ocuila, Durango, Mexico (USNM 1138230)	KF972420
<i>Trygonia chuviscarae</i>	chuviscarae1	Bahneario de San Diego de Alcalá, Chihuahua	JF776782 ^c
	chuviscarae2	Bahneario de San Diego de Alcalá, Chihuahua	JF776783 ^c
	chuviscarae3	Bahneario de San Diego de Alcalá, Chihuahua	JF776788 ^c
<i>Trygonia julimesensis</i>	julimesensis1	Springs (northern) along east side of Río Conchos, south-southeast of Julimes, Chihuahua	JF776789 ^c
	julimesensis52A (n = 3)	Spring complex southeast of Julimes, southernmost spring (outflow), Chihuahua (USNM 1160586)	KF972421
	julimesensis52D (n = 1)	Spring complex southeast of Julimes, southernmost spring (outflow), Chihuahua (USNM 1160586)	KF972422
<i>Trygonia minckleyi</i>	minckleyi1	Bahneario de San Diego de Alcalá, Chihuahua	JF776780 ^c
	minckleyi2	Bahneario de San Diego de Alcalá, Chihuahua	JF776781 ^c
	minckleyi53A (n = 1)	Spring complex southeast of Julimes, confluence of 2 southernmost springs, Chihuahua (USNM 1177598)	KF972423
	minckleyi53D (n = 1)	Spring complex southeast of Julimes, confluence of 2 southernmost springs, Chihuahua (USNM 1177598)	KF972424
<i>Phreatela marginata</i>		Tributary of Thirteen Mile Creek, Tasmania, Australia	AF129331 ^a

^aHershbler et al. 1999

^bHershbler et al. 2011a

^cHershbler et al. 2011b

Ahumada, Chihuahua, Mexico, 30.4158° N, 106.5917° W. J.J.L., 13 April 1973. Paratypes (from same lot), USNM 873290, USNM 1220955.

ETYMOLOGY.—The species name is a geographic epithet derived from [Ojo de] Santa Rosa.

REFERRED MATERIAL.—MEXICO. Chihuahua: BellMNH 21164, BellMNH 21165, BellMNH 21166, BellMNH 21167, BellMNH 21263, BellMNH 21293, BellMNH uncat., UTEP 979, *ibid.*, coll. D.W. Taylor and Artie L. Metcalf, 28 August 1969; DWT, 4 September 1969; DWT and ALM, 28 August 1969; DWT and ALM, 28 August 1969; DWT and ALM, 4 September 1969; DWT, 4 September 1969; DWT and ALM, 4 September 1969; ALM, 28 August 1969, respectively.

DIAGNOSIS.—Shell medium-sized, elongate-conic; penis having 3 distal and 2 basal papillae on the inner edge. Distinguished from geographically proximal and closely similar *T. contrerasi* by its smaller (3.09 vs. 4.92 mm mean shell height, $t = -12.758$, $df = 13.7$, $P < 0.01$; $n = 13$ for *T. contrerasi*), wider shell (0.513 vs. 0.412 SW/SH, $t = 11.347$, $df = 26.0$, $P < 0.01$; $n = 13$ for *T. contrerasi*); thicker apertural lip; and larger number of penial papillae.

DESCRIPTION.—Shell (Fig. 2A–C) up to 4.2 mm tall, large females having up to 6.5 whorls; spire height 110%–144% width of shell, males smaller than females. Teleoconch whorls medium convex, sometimes weakly shouldered. Sculpture of numerous prominent spiral cords, elements weakening on body whorl. Aperture slightly angled adapically, parietal lip usually incomplete, adnate when complete, umbilicus absent or a narrow chink. Outer lip orthocline or prosocline. Periostracum tan. Shell measurements and whorl counts for the holotype and paratypes are summarized in Table 2.

Edges of last 0.25–0.5 operculum whorl weakly frilled (Fig. 2F); inner side with edges of muscle attachment scar slightly thickened, sometimes having a weak rim (Fig. 2G). Radula (Fig. 2J–L): dorsal edge of central teeth concave, basal tongue U-shaped, median cusps elongate, distally pointed, parallel-sided proximally, lateral cusps 4–5, basal cusps 1–2 (innermost larger; Fig. 2K). Lateral teeth having 2–3 cusps on inner and 3–4 cusps on outer side, length of outer wing about 200% width of cutting edge, central cusp pointed (Fig. 2L). Inner marginal teeth with 16–24 cusps, outer

marginal teeth with 31–37 cusps. Radula data are from USNM 1220955.

Penis (Fig. 3A) having 3 distal and 2 basal papillae on inner edge (42 of 60 specimens from 2 samples); 8 specimens differed in also having a medial papilla, 9 specimens had a single basal papilla, and 1 specimen had 2 medial papillae. Distal bulb of penis expanded laterally on inner side, black; stylet small. Penial duct undulating in proximal half of penis. Penial data are from USNM 873290, BellMNH uncat.

DISTRIBUTION AND HABITAT.—*Tryonia santarosae* was distributed in a single spring (Ojo de Santa Rosa) that historically flowed to the Rio del Carmen (Wislizenus 1848). When visited in April 1973, the spring formed a pool (29 °C) that was surrounded by a stone wall (Fig. 4A); there was no obvious spring run. Snails were abundant on aquatic vegetation and other substrates in association with another locally endemic rissooidean gastropod, *Pyrgulopsis chihuahua*. Ojo de Santa Rosa, which was an important stopping point for early travelers on the Chihuahuan extension of the Santa Fe trail, was dry when visited on 21 September 1990 (probably due to locally extensive groundwater pumping). Thus we presume that *T. santarosae* is now extinct. Jackson's (2006:112) guide to the Santa Fe historic trail contains an undated photograph of the now-dry site of this spring.

Tryonia shikuei, new species

TYPES.—Holotype, USNM 1220952, Ojo de Federico, 11.0 km southeast of Ascensión, Chihuahua, Mexico, 31.0222° N, 107.9056° W. Artie L. Metcalf (ALM), 19 October 1979. Paratypes (from same lot), USNM 1220953, UTEP 7724.

ETYMOLOGY.—Named after Shi-Kuei Wu, curator emeritus, Museum of Natural History, University of Colorado at Boulder, in honor of his contributions to the study of western North American freshwater mollusks (e.g., Wu 1989) and for the unwavering support that he has provided to other students of this fascinating fauna.

REFERRED MATERIAL.—MEXICO. Chihuahua: USNM 1220954, UTEP 7023, Ojo de San Juan, ca. 4.8 km southeast of Ascensión, 31.0508° N, 107.9406° W, coll. ALM, 7 April 1979.

DIAGNOSIS.—Shell medium-sized, elongate-conic; penis having 2 distal and 1 basal papillae

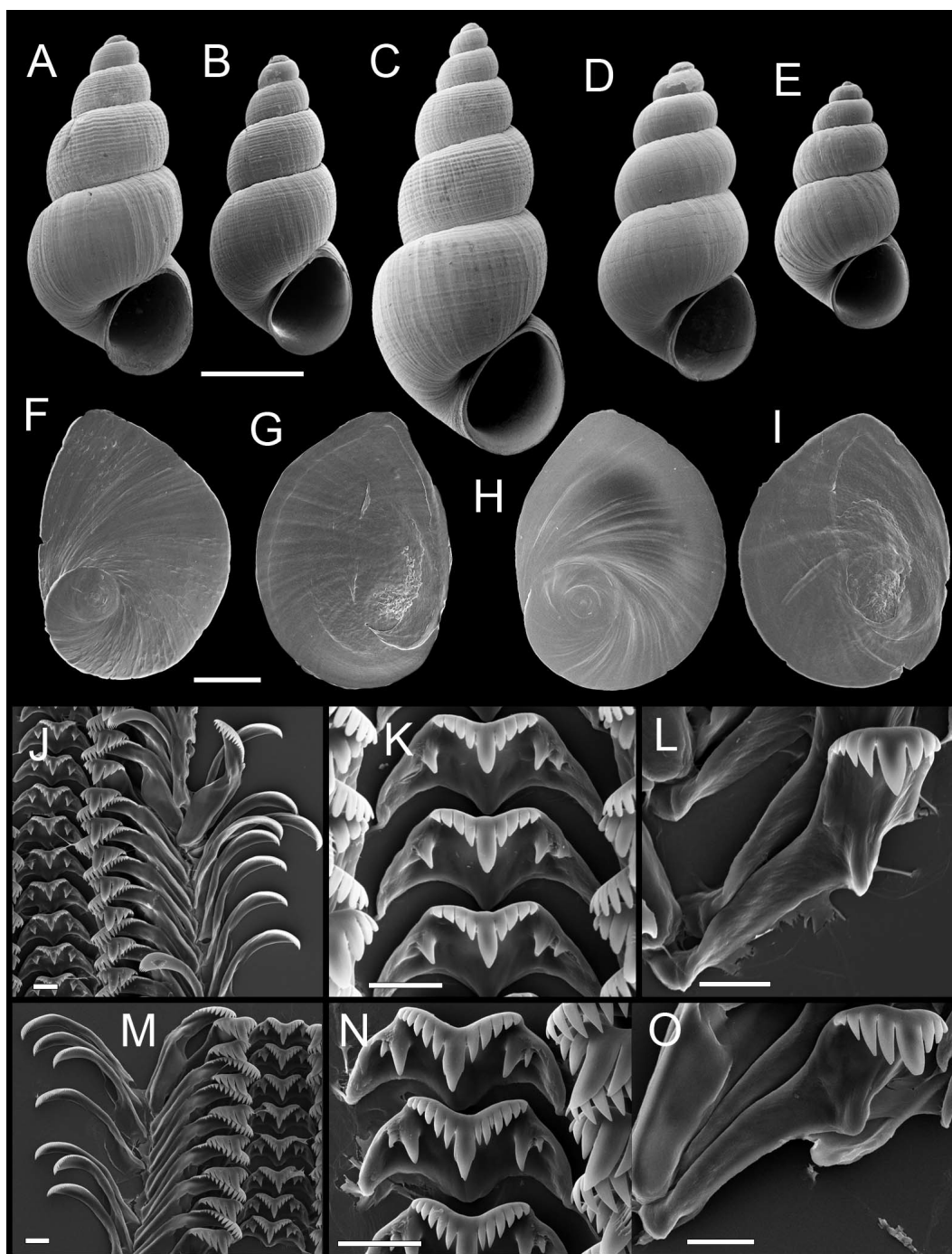


Fig. 2. Scanning electron micrographs of shells, opercula, and radula: A, holotype, *Tryonia santarosae*, USNM 873280; B, shell (male), *T. santarosae*, UTEP 979; C, shell, *T. santarosae*, BellMNH 21167; D, holotype, *T. shikueii*, USNM 1220952; E, shell (male), *T. shikueii*, USNM 1220953; F–G, operculum (outer, inner sides), *T. santarosae*, USNM 1220955; H–I, operculum (outer, inner sides), *T. shikueii*, USNM 1220953; J, portion of radular ribbon, *T. santarosae*, USNM 1220955; K, central radular teeth, *T. santarosae*, USNM 1220955; L, lateral radular tooth, *T. santarosae*, USNM 1220955; M, portion of radular ribbon, *T. shikueii*, USNM 1220953; N, central radular teeth, *T. shikueii*, USNM 1220953; O, lateral radular tooth, *T. shikueii*, USNM 1220953. Scale bars: A–E, 1.0 mm; F–I, 100 μ m; J–O, 10 μ m.

TABLE 2. Shell measurements (mm), whorl counts, and shell ratios for new species described herein. WH = total number of shell whorls, SH = shell height, SW = shell width, HBW = body whorl height, WBW = body whorl width, AH = aperture height, AW = aperture width.

	WH	SH	SW	HBW	WBW	AH	AW
<i>T. santarosae</i> , holotype, USNM 873280	5.5	3.52	1.68	2.06	1.64	1.14	0.90
<i>T. santarosae</i> , paratypes, USNM 1220955 ($n = 15$)							
Mean	*	3.09	1.59	1.90	1.49	1.11	0.90
SD		0.14	0.08	0.09	1.00	0.05	0.05
Range		2.92–3.46	1.46–1.72	1.75–2.03	1.32–1.67	1.01–1.18	0.80–0.98
<i>T. shikueii</i> , holotype, USNM 1220952	6.0	3.33	1.58	1.85	1.53	1.09	0.87
<i>T. shikueii</i> , paratypes, USNM 1220953 ($n = 10$)							
Mean	5.05	3.03	1.45	1.69	1.38	0.95	0.79
SD	0.46	0.35	0.09	0.09	0.08	0.07	0.04
Range	4.75–6.25	2.61–3.73	1.32–1.63	1.55–1.83	1.24–1.49	0.83–1.08	0.74–0.87

*Shell apices eroded.

on the inner edge. Distinguished from geographically proximal and closely similar *T. hertleini* by the larger shell umbilicus and much smaller basal papilla on the inner edge of the penis. Readily differentiated from congeners distributed in the upper portion of the Rio Casas Grandes basin (*T. peregrina*, *T. zaragozae*) by its smaller size (3.03 vs. 4.08 mm mean shell height, $t = -7.186$, $df = 19.7$, $P < 0.01$; $n = 13$ for *T. peregrina*; 3.03 vs. 4.20 mm mean shell height, $t = -9.390$, $df = 14.2$, $P < 0.01$; $n = 15$ for *T. zaragozae*) and absence of papillae on the outer edge of the penis.

DESCRIPTION.—Shell (Fig. 2D–E) up to 3.7 mm tall, large females having up to 6.25 whorls; spire height 120%–170% width of shell, males smaller than females. Teleoconch whorls medium convex, evenly rounded. Sculpture of numerous spiral threads. Aperture strongly angled adapically, parietal lip usually complete, adnate or slightly disjunct, umbilicus perforate. Outer lip orthocone. Periostracum tan. Shell measurements and whorl counts for the holotype and paratypes are summarized in Table 1.

Edges of last 0.25–0.5 operculum whorl weakly frilled on outer side (Fig. 2H), inner side smooth or with edges of muscle attachment scar slightly thickened (Fig. 2I). Radula (Fig. 2M–O): dorsal edge of central teeth concave, basal tongue U-shaped, median cusps elongate, distally pointed, parallel-sided proximally, lateral cusps 5–6, basal cusps 2–3, usually 2 (innermost larger; Fig. 2N). Lateral teeth having 3–4 cusps on inner side and 4–5 cusps on outer side, length of outer wing about

160% width of cutting edge, central cusp pointed (Fig. 2O). Inner marginal teeth with 17–23 cusps, outer marginal teeth with 24–34 cusps. Radula data are from UTEP 7724.

Penis (Fig. 3B) having 2 distal and 1 basal papillae on inner edge (30 of 30 specimens from one sample). Distal bulb of penis expanded laterally on inner side, black; stylet small. Penial duct undulating along most of length. Penial data are from UTEP 7724.

DISTRIBUTION AND HABITAT.—*Tryonia shikueii* was distributed in 2 closely proximal springs in the lower Rio Casas Grandes basin. Metcalf did not take habitat notes when he briefly visited and sampled these localities in 1979 (letter to RH, 9 August 2000). Ichthyologist Robert Rush Miller recorded water temperatures of 27 °C (Ojo de Federico, 29 May 1978) and 23 °C (Ojo de San Juan, 5 April 1982) at these springs (RRM field notes, localities M78-15, M82-84, respectively). Ojo de Federico was dry when visited on 6 December 1998, and Ojo de San Juan was dry when visited on 10 November 2012. Local inquiry indicated that these 2 springs failed in the middle of the 1980s. A recent photograph of the type locality area (site of Ojo de Federico) is shown in Fig. 4B. Inasmuch as there are no other (still flowing) springs in this overdrafted (NWCM 2008, annex D) portion of the Rio Casas Grandes basin, we consider it likely that *T. shikueii* is extinct.

REMARKS.—The Ojo de San Juan population is known only from dry shells that are somewhat eroded, but otherwise closely similar to the type material of *T. shikueii*.

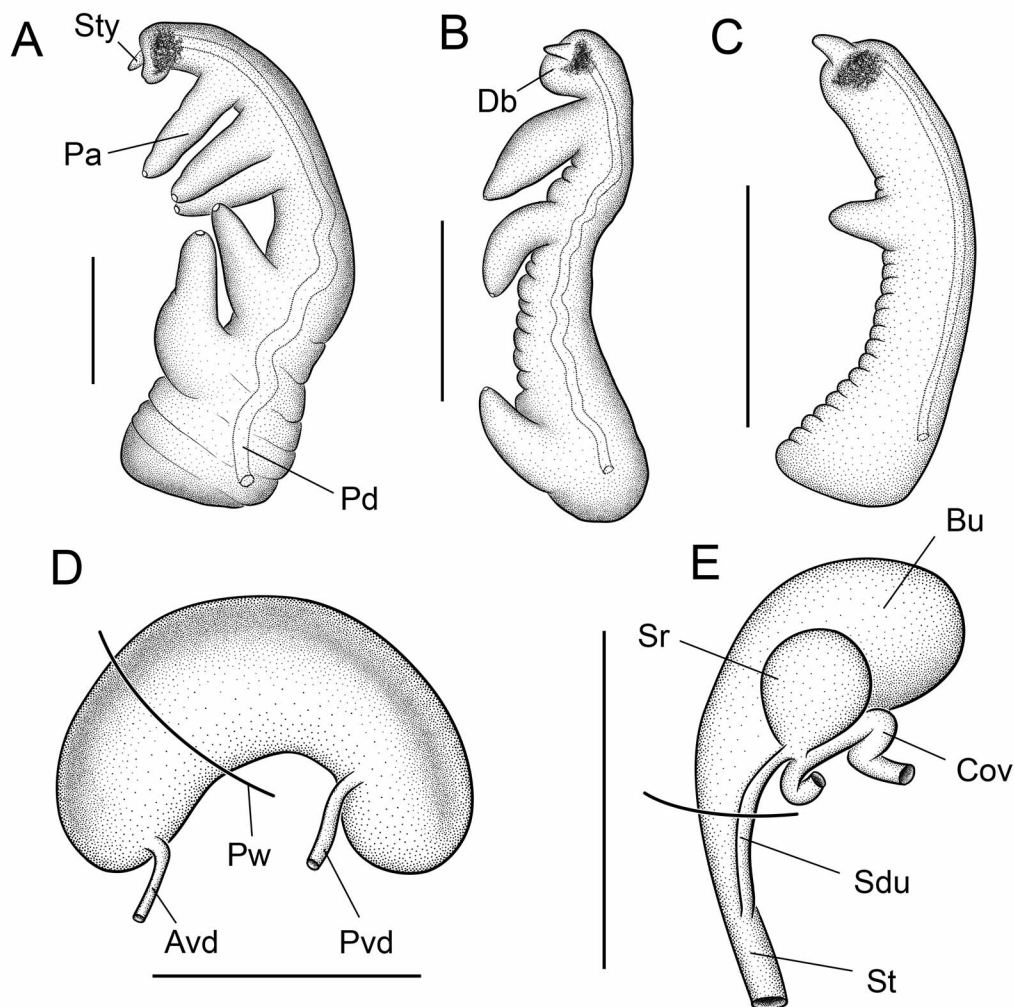


Fig. 3. Reproductive anatomy: **A**, penis, *Tryonia santarosae*, USNM 873290; **B**, penis, *T. shikueii*, UTEP 7724; **C**, penis, *Juturnia brunei*, BellMNH 21270; **D**, prostate gland (viewed from left side), *J. brunei*, BellMNH 21270; **E**, sperm pouches and associated ducts, *J. brunei*, BellMNH 21270. Abbreviations: Avd, anterior vas deferens; Bu, bursa copulatrix; Cov, coiled oviduct; Db, distal bulb; Pa, papilla; Pd, penial duct; Pvd, posterior vas deferens; Pw, posterior wall of pallial cavity; Sdu, sperm duct; Sr, seminal receptacle; St, sperm tube; Sty, stylet. Scale bars = 250 μ m.

Tryonia allendae Hershler et al., 2011

Tryonia allendae Hershler et al., 2011b: 26, figs. 5M, 11D–E, H–I, M–O (type locality, El Ojito, southwest of Talamantes, Rio Conchos basin, Chihuahua, Mexico).

REFERRED MATERIAL.—MEXICO. Chihuahua: USNM 854938, USNM 873265, USNM 873274, USNM 873275, USNM 874122, unnamed spring at San Gregorio, just north of Mexico Highway 45, ca. 65 km west of Ciudad Jiminez, 27.0116° N, 105.4967° W, coll. RH and JJJ, 10 December 2012; JJJ et al., 31

August 1971; JJJ et al., 31 August 1971; JJJ et al., 31 August 1971; JJJ et al., 6 May 1991, respectively.

Shell: WH, 5.25–6.00; SH, 2.69–3.67 mm; SW, 1.36–1.81 mm; HBW, 1.63–2.14 mm; WBW, 1.20–1.69 mm; AH, 1.00–1.29 mm; AW, 0.81–1.04 mm (USNM 873274, $n = 16$). Radula: central teeth, lateral cusps, 3–6; central teeth, basal cusps, 2; lateral teeth, cusps on outer side, 3–4; lateral teeth, cusps on inner side, 2–3; inner marginal teeth cusps, 22–31; outer

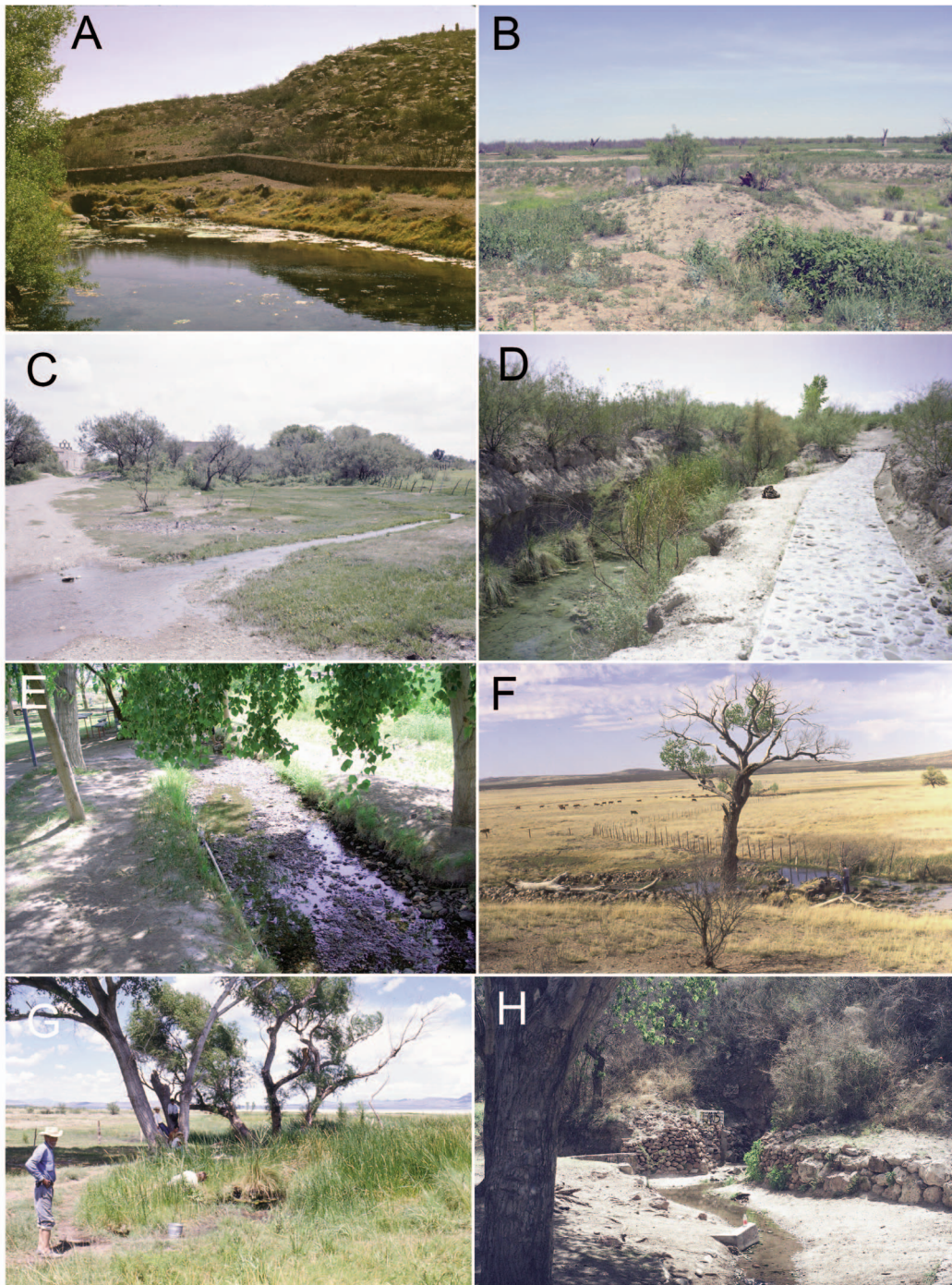


Fig. 4. Habitat photographs: **A**, Ojo de Santa Rosa, type locality of *Tryonia santarosae* (photograph taken on 13/iv/1973); **B**, former site of Ojo de Federico, type locality of *T. shikueii* (10/xi/2012); **C**, outflow of the springs at Ojo de San Gregorio, *T. allendae* habitat (31/viii/1971); **D**, outflow of (southernmost) spring southeast of Julimes, *T. julimesensis* and *T. minckleyi* habitat (20/ix/2011); **E**, confluence of 2 southern springs southeast of Julimes, *T. julimesensis* and *T. minckleyi* habitat (21/ii/2012); **F**, spring at El Nariz, Chihuahua, *T. peregrina* habitat (4/iv/1991), **G**, spring north of La Laguna, Chihuahua, *Tryonia* sp. habitat (28/viii/1971), **H**, spring at San Pedro de Ocuila, Durango, *Pseudotryonia pasajae* habitat (9/iii/2010).

marginal teeth cusps, 26–35 (USNM 873274). Penis: 2 distal papillae on the inner edge and one basal papilla on the outer edge ($n = 30$, USNM 873274).

The spring at San Gregorio, sometimes referred to as El Ojo de San Gregorio, is located about 12.7 km north-northwest of the other known locality of *T. allendae* in the Rio Parral watershed (Rio Conchos basin). This site consists of several closely proximal spring sources that discharge into a reservoir. *Tryonia allendae* was most abundant in slow current pools in the runoff from the reservoir in 1971 (Fig. 4C); snails were found on various substrates. The temperature in the headsprings was 27 °C when visited in April 1991. When visited in 1998, the runoff area below the reservoir and a small spring at the southwestern edge of the complex were dry; however, the headsprings above the old hacienda buildings were still flowing. *Tryonia allendae* was abundant in this area. A mosquitofish, *Gambusia alvarezi*, is endemic to this spring complex (Hubbs and Springer 1957).

REMARKS.—We were unable to successfully extract and amplify DNA from specimens from the San Gregorio population, despite multiple attempts.

Tryonia julimesensis Hershler et al., 2011

Tryonia julimesensis Hershler et al., 2011b: 24, figs. 5L, 11A–C, F–G, J–L (type locality, unnamed springs along east side of Rio Conchos, south-southeast of Julimes, Rio Conchos basin, Chihuahua, Mexico).

REFERRED MATERIAL.—MEXICO. Chihuahua: USNM 1152480, USNM 1160586, spring complex southeast of Julimes, southernmost spring (outflow), 28.4093° N, 105.4239° W, coll. MMB, EWC and Lilia Vela-Valladares, 22 May 2011, JJJ, 20 September 2011, respectively. USNM 1220958, spring complex southeast of Julimes, at confluence of 2 southernmost springs, 28.40983° N, 105.4237° W, JJJ, 21 February 2012.

Shell: WH, 4.75–5.00; SH, 2.50–2.90 mm; SW, 1.43–1.58 mm; HBW, 1.55–1.80 mm; WBW, 1.24–1.40 mm; AH, 0.87–1.00 mm; AW, 0.76–0.92 mm (USNM 1160586, $n = 10$). Radula: central teeth, lateral cusps, 4–5; central teeth, basal cusps, 2; lateral teeth, cusps on outer side, 3–5; lateral teeth, cusps on inner side, 3–4; inner marginal teeth cusps, 16–19; outer marginal teeth cusps, 16–20 (USNM 1160586). Penis: no males were found in the small samples from these new localities.

REMARKS.—*Tryonia julimesensis* originally was discovered in the outflow of the 2 northernmost springs in the Julimes spring complex in 1991 (Fig. 5). When revisited in 2001, these springs were highly modified (excavated) and no snails were found, leading us to suggest that *T. julimesensis* had become extinct (Hershler et al. 2011b). *Tryonia julimesensis* recently was found in the southernmost spring in this complex, locally known as El Pandeño (Fig. 5), which JJJ and RH did not access during their earlier field surveys. Snails were found in the outflow of El Pandeño on mud that was slightly covered with algae, as well as on rocks and on submerged portions of a cattail stand (Fig. 4D). The water temperature at this site, which is the type locality for an endemic pupfish, *Cyprinodon julimes* (De la Maza-Benignos and Vela-Valladares 2009), was 40 °C on 20 September 2011. Snails were also collected where this spring run joins the outflow of another spring that originates to the east near the paved road (Fig. 5). *Tryonia julimesensis* was found at this locality on flocculent silt in 40–45 cm deep water that had a temperature of 44 °C. The amount of snail habitat at these sites appears to be less than what was previously available in the northern springs.

Specimens from the recently discovered localities have larger (2.68 vs. 1.69 mm mean shell height, $t = -20.236$, $df = 18.4$, $P < 0.01$) and narrower (0.558 vs. 0.645 SW/SH, $t = 9.606$, $df = 23.0$, $P < 0.01$) shells than previously described *T. julimesensis* (data for the latter from Hershler et al. 2011b). The 2 haplotypes that were detected in specimens (4) from the southernmost spring differed from the previously published *T. julimesensis* sequence by only 0–1 base pairs. The 3 *T. julimesensis* sequences formed a strongly supported (97% posterior probability) clade in the Bayesian tree (Fig. 6).

Tryonia minckleyi Hershler et al., 2011

Tryonia minckleyi Hershler et al., 2011b: 23–24, Figs. 5K, 10D–E, H–I, M–O (type locality, Balneario de San Diego de Alcalá, Rio Conchos basin, Chihuahua, Mexico).

REFERRED MATERIAL.—MEXICO. Chihuahua: USNM 874131, USNM 905272, Ojos de Julimes, spring complex southeast of Julimes, northern springs (outflows), 28.4015° N, 105.4226° W, coll. JJJ et al., 5 May 1991.

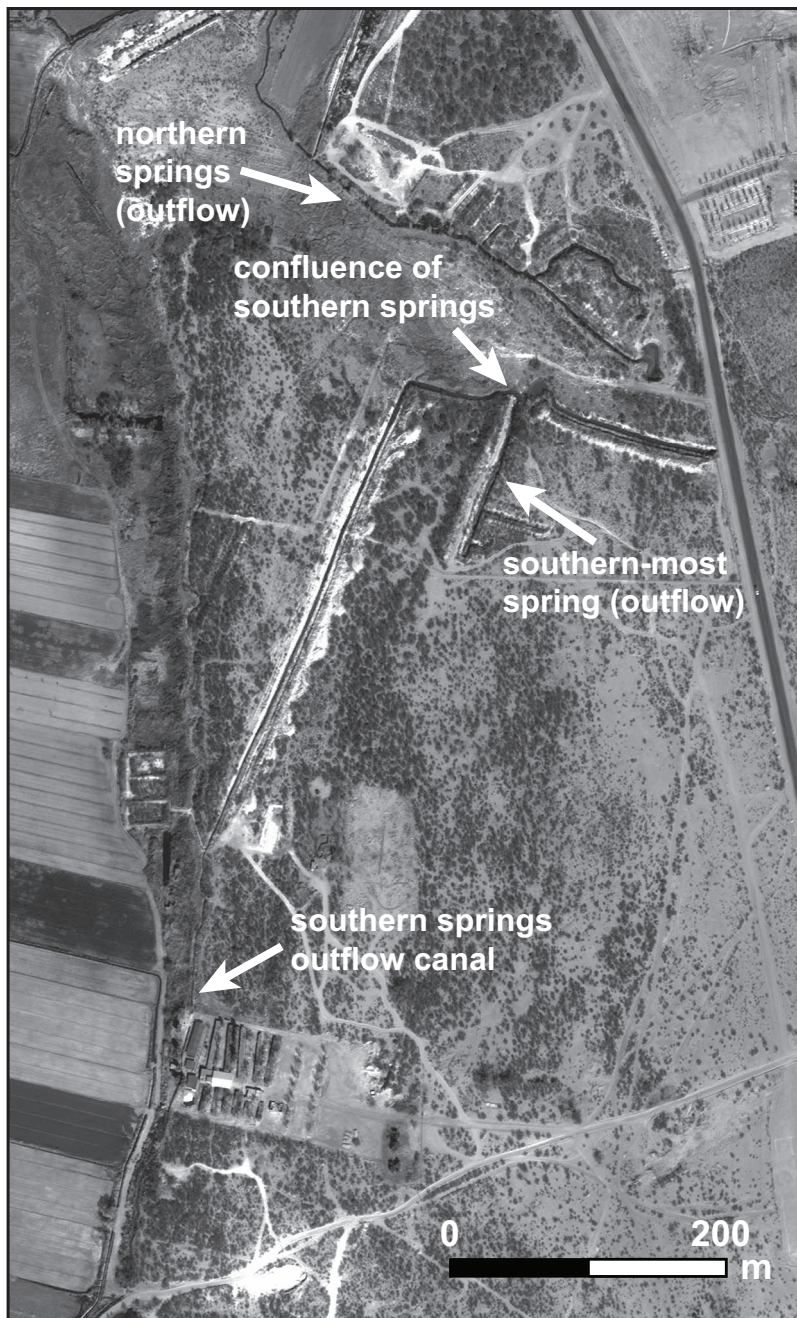


Fig. 5. DigitalGlobe satellite image of spring complex south of Julimes (31/i/2012).

USNM 1220957, spring complex southeast of Julimes, southernmost spring outflow, 28.4093° N, 105.4239° W, coll. JJL, 20 September 2011. USNM 1177598, spring complex southeast of Julimes, confluence of 2 southernmost springs,

28.40983° N, 105.4237° W, JJL, 21 February 2012. USNM 1152479, spring complex south-east of Julimes, outflow canal of southern springs, 28.4055° N, 105.4265° W, MMB, EWC, and Lilia Vela-Valladares, 21 May 2011.

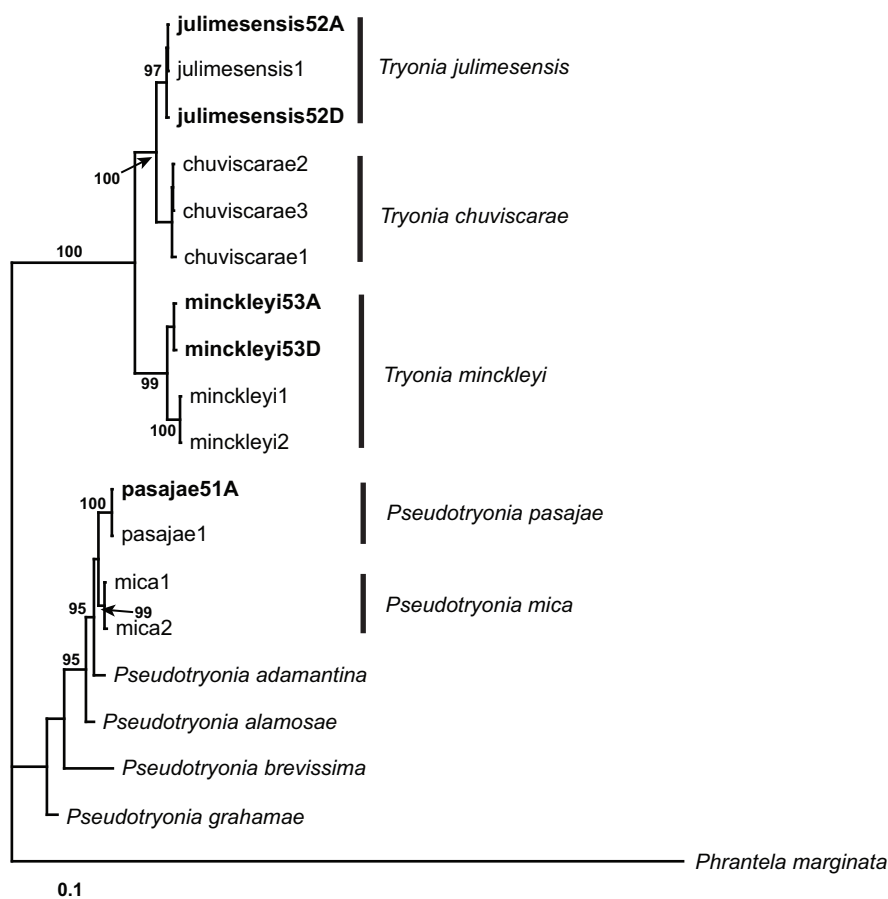


Fig. 6. Bayesian tree based on the mtCOI data set. Posterior probabilities are provided when >95%. Terminals and specimen codes are labeled as in Table 1. The new sequences generated for this study are in boldface type.

Shell: WH, 4.75–5.50; SH, 2.14–2.89 mm; SW, 1.02–1.59 mm; HBW, 1.32–1.69 mm; WBW, 0.95–1.33 mm; AH, 0.74–0.97 mm; AW, 0.61–0.78 mm (USNM 874131, $n = 29$). Radula: central teeth, lateral cusps, 4–5; central teeth, basal cusps, 1–2; lateral teeth, cusps on outer side, 4–5; lateral teeth, cusps on inner side, 3–4; inner marginal teeth cusps, 18–25; outer marginal teeth cusps, 23–31 (USNM 874131). Penis: 2 distal papillae on the inner edge and 1–2 papillae on the outer edge (27 of 28 specimens from 2 samples, USNM 874131, USNM 1177598); one specimen had 3 papillae on the outer edge.

Tryonia minckleyi has been found in both the northern and southern springs (sympatrically with *T. julimesensis*) southeast of Julimes, as well as in the (southern) outflow canal well below the spring sources (Fig. 4E, 5). We

suspect that the population in the type locality area has been extirpated as a result of the extensive modification of the northern head-springs (Hershler et al. 2011b); we also note in this context that the outflow of these springs is subject to disturbance from extensive recreational activities (bathing).

REMARKS.—The Julimes spring complex is about 24 km south-southeast of the other known locality of *T. minckleyi*. Specimens from this site differed from those from the type locality in having a narrower shell (0.485 vs. 0.502 SW/SH, $t = -2.319$, $df = 28.1$, $P = 0.028$; data for the latter from Hershler et al. 2011b) and more distally positioned papillae on the outer edge of the penis. We did not discern other diagnosable morphological characters between specimens from these 2 areas. The 2 haplotypes that were detected in specimens

(2) from the Julimes spring differed from the 2 previously published *T. minckleyi* sequences by 12 base pairs and formed a weakly supported (posterior probability = 83%) clade in the Bayesian tree (Fig. 6). Collectively, the 4 sequences formed a strongly supported clade (99%). Based on this total sum of evidence, we are treating the Julimes population as a differentiated form of *T. minckleyi* rather than as a distinct species.

Tryonia peregrina Hershler et al., 2011

Tryonia peregrina Hershler et al., 2011b: 7–9, figs. 3, 4A–D, H–I, M–O, 5A (type locality, Ojo Vareleño, 2.4 km northwest of Casas Grandes, Chihuahua, Mexico).

REFERRED MATERIAL.—MEXICO. Chihuahua: USNM 874012, unnamed (main) spring at La Nariz, 30.2374° N, 107.3622° W, coll. JJJ and Phil Hines, 17 September 1990. USNM 874119, USNM 874127, USNM 874255, USNM 892113, unnamed spring ca. 100 m north of the above, coll. JJJ and PH, 17 September 1990.

Shell: WH, 6.00–6.75; SH, 3.66–4.74 mm; SW, 1.55–1.83 mm; HBW, 1.95–2.33 mm; WBW, 1.44–1.90 mm; AH, 1.09–1.27 mm; AW, 0.91–1.06 mm (USNM 84012, $n = 11$). Radula: central teeth, lateral cusps, 4–6; central teeth, basal cusps, 1–2; lateral teeth, cusps on outer side, 4–5; lateral teeth, cusps on inner side, 4–6; inner marginal teeth cusps, 17–27; outer marginal teeth cusps, 32–40 (USNM 874012). Penis: 2 distal and one basal papilla on the inner edge and one basal papilla on the outer edge (23 of 23 specimens from 2 samples, USNM 874012, USNM 874127).

The springs at Rancho La Nariz were located ca. 28 km northeast of Galeana in the Rio Santa Maria del Carmen basin about 60 km to the east-southeast of the type locality of *T. peregrina*. Snails were found in the main spring (25 °C, 17 September 1990) on tree roots and on aquatic vegetation and (more abundantly) in a second spring (just to the north) on detritus, tree roots, and aquatic vegetation. The site was somewhat impacted by livestock grazing at that time (Fig. 4F). The springs were dry when the site was revisited in 1998, apparently the result of groundwater pumping to irrigate a large agricultural plot at Las Pastañas (a few kilometers to the south).

Tryonia sp.

REFERRED MATERIAL.—MEXICO. Chihuahua: USNM 873263, unnamed spring just north

of La Laguna, east of Mexico Highway 45, 29.4642° N, 106.3803° W, coll. JJJ and Charles O. Minckley, 28 August 1971.

The only sample from this locality was poorly preserved, preventing detailed taxonomic study. The specimens are similar to *T. peregrina* in having medium-sized, elongate conic shells and 2 distal and one basal papillae on the inner edge and one basal papilla on the outer edge of the penis ($n = 3$, USNM 873263). The closest *T. peregrina* locality to La Laguna is 150 km to the west-southwest (Yepomera).

This small spring was located in an isolated basin about 90 km north of Chihuahua City. *Tryonia* was found in black detritus at the spring source in 1971 (Fig. 4G); water temperature was not recorded. When visited in 1990, the spring was dry.

Genus *Pseudotryonia* Hershler, 2001

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

Diagnosis: Hershler (2001)

Pseudotryonia pasajae Hershler et al., 2011

Pseudotryonia pasajae Hershler et al., 2011a: 14, 16, figs. 2E–F, I–J, M–O, 3E–H, 4D–E (type locality, unnamed spring at El Tanque [second pool below spring source], west-northwest of Cuenca de Cenicerros, Rio Nazas basin, Durango, Mexico).

REFERRED MATERIAL.—MEXICO. Durango: USNM 1138230, unnamed spring, San Pedro de Ocuila, 24.8292° N, 103.695° W, coll. JJJ, 9 March 2010.

Shell: WH, 4.25–4.75; SH, 2.16–2.43 mm; SW, 1.35–1.48 mm; HBW, 1.52–1.77 mm; WBW, 1.21–1.34 mm; AH, 0.85–0.98 mm; AW, 0.74–0.98 mm (USNM 1138230, $n = 10$). Radula: central teeth, lateral cusps, 3–5; central teeth, basal cusps, 1–2; lateral teeth, cusps on outer side, 4–5; lateral teeth, cusps on inner side, 3–4; inner marginal teeth cusps, 19–24; outer marginal teeth cusps, 18–27 (USNM 1138230). Penis: single distal papilla on both the inner and outer edges (27 of 30 specimens from one sample, USNM 1138230); 3 specimens did not have a papilla on the inner edge.

This spring is located on the hill behind (to the south of) the village of San Pedro de Ocuila (upper Rio Nazas basin). Snails were found in the uppermost 3–4 m of this spring (25 °C, 9 March 2010), which flows out of a modified tunnel (Fig. 4H). Specimens were

most common on hard substrates, including rock, but were also found in coarse sand. Specimens were not found below the confluence with a nearby spring (note that the spring run flows about 40 m before emptying into cement collection boxes) or in the outflow of the second spring (the source of this spring, which is covered by a cement top, was not sampled).

REMARKS.—The spring at San Pedro de Ocuila is about 10.5 km southeast of the other known locality of *P. pasajae*. Specimens from this new population were differentiated by their smaller (2.31 vs. 3.66 mm mean shell height, $t = 21.478$, $df = 36.3$, $P < 0.01$, data for the El Tanque population from Hershler et al. 2011a) and narrower (0.606 vs. 0.513 mean SW/SH, $t = -13.503$, $df = 13.3$, $P < 0.01$, data for the El Tanque population from Hershler et al. 2011a) shell, less angular teleoconch whorls, and weaker shell sculpture. The mtCOI sequence detected in the new population was identical to that reported for specimens from the type locality.

***Juturnia brunei* (Taylor, 1987),
new combination**

Tryonia (*Paupertryonia*) *brunei* Taylor, 1987: 44–45, Fig. 22 (type locality, outflow of Phantom Lake Spring at Joe Kingston Ranch, Jeff Davis County, Texas).

MATERIAL EXAMINED.—TEXAS. Jeff Davis County: BellMNH 21271, BellMNH 21277, BellMNH 21323, Phantom Lake, spring outflow, Kingston Ranch, coll. DWT, 5 November 1981, 30 October 1984, 10 April 1965. BellMNH 21252, BellMNH 21270, Phantom Spring, lateral ditch at Kingston Ranch, coll. DWT, 25 June 1968, 5 November 1981. BellMNH 21256, Phantom Spring outflow, first lateral north, coll. DWT, 25 June 1968.

GENUS REALLOCATION.—Our study of Taylor's original alcohol-preserved collections indicates that this species belongs to the genus *Juturnia* based on the nonglandular nature of the penial lobe (Fig. 3C), insertion of the posterior vas deferens into the ventral edge of the prostate gland (Fig. 3D), weakly coiled and unpigmented renal oviduct (Fig. 3E), and subglobular shape of the seminal receptacle (Fig. 3E). Taylor's (1987) description and illustration of a glandular papilla in *J. brunei* appears to have been in error as we did not observe this feature in any of the males that we examined ($n = 30$, BellMNH 21270).

DIAGNOSIS.—Shell medium-sized, elongate-conic; penis having a distal, nonglandular lobe on the inner edge. Differs from *J. kosteri*, which also is distributed in the Pecos River basin, in its smaller (3.39 vs. 4.05 mm mean shell height, $t = -7.6504$, $df = 54.0531$, $P < 0.01$, data from Taylor 1987) and narrower (0.401 vs. 0.582 mean SW/SH, $t = -23.3605$, $df = 51.334$, $P < 0.01$, data from Taylor 1987) shell; larger number of basal cusps on the central radular teeth; smaller number of cusps on the outer marginal teeth; and more well-defined basal process on the lateral teeth.

DESCRIPTION OF RADULA.—Radula (Fig. 7A–C): dorsal edge of central teeth concave, basal tongue V-shaped, median cusps elongate, distally pointed, parallel-sided proximally, lateral cusps 5–6, basal cusps 2–3 (innermost larger) (Fig. 7B). Lateral teeth having 4–6 cusps on inner and 4–7 cusps on outer side, length of outer wing about 200% width of cutting edge, central cusp pointed (Fig. 7C). Inner marginal teeth with 25–31 cusps, outer marginal teeth with 19–27 cusps. Radula data are from BellMNH 21270.

Radular data were not included in Taylor's (1987) description of *J. brunei* and thus are detailed above. This species was not treated in the first author's revision of *Tryonia* (Hershler 2001) because anatomical material, which needs to be studied to assign species of *Tryonia* sensu lato to a genus (Hershler 2001), was not available at that time.

Phantom Lake spring (Fig. 8) historically discharged from a limestone cave into a small lake and cienega prior to diversion of the outflow into a concrete-lined irrigation canal system during the middle of the last century (Simonds 1996). *Juturnia brunei* had a considerably narrower distribution within this spring system than another locally endemic cochlidiopid gastropod, *T. cheatumi*, which was recently ruled as endangered by the USFWS (2013). Taylor (1987) collected *J. brunei* on thin mud in the first north lateral canal (off of the main canal), another north lateral canal just east of the Joe Kingston ranch house driveway, and (rarely) in the main canal just below the ranch house (Fig. 8). These habitats may have been supported by a spring to the northeast of Phantom Cave (shown on the USGS 1:24,000 Toyahvale [1972] topographic map; also see Fig. 8) that flowed into a small pond that had ditches leading to a pecan grove

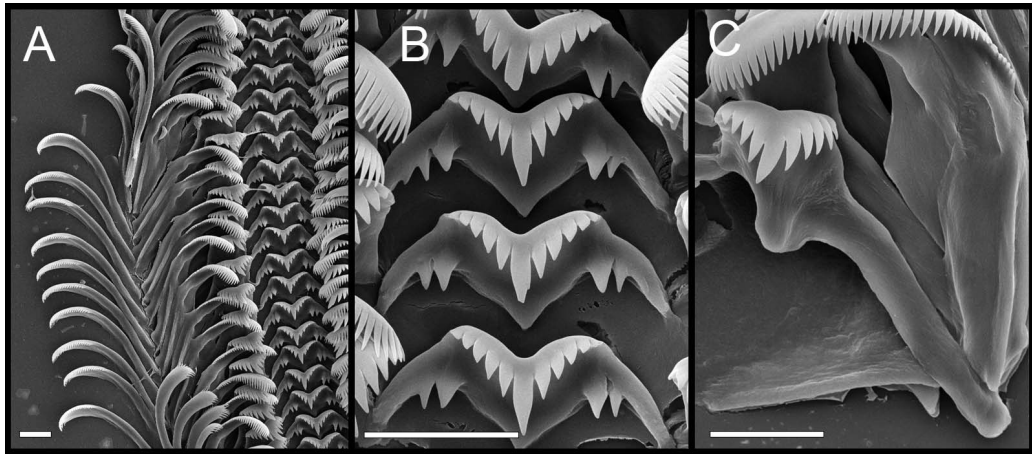


Fig. 7. Scanning electron micrographs of shells, opercula, and radula of *Juturnia brunei*, BellMNH 21270: A, portion of radular ribbon; B, central radular teeth; C, lateral and inner marginal radular teeth. Scale bars: A–C, 10 μ m.

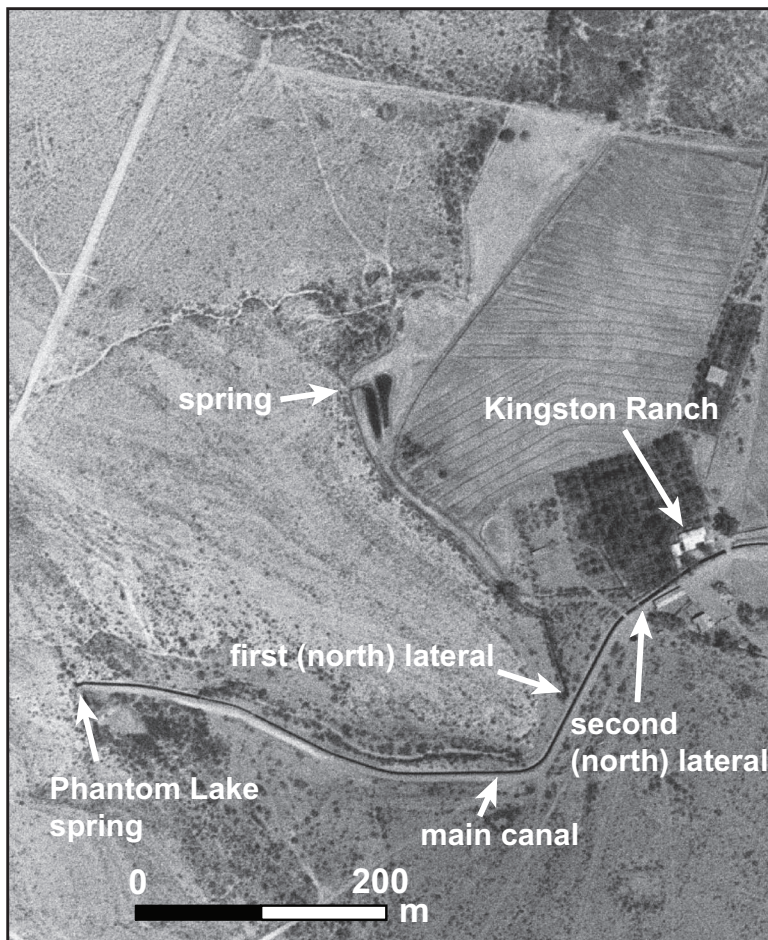


Fig. 8. Aerial photograph of Phantom Lake spring and canal system (2/iv/1971).

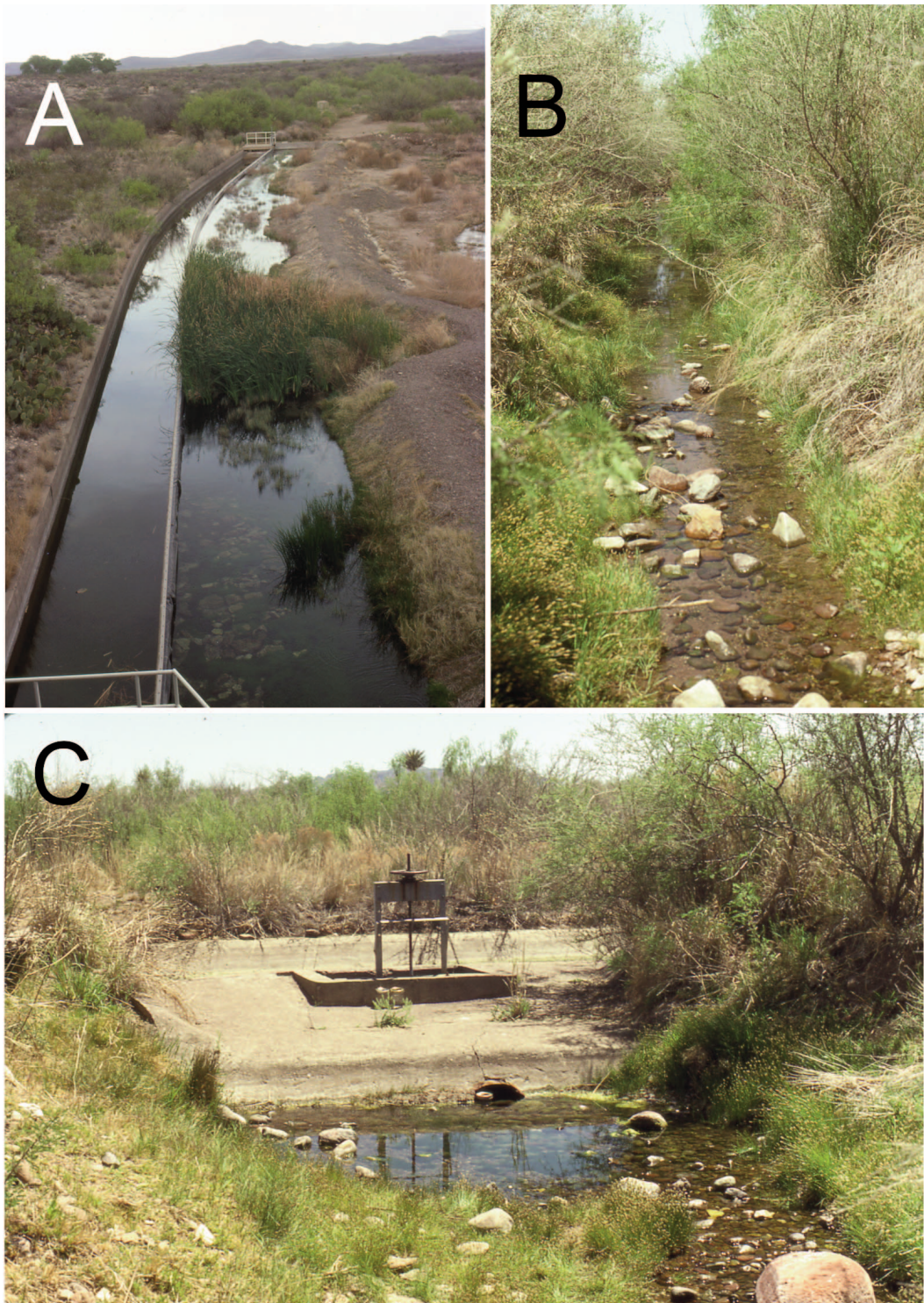


Fig. 9. Photographs of sampling localities in Phantom Lake spring system: A, main canal, looking east (downflow) (3/iv/1995); B, first (north) lateral canal, straight reach ca. 20 m up from main canal (20/iv/1993); C, small pool at beginning of first (north) lateral canal (20/iv/1993).

(behind the ranch house). When irrigation was taking place, the water from this spring potentially was returned to these lateral canals and to the main canal near the ranch house. *Juturnia brunei* has not been found since Taylor collected it in 1984. RH and JJJL intensively searched for this species at Taylor's collecting localities and other parts of the Phantom Lake spring system on several occasions after 1990 without success (although *T. cheatumi* was found abundantly); photographs of several of these sampling sites are shown in Fig. 9A–C. The second spring was dry when visited in 1993 (note that Phantom Lake spring ceased to flow around 2001; water is currently pumped out of a pool in Phantom Cave to maintain the epigeal aquatic habitats; USBR 2011). Based on this body of evidence, we concur with Allen (2001) and Besse (2002) that *J. brunei* is likely extinct. Besse's (2002:x) reference to the "small side spring" inhabited by *J. brunei* is incorrect and probably refers to the lateral canals where this snail once lived. The USFWS (2009:422) recently decided not to initiate a status review of *J. brunei* in response to a listing proposal (Forest Guardians 2007), owing to the minimal information provided for this species.

DISCUSSION

Our findings increase the number of species of *Tryonia* sensu lato in the Chihuahuan Desert to 28, further highlighting this area as one of the major centers of diversity of the predominantly New World cochliopid radiation (Hershler and Thompson 1992). The results of this study also provide additional insight into the complex biogeographic history of the regional cochliopid fauna. As previously detailed, *Tryonia* sensu lato is composed of 6 anatomically distinctive lineages (genera) that are variously nested within the North American cochliopid fauna (Hershler et al. 2011b: fig. 9). Two pairs of these lineages were previously found to co-occur sympatrically (*Eremopyrgus*–*Tryonia*, Hershler et al. 2011a; *Minckleyella*–*Pseudotryonia*, Hershler et al. 2011b); our reallocation of *T. brunei* to *Juturnia* has revealed a third such instance of sympatry (with *Tryonia* at Phantom Lake spring). Our findings also delineate a sister relationship between *T. minckleyi* populations of the San Diego and Julimes springs paralleling that of

T. chuwiscarae (San Diego) and *T. julimesensis* (Julimes). *Tryonia chuwiscarae* and *T. julimesensis* differ by $2.8\% \pm 0.6\%$ for COI, whereas the disjunct populations of *T. minckleyi* differ by $2.0\% \pm 0.5\%$, suggesting that they may have diverged more recently than the former.

Our findings also further highlight the imperiled status of the regional assemblage of *Tryonia* sensu lato, which includes 26 species that are (or were) narrowly distributed in only one or 2 springs or local spring complexes. The 2 new species of *Tryonia*, *J. brunei*, and the new populations of *T. allendae*, *T. peregrina*, and *T. sp.* reported herein all became extinct after 1970. Groundwater mining appears to have been the culprit in these cases. The remaining extant populations of *Tryonia* sensu lato in the Chihuahuan Desert are threatened not only by this component of water development, but also by urbanization, pollution, diversion of spring flow, and recreational activities.

Our taxonomic study of this fauna was based in large part on fieldwork conducted over a period of more than 40 years by JJJL. Beginning in 1968, previously described taxa were re-collected, and in the process, new populations and species were discovered, especially during an extended exploration of Chihuahua, Coahuila, Durango, Nuevo Leon, San Luis Potosi, and Zacatecas (with W.L. Minckley and 4 other associates) in 1971. During the early stage of this fieldwork, U.S. Army Map Service maps (1:250,000) and National Oceanic and Atmospheric Administration (NOAA) Sectional Aeronautical Charts (1:500,000) were used to locate springs. More recently, we relied on the 1:50,000 topographic maps published by the Instituto Nacional de Estadística Geografía e Informática (INEGI), which have proven invaluable in finding obscure spring sources (and obtaining correct Mexican place names). Springs also were located based on local inquiry (while in the field); suggestions and field notes from colleagues (e.g., Artie L. Metcalf, Robert R. Miller, Dwight W. Taylor); and consultation of historic diaries, memoirs, and modern travelogues following the Royal Road (Chihuahua Trail).

Although many of the regional topographic maps have been 80%–90% field-evaluated for the springs indicated on the sheets, others have been less investigated and thus discovery

of additional species of *Tryonia* sensu lato, especially in the Mexican portion of the Chihuahuan Desert, is likely. The area east of the Rio Conchos Valley, the part of the old Royal Road from Hacienda de Dolores to Mapimi (Chihuahua), and portions of the Rio Nazas and Rio Papigochic drainages in particular have been little surveyed for cochliopid gastropods and other freshwater mollusks. Given the widespread extirpation of spring-fed habitats in municipal areas of Mexico owing to groundwater withdrawals, the most likely prospects for additional species discovery are in little-developed regions.

Note that the type locality area for one of the species described in a previous contribution to this series, *Chorrobrius crassilabrum*, was incorrectly identified as being in the Rio Salado drainage (Hershler et al. 2011a). Further inspection of pertinent INEGI maps indicates that this area instead is in the Rio Salinas watershed (Rio Grande basin) as noted by Miller et al. (2005:124) per the distribution of *Gila modesta*.

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LITERATURE CITED

- ALLEN, N. 2001. Deterioration of Phantom Lake Spring, Jeff Davis County, Texas. *Proceedings of the Desert Fishes Council* 32:50–51.
- BESSE, H.C. 2002. Introduction. Pages i–xvi in G. Brune, author, *Springs of Texas*. Volume 1. 2nd edition. Texas A&M University Press, College Station, TX.
- BUCKLIN, A. 1992. Use of formalin-preserved samples for molecular analysis. *Newsletter of Crustacean Molecular Techniques* 2:3.
- DE LA MAZA-BENIGNOS, M., AND L. VELA-VALLADARES. 2009. *Cyprinodon julimes* De la Maza-Benignos & Vela-Valladares, sp. nov. Pages 185–187 in M. De la Maza-Benignos, editor, *Los Peces del Rio Conchos*. Alianza WWF – FGRA y Gobierno del Estado de Chihuahua, Chihuahua.
- FOLMER, O., M. BLACK, W. HOEH, R. LUTZ, AND R. VRIJENHOEK. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3:294–299.
- FOREST GUARDIANS. 2007. A petition to list all critically imperiled or imperiled species in the southwest United States as threatened or endangered under the Endangered Species Act, 16 U.S.C. §§ 1531 et seq. Santa Fe, New Mexico; [cited 20 August 2013]. 55 pp. Available from http://www.wildearthguardians.org/site/DocServer/petition_protection-475-species_6-21-07.pdf?docID=1442&AddInterest=1262
- HERSHLER, R. 1989. Springsnails (Gastropoda: Hydrobiidae) of Owens and Amargosa River (exclusive of Ash Meadows) drainages, Death Valley system, California–Nevada. *Proceedings of the Biological Society of Washington* 102:176–248.
- _____. 2001. Systematics of the North and Central American aquatic snail genus *Tryonia* (Rissooidea: Hydrobiidae). *Smithsonian Contributions to Zoology* 612:1–53.
- HERSHLER, R., H.-P. LIU, T.J. FREST, AND E.J. JOHANNES. 2007. Extensive diversification of pebblesnails (Lithoglyphidae: *Fluminicola*) in the upper Sacramento River basin, northwestern United States. *Zoological Journal of the Linnean Society of London* 149: 371–422.
- HERSHLER, R., H.-P. LIU, AND J.J. LANDYE. 2002b. A new species of *Eremopyrgus* (Hydrobiidae: Cochliopinae) from the Chihuahuan Desert, Mexico: phylogenetic relationships and biogeography. *Journal of Molluscan Studies* 68:7–15.
- _____. 2011a. Two new genera and four new species of freshwater cochliopid gastropods (Rissooidea) from northeastern Mexico. *Journal of Molluscan Studies* 77:8–23.
- _____. 2011b. New species and records of springsnails (Caenogastropoda: Cochliopidae: *Tryonia*) from the Chihuahuan Desert (Mexico and United States), an imperiled biodiversity hotspot. *Zootaxa* 3001:1–32.
- HERSHLER, R., H.-P. LIU, AND M. MULVEY. 1999. 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., H.-P. LIU, AND C.A. STOCKWELL. 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., AND E.G. THOMPSON. 1992. A review of the aquatic gastropod subfamily Cochliopinae (Prosobranchia: Hydrobiidae). *Malacological Review Supplement* 5:1–140.
- HUBBS, C., AND V.G. SPRINGER. 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., AND F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755.
- JACKSON, H. 2006. *Following the Royal Road: a guide to the historic Camino Real de Tierra Adentro*. University of New Mexico Press, Albuquerque, NM.
- MILLER, R.R., W.L. MINCKLEY, AND S.M. NORRIS. 2005. *Freshwater fishes of México*. University of Chicago Press, Chicago, IL.
- [NWC] NATIONAL WATER COMMISSION OF MEXICO. 2008. *Statistics of water in Mexico*. 2008 edition. Comisión Nacional de Agua, Mexico City, Mexico; [cited 5 August 2013]. 227 pp. Available from: <http://www.conagua.gob.mx/english07/publications/SGP-20StatisticsLow.pdf>
- NYLANDER, J.A.A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.
- SIMONDS, W.J. 1996. Balmorhea Project. Historic reclamation projects. U.S. Department of the Interior, Bureau of Reclamation; [cited 12 August 2013]. 20 pp. Available from http://www.usbr.gov/projects/ImageServer?imgName=Doc_1260211017894.pdf
- [SSI] SYSTAT SOFTWARE, INC. 2004. Systat® for Windows®. Richmond, CA.
- TAMURA, K., D. PETERSON, N. PETERSON, G. STECHER, M. NEI, AND S. KUMAR. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28:2731–2739.
- TAYLOR, D.W. 1987. Fresh-water molluscs from New Mexico and vicinity. *New Mexico Bureau of Mines and Mineral Resources Bulletin* 116:1–50.
- [USBR] UNITED STATES DEPARTMENT OF THE INTERIOR, BUREAU OF RECLAMATION. 2011. Final environmental assessment, ciénega wetland rehabilitation at Phantom Lake Spring, Toyavale, Texas. Albuquerque Area Office, Albuquerque, NM. 47 pp.
- [USFWS] UNITED STATES FISH AND WILDLIFE SERVICE. 2009. Endangered and threatened wildlife and plants; partial 90-day finding on a petition to list 475 species in the southwestern United States as threatened or endangered with critical habitat. *Federal Register* 74:419–427.
- _____. 2013. Endangered and threatened wildlife and plants; determination of endangered species status for six West Texas aquatic invertebrates. *Federal Register* 78:41228–41258.
- WISLIZENUS, A. 1848. *Memoir of a tour to northern Mexico connected with Col. Doniphan's expedition in 1846 and 1847*. Tippin and Streeper, Washington, DC. 30th Congress Senate Miscellaneous Document No. 26:1–141 + appendix, maps.
- WU, S.-K. 1989. *Colorado freshwater mollusks*. Natural History Inventory of Colorado, No. 11. University of Colorado Museum, Boulder, CO. 117 pp.

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