



Two new, possibly threatened species of *Pyrgulopsis* (Gastropoda: Hydrobiidae) from southwestern California

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Abstract

Here we describe (for conservation purposes) two new species of *Pyrgulopsis* from southwestern California based on morphologic and molecular (mtCOI) evidence. *Pyrgulopsis castaicensis* n. sp. is endemic to a single spring in the upper portion of the Santa Clara River basin and may be threatened by the proposed development of a master-planned community (Newhall Ranch) near Santa Clarita Valley. This snail differs from two morphologically similar regional congeners (*P. micrococcus* [Pilsbry in Stearns, 1893], *P. stearnsiana* [Pilsbry, 1899]) by its larger terminal gland, simple oviduct coil and mtCOI sequences (6.1–9.3% for *P. micrococcus*, 3.5–8.2% for *P. stearnsiana*). *Pyrgulopsis milleri* n. sp. is distributed in spring-fed waters along a short reach of upper Tule River drainage and threatened by surface water diversion and its close proximity to a major road (CA 190). *Pyrgulopsis milleri* differs from closely similar and geographically proximal *P. stearnsiana* in its broader central cusps on the central radular teeth, shorter pallial section of the albumen gland, greater overlap of the bursa copulatrix by the albumen gland, simple anterior vas deferens, usual absence of a terminal gland and mtCOI sequences (2.8–8.4%).

Key words: Santa Clara River, Tule River, springsnails, mitochondrial DNA, conservation

Introduction

Pyrgulopsis Call & Pilsbry, 1886 (Gastropoda: Hydrobiidae) is the largest genus of aquatic mollusks in North America, with 129 currently recognized species (Hershler & Liu 2009). This genus is composed of small (ca. 2–8 mm shell height) species whose rather uniform shells mask a striking anatomical (e.g., penial morphology) radiation (Taylor 1987; Hershler 1994; Hershler 1998). *Pyrgulopsis* typically lives in small, spring-fed habitats (e.g., Hershler 1998) and is distributed within much of the continent west of longitude 97°W (Hershler *et al.* 2008, fig. 1).

Although *Pyrgulopsis* has been intensively studied since 1994 (see Hershler & Liu 2009 and references cited therein), its species diversity is still poorly known because the genus has not been surveyed and taxonomically investigated in detail across much of its broad geographic range (Hershler & Liu 2009). One of the least studied geographic subunits of *Pyrgulopsis* is that of southwestern California, here treated as the San Joaquin-Tulare basin and coastal drainage from San Francisco Bay southward. Only seven currently recognized species and few records have been reported from this large region, which encompasses five major drainages having a total watershed area of >150,000 km² (Seaber *et al.* 1994). Five of these species — *P. diablensis* Hershler, 1995; *P. giulianii* Hershler & Pratt, 1990; *P. greggi* Hershler, 1995; *P. micrococcus* (Pilsbry in Stearns, 1893); *P. taylori* Hershler, 1995 — have narrowly localized distributions within the region (Hershler & Pratt 1990; Hershler 1995) while the other two — *P. californiensis* (Gregg & Taylor, 1965), *P. stearnsiana* (Pilsbry, 1899) — range widely (Gregg & Taylor 1965; Taylor 1981; Hershler 1994).

During the course of ongoing revisionary studies of *P. californiensis* and *P. stearnsiana* (prompted by genetic evidence that both are non-monophyletic; Hershler & Liu 2008) we discovered an undescribed species in the western Sierra Nevada and another in the Transverse Ranges. As per our other recent taxonomic studies of the western North American Hydrobiidae (e.g., Hershler *et al.* 2007), we are treating these snails as new species because they are morphologically diagnosable, and phylogenetically independent and substantially divergent (based on sequence data) relative to other members of the genus (see below). Both of these species are distributed in watersheds (upper Tule River, Santa Clara River, respectively) that have been assigned to the highest priority category (I) for restoration activities in California (CSWRCB 1998). Both also appear to be locally endemic and vulnerable to extirpation, which are among the criteria used by the State of California to list “special animals” having the greatest need of protection (CDFG 2009). We describe these possibly threatened novelties here so that they can be formally recognized and appropriately considered in local and regional conservation planning.

Material and methods

Snails were collected by hand or with a fine sieve. Specimens used for anatomical study were relaxed with menthol crystals and fixed in dilute formalin. Snails used for mtDNA sequencing were preserved in 90% ethanol in the field. UTM x-y coordinates (NAD83 datum, zone 11) are provided when available for a given sample. Types and other material of the two new species were deposited in the National Museum of Natural History (USNM) and Santa Barbara Museum of Natural History (SBMNH) collections.

Variation in the number of cusps on the radular teeth was assessed using the method of Hershler *et al.* (2007). Other methods of morphological study and descriptive terminology are from recent taxonomic studies of *Pyrgulopsis* (Hershler 1998; Hershler *et al.* 2003a). Shell measurement and whorl count data were compiled and analyzed using Systat for Windows 11.00.01 (SSI 2004).

Our molecular phylogenetic analysis included the two new species, the seven species which had previously been recorded from southwestern California (listed above), four other members of the genus which live in closely proximate portions of the southwestern Great Basin (*P. erythropoma* [Pilsbry, 1899]; *P. longinqua* [Gould, 1855]; *P. owensensis* Hershler, 1989; *P. wongi* Hershler, 1989) and two representatives of the closely related (per Liu & Hershler 2005) eastern North American genus *Floridobia* Thompson & Hershler (*F. floridana* [Frauenfeld, 1863], *F. winkleyi* [Pilsbry, 1912]). Prior to the final analyses we performed a comprehensive (unpublished) survey of mtDNA variation within *Pyrgulopsis* to confirm that close relatives of the two new species had not been omitted. Trees were rooted with the type species of *Floridobia*, *F. floridana*.

Genomic DNA was extracted from entire snails using a CTAB protocol (Bucklin 1992). A partial (658 bp) segment of mitochondrial cytochrome c oxidase subunit I (mtCOI) corresponding to “Folmer’s fragment” (Folmer *et al.* 1994) was amplified and sequenced with primers LCO1490 and HCOI2198 following the protocols of Liu *et al.* (2003). One to four specimens were sequenced from each sample. Sequences were determined for both strands and then edited and aligned using Sequencher™ version 4.8. Sample information and GenBank accession numbers for sequenced specimens are in Table 1; the locations of *Pyrgulopsis* sampling sites are shown in Figure 1.

Sequence divergences (uncorrected p distance) within and between phylogenetic lineages were calculated using MEGA4 (Tamura *et al.* 2007); standard errors were estimated by 1000 bootstrap replications with pairwise deletion of missing data. In order to provide a readable tree and reduce computation time, only one sequence of each haplotype per population sample was used in the phylogenetic analyses. Base compositional differences were first evaluated using the X² 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. Bayesian inference was performed using MrBayes 3.12 (Ronquist & Huelsenbeck 2003). In the initial analysis the burnin was set at 10% (10,000 generations) of the chain length (100,000 generations). Three runs were conducted in MrBayes using the model selected by MrModeltest and the default random tree

option to determine when the log likelihood scores reached a stable value (by plotting the log likelihood scores of sample points against generation time). The log likelihood scores started at around -5100 and quickly converged upon a stable value of about -2950 after approximately 10,000 generations in all three preliminary runs. For the final run Metropolis-coupled Markov chain Monte Carlo simulations were performed with four chains for 1,000,000 generations and Markov chains were sampled at intervals of 10 generations to obtain 100,000 sample points. The sampled trees with branch lengths were used to generate a 50% majority rule consensus topology with the first 5,000 trees, equal to 50,000 generations, removed to ensure that the chain sampled a stationary portion.

TABLE 1. Specimen codes, number of sequenced specimens (*n*), locality details and GenBank accession numbers. ^aTwo new sequences same as DQ364019; ^bnew sequence same as AY627922; ^cthree new sequences same as AY367484; ^dnew sequence same as AF520943; ^esequence same as AY367470; ^ftwo new sequences same as AF367490; ^gthree new sequences same as AY627956; ^hLiu & Hershler (2005); ⁱHershler & Liu (2008); ^jLiu *et al.* (2003); ^kHershler *et al.* (2003b).

Species	Specimen code	n	Locality	GenBank
<i>californiensis</i>	1A	2	Spring tributary to Campo Creek, Southern California coastal drainage, San Diego County, California	AY627924 ^h
<i>californiensis</i>	2A	3 ^a	Spring tributary to Lytle Creek, Santa Ana River basin, San Bernardino County, California	DQ364019 ^j
<i>californiensis</i>	3A	1	Spring tributary to Snow Creek, Southern California coastal drainage, Riverside County, California	AY367481 ^j
<i>castaicensis</i> sp. nov.	4A	3	Middle Canyon Spring, Southern California coastal drainage, Los Angeles County, California	GQ275097
<i>diablensis</i>	5AA	2 ^b	Stream, Del Puerto Canyon, San Joaquin River basin, Stanislaus County, California	AY627922 ^h
<i>erythropoma</i>	6AA	4 ^c	Kings Pool outflow, Ash Meadows, Amargosa River basin, Nye County, Nevada	AY367484 ^j
<i>giulianii</i>	7A	1	Stream, Sand Canyon, Indian Wells Valley, Kern County, California	AF520937 ^k
<i>greggi</i>	8A	3 ^d	Grapevine Creek, Kern River basin, Kern County, California	AF520943 ^k
-	8AA	2		GQ275088
<i>longinqua</i>	9B	1	Spring west-southwest of Hunters Spring, Salton basin, Riverside County, California	DQ364006 ⁱ
<i>micrococcus</i>	10A	1	Cushenbury Springs, Southern Mojave basin, San Bernardino County, California	AY367461 ^j
<i>micrococcus</i>	11A	1	Springs at Big Bear Ranger Station, Southern Mojave basin, San Bernardino County., California	AY367462 ^j
<i>micrococcus</i>	12A	2	Springs at Thurman Flats, Southern California coastal drainage, San Bernardino County, California	AY367463 ^j
<i>micrococcus</i>	13A	1	Spring north of Tecopa Hot Springs, Amargosa River basin, Inyo County, California	AY367469 ^j
-	13B	1		AY367470 ^j
-	13C	1 ^e		AY367471
<i>milleri</i> sp. nov.	14B	3	Pierpoint Spring, Tulare-Buena Vista Lakes drainage, Tulare County, California	GQ275096
<i>owensensis</i>	15C	1	Stream in canyon south of Piute Creek, Owens River basin, Mono County, California	AF520922 ^k

to be continued.

TABLE 1. (continued.)

Species	Specimen code	n	Locality	GenBank
-	15AA	2		GQ275089
-	15AB	1		GQ275090
<i>stearnsiana</i>	16	2	Springs in Wild Cat Canyon, San Francisco Bay drainage, Contra Costa County, California	AF520925 ^k
<i>stearnsiana</i>	17AA	4	San Domingo Creek, San Joaquin River basin, Calaveras County, California	GQ275091
<i>stearnsiana</i>	18A	1	Partington Creek, Central California coastal drainage, Monterey County, California	AF367489 ^j
-	18AA	1		GQ275092
<i>stearnsiana</i>	19A	3 ^f	Stream in Colson Canyon, Central California coastal drainage, Santa Barbara County, California	AF367490 ^j
<i>stearnsiana</i>	20A	4	Spring tributary to Sisar Creek, Southern California coastal drainage, Ventura County, California	GQ275093
<i>stearnsiana</i>	21AA	3	Stream, Little Sycamore Canyon, Southern California coastal drainage, Ventura County, California	GQ275094
<i>taylori</i>	22A	1	Spring tributary to San Luis Obispo Creek, California central coastal drainage, San Luis Obispo County, California	AY627923 ^h
-	22AA	3		GQ275095
<i>wongi</i>	23A	4 ^s	Spring in Lower Pine Creek Canyon, Owens River basin, Inyo County, California	AY627956 ^h
<i>F. floridana</i>	-	1	Juniper Springs, St. Johns River basin, Marion County, Florida	AF520916 ^k
<i>F. winkleyi</i>	-	1	Dunstan River salt marsh, Saco River basin, Cumberland County, Maine	AF520917 ^k

Results

Molecular analysis. Thirty-two specimens were newly sequenced for this study. New sequences were deposited in GenBank under accession numbers GQ275088–GQ275097 (Table 1; note that only one sequence per haplotype per population was deposited in GenBank). A total of 658 bp of COI was analyzed, of which 182 sites (27.66%) were variable and 143 (21.73%) were parsimony-informative. Average base frequencies were 25.1% A, 36.6% T, 19.9% C and 18.4% G. There was no significant base frequency bias among species ($X^2 = 12.64$, $df = 90$, $P = 1.00$).

MrModeltest selected the general time-reversible (GTR) model, with some sites assumed to be invariable and with variable sites assumed to follow a discrete gamma distribution (e.g., GTR + I + G), as the best fit for the combined dataset using the Akaike Information Criterion. The GTR + I + G model was used for the Bayesian analysis. The phylogenetic relationships of the two new species were not well supported (Figure 2). The new species from the Santa Clara River basin, *P. castaicensis* (described below), was depicted as sister to a population of *P. stearnsiana* (20A) that also lives in this watershed. The new species from the Tule River basin, *P. milleri* (described below), was sister to a coastal population of *P. stearnsiana* (21AA). Note that each of the three species that were depicted as non-monophyletic (*P. californiensis*, *P. micrococcus*, *P. stearnsiana*) are composites requiring revision (Liu *et al.* 2003; Hershler & Liu unpublished).

We did not detect any sequence variation within the small samples ($n = 3$) that were analyzed for both of the new species. The sequence divergence between these snails and other congeners included in the analysis ranged from 2.8–9.3%. Additional genetic results are detailed in the “Remarks” sections below.

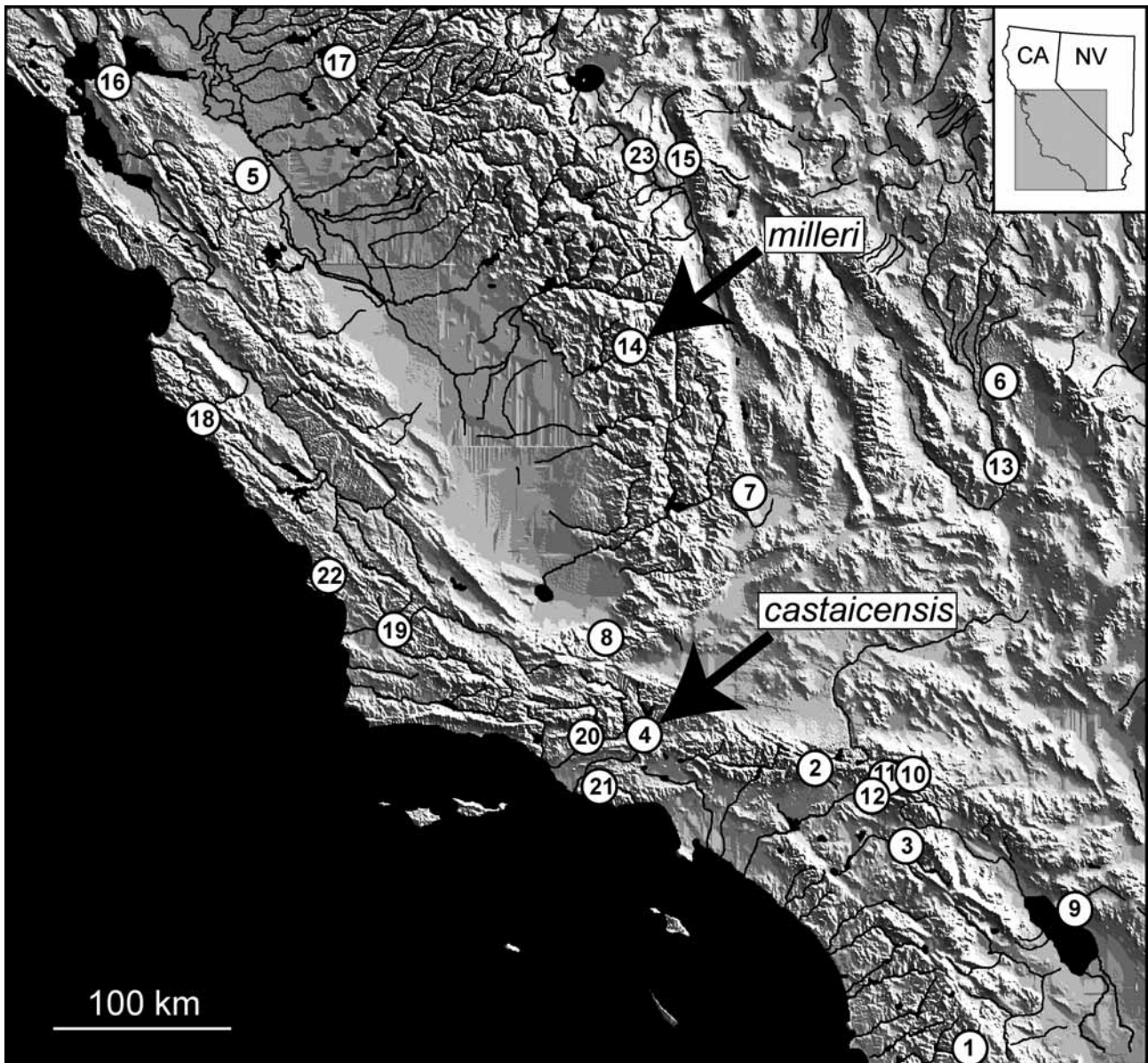


FIGURE 1. Map of southwestern California and an adjacent portion of Nevada showing the collecting localities for *Pyrgulopsis* samples used in the molecular analysis.

Systematics

Family Hydrobiidae

Subfamily Nymphophilinae

Genus *Pyrgulopsis* Call & Pilsbry, 1886

Type species: *Pyrgula nevadensis* Stearns, 1883, by original designation

Diagnosis: Liu & Hershler 2005: 296.

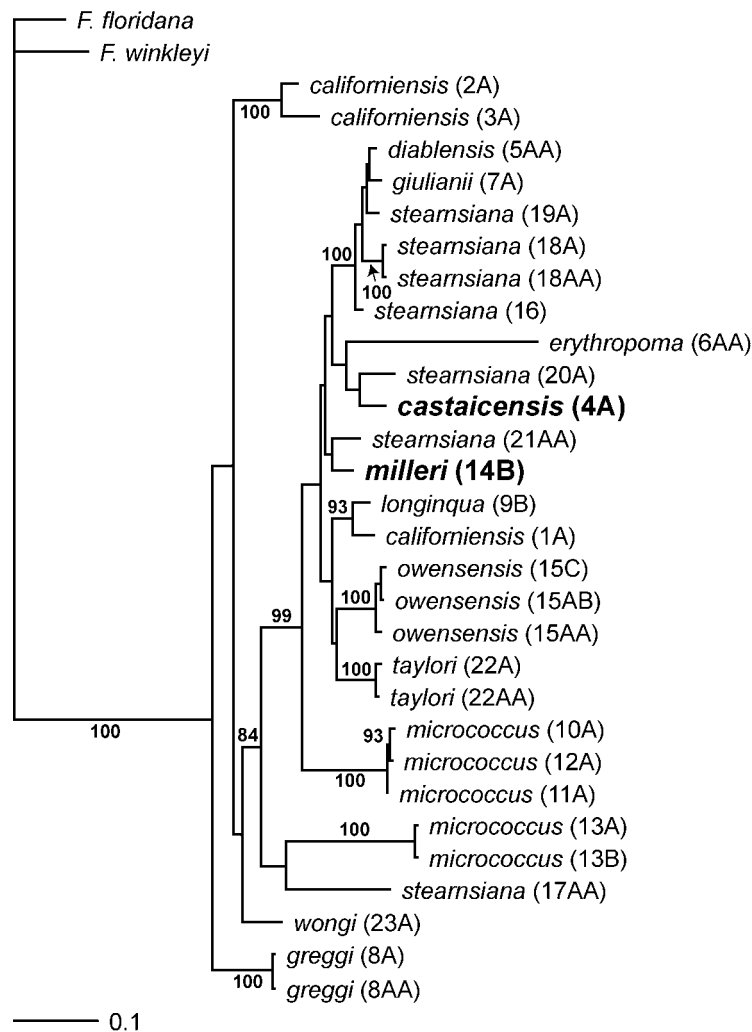


FIGURE 2. Bayesian tree based on the COI dataset. Posterior probabilities for nodes are shown when >80%.

***Pyrgulopsis castaicensis* sp. nov.**

(Figs 3–5)

Types. Holotype (Fig. 3A), USNM 1120442, Middle Canyon Spring, ca. 1.46 km southwest of Castaic Junction, Santa Clara River drainage, Los Angeles County, California (351542 E, 3810993 N), 21/x/2008, Robert Hershler. Paratypes, USNM 1132532 (from same lot, 60 dry shells).

Etymology. A geographic epithet referring to the distribution of this species near the mouth of Castaic Creek.

Referred material. CALIFORNIA. Los Angeles County. USNM 1097788., Middle Canyon Spring, ca. 20 m below spring source, 351596 E, 3810937 N, 26/vi/2006, Mark Elvin & Douglas Threlhoff.

Diagnosis. A small species of *Pyrgulopsis* having a medium-spined, sub-globose to narrow-conic shell with medium to highly convex whorls. Penis having a small lobe and short filament; penial ornament consisting of a well developed terminal gland.

Description. Shell (Fig. 3A–C) usually ovate- or narrow-conic, rarely sub-globose (smallest individuals); height about 1.3–2.5 mm; whorls 3.5–4.5. Periostracum tan, thin. Protoconch (Fig. 3D) near planispiral, about 1.3 whorls, diameter about 365 μ m, initial 0.5 whorl (Fig. 3E) weakly wrinkled, otherwise smooth. Teleoconch whorls medium or highly convex, strongly shouldered; sculpture of well developed, collabral growth lines; last 0.5 whorl usually slightly loosened. Aperture medium-sized, ovate, slightly angled adapically. Inner lip usually disjunct, rarely narrowly adnate; thin or slightly thickened internally, without

columellar shelf; outer lip thin, orthocline or weakly prosocline. Umbilicus perforate in specimens having an adnate inner lip.

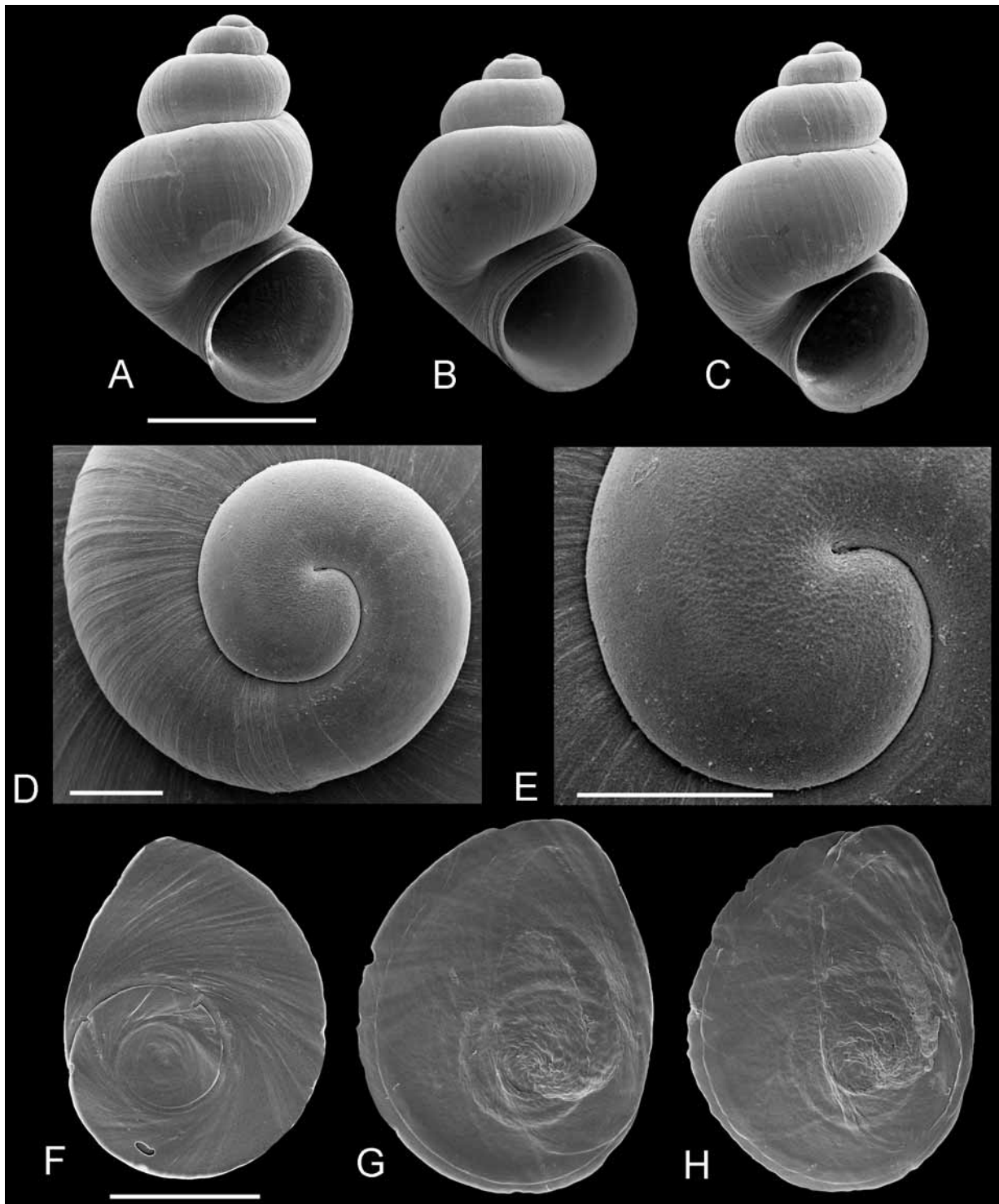


FIGURE 3. *P. castaicensis* sp. nov. A–C. Shells. Scale = 1.0 mm. A. Holotype, USNM 1120442. B–C. Paratypes, USNM 1132532. D. Shell apex, USNM 1132532. Scale = 100 μ m. E. Close-up of protoconch sculpture, USNM 1132532. Scale = 100 μ m. F–H. Opercula, USNM 1132532. Scale = 250 μ m. F. Outer side, showing frilled whorl. G–H. Inner side, showing variation in thickening of attachment scar margin.

Operculum (Fig. 3F) thin, flat, light amber (nuclear region slightly darker), multispiral with sub-central nucleus; last 0.5–0.75 whorl weakly frilled on outer side; attachment scar border on inner side of operculum (Fig. 3G–H) nearly smooth to slightly thickened almost all around. Radula taenioglossate (Fig. 4A), having

about 43 well-formed rows of teeth. Central teeth (Fig. 4B) about 17 μm wide, cutting edge concave; lateral cusps 4–6; central cusp narrow, pointed, parallel-sided proximally; basal cusp 1, small; basal tongue V-shaped, about as long as lateral margins. Lateral tooth (Fig. 4C) face rectangular; central cusp large, narrow, pointed; lateral cusps 3–4 (inner), 4–5 (outer); outer wing medium width, flexed, straight, about 250% length of cutting edge; basal tongue weakly developed. Inner marginal teeth (Fig. 4C–E) having 22–26 cusps. Outer marginal teeth (Fig. 4F) having 24–28 cusps; inner edge having long, rectangular wing. Radular data were from USNM 1097788.

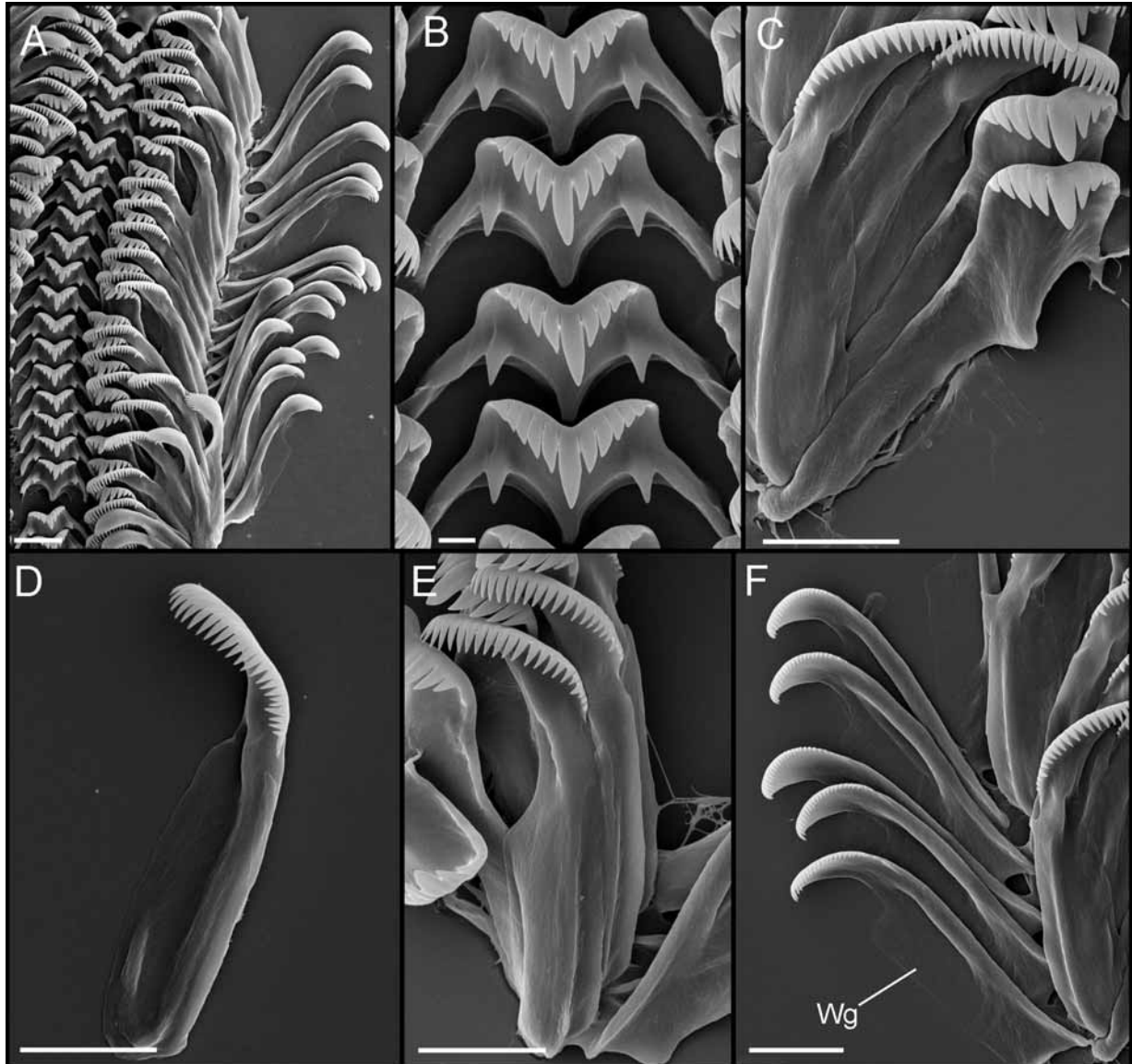


FIGURE 4. *P. castaicensis* sp. nov., USNM 1097788. A. Portion of radula ribbon. Scale = 10 μm . B. Central radular teeth. Scale = 2 μm . C. Lateral and inner marginal teeth. Scale = 10 μm . D–E. Inner marginal teeth. Scales = 10 μm . F. Outer marginal teeth. Scale = 10 μm . Wg = wing.

Head-foot generally lightly pigmented. Cephalic tentacles pale or having central, longitudinal brown streak. Snout light brown or black, pigment weaker or absent centrally; distal lips pale. Sole of foot pale. Pallial roof, visceral coil dark brown or black dorsally. Ctenidium well developed, positioned a little in front of pericardium; ctenidial filaments about 15, rather small, taller than wide, without pleats. Osphradium narrow or elongate, positioned centrally along ctenidium. Prostate gland small, bean-shaped, about 33% of length in pallial roof. Anterior vas deferens opening from ventral edge of prostate gland a little in front of (posterior) pallial wall, section of duct on columellar muscle having prominent bend. Penis (Fig. 5A–B) medium sized; base rectangular, slightly expanded distally; inner edge having weak folds; filament short,

narrow, tapering, slightly oblique; lobe short, broad, slightly tapered distally, oblique. Terminal gland usually narrow, crescent-shaped, overlapping ventral and (to a lesser extent) dorsal edges of lobe; gland divided into two small, circular units in one specimen. Penial duct narrow, nearly straight. Proximal half of penial filament containing a dense core of black pigment; penis otherwise pigmented with scattered black granules. Female glandular duct and associated structures shown in Figure 5C–E. Coiled oviduct a medium-sized, posterior-oblique loop. Bursa copulatrix small, ovate, horizontal, largely overlapped by albumen gland. Bursal duct slightly longer and considerably narrower than bursa, opening from distal edge, partly embedded in albumen gland. Seminal receptacle small to medium-sized, sac-shaped, sometimes folded, positioned near antero-ventral edge of bursa, duct short to medium length. Albumen gland longer and wider than capsule gland, having very short pallial section. Capsule gland composed of two tissue sections. Genital aperture a terminal slit.

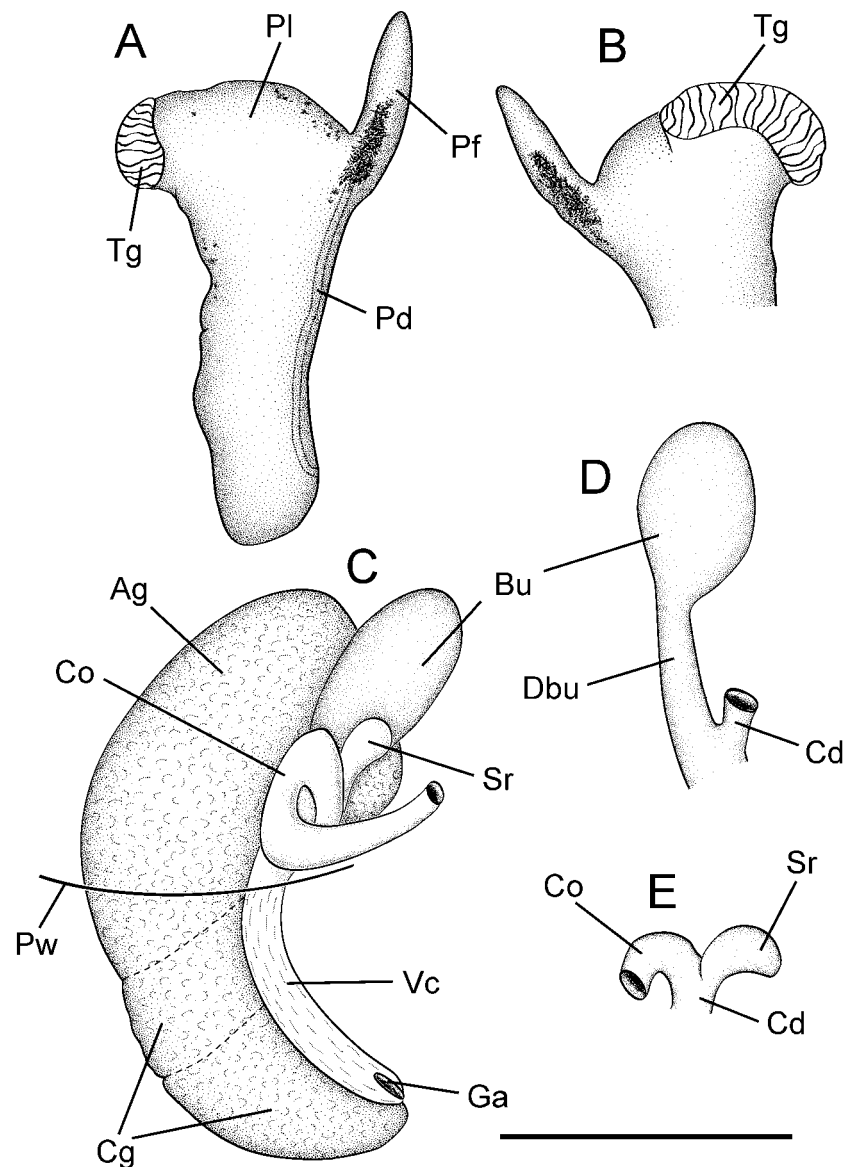


FIGURE 5. *P. castaicensis* sp. nov., USNM 1097788. Scale = 0.25 mm. A–B. Penis. A. Dorsal surface. B. Ventral surface of distal portion, showing extent of terminal gland. C. Female glandular oviduct and associated structures (viewed from the left side). D. Bursa copulatrix and its duct. E. Seminal receptacle and its duct. Ag = albumen gland, Bu = bursa copulatrix, Cd = common duct of seminal receptacle and oviduct, Cg = capsule gland, Co = coiled oviduct, Dbu = bursal duct, Ga = genital aperture, Pd = penial duct, Pf = penial filament, Pl = penial lobe, Pw = posterior wall of pallial cavity, Tg = terminal gland, Vc = ventral channel, Vg = ventral gland.

Shell measurements (mean \pm standard deviation in parentheses): height 1.93–2.45 mm (2.16 \pm 0.15), width 1.29–1.65 mm (1.44 \pm 0.10), body whorl height 1.43–1.79 mm (1.62 \pm 0.09), body whorl width 1.13–1.44 mm (1.27 \pm 0.08), aperture height 0.82–1.03 mm (0.90 \pm 0.06), aperture width 0.75–0.91 mm (0.82 \pm 0.04), shell width/height 0.61–0.71 (0.67 \pm 0.03), body whorl height/shell height 0.69–0.79 (0.75 \pm 0.02), aperture height/shell height 0.36–0.46 (0.42 \pm 0.02) (from paratype lot, USNM 1132532, $n = 30$).

Measurements of holotype: height 2.50 mm, width 1.70 mm, body whorl height 1.81 mm, body whorl width 1.39 mm, aperture height 1.05 mm, aperture width 0.92 mm, shell width/height 0.68, body whorl height/shell height 0.72, aperture height/shell height 0.42, 4.25 whorls.

Distribution, habitat and conservation status. *Pyrgulopsis castaicensis* appears to be endemic to the type locality area based on the first author's regional fieldwork and study of pertinent institutional collections, and recent surveys of proximate portions of the Santa Clara River basin (Swift 2009). The small, shallow spring-fed stream inhabited by this species is situated on a terrace of the Santa Clara River and shaded by a tall canopy and dense riparian vegetation (Dudek 2007). *Pyrgulopsis castaicensis* was rather abundant on plant debris and the sandy bottom of the stream.

The type locality area is located along the northeastern edge of the property slated for development. The resources management plan for this development incorporates an adaptive management framework to ensure protection of the spring habitat of *P. castaicensis* (USACE & CDFG 2009); the California Department of Fish and Game is overseeing the implementation of the plan and the conservation program associated with the development. Although its spring habitat will be protected under this plan as a "unique landscape feature," *P. castaicensis* nonetheless may be threatened by the extensive alteration of local topography and watershed associated with this development (GSI Water Solutions Inc. 2007; Dudek 2008a).

Remarks. The spring that constitutes the type locality of *P. castaicensis* is not shown on USGS topographic or BLM land management maps, but has been referred to as Middle Canyon Spring in numerous documents relating to the Newhall Ranch development plan (e.g., Dudek 2007; Dudek 2008b; USACE & CDFG 2009). The species described herein as *P. castaicensis* was mentioned (as an undescribed species of *Pyrgulopsis*) in several of these documents (e.g., Dudek 2007; Swift 2009; USACE & CDFG 2009).

The penial ground plan of *P. castaicensis* – narrow, distally bifurcate, ornamented with a terminal gland along the edge of the lobe – is a common (Hershler & Sada 2002) and apparently iteratively evolved (Liu & Hershler 2005) morphology within the genus that is shared by two congeners that are distributed in fairly close proximity (ca. 45–150 km) to this new species (*P. micrococcus*, eastern Transverse Ranges; *P. stearnsiana*, lower Santa Clara River basin). *Pyrgulopsis castaicensis* is distinguished from *P. micrococcus* by its larger terminal gland (on the penis), simple oviduct coil (lacking a proximal kink or loop), more anteriorly positioned seminal receptacle, and mtCOI sequences (6.1–9.3% divergence) (see Hershler & Sada 1987; Hershler 1989; Hershler 1994 for morphological descriptions of the latter). *Pyrgulopsis castaicensis* is differentiated from *P. stearnsiana* by its more convex teleoconch whorls, larger penial lobe, larger terminal gland, simple oviduct coil, shorter pallial section of albumen gland and smaller (relative to bursa copulatrix) seminal receptacle (see Hershler 1994 for description of the latter). *Pyrgulopsis castaicensis* was most similar in its mtCOI sequences to *P. stearnsiana* from the San Francisco Bay area (3.50% divergence) among all of the samples included in our study and differed from other specimens of this species by 3.8–8.2%. This level of sequence divergence falls within the range previously documented for >60 other species of *Pyrgulopsis* (1.1–13.1%; Liu & Hershler 2005). *Pyrgulopsis castaicensis* thus is recognized as a new species because it is morphologically diagnosable and because our sequence data indicate that it is phylogenetically independent and substantially divergent.

***Pyrgulopsis milleri* sp. nov.**

(Figs 6–8)

Types. Holotype (Fig. 6A), SBMNH 83651, creek 0.7 mile (1.13 km) east of Pierpoint Spring on (California) Hwy 190, approximately 15.6 miles (25.1 km) east of Springville, Tule River drainage, Tulare County,

California, Walter B. Miller, 1/viii/1964. Paratypes, SBMNH 74688, USNM 1132568 (from same lot, 211 + 6 dry shells, respectively).

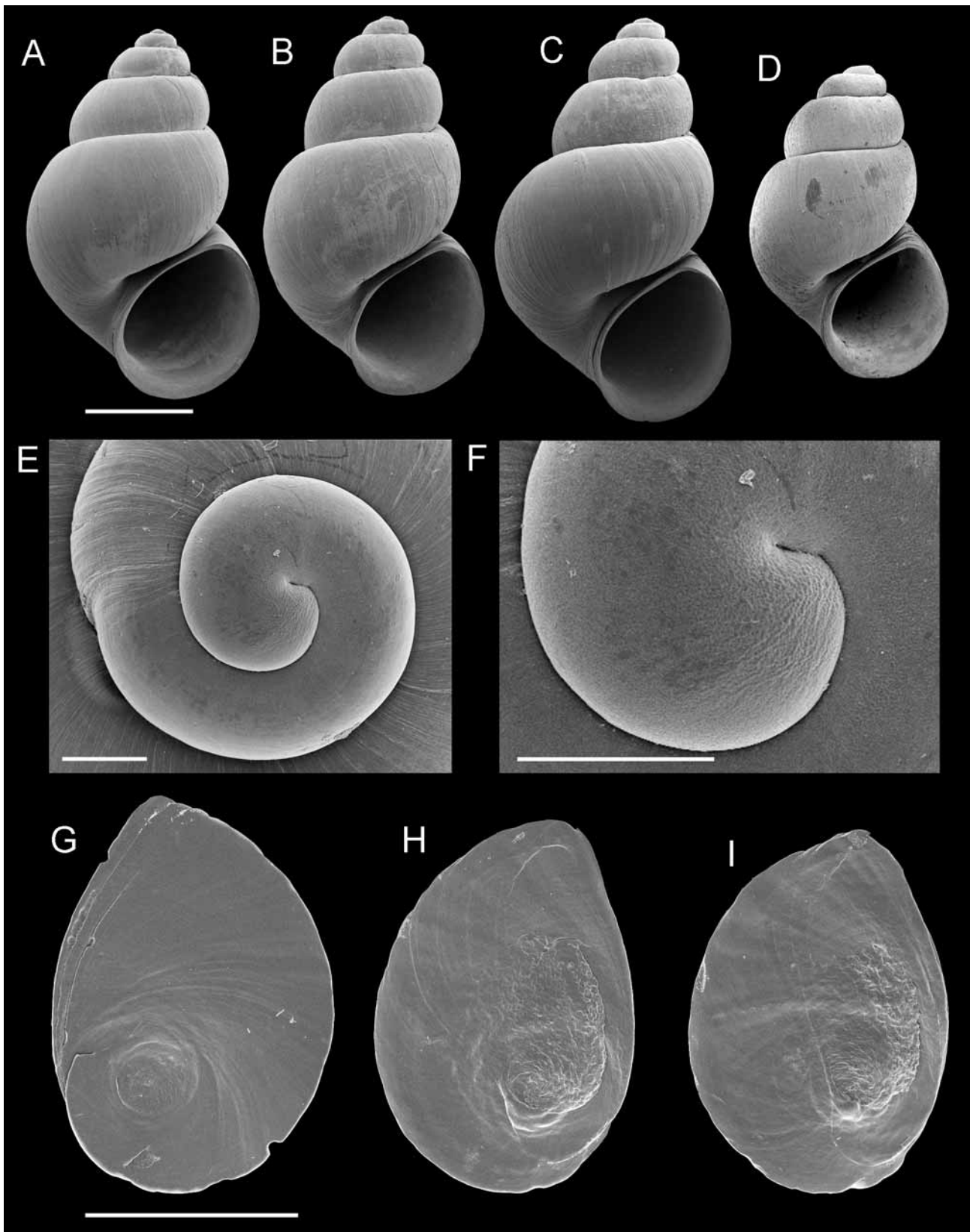


FIGURE 6. *P. milleri* sp. nov. A–D. Shells. Scale = 1.0 mm. A. Holotype, SBMNH 83651. B–C. Paratypes, SBMNH 74688. D. SBMNH 74687. E. Shell apex, SBMNH 74688. Scale = 100 µm. F. Close-up of protoconch sculpture, SBMNH 74688. Scale = 100 µm. G–I. Opercula, USNM 90527. Scale = 500 µm. G. Outer side. H–I. Inner side, showing variation in thickening of attachment scar margin.

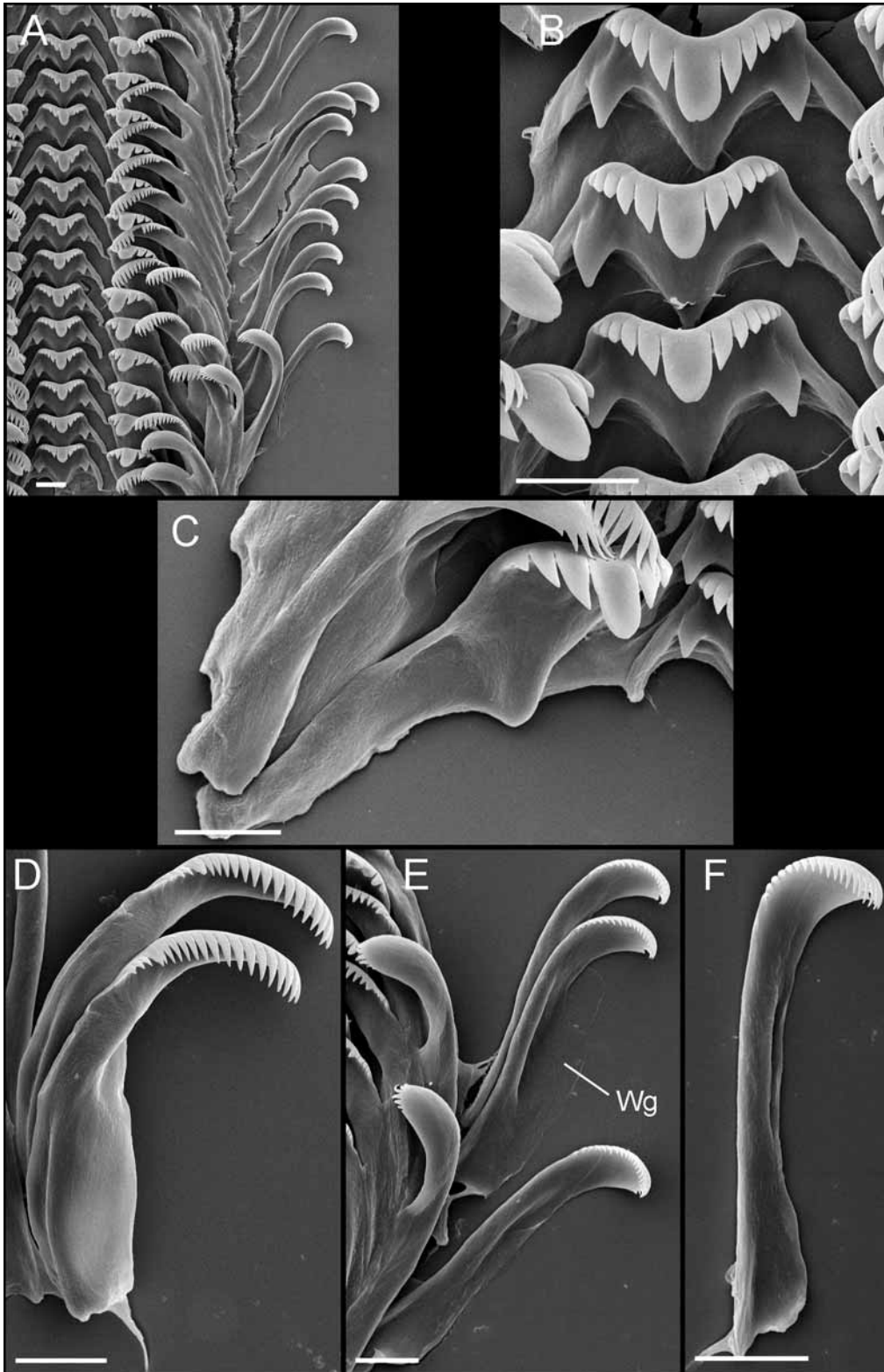


FIGURE 7. *P. milleri* sp. nov., USNM 90527, scales = 10 μ m. A. Portion of radula ribbon. B. Central radular teeth. C. Lateral tooth. D. Inner marginal teeth. E–F. Outer marginal teeth. Wg = wing.

Etymology. In honor of deceased zoologist Walter B. Miller, an intrepid collector of western North American nonmarine mollusks (Hochberg & Roth 2001) who first discovered this novelty in 1964.

Referred material. CALIFORNIA. Tulare County: SBMNH 74687, Pierpoint Spring, 6.5 miles (10.46 km) east of Tule River power house, 353028 E, 4000829 N (approximate coordinates), Walter B. Miller, 31/

vii/1964. USNM 905257, *ibid.*, Robert Hershler, 26/vi/2000. SBMNH 74540, spring along Hwy 190 7 miles (11.23 km) east of Tule hydroelectric plant, Walter B. Miller, 25/i/1963.

Diagnosis. A medium-sized species having ovate- to narrow-conic shell with weakly convex whorls. Penis usually alobate; filament medium length; penial ornament usually consisting of a small ventral gland.

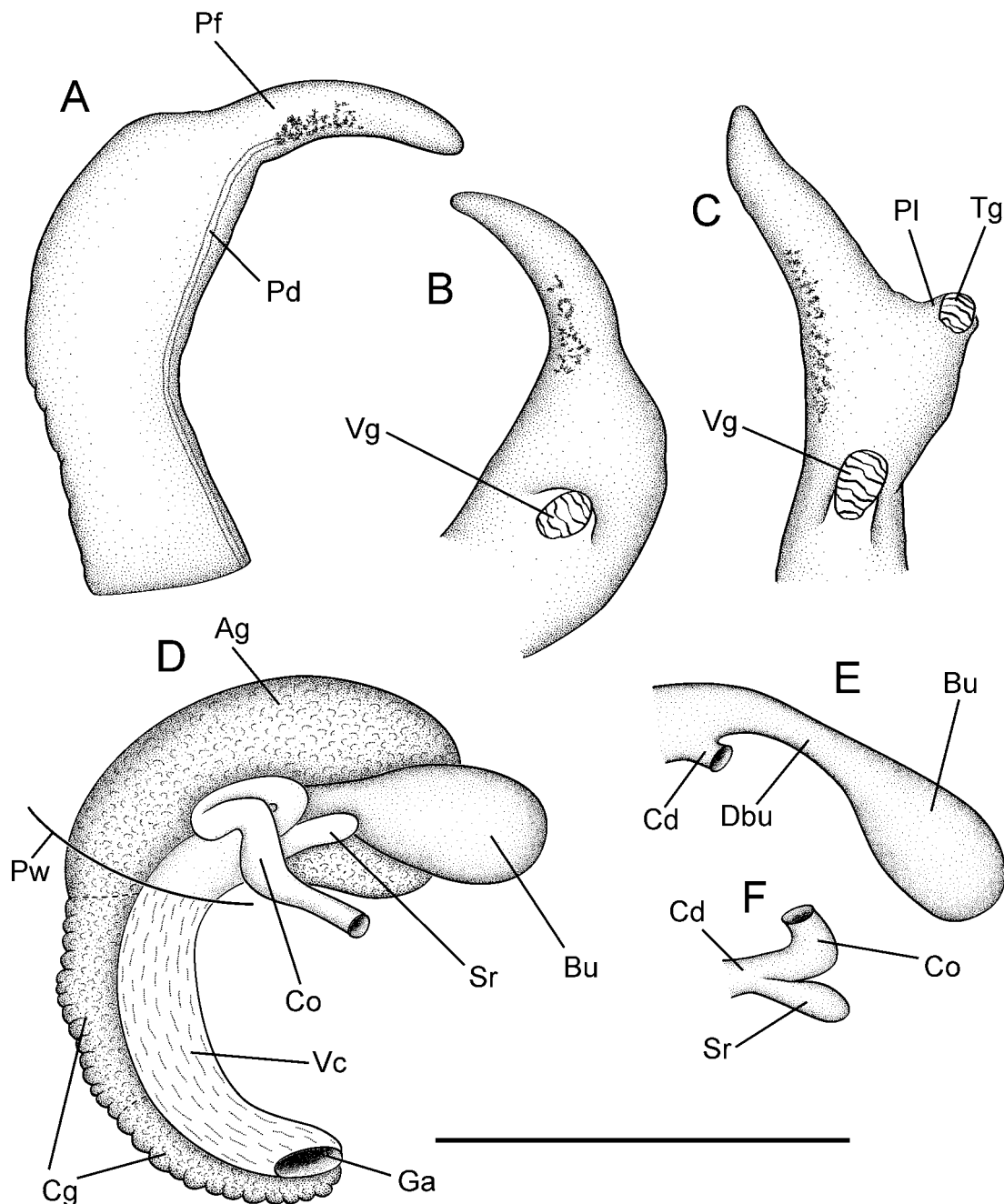


FIGURE 8. *P. milleri* sp. nov., USNM 90527. Scale = 0.5 mm. A–C. Penis. A. Dorsal surface. B–C. Ventral surface, showing ventral and terminal glands. D. Female glandular oviduct and associated structures (viewed from the left side). E. Bursa copulatrix and its duct. F. Seminal receptacle and its duct. Ag = albumen gland, Bu = bursa copulatrix, Cd = common duct of seminal receptacle and coiled oviduct, Cg = capsule gland, Co = coiled oviduct, Dbu = bursal duct, Ga = genital aperture, Pd = penial duct, Pf = penial filament, Pl = penial lobe, Pw = posterior wall of pallial cavity, Sr = seminal receptacle, Tg = terminal gland, Vc = ventral channel, Vg = ventral gland.

Description. Shell (Fig. 6A–D) ovate- or narrow-conic, height about 2.5–4.0 mm; whorls 4.25–5.25. Periostracum tan, thin, often covered with dark deposits. Protoconch (Fig. 6E) near planispiral, 1.3–1.5 whorls, diameter about 380 μ m, surface weakly wrinkled near apex (Fig. 6F), otherwise smooth. Teleoconch

whorls weakly convex, sometimes narrowly shouldered, last 0.25 whorl rarely slightly loosened; sculpture of collabral growth lines. Aperture ovate, angled adapically. Inner lip complete, usually adnate, slightly thickened internally; columellar shelf absent; outer lip thin, weakly prosocline. Umbilicus narrow.

Operculum (Fig. 6G) thin, flat, light amber, multispiral with eccentric nucleus; last quarter whorl frilled on outer side; inner side (Fig. 6H–I) having attachment scar border slightly thickened almost all around. Radula taenioglossate (Fig. 7A), having about 55 well-formed rows of teeth. Central teeth (Fig. 7B) about 28 μm wide, cutting edge concave; lateral cusps 3–6; central cusp large, U-shaped; basal cusp 1, small; basal tongue V-shaped, about as long as lateral margins. Lateral tooth (Fig. 7C) face rectangular, angled; central cusp large, U-shaped; lateral cusps 2–4 (inner), 3–5 (outer); outer wing medium width, straight, about 200% length of cutting edge; basal tongue well developed. Inner marginal teeth (Fig. 7D) having 17–21 cusps. Outer marginal teeth (Fig. 7E–F) having 19–28 cusps; inner edge having long, rectangular wing. Radula data were from USNM 905257.

Head-foot generally pale. Snout usually pale, but sometimes light to dark brown. Pallial roof, visceral coil dark brown or black. Ctenidium well developed, positioned a little in front of pericardium; ctenidial filaments about 20, broadly triangular. Osphradium narrow, positioned slightly posterior to middle of ctenidium. Prostate gland large, bean-shaped, with about 40% of length in pallial roof. Anterior vas deferens opening from ventral edge of prostate gland a little in front of posterior pallial wall, section of duct on columellar muscle straight. Penis (Fig. 8A–C) medium-sized, base rectangular, inner edge smooth; filament short or medium length, tapering, slightly oblique; lobe usually absent, nub-like when present (Fig. 8C). Terminal gland (observed in one specimen) small, ovate, positioned on ventral surface of lobe. Ventral gland small, ovate, centrally positioned, borne on raised swelling. Penial duct narrow, nearly straight. Penis entirely pale or with filament variably pigmented by black granules. Female glandular oviduct and associated structures shown in Figure 8D–F. Coiled oviduct a small, proximally kinked, posterior-oblique loop. Bursa copulatrix small, narrowly ovate, horizontal, about 50% overlapped by albumen gland. Bursal duct about as long as bursa, medium width, opening from distal edge, partly embedded in albumen gland. Seminal receptacle small, pouch-shaped, positioned along ventral edge of proximal bursal duct; duct short. Albumen gland a little shorter than capsule gland, having very short pallial section. Capsule gland composed of a single tissue section. Genital aperture a terminal slit.

Shell measurements (mean \pm standard deviation in parentheses): height 3.00–4.00 mm (3.42 ± 0.35), width 2.00–2.50 mm (2.17 ± 0.12), body whorl height 2.25–2.75 mm (2.45 ± 0.15), body whorl width 1.70–2.19 mm (1.87 ± 0.13), aperture height 1.36–1.61 mm (1.46 ± 0.07), aperture width 1.15–1.41 mm (1.25 ± 0.06), shell width/height 0.58–0.69 (0.64 ± 0.03), body whorl height/shell height 0.67–0.76 (0.72 ± 0.02), aperture height/shell height 0.39–0.47 (0.43 ± 0.02) (paratypes, SBMNH 74688, $n = 30$). Height 2.91–3.46 mm (3.12 ± 0.14), width 1.77–2.16 mm (1.97 ± 0.08), body whorl height 2.10–2.57 mm (2.30 ± 0.10), body whorl width 1.52–1.80 mm (1.65 ± 0.07), aperture height 1.23–1.57 mm (1.39 ± 0.07), aperture width 1.08–1.31 mm (1.16 ± 0.06), shell width/height 0.57–0.66 (0.63 ± 0.02), body whorl height/shell height 0.68–0.77 (0.74 ± 0.02), aperture height/shell height 0.39–0.48 (0.45 ± 0.02) (SBMNH 74687, $n = 30$).

Measurements of holotype: height, 3.70 mm, width 2.36 mm, body whorl height 2.67 mm, body whorl width 2.01 mm, aperture height 1.59 mm, aperture width 1.39 mm, shell width/height 0.64, body whorl height/shell height 0.72, aperture height/shell height 0.43, 5.0 whorls.

Distribution, habitat and conservation status. *Pyrgulopsis milleri* is known only from its type locality area, which consists of spring-fed waters along a very short (ca. 1.0 km) reach of the South Fork of the Middle Fork Tule River drainage. Pierpoint Spring spills down a rock face into a ditch right alongside Hwy 190; part of its flow issues from a pipe (see Jenkins & Jenkins 1995:153 for a photograph of this spring). The first author collected *P. milleri* from aquatic vegetation in this ditch; density was low. There is no information on the habitat and current status of the other two populations which Miller sampled during the early 1960's. (The first author did not attempt to access these sites during his brief visit to the area in 2000.)

Pyrgulopsis milleri is distributed within a parcel of patented (private) land that is nested within the Giant Sequoia National Monument (Sequoia National Forest). This species currently has no protection and may be threatened by the diversion of Pierpoint Spring, physical disturbance (e.g., trampling, pollution) associated

with the frequent use of this spring as a drinking water supply by travelers on CA Highway 190 (Jenkins & Jenkins 1995:153), and the planned local widening of this road (CDOT 2009).

Remarks. *Pyrgulopsis milleri* differs from closely similar *P. stearnsiana*, which ranges into the lower portion of the Tule River basin (Hershler unpublished), in its broader central cusps on the central radular teeth, shorter pallial section of the albumen gland, greater overlap of the bursa copulatrix by the albumen gland, absence of a bend or loop in the anterior vas deferens, presence of a ventral gland on the penis and absence (except in one specimen) of a terminal gland. *Pyrgulopsis milleri* was most similar to *P. stearnsiana* from the Santa Clara River basin (21AA) in its mtCOI sequences (2.8% divergence) and differed from other specimens of this species by 3.3–8.4%. Thus, as with *P. castaicensis* (described above), both morphological and genetic evidence indicate that this snail is a new species.

Based on the road distances provided by Miller we infer that Pierpoint Spring is the well known (unnamed) spring that is shown on USGS maps along the north side of Hwy 190 about 0.6 km east of the Pierpoint Springs Resort and 1.6 km east of Camp Nelson.

Acknowledgments

The first author thanks Matt Carpenter (Newhall Land) for permission to sample Middle Canyon Spring and for assistance in the field. Additional collections from this site were provided by Douglas Threlhoff. We thank Erich Hochberg and Paul Scott for the loan of SBMNH material that was used in this study. Yolanda Villacampa measured shells and prepared scanning electron micrographs. Karolyn Darrow inked the anatomical drawings. The first author's fieldwork was supported, in part, by an award from the Smithsonian Institution's Research Opportunity Fund. This manuscript was improved by comments and corrections provided by Matt Carpenter and two anonymous reviewers.

References

- Bucklin, A. (1992) Use of formalin-preserved samples for molecular analysis. *Newsletter of Crustacean Molecular Techniques*, 2, 3.
- CDFG (California Department of Fish and Game) (2009) Biogeographic Data Branch California Natural Diversity Database Special Animals (901 taxa). Available from: <http://www.dfg.ca.gov/biogeodata/cnddb/pdfs/SPAnimals.pdf> (accessed 7 July 2009).
- CDOT (California Department of Transportation) (2009) Status of projects. Central Region. District 06. Available from <http://www.dot.ca.gov/dist6/ppm/docs/sop/d6sop.pdf> (accessed 6 August 2009).
- CSWRCB (California State Water Resources Control Board) (1998) Final California unified watershed assessment. Sacramento, California. 15 pp + 3 maps.
- Call, R.E. & Pilsbry, H.A. (1886) On *Pyrgulopsis*, a new genus of rissoid mollusk, with descriptions of two new forms. *Proceedings of the Davenport Academy of Natural Sciences*, 5, 9–14.
- Dudek [and Associates] (2007) (Draft) Survey and status report for the Middle Canyon Spring. Report prepared for the Newhall Land and Farming Company, Valencia, California. Available from <http://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=11139> (accessed 29 July 2009).
- Dudek [and Associates] (2008a) Newhall Ranch resource management and development plan: wildlife habitat buffers and connectivity white papers. Available from <http://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=11147> (accessed 3 August 2009).
- Dudek [and Associates] (2008b) Draft Newhall Ranch resource management and development plan. Prepared for the Newhall Land and Farming Company, Valencia, California. Available from <http://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=11051> (part 1) and <http://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=11052> (part 2) (accessed 29 July 2009).
- 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.
- Frauenfeld, G. von (1863) Vorläufige Aufzählung der Arten der Gattungen *Hydrobia* Htm. Und *Ammicola* Gld. Hldm. In der kaiserlichen und Cuming's Sammlung. *Verhandlungen der Kaiserlich-Königlichen Zoologischen-Botanischen*

- Gesellschaft in Wien*, 13, 1017–1032.
- Gould, A.A. (1855) New species of land and freshwater shells from western (N.) America. *Proceedings of the Boston Society of Natural History*, 5, 127–130.
- Gregg, W.O. & Taylor, D.W. (1965) *Fontelicella* (Prosobranchia: Hydrobiidae), a new genus of west American freshwater snails. *Malacologia*, 3, 103–110.
- GSI Water Solutions, Inc. (2007) Middle Canyon Spring hydrogeologic assessment and impact evaluation report. Prepared for the Newhall Land and Farming Company, Valencia, California. Available from <http://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=11067> (accessed 30 July 2009).
- 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.
- Hershler, R. (1994) A review of the North American freshwater snail genus *Pyrgulopsis*. *Smithsonian Contributions to Zoology*, 554, 1–115.
- Hershler, R. (1995) New freshwater snails of the genus *Pyrgulopsis* (Rissooidea: Hydrobiidae) from California. *The Veliger*, 38, 343–373.
- Hershler, R. (1998) A systematic review of the hydrobiid snails (Gastropoda: Rissooidea) of the Great Basin, western United States. Part I. Genus *Pyrgulopsis*. *Veliger*, 41, 1–132.
- Hershler, R. & Liu, H.-P. (2008) Ancient vicariance and recent dispersal of springsnails (Hydrobiidae: *Pyrgulopsis*) in the Death Valley system, California-Nevada. In: Reheis, M.C., Hershler, R. & Miller, D.M. (Eds), *Late Cenozoic Drainage History of the Southwestern Great Basin and Lower Colorado River Region*. *Geological Society of America Special Paper*, 439, 91–101.
- Hershler, R. & Liu, H.-P. (2009) New species and records of *Pyrgulopsis* (Gastropoda: Hydrobiidae) from the Snake River basin, southeastern Oregon: further delineation of a highly imperiled fauna. *Zootaxa*, 2006, 1–22.
- Hershler, R. & Pratt, W.L. (1990) A new *Pyrgulopsis* (Gastropoda: Hydrobiidae) from southeastern California, with a model for historical development of the Death Valley hydrographic system. *Proceedings of the Biological Society of Washington*, 103, 279–299.
- Hershler, R. & Sada, D. (1987) Springsnails (Gastropoda: Hydrobiidae) of Ash Meadows, Amargosa basin, California-Nevada. *Proceedings of the Biological Society of Washington*, 100, 776–843.
- Hershler, R. & Sada, D.W. (2002) Biogeography of Great Basin aquatic snails of the genus *Pyrgulopsis*. In: Hershler, R., Madsen, D.B. & Currey, D. R. (Eds), *Great Basin Aquatic Systems History*. *Smithsonian Contributions to the Earth Sciences*, 33, 255–276.
- Hershler, R., Liu, H.P. & Gustafson, D.L. (2008) A second species of *Pyrgulopsis* (Hydrobiidae) from the Missouri River basin, with molecular evidence supporting faunal origin through Pliocene stream capture across the northern Continental Divide. *Journal of Molluscan Studies*, 74, 403–413.
- Hershler, R., Liu, H.-P. & Thompson, F.G. (2003b) Phylogenetic relationships of North American nymphophiline gastropods based on mitochondrial DNA sequences. *Zoologica Scripta*, 32, 357–366.
- Hershler, R., Frest, T.J., Liu, H.-P. & Johannes, E.J. (2003a) Rissooidean snails from the Pit River basin, California. *The Veliger*, 46, 275–304.
- Hershler, R., Liu, H.-P., Frest, T.J. & Johannes, E.J. (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.
- Hochberg, F.G. & Roth, B. (2001) Walter B. Miller (1918–2000): molluscan taxa and bibliography. *The Veliger*, 44: 95–98.
- Jenkins, J.C. & Jenkins, R.J. (1995) *Exploring the Southern Sierra: west side*. Wilderness Press, Berkeley, California. 256 pp.
- Liu, H.-P. & Hershler, R. (2005) Molecular systematics and radiation of western North American nymphophiline gastropods. *Molecular Phylogenetics and Evolution*, 34, 284–298.
- 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.
- Nylander, J.A.A. (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Pilsbry, H.A. (1899) Catalogue of the Amnicolidae of the western United States. *Nautilus*, 12, 121–127.
- Pilsbry, H.A. (1912) A new species of *Ammicola*. *Nautilus*, 26, 1.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
- Seaber, P.R., Kapinos, F.P. & Knapp, G.L. (1994) Hydrologic unit maps. *United States Geological Survey Water-Supply Paper*, 2294, 1–63.
- Stearns, R.E.C. (1883) Description of a new hydrobiinoid gastropod from the mountain lakes of the Sierra Nevada, with remarks on allied species and the physiographic features of said region. *Proceedings of the Academy of Natural*

Sciences of Philadelphia, 35, 171–176.

- Stearns, R.E.C. (1893) Report on the land and fresh-water shells collected in California and Nevada by the Death Valley Expedition, including a few additional species obtained by Dr. C. Hart Merriam and assistants in parts of the southwestern United States. *North American Fauna*, 7, 269–283.
- Swift, C. (2009) Summary of the recent reconnaissance survey work conducted to detect freshwater snail presence in selected habitat locations on Newhall Ranch. Memorandum submitted to Matt Carpenter, The Newhall Land and Farming Company, Valencia, California. Available from <http://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=11296> (accessed 29 July 2009).
- SSI (Systat Software, Inc.) 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. (1981) Freshwater mollusks of California: a distributional checklist. *California Fish and Game*, 67, 140–163.
- Taylor, D.W. (1987) Fresh-water molluscs from New Mexico and vicinity. *New Mexico Bureau of Mines & Mineral Resources Bulletin*, 116, 1–50.
- USACE (United States Army Corps of Engineers) & CDFG (California Department of Fish and Game) (2009) Newhall Ranch resource management and development plan and spineflower conservation plan. Draft joint environmental.