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New species and records of *Pyrgulopsis* (Gastropoda: Hydrobiidae) from the Snake River basin, southeastern Oregon: further delineation of a highly imperiled fauna

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Abstract

Here we describe two new species from southeastern Oregon based on morphologic and molecular (mtCOI) evidence. *Pyrgulopsis fresti* **n. sp.**, commonly known as the "Owyhee hot springsnail" and long considered to be distinct and critically imperiled, lives in thermal springs along a short reach of the Owyhee River above Three Forks. This snail differs from other regional species in its squat shell; penial ornament consisting of a large, disc-shaped ventral gland; absence of a seminal receptacle; and mtCOI sequences. *Pyrgulopsis owyheensis* **n. sp.** ranges among five disjunct groups of springs in the Owyhee and Malheur river drainages and is occasionally sympatric with *P. fresti*. This snail is closely similar to another regional congener, *P. intermedia* (Tryon, 1865), but is smaller and further differentiated by its typically disjunct inner shell lip, longer and narrower penial filament, more distally positioned ventral gland of penis, and mtCOI sequences. The type locality (Owyhee Spring) population of *P. owyheensis* is genetically differentiated from the other geographical subunits of this species (1.5–1.8% sequence divergence) and should perhaps be managed as a separate conservation unit. New records are provided for *P. intermedia* which extends the range of this conservation priority species into the lower Owyhee River basin. We also show that the "Malheur springsnail," which has been listed in various conservation-related publications and documents, is the same as *P. intermedia*. This study provides critical information for the conservation of springsnails in southeastern Oregon and underscores the need for additional field surveys in the region.

Key words: springs, Owyhee River, Malheur River, springsnails, mitochondrial DNA, conservation

Introduction

Pyrgulopsis Call & Pilsbry, 1883, with 127 currently recognized congeners (Hershler *et al.* 2007a), is the most species-rich genus of freshwater mollusks in North America. These tiny, gill-breathing gastropods, commonly known as springsnails, are distributed in springs and other perennial waters throughout much of western North America and in portions of the Missouri River basin, Rio Grande basin, and the internal drainage of northeastern Mexico (Hershler & Gustafson 2001; Hershler & Sada 2002). *Pyrgulopsis* was last reviewed by Hershler (1994); 72 additional species have subsequently been described (Hershler 1995, 1998; Hershler & Sada 2000; Hershler & Gustafson 2001; Hershler *et al.* 2003a; Hershler *et al.* 2007a), the majority of which (50/72, 69%) are endemic to the Great Basin. In spite of this recent surge in descriptive studies, our knowledge of the diversity and distribution of *Pyrgulopsis* is far from complete as many portions of the broad geographic range of this genus are poorly sampled and/or contain possibly undescribed species which have not been formally treated (e.g., Frest & Johannes 1995, 2000; Liu *et al.* 2003; Liu & Hershler 2008). A more complete characterization of these faunas is needed to aid conservation initiatives that are currently being

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directed towards *Pyrgulopsis* and its fragile aquatic habitats.

One of the lesser studied parts of the West from this perspective is southeastern Oregon, which includes portions of the Snake River (Owyhee and Malheur River drainages) and Great Basins. The few previously published records of *Pyrgulopsis* from this large region consist of several collections of *P. intermedia* (Tryon, 1865) from its type locality area (Crooked Creek, Owyhee River drainage) and closely proximal Barren Valley (Great Basin), and a single occurrence of *P. robusta* (Walker, 1908) in the South Fork of the Malheur River (Taylor & Smith 1981; as *Fontelicella hendersoni* [Pilsbry, 1933]) (Fig. 1). Additional, taxonomically unstudied springsnail populations have also been found in several springs in the middle Owyhee River drainage (above the mouth of Crooked Creek) over the past 45 years. These include two putatively undescribed species - the "Owyhee hot springsnail" (less frequently referred to as the "Three Forks pyrg"), which was discovered in the large spring complex on the Owyhee River just above Three Forks (Tudor Warm Springs); and the "Malheur springsnail," which lives in the headspring of Crooked Creek (Frest & Johannes 1995). These purported novelties (and *P. intermedia*) are currently ranked as critically imperiled (G1) by NatureServe (2008) and threatened or endangered throughout their range (List 1) by the Oregon Natural Heritage Information Center (ONHIC 2007).

One of us (RH) recently collected fresh material from most of these sites and also sampled previously unknown *Pyrgulopsis* populations in the lower Owyhee River and Malheur River drainages. Herein we describe the Owyhee hot springsnail and a second (previously unrecognized) new congener based on morphologic and genetic studies of these specimens and existing museum holdings. We show that the Malheur springsnail is the same as *P. intermedia* and provide new records that significantly extend the geographic range of this species. Our findings, together with our recent revision of the congeners previously assigned to the subgenus *Natricola* Gregg & Taylor, 1965 (Hershler & Liu 2004a), fill one of the data gaps that was identified in the State of Oregon's current conservation strategy (determination of the taxonomic status of the *Pyrgulopsis* fauna in the southeastern part of the state; ODFW 2006: 368–369); and can be used to help develop the conservation measures for the Owyhee hot springsnail (*P. fresti*) and *P. intermedia* that are called for in the southeastern Oregon resource management plan (SEORMP) (USDI, BLM 2001). (Note that the morphologic and genetic evidence provided by Hershler & Liu [2004a] demonstrates that one of the other putatively undescribed species that was flagged in this conservation document, the "Lake Abert springsnail," is conspecific with *P. robusta*.)

Material and methods

Anatomical study was based on specimens that 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; otherwise township (T.), range (R.) and section (sec.) data are given. The large thermal spring complex on the Owyhee River at the mouth of Warm Springs Canyon, which is unnamed on current United States Geological Survey topographic maps, is referred to herein as Tudor Warm Springs following SWRB (1967) and USDI, GNIS (2008). Types and other specimens collected during the course of this project were deposited in the National Museum of Natural History (USNM) collection; relevant holdings from the James Ford Bell Museum of Natural History (JFBM) were also studied.

Variation in the number of cusps on the radular teeth was assessed using the method of Hershler *et al.* (2007b). 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 two congeners which had previously been recorded from the Owyhee River basin (*P. intermedia*, *P. robusta*), and five species from proximate Klamath basin (*P. archimedis* Berry), Pit River (*P. rupinicola* Hershler *et al.*) and Great Basin (*P.*

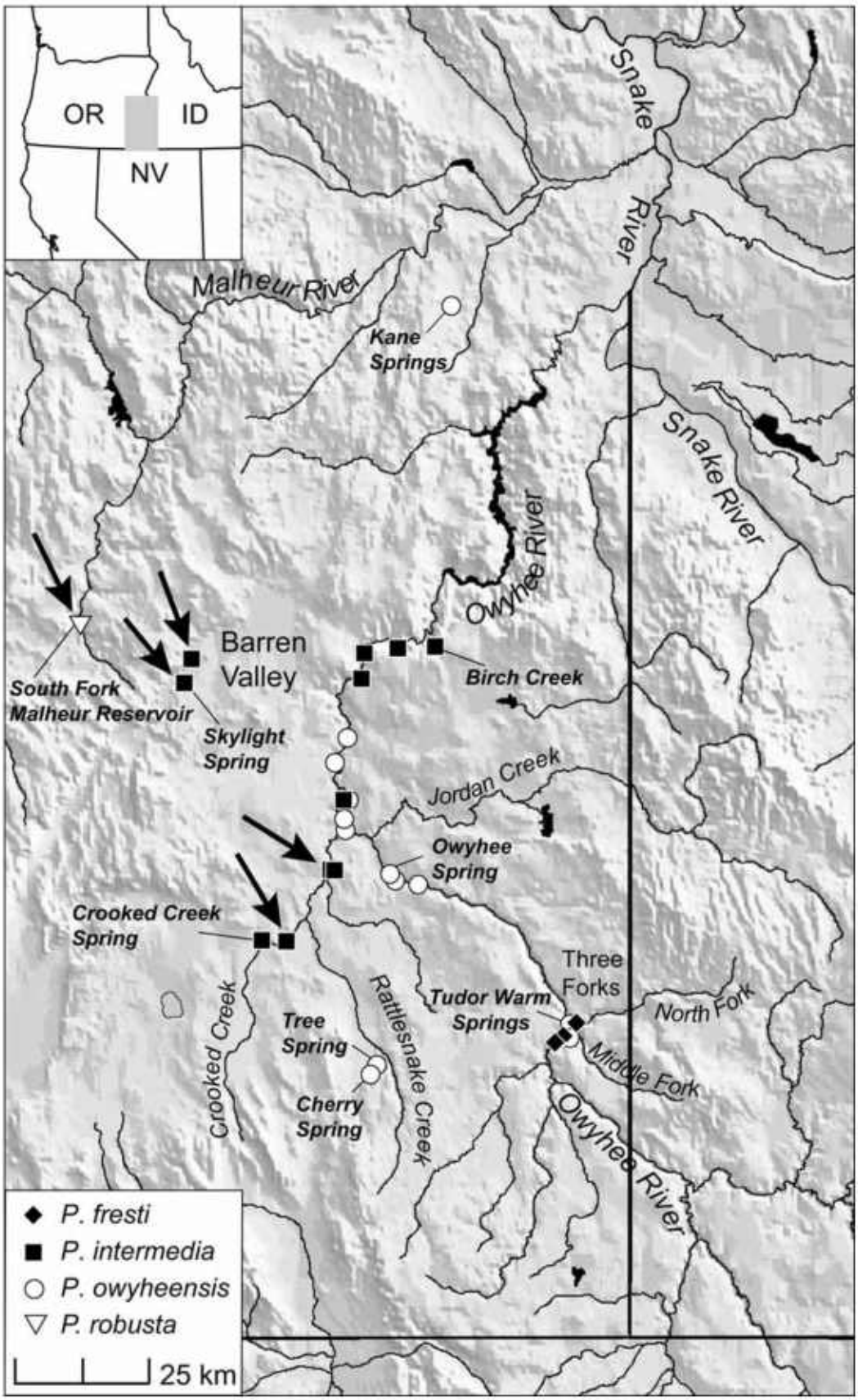

FIGURE 1. Map showing the distributions of *Pyrgulopsis* species in southeastern Oregon. Arrows indicate previously documented localities; all others are newly reported herein. Some of the symbols refer to multiple, closely proximal localities.

gibba Hershler, *P. imperialis* Hershler, *P. militaris* Hershler) drainages. Prior to our 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. One–seven specimens were sequenced from each sample. Trees were rooted with an eastern North American nymphophiline, *Marstonia agarhecta* Thompson, 1969 (following Liu & Hershler 2005). 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). Sequences were determined for both strands and then edited and aligned using Sequencher™ version 4.8. Locality details and GenBank accession numbers for sequenced specimens are in Table 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 X^2 test. MrModeltest 2.3 (Nylander 2004) was used to obtain an appropriate substitution model (using the Akaike Information Criterion) and parameter values for the analyses. Phylogenetic analyses based on distance, parsimony, and maximum-likelihood methods were generated using PAUP*4.0b10 (Swofford 2000). Bayesian inference was performed using MrBayes 3.12 (Ronquist & Huelsenbeck 2003). An appropriate genetic distance measure was used to generate a neighbor-joining (NJ) tree (Saitou & Nei 1987). Maximum-parsimony (MP) analyses were conducted with equal weighting, using the heuristic search option with tree bisection reconnection branch-swapping and 100 random additions. The appropriate model (selected by Modeltest) was used for the maximum likelihood analyses (ML). A NJ tree with the appropriate genetic distance model was used as the initial topology for branch-swapping. Node support was evaluated by 10,000 bootstrap pseudo-replicates in all but the ML analysis, in which support was based on 100 replications. In the initial Bayesian 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 Modeltest and the default random tree option to determine when the log likelihood sum reached a stable value (by plotting the log-likelihood scores of sample points against generation time). The ln likelihood scores started at around –9,100 and quickly converged upon a stable value of about –2,750 after approximately 12,000 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.

Results

Molecular analysis. Seventy-two specimens were sequenced. New sequences were deposited in GenBank under accession numbers FJ17247–FJ172502 (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 171 sites (25.99%) were variable and 123 (18.69%) were parsimony-informative. Average base frequencies were 25.5% A, 37.0% T, 19.4% C, 18.1% G. There was no significant base frequency bias among species ($X^2 = 30.07$, df = 228, $P = 1.00$).

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 optimized parameters were base frequencies of A = 0.2610, C = 0.1982, G = 0.1776, T = 0.3632; Rmat = {3.2184 15.4202 0.4737 1.2347 16.1059}; shape of gamma distribution = 0.2440; and proportion of invariant sites = 0.2668. GTR distance was used to generate a NJ tree and GTR + I +G was used for the ML and Bayesian analyses.

TABLE 1. Specimen codes, localities, and GenBank accession numbers. The number of sequenced specimens (*n*) is given for samples that are newly reported herein. [a]Hershler *et al.* 2003a; [b]Hershler *et al.* 2003b; [c]Hershler & Liu 2004a; [d]Hershler & Liu 2004b.

Species	Specimen code	Locality (Owyhee River basin, Malheur County, Oregon unless otherwise specified)	Accession number
archimedis	D1A, D	Fall River, Pit River basin, Shasta Co., CA	AY197577[a], AY426357[d]
	D7A, C	Pit River at confluence with Hat Creek, Pit River basin, Shasta Co., CA	AY197580[a], AY426358[d]
	D8B	Baum Lake, Pit River basin, Shasta Co., CA	AY197579[a]
	D31A, B	Fifth Link River spring, Klamath basin, Klamath Co., OR	AY197586[a], AY426356[d]
	P50A, C	Upper Klamath Lake, Klamath basin, Klamath Co., OR	AF520950[b], AY426355[d]
fresti **sp. nov.**	IP68A-C (*n*=3)	Tudor Warm Springs, east side of river, fourth spring from north	FJ172470, FJ172471, FJ172472
	IP69B (*n*=1)	Tudor Warm Springs, west side of river, first spring north of mouth of Warm Springs Canyon	FJ172473
	P176B (*n*=1)	Tudor Warm Springs, east side of river, second spring from north (coll. 25/ix/2002)	FJ172474
	P207A (*n*=2)	Spring tributary to Owyhee River upflow from Tudor Warm Springs	FJ172476
	P208A, E (*n*=5)	Spring complex along Owyhee River upflow from Tudor Warm Springs	FJ172477, FJ172478
	P210A (*n*=3)	Tudor Warm Springs, east side of river, third spring from north	FJ172480
	P212A (*n*=2)	Tudor Warm Springs, east side of river, second spring from north (11/v/2007)	FJ172483
gibba	P134B, D	Springs west of Fee Reservoir, Surprise Valley (Great Basin), Lassen Co., CA	AY197603[a], AY426359[d]
imperialis	P140A, C	Spring, Thacker Pass, Kings River Valley (Great Basin), Humboldt Co., NV	AY379450[c], AY426350[d]
intermedia	P1B, E	Crooked Creek, Hwy 95 crossing	AY379442[c], AY426351[d]
	P2B, C	Crooked Creek at Crooked Creek State Wayside (19/v/2000)	AY426352[d], AY426353[d]
	P4B, C	Skylight Spring, Barren Valley (Great Basin), Malheur Co., OR	AY379444[c], AY379445[c]
	IP60A-D (*n*=4)	Crooked Creek at Crooked Creek State Wayside (16/vii/2006)	FJ172460, FJ172461, FJ172462, FJ172463
	IP67A, C (*n*=2)	Spring tributary to Birch Creek	FJ172468, FJ172469
	P217A (*n*=4)	Spring on Owyhee River, above Long Sweetwater rapids	FJ172488
	P222A, C (*n*=2)	Spring on hillside, Jackson Hole	FJ172496, FJ172497
	P223A, B (*n*=2)	Mouth of Rinehart Creek	FJ172498, FJ172499
	P224C (*n*=1)	Spring west of Two Mile Spring	FJ172500
militaris	P147A, C	Spring west of Soldier Meadow Ranch, Black Rock Desert (Great Basin), Humboldt Co., NV	AY197596[a], AY426362[d]
owyheensis **sp. nov.**	IP59A, C, D (*n*=3)	Owyhee Spring	FJ172457, FJ172458, FJ172459

to be continued.

TABLE 1. (continued)

Species	Specimen code	Locality (Owyhee River basin, Malheur County, Oregon unless otherwise specified)	Accession number
	IP62A-D (*n*=4)	Tree Spring	FJ172464, FJ172465, FJ172466, FJ172467
	P177B (*n*=1)	Tudor Warm Springs, east side of river, second spring from north (25/ix/2002)	FJ172475
	P209A (*n*=5)	Kane Springs, Malheur River basin, Malheur Co., OR	FJ172479
	P211A, B (*n*=2)	Tudor Warm Springs, east side of river, third spring from north	FJ172480, FJ172481
	P213A (*n*=1)	Tudor Warm Springs, east side of river, second spring from north (11/v/2007)	FJ172484
	P215A (*n*=2)	Spring tributary to Owyhee River upflow from Tudor Warm Springs	FJ172485
	P216A, B (*n*=4)	Spring on Owyhee River, below mouth of Crooked Creek	FJ172486, FJ172487
	P218A-C (*n*=4)	Spring on Owyhee River, above Long Sweetwater rapids	FJ172489, FJ172490, FJ172491
	P219A; P220B, C (*n*=7)	Weeping Wall springs, just below mouth of Granite Creek	FJ172492, FJ172493, FJ172494
	P221B (*n*=3)	Spring on Owyhee River, above Artillery rapids	FJ172495
	P225A, P226A (*n*=4)	Seep wall on Owyhee River, just above mouth of Crooked Creek	FJ172501, FJ172502
robusta	D33C, D	XL Spring, Abert Lake basin (Great Basin), Lake Co., OR	AY426348[d], AY426349[d]
	P3B, D	Hughet Spring, Harney basin (Great Basin), Harney Co., OR	AY379430[c], AY379431[c]
	P5B, C	South Fork Malheur Reservoir, Malheur Cave Road crossing, Malheur River basin, Harney Co., OR	AY379432[c], AY379433[c]
	P179A, B	Snake River, Glenns Ferry, Snake River basin, Elmore Co., ID	AY379426[c], AY379427[c]
rupinicola	D3E	Sucker Springs Creek, Pit River basin, Shasta Co., CA	AY197591[a]

In the MP and NJ analyses snails from the Owyhee and Malheur River basins were placed in four distinct and well supported clades corresponding to *P. intermedia*, *P. robusta* and the two morphologically distinctive species described below (*P. fresti*, *P. owyheensis*). The MP tree is shown in Figure 2. Although sequences from one of the new species (*P. owyheensis*) formed a strongly support clade in the MP (98%, Fig. 2) and NJ (100%) analyses, they were basally positioned as a paraphyletic assemblage in the Bayesian and ML trees. Although this new snail was not consistently depicted as monophyletic, we treat it as a single taxon based on its limited morphological variation and absence of molecular evidence of well supported subdivision. We do, however, recognize that one population of this species (IP59) is well differentiated genetically and discuss the conservation implications of this finding below. *Pyrgulopsis intermedia* was consistently depicted (with medium to high support) as sister to *P. robusta*; the relationships of the new species were not well resolved in any of the analyses. The mean uncorrected sequence divergence was low within both of the new species (*P. fresti*, 0.9±0.2%; *P. owyheensis*, 0.6±0.1%) and ranged from 5.8–9.0% between these and other congeners included in the analysis (Table 2). Additional genetic results are detailed in the "Remarks" sections below.

TABLE 2. Mean (uncorrected) COI sequence divergence between species with standard errors.

	archimedis	*fresti*	*gibba*	*imperialis*	*intermedia*	*militaris*	*owyheensis*	*robusta*	*rupinicola*
archimedis	0.002 ±0.001								
fresti	0.079±0.010	0.009±0.002							
gibba	0.072±0.010	0.081±0.011	0.000±0.000						
imperialis	0.064±0.009	0.082±0.011	0.069±0.010	0.003±0.002					
intermedia	0.060±0.009	0.081±0.010	0.068±0.010	0.037±0.007	0.002±0.001				
militaris	0.065±0.009	0.090±0.010	0.083±0.011	0.082±0.011	0.077±0.011	0.007±0.003			
owyheensis	0.063±0.010	0.080±0.010	0.072±0.010	0.071±0.010	0.069±0.010	0.071±0.010	0.006±0.001		
robusta	0.063±0.009	0.085±0.011	0.066±0.010	0.037±0.007	0.027±0.006	0.079±0.011	0.074±0.010	0.003±0.001	
rupinicola	0.029±0.007	0.070±0.010	0.068±0.010	0.053±0.009	0.058±0.010	0.066±0.010	0.058±0.010	0.054±0.009	
M. agarhecta	0.166±0.015	0.165±0.015	0.154±0.015	0.166±0.015	0.161±0.014	0.171±0.015	0.153±0.014	0.168±0.015	0.155±0.015

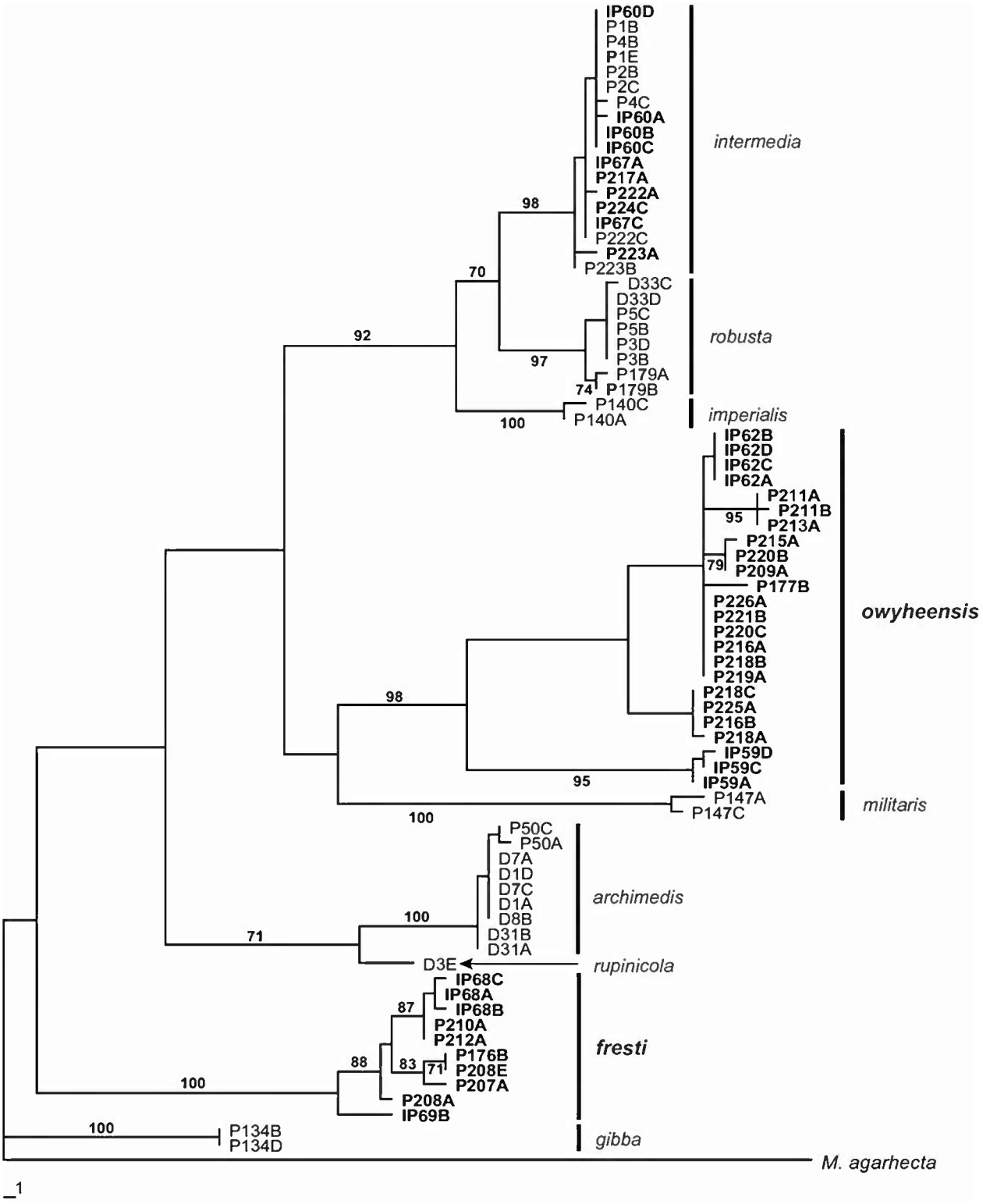

FIGURE 2. Maximum parsimony (MP) tree based on the COI dataset (TL = 322, CI = 0.64). Bootstrap support for nodes is provided when >70%. Bold-faced sequences are newly reported herein. Scale bar = one substitutional change per 100 nucleotide positions.

Systematics

Family Hydrobiidae

Subfamily Nymphophilinae

Genus *Pyrgulopsis* Call & Pilsbry, 1886

Type species: *Pyrgula nevadensis* Stearns, 1883, by original designation
Diagnosis: Liu & Hershler 2005: 296.

Pyrgulopsis fresti sp. nov.
(Figs 3–5)

Types. Holotype (Fig. 3A), USNM 1102148, Tudor Warm Springs, east side of Owyhee River, second spring from north, Malheur County, Oregon (484936 E, 4708680 N), 11/v/2007, Robert Hershler, William H. Clark & Shaney Rockefeller. Paratypes, USNM 1116914 (from same lot, 751 specimens); USNM 1102161 (453 specimens), 12/v/2007, Robert Hershler & William H. Clark.

Etymology. In honor of recently deceased colleague Terrence J. Frest, who made tremendous contributions to the study and conservation of the nonmarine mollusks of the Pacific Northwest through his extensive fieldwork, documentation of previously little known faunas, and staunch environmental advocacy.

Referred material. OREGON. Malheur County. USNM 1097817, spring tributary to Owyhee River upflow from Tudor Warm Springs, 484401 E, 4707220 N, 11/x/2006. USNM 1097818, spring complex along Owyhee River upflow from Tudor Warm Springs, 483313 E, 4705913 N, 11/x/2006. USNM 873474, above Three Forks, T. 35 S, R. 45 E, E 1/2 SW 1/4 sec. 3, 15/i/1987. USNM 892084, Tudor Warm Springs, east side of river, second spring from north, 485020 E, 4708400 N, 23/ix/1989. USNM 1071457, *ibid.*, 485060 E, 4708420 N, 25/ix/2002. USNM 1102151, Tudor Warm Springs, east side of river, third spring from north, 484935 E, 4708661 N, 12/v/2007. USNM 892082, Tudor Warm Springs, east side of river, fourth spring from north, 485040 E, 4708330 N, 23/ix/1989. USNM 1092856, *ibid.*, 484949 E, 4708640 N, 19/vii/2006. USNM 1102147, *ibid.*, 484950 E, 4708634 N, 11/v/2007. USNM 892083, Tudor Warm Springs, west side of river, first spring north of mouth of Warm Springs Canyon, 484920 E, 4708520 N, 23/ix/1989. USNM 1092860, Tudor Warm Springs, west side of river, second spring north of mouth of Warm Springs Canyon, 484853 E, 4708749 N, 19/vii/2006. USNM 1102146, *ibid.*, 484851 E, 4708753 N, 11/v/2007.

Diagnosis. A small species of *Pyrgulopsis* having a low-spired, globose or trochiform shell with medium to highly convex whorls. Penis having a small lobe and short filament; penial ornament consisting of a large, disc-shaped ventral gland.

Description. Shell subglobose or trochiform (Fig. 3A–C); height about 1.3–2.7 mm; whorls 3.0–4.0. Periostracum light brown, thin. Protoconch near planispiral, often eroded, about 1.3 whorls, diameter about 330 µm, surface weakly wrinkled near apex, otherwise smooth (Fig. 3D–E). Teleoconch whorls medium or highly convex, variably shouldered; sculpture of strong, collabral growth lines. Aperture large, broadly ovate, slightly angled adapically. Inner lip usually adnate, rarely slightly disjunct, often nearly straight, thin or slightly thickened internally, without columellar shelf; outer lip thin, prosocline. Umbilicus narrow or perforate.

Shell measurements (mean ± standard deviation in parentheses): height 2.06–2.67 mm (2.24±0.14), width 1.79–2.26 mm (1.98±0.12), body whorl height 1.87–2.27 mm (2.02±0.10), body whorl width 1.38–1.71 mm (1.54±0.08), aperture height 1.18–1.63 mm (1.33±0.09), aperture width 1.09–1.34 mm (1.20±0.06), shell width/height 0.82–0.97 (0.88±0.04), body whorl height/shell height 0.85–0.94 (0.90±0.02), aperture height/shell height 0.55–0.65 (0.60±0.03) (paratypes, USNM 1116914, $n = 29$).

Measurements of holotype: height 2.67 mm, width 2.03 mm, body whorl height 2.16 mm, body whorl

width 1.74 mm, aperture height 1.30 mm, aperture width 1.22 mm, shell width/height 0.76, body whorl height/shell height 0.81, aperture height/shell height 0.49, 4.0 whorls.

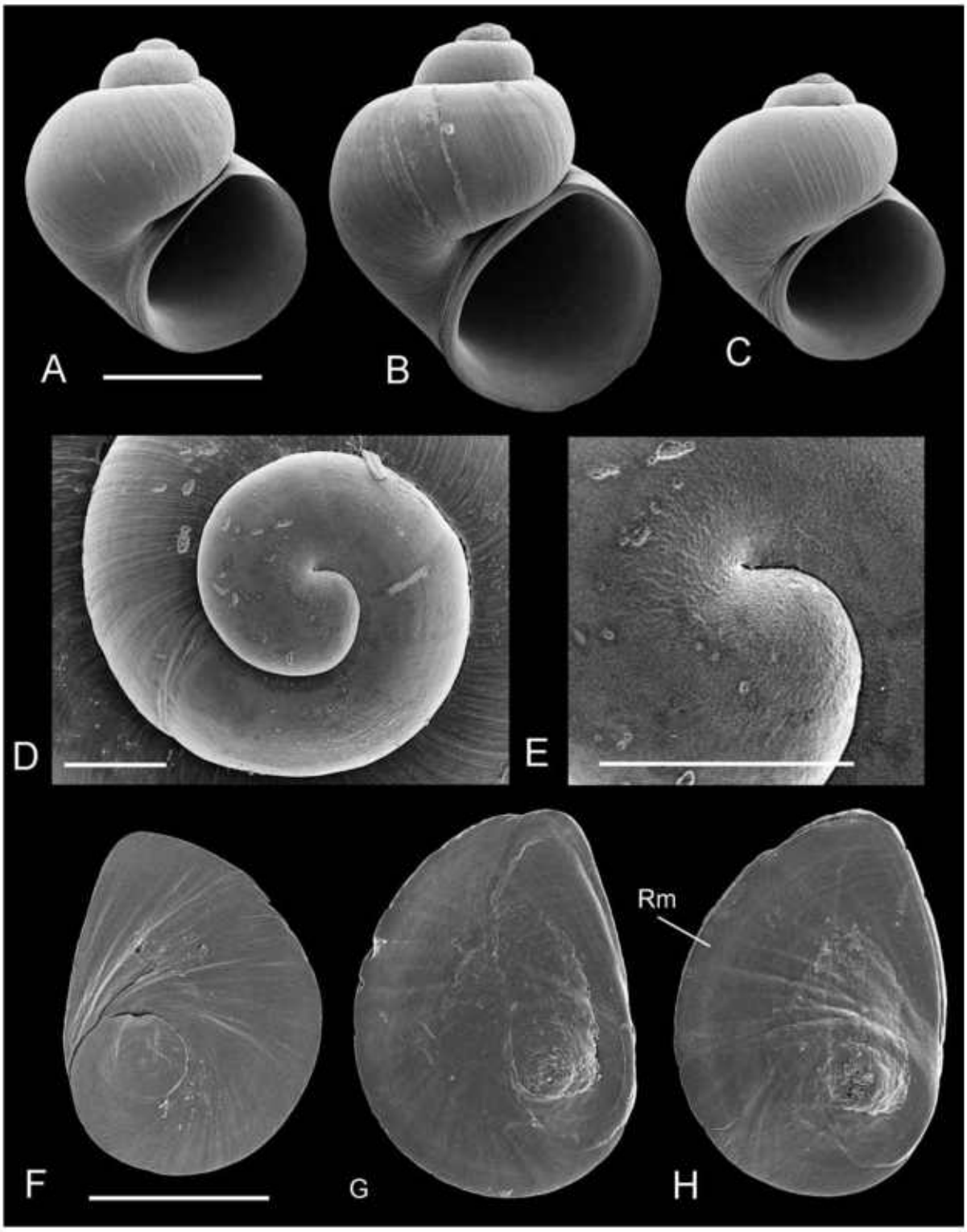

FIGURE 3. *P. fresti* **sp. nov**. A–C. Shells. Scale bar = 1.0 mm. A. Holotype, USNM 1102148. B. Paratype, USNM 1116914. C. USNM 1092860. D. Shell apex, USNM 1116914. Scale bar = 100 µm. E. Close-up of protoconch sculpture, USNM 1116914. Scale bar = 100 µm. F–H. Opercula. Scale bar = 500 µm. F. Outer side, showing frilled whorl, USNM 1102161. G–H. Inner side, showing variation in thickening of attachment scar margin, USNM 1116914, USNM 1102161. Rm = rim.

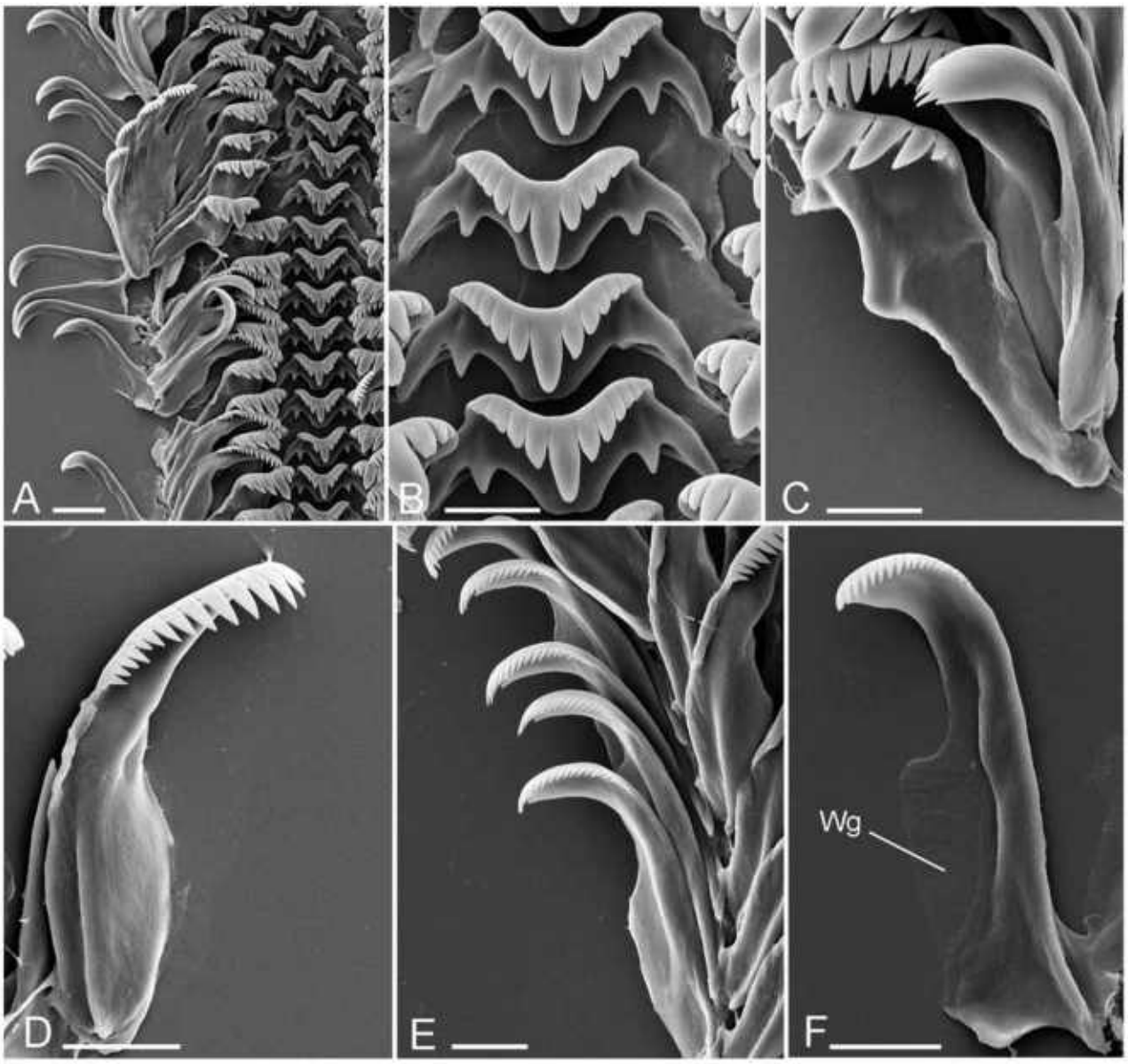

FIGURE 4. *P. fresti* **sp. nov.**, USNM 1102161. A. Portion of radula ribbon. Scale bar = 20 µm. B. Central radular teeth. Scale bar = 10 µm. C. Lateral and inner marginal teeth. Scale bar = 10 µm. D. Inner marginal tooth. Scale bar = 10 µm. E. Outer marginal teeth. Scale bar = 10 µm. .F. Outer marginal tooth, showing outer wing. Scale bar = 10 µm. Wg = wing.

Operculum thin, flat, amber-colored, multispiral with eccentric nucleus; last half whorl weakly frilled on outer side (Fig. 3F); inner side sometimes having weakly rimmed along outer edge, attachment scar border smooth to slightly thickened almost all around (Fig. 3G–H). Radula taenioglossate (Fig. 4A), with about 55 well-formed rows of teeth. Central teeth (Fig. 3B) about 33 µm wide, cutting edge strongly concave; lateral cusps 5–8; central cusp narrow, pointed, sometimes parallel-sided proximally; basal cusp 1, small; basal tongue U-shaped, about as long as lateral margins. Lateral tooth (Fig. 4C) face rectangular, angled; central cusp large, pointed, parallel-sided proximally; lateral cusps 2–4 (inner), 3–5 (outer); outer wing rather broad, straight, about 145% length of cutting edge; basal tongue weakly developed. Inner marginal teeth having 15–20 cusps (Fig. 4D). Outer marginal teeth having 15–21 small cusps (Fig. 4E); inner edge having long, rectangular wing (Fig. 4F).

Head-foot generally dark brown. Cephalic tentacles light brown dorsally, proximal half sometimes darker,

ventral surfaces nearly pale. Distal lips of snout pale. Sole of foot pale or grey. Pallial roof, visceral dark brown or black dorsally. Ctenidium well developed, positioned a little in front of pericardium; ctenidial filaments about 18, rather small. Osphradium narrow, positioned posterior to middle of ctenidium. Prostate gland small, bean-shaped, almost entirely visceral. Anterior vas deferens opening from ventral edge of prostate gland a little in front of pallial wall, section of duct on columellar muscle having prominent bend. Penis (Fig. 5A–B) large, base rectangular, inner edge weakly folded proximally; filament short, tapering, slightly oblique, distal tip sometimes folded; lobe short, rectangular or tapering, horizontal or slightly oblique. Ventral gland large, disc-shaped, slightly elevated, composed on numerous small units, proximally positioned (Fig. 5B). Penial duct very narrow, nearly straight. Penial filament containing a dense core of black pigment; penis otherwise pigmented with a few black granules distally. Female glandular oviduct and associated structures shown in Figure 5C–D. Coiled oviduct a circular or vertical loop. Bursa copulatrix small, ovate, horizontal or slightly oblique, largely or entirely overlapped by and sometimes partly embedded within albumen gland. Bursal duct slightly shorter to slightly longer than bursa, opening from distal edge. Seminal receptacle absent. Albumen gland longer than capsule gland, entirely visceral or with very short pallial section. Capsule gland composed of two distinct tissue sections. Genital aperture a terminal slit.

Distribution and habitat. *Pyrgulopsis fresti* is distributed along a short reach of the Owyhee River above Three Forks (Fig. 1). This species was previously thought to be endemic to Tudor Warm Springs (Frest & Johannes 1995, as Owyhee hot springsnail). The two additional populations reported herein were discovered during a survey that was focused on riverine hydrobiids (Myler & Hopper 2006). Additional surveys of seeps and springs along the Owyhee River in southeastern Oregon will be needed to accurately delineate the geographic range of this species. *Pyrgulopsis fresti* lives on loose cobble and basalt rock faces in very shallow, thermal (ca. 24–33° C.) spring runs and is sometimes found in sympatry with *P. owyheensis*; additional ecological details are provided by Frest & Johannes (1995).

Remarks. *Pyrgulopsis fresti* is readily distinguished from other congeners living in the Owyhee River basin and adjacent drainages by its broad, low-spired shell and distinctive glandular ornament on its penis. It also differs from these species in lacking a seminal receptacle, a character which it uniquely shares with *P. arizonae* (Taylor, 1987) (see Taylor 1987, fig. 15d, e), and in its mitochondrial DNA sequences (Table 2). *Pyrgulopsis fresti* is further differentiated from *P. bruneauensis* Hershler, 1990, the only other congener endemic to thermal springs in the Snake River basin, by its larger penial lobe and shorter penial filament (compare Fig. 5B with Hershler, 1990, fig. 6). The COI sequence divergence between these two species is 7.7–8.9% (Liu & Hershler unpublished). Eight congeners which live in drainages well to the south of the Snake River basin have penial ornament similar to that of *P. fresti* (Hershler & Sada, 2002:271, "species group 2"). As noted in the Diagnosis (above), one of these species (*P. arizonae*) uniquely shares with *P. fresti* the absence of a seminal receptacle. However, the COI sequence divergence between these two species is 8.8–9.2% (Liu & Hershler unpublished), which suggests that they are not close relatives. None of the remaining seven species is closely related to *P. fresti* either based on our molecular evidence (Liu & Hershler unpublished). *Pyrgulopsis fresti* appears to be another example of a morphologically and genetically divergent, narrowly endemic springsnail lineage associated with thermal spring habitats (Liu & Hershler 2005).

Specimens from the Tudor Warm Springs localities varied somewhat in terms of spire height, whorl convexity and thickening of the inner shell lip (Fig. 3A–C). The few individuals collected from the two upriver sites (not figured) differed from the above in having weaker shouldering of the teleoconch whorls. The single sequenced specimen from the west side of the river at Tudor Warm Springs (IP69) differed from those collected on the east side (IP68, P176, P210, P212) by 2.1% sequence divergence and from specimens obtained from the two upriver sites (P207, P208) by 2.0% (1.6–2.5%). (The other two groups differed from each other by only 0.9% [0.6–1.2%].) This level of divergence falls within the range of values documented for other congeners (1.1-13.1% for COI, Liu & Hershler 2005). Additional sequencing may provide the basis for recognizing the western Tudor Warm Springs population as a distinct conservation unit.

Radular count data were from USNM 1092860, USNM 1102161.

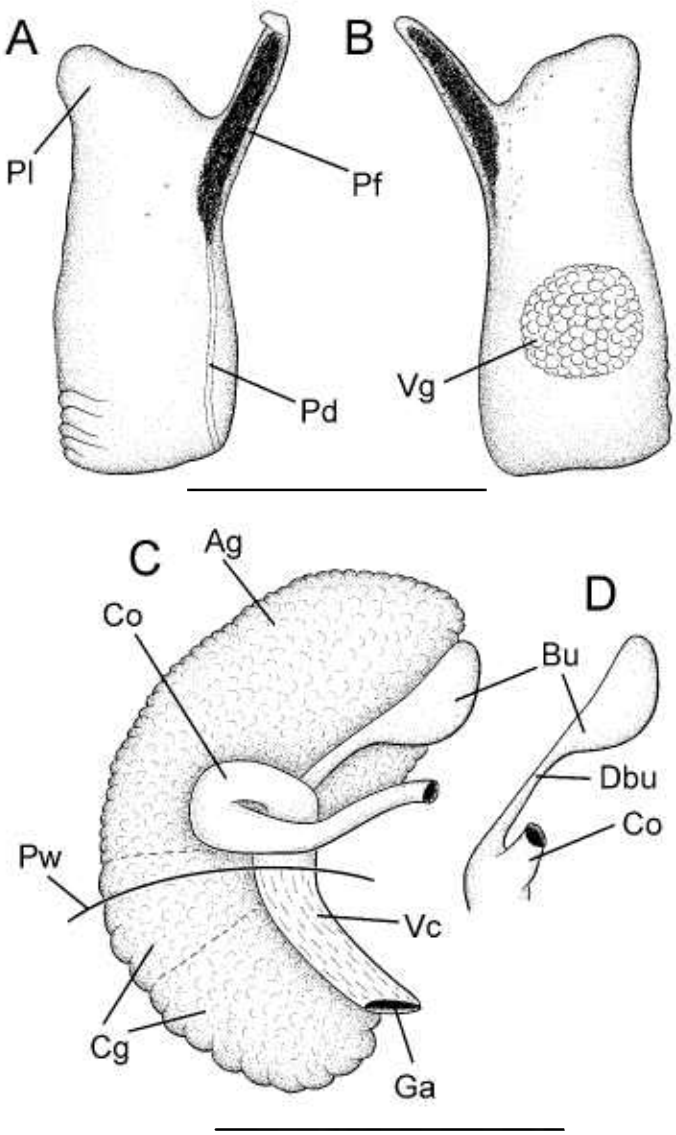

FIGURE 5. *P. fresti* **sp. nov.**, USNM 1102161. A–B. Penis. Scale bar = 0.5 mm. A. Dorsal surface. B. Ventral surface. C. Female glandular oviduct and associated structures (viewed from the left side). Scale bar = 0.5 mm. D. Bursa copulatrix and its duct. Scale as in "C." Ag = albumen gland, Bu = bursa copulatrix, 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, Vc = ventral channel, Vg = ventral gland.

***Pyrgulopsis owyheensis* sp. nov.**
(Figs 6–9)

Types. Holotype (Fig. 6A), USNM 883435, Owyhee Spring, T. 32 S, R. 42 E, SE 1/4 sec. 6, Gary L. Vinyard, 27/vii/1993. Paratypes, USNM 1116915 (from same lot, 2283 specimens).

Etymology. A geographic epithet referring to the distribution of this species in the Owyhee Uplands region.

Referred material. OREGON. Malheur County. USNM 883448, Cherry Spring, T. 36 S, R. 43 E, sec. 14, 29/vii/1993. USNM 883437, Tree Spring, T. 35 S, R. 41 E, sec. 36, 29/vii/1993. USNM 1092853, 1092854, *ibid.*, 447555 E, 4700938 E, 16/vii/2006, 18/vii/2006. USNM 1106163, USNM 1102150, *ibid.*, 447559 E,

4700915 E, 11/v/2007, 13/v/2007. USNM 1115387, spring tributary to Owyhee River upflow from Tudor Warm Springs, 484401 E, 4707220 N, 11/x/2006. USNM 1070694, USNM 1071458, USNM 1115399, Tudor Warm Springs, east side of river, second spring from north, 485060 E, 4708420 N, 25/ix/2002. USNM 1115389, USNM 1115400, *ibid.*, 484936 E, 4708680 N, 11/v/2007, 12/v/2007. USNM 1115390, Tudor Warm Springs, east side of river, third spring from north, 484935 E, 4708661 N, 12/v/2007. USNM 1115401, Tudor Warm Springs, west side of river, second spring north of mouth of Warm Springs Canyon, 484853 E, 4708749 N, 19/vii/2006. USNM 1071259, springs on south side of Owyhee River, 0.32 km west of Sand Hollow, 455620 E, 4736750 N, 24/ix/2002. USNM 883450, spring 1.6 km east-southeast of Owyhee Spring, T. 32 S, R. 42 E, sec. 6, 27/vii/1993. JFBM 20915, Owyhee Spring, T. 32 S, R. 42 E, sec. 6, 18/v/1982. USNM 1070697, USNM 1071456, *ibid.*, 450500 E, 473600 N, 26/ix/2002. USNM 1092819, Owyhee Spring, first spring south of main spring, 450415 E, 4737821 N, 16/vii/2006. USNM 1102149, USNM 1102162, *ibid.*, 450422 E, 4737819 N, 11/v/2007, 13/v/2007. USNM 1107068, seep wall on west side of Owyhee River, just above mouth of Crooked Creek, 443352 E, 4747342 N, 5/ix/2007. USNM 1107070, spring on east side of Owyhee River below mouth of Crooked Creek, 443026 E, 4749973 N, 5/ix/2007. USNM 1115388, spring on east side of Owyhee River, above Long Sweetwater rapids, 442355 E, 4753770 N, 6/ix/2007. USNM 1107073, Weeping Wall springs, west side of Owyhee River, just below mouth of Granite Creek, 440473 E, 4760771 N, 6/ix/2007. USNM 1107074, spring on east side of Owyhee River, above Artillery rapids, 443011 E, 4765226 N, 7/ix/2007. USNM 1102152, Kane Springs, 462846 E, 4846953 N, 15/v/2007.

Diagnosis. A small to medium-sized species having an ovate to narrow conic shell with medium to highly convex whorls. Penis having a medium-sized lobe and medium length filament; penial ornament consisting of a transverse terminal gland; a penial gland; and a well-developed, distally positioned ventral gland.

Description. Shell (Fig. 6A–I) ovate to narrow conic, height about 1.6–3.3 mm; whorls, 3.50–4.75. Periostracum tan or dark brown, thin. Protoconch (Fig. 6J–K) near planispiral, about 1.5 whorls, diameter about 350 µm, surface entirely smooth or weakly wrinkled on initial 0.75 whorl. Teleoconch whorls medium or highly convex, narrowly and sometimes distinctively shouldered (Fig. 6A), last 0.5–0.25 whorl sometimes slightly loosened (Fig. 6E); sculpture of collabral growth lines. Aperture ovate, angled adapically. Inner lip usually disjunct, rarely adnate, usually thickened internally; columellar shelf absent; outer lip thin, weakly prosocline or orthocline. Umbilicus narrow or perforate; umbilical area sometimes having a narrow, adapertural groove.

Shell measurements (mean ± standard deviation in parentheses): height 2.13–2.78 mm (2.53±0.16), width 1.79–2.19 mm (2.00±0.11), body whorl height 1.87–2.39 mm (2.13±0.12), body whorl width 1.43–1.89 mm (1.69±0.10), aperture height 1.19–1.41 mm (1.29±0.06), aperture width 1.05–1.32 mm (1.17±0.07), shell width/height 0.69–0.91 (0.79±0.05), body whorl height/shell height 0.78–0.88 (0.84±0.02), aperture height/shell height 0.46–0.56 (0.51±0.03) (USNM 1116915, $n = 30$).

Measurements of holotype: height 2.29 mm, width 1.99 mm, body whorl height 1.97 mm, body whorl width 1.45 mm, aperture height 1.34 mm, aperture width 1.19 mm, shell width/height 0.87, body whorl height/shell height 0.86, aperture height/shell height 0.58, 3.75 whorls.

Operculum thin, flat, amber-colored, multispiral with eccentric nucleus; last half whorl usually frilled on outer side (Fig. 7A–B); inner side sometimes having a distinct rim along outer edge (Fig. 7C), attachment scar border slightly thickened along inner edge to strongly thickened almost all around (Fig. 7C–F). Radula taenioglossate (Fig. 8A), with about 50 well-formed rows of teeth. Central teeth (Fig. 8B) about 27 µm wide; cutting edge concave, sometimes strongly so; lateral cusps 4–6; central cusp narrow, considerably longer than lateral cusps, pointed, parallel-sided proximally; basal cusp 1, small; basal tongue V-shaped, length about equal to lateral margins. Lateral tooth (Fig. 8C) face rectangular, angled; central cusp large, hoe-shaped; lateral cusps 2–3 (inner), 2–4 (outer); outer wing rather broad, straight, about 160% length of cutting edge; basal tongue well developed. Inner marginal teeth (Fig. 8D) having 17–22 cusps. Outer marginal teeth (Fig. 8E) having 23–36 small cusps; a rectangular wing along inner edge was seen in some specimens (Fig. 8F).

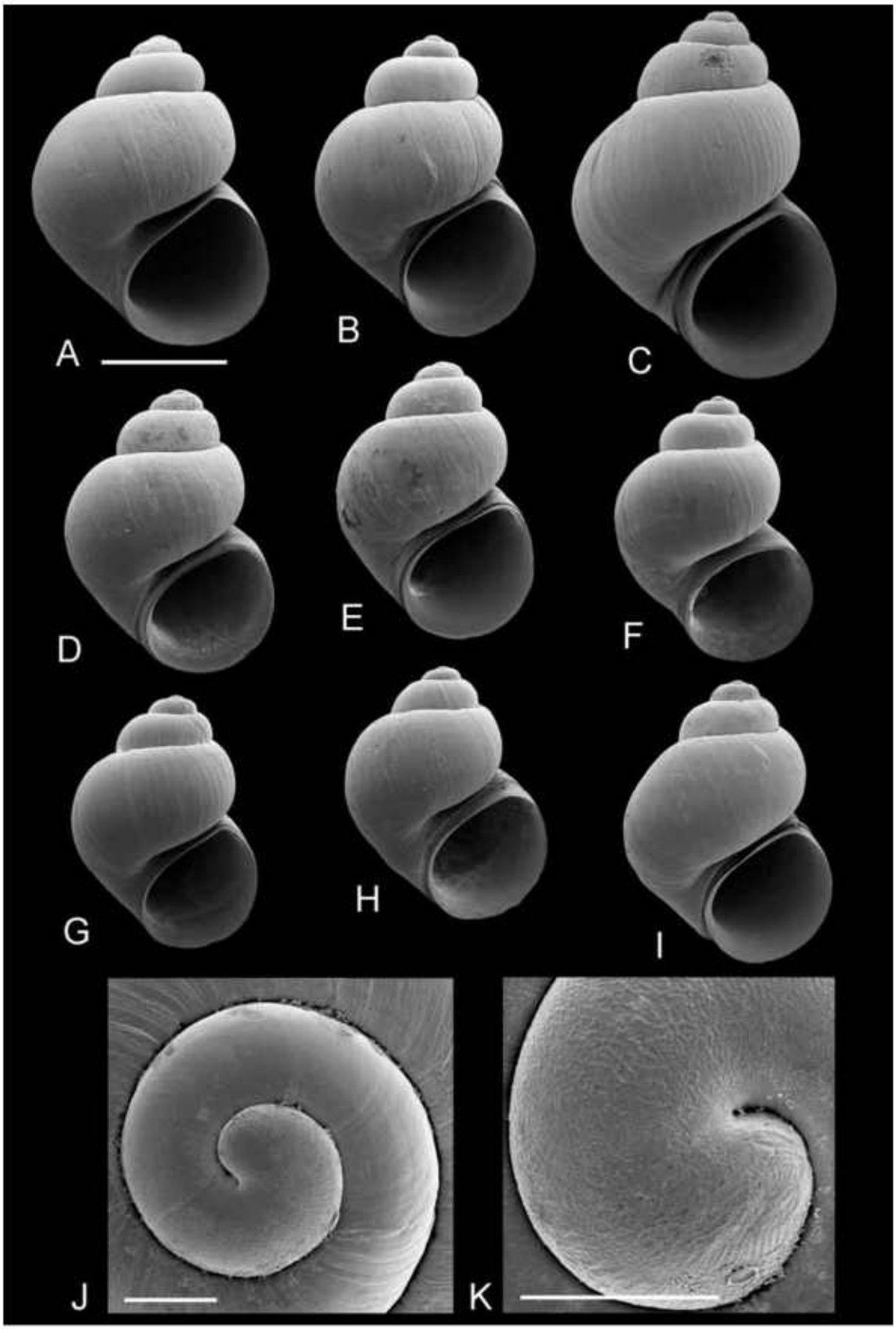

FIGURE 6. *P. owyheensis* **sp. nov**. A–I. Shells. Scale bar = 1.0 mm. A. Holotype, USNM 883435. B, USNM 1071259. C. USNM 1092854. D. USNM 1115390. E. USNM 1115400. F. USNM 1115388. G. USNM 1107074. H. USNM 1107073. I. USNM 1102152. J. Shell apex, USNM 1102152. Scale bar = 100 µm. K. Close-up of protoconch sculpture, USNM 1102152. Scale bar = 100 µm.

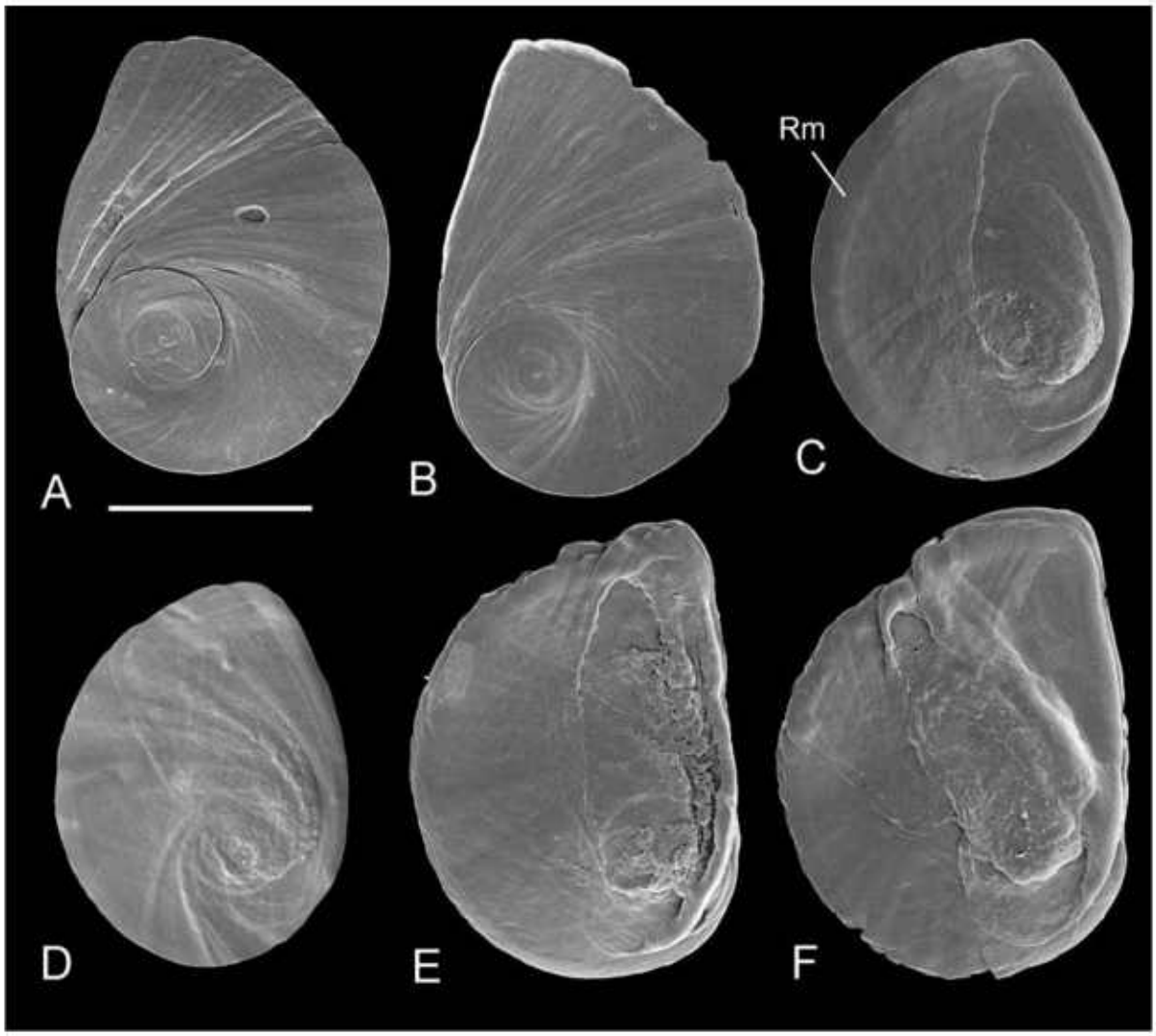

FIGURE 7. *P. owyheensis* **sp. nov**. A–F. Opercula. Scale bar = 500 µm. A–B. Outer side, showing frilled whorls, USNM 1116915, USNM 1092854. C–F. Inner side, showing variation in thickening of attachment scar margin, USNM 1116915, USNM 1102152, USNM 1092854, USNM 1092854. Rm = rim.

Head-foot generally dark brown. Cephalic tentacles pale dorsally except for central pigmented strips, ventral surfaces pale. Distal lips of snout light brown or pale. Sole of foot pale or grey. Pallial roof, visceral dark brown. Ctenidium well developed, positioned a little in front of pericardium; ctenidial filaments about 15. Oshpradium narrow, positioned posterior to middle of ctenidium. Prostate gland small, pea-shaped, with about 33% of length in pallial roof. Anterior vas deferens opening from ventral edge of prostate gland a little in front of pallial wall, section of duct on columellar muscle having weak bend. Penis (Fig. 9A–D) medium to large, base elongate-rectangular, inner edge smooth; penial filament medium length, tapering, oblique; lobe medium-sized, rectangular or tapering, oblique. Terminal gland narrow, transverse, overlapping dorsal and ventral edges of lobe. Penial gland overlapping up to 50% of filament length, slightly overlapping penis posteriorly. Ventral gland well developed, narrow, distally positioned, borne on short stalk (Figs 9B, D). Penial duct narrow, straight. Penial filament containing a dense core of black pigment; penis otherwise pigmented with scattered black granules. Female glandular oviduct and associated structures shown in Figure 9E–G. Coiled oviduct a large, posteriorly oblique, proximally kinked loop. Bursa copulatrix small, narrowly ovate, horizontal, largely overlapped by albumen gland. Bursal duct about as long as and slightly narrower than bursa, opening from distal edge, sometimes pigmented with black granules near edges. Seminal receptacle small, pouch-shaped, positioned near antero-ventral edge of bursa; duct short. Albumen gland

about as long as capsule gland, entirely visceral or with very short pallial section, sometimes pigmented with scattered black granules. Capsule gland composed of a single tissue section. Genital aperture a sub-terminal slit.

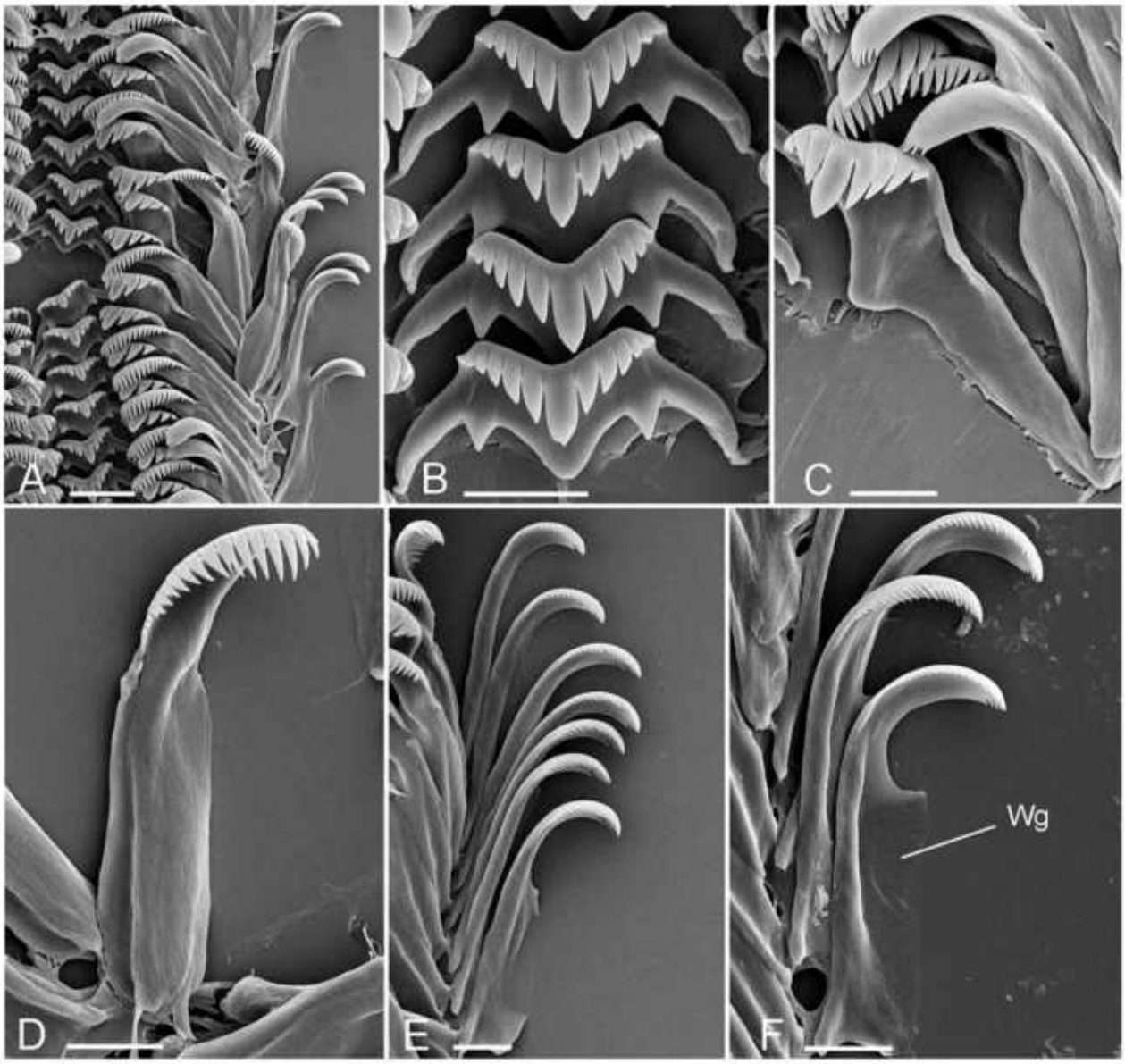

FIGURE 8. *P. owyheensis* **sp. nov.** A. Portion of radula ribbon, USNM 1116915. Scale bar = 20 µm. B. Central radular teeth, USNM 1116915. Scale bar = 10 µm. C. Lateral and inner marginal teeth, USNM 1116915. Scale bar = 10 µm. D. Inner marginal tooth, USNM 1116915. Scale bar = 10 µm. E. Outer marginal teeth, USNM 1116915. Scale bar = 10 µm. F. Outer marginal tooth, showing outer wing, USNM 1092854. Scale bar = 10 µm. Wg = wing.

Distribution and habitat. *Pyrgulopsis owyheensis* is disjunctly distributed among five small areas in southeastern Oregon (Owyhee River near Three Forks, Rattlesnake Creek drainage, Owyhee Spring area, lower Owyhee River, Malheur River drainage) (Fig. 1). Additional sampling will be needed to determine whether the intervening areas are also inhabited by this species. *Pyrgulopsis owyheensis* lives in spring sources and stream outflows, some of which are thermal, and is found on a variety of hard substrates. This species was collected together with *P. intermedia* in a spring along the Owyhee River above Long Sweetwater rapids, and with the invasive New Zealand mudsnail, *Potamopyrgus antipodarum* (Gray) (USNM 1107078) at a seep wall above the mouth of Crooked Creek.

Remarks. *Pyrgulopsis owyheensis* differs from closely similar *P. intermedia* in size (shell height about half of that of the latter when in sympatry; Figs 6F, 10A) and in its typically disjunct inner shell lip, longer and narrower penial filament, more distally positioned ventral gland of penis, and mitochondrial DNA sequences (6.9±0.01%, Table 2). None of our phylogenetic analyses supported a close relationship between these species (e.g., Fig. 2).

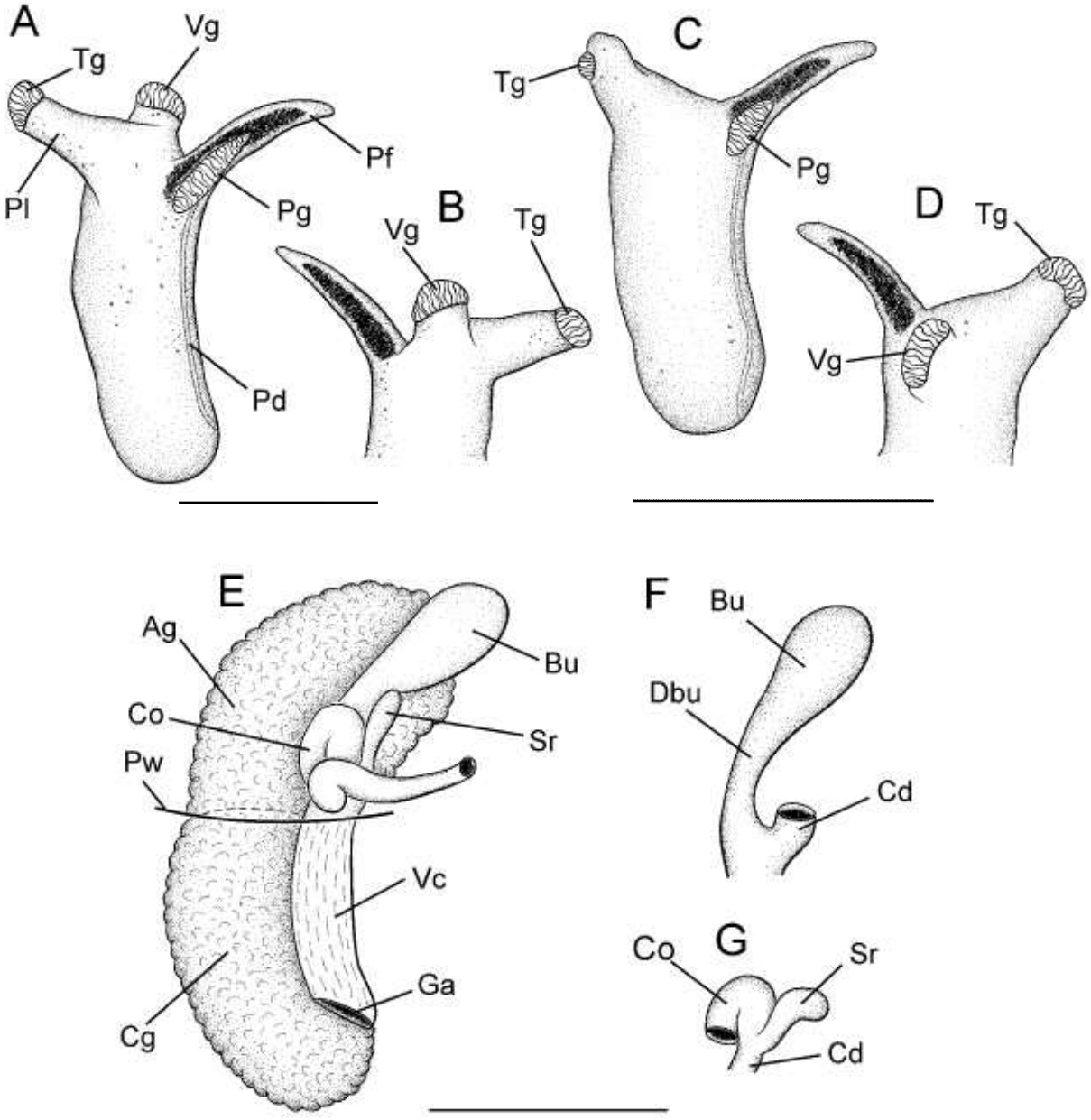

FIGURE 9. *P. owyheensis* **sp. nov.**. A–B. Penis, USNM 1092819. Scale bar = 0.25 mm. A. Dorsal surface. B. Ventral surface. C–D. Penis, USNM 1102152. Scale bar = 0.5 mm. C. Dorsal surface. D. Ventral surface. E. Female glandular oviduct and associated structures (viewed from the left side), USNM 1092819. Scale bar = 0.25 mm. F. Bursa copulatrix and its duct. Scale as in "E." G. Seminal receptacle and its duct. Scale as in "E." 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, Pg = penial gland, Pl = penial lobe, Pw = posterior wall of pallial cavity, Sr = seminal receptacle, Tg = terminal gland, Vc = ventral channel, Vg = ventral gland.

Shell variation within *P. owyheensis* (Fig. 6A–I) was minor, involving slight differences in size and shape (Fig. 6). The sequence divergence between specimens from the type locality (Owyhee Spring) and the other

four areas inhabited by this species ranged from 1.5–1.8% whereas the latter differed from each other by only 0.3–0.8%. This suggests the possibility of incipient speciation in the Owyhee Spring area and a need to manage these populations as a distinct conservation unit.

Radular count data were from USNM 1092854, USNM 1102152, USNM 1116915.

Pyrgulopsis intermedia (Tryon, 1865)

(Figs 10–11)

Pomatiopsis intermedia Tryon, 1865: 220 [type locality, Owyhee R., S. E. Oregon; original label states Crooked Creek, Owyhee, southeast Oregon].

Pyrgulopsis intermedia (Tryon, 1865).—Hershler 1994: 42, 44, figs 17a–d, 36c, 47b (synonymy, diagnosis, description).

Referred material. OREGON. Malheur County. JFBM 20497, JFBM 20507, Crooked Creek Spring, ca. 16 km southwest of Burns Junction, T. 33 S, R. 39 E, sec. 22, 23/v/1963, 19/v/1982. USNM 1075362, spring tributary to Birch Creek, 459964 E, 4781865 N, 25/iv/2003. USNM 1075684, *ibid.*, 459871 E, 4782621 N, 25/vi/2003. USNM 1092855, *ibid.*, 460058 E, 4781808 N, 18/vii/2006. USNM 1107071, spring on east side Owyhee River, above Long Sweetwater rapids, 442355 E, 4753770 N, 6/ix/2007. USNM 1107076, spring on hillside, Jackson Hole, 447566 E, 4781838 N, 8/ix/2007. USNM 1107079, spring west of Two Mile Spring, 453320 E, 4782496 N, 9/ix/2007. USNM 1107077, mouth of Rinehart Creek, west side of Owyhee River, 449007 E, 4783611 N, 9/ix/2007.

Distribution and habitat. Crooked Creek (Owyhee River basin), Barren Valley (Great Basin), plus new records from the Crooked Creek headspring and along the lower Owyhee River, southeastern Oregon (Fig. 1). This species lives in springs and small streams. It was collected in association with *Potamopyrgus antipodarum* (USNM 1107069) at the mouth of Rinehart Creek.

Remarks. In an unpublished checklist, Taylor (1977) contended that the type locality of *Fontelicella intermedia* (= *Pyrgulopsis intermedia*) may have been incorrectly attributed to Crooked Creek (Owyhee River drainage) instead of Crooked Creek (Deschutes River drainage) and consequently provided a new species name, *Fontelicella malhorica*, for the congener that lives in the headspring of the former stream. (Note that Taylor apparently changed his mind on this subject as he later stated that *P. intermedia* lives in Crooked Creek, Owyhee River drainage [Taylor 1985:309–310].) In an unpublished report, Frest & Johannes (1995:198, 202) listed this *nomen nudum* (to which they applied a common name, "Malheur springsnail") as a different species than *P. intermedia* and indicated that both of these snails live in Crooked Creek, Owyhee River drainage. *Pyrgulopsis intermedia* and *P.* sp. nov. (Malheur springsnail) have subsequently been listed in various conservation-related documents and publications (cited in the Introduction; also see Wisdom *et al.* 2003; Brown *et al.* 2007). Taylor's (1977) manuscript name is not valid because his checklist does not constitute published work under ICZN Articles 8.1 and 9.7 and the only subsequent citation (Frest & Johannes 1995) was also in an unpublished venue. (Also note in this context that the name was not accompanied by a description or definition.) There is no reason to suspect that Tryon (1865) made an error in reporting the type locality of *P. intermedia* as his types well conform to snails now living in Crooked Creek, Owyhee River drainage (Hershler 1994). Snails (collected by Taylor) from Crooked Creek Spring (JFBM 20497, JFBM 20507, Fig. 10A) conform morphologically to *P. intermedia* in all respects and our genetic studies also suggest that only one congener lives in Crooked Creek. Thus, there is no basis for recognizing the Malheur springsnail as a validly described and/or distinct species and this name therefore should be removed from conservation watch lists and other databases.

Snails from the newly reported localities are referable to *P. intermedia* based on their relatively large (>3.3 mm), solid, ovate conic shell (Fig. 10A–D) with prominent spire; and details of penis shape and glandular ornament (Fig. 11A–B), particularly the short penial filament and medially positioned ventral gland. Samples from Crooked Creek (IP60) and the lower Owyhee River drainage (IP67, P217, P222, P223, P224) that were

newly sequenced for this project were closely similar (0.2% divergence) to previously analyzed specimens from Crooked Creek (P1, P2) and Barren Valley (P4). The mean uncorrected sequence divergence within *P. intermedia* was quite low (0.2%), ranging from 0–0.8%.

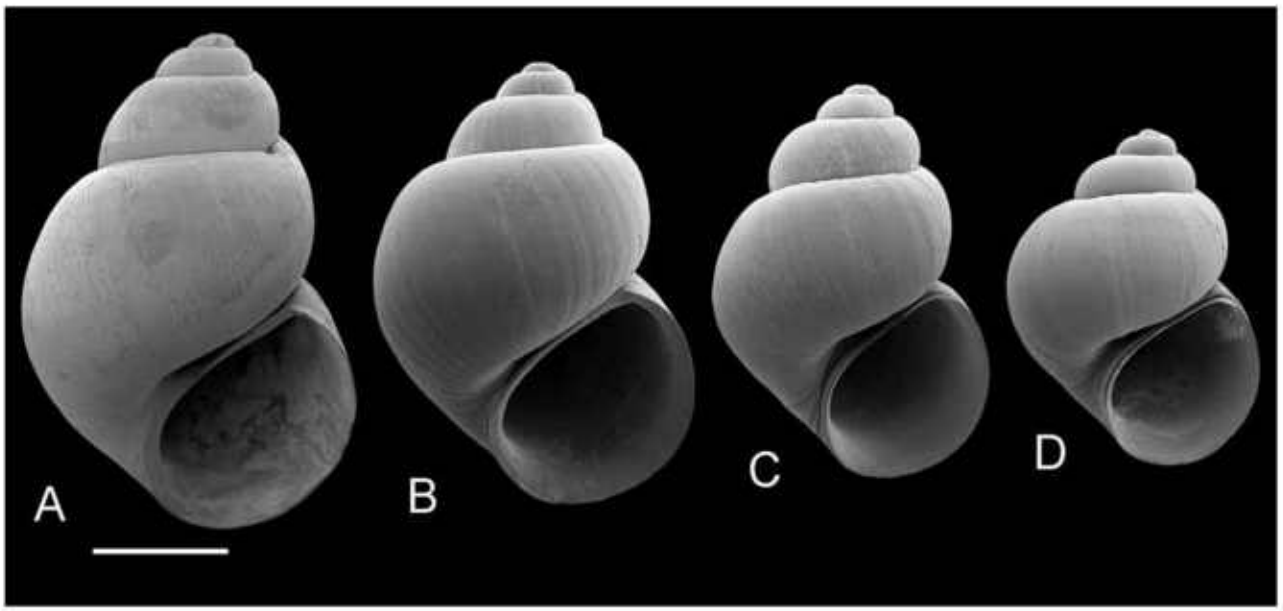

FIGURE 10. *P. intermedia* (Tryon). A–D. Shells. Scale = 1.0 mm. A. JFBM 20507. B. USNM 1107071. C. USNM 1107076. D. USNM 1075362.

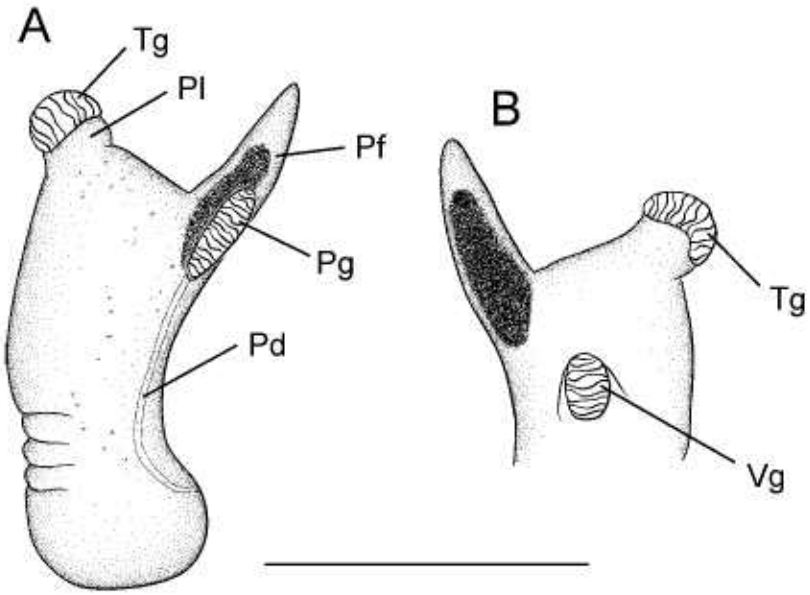

FIGURE 11. *P. intermedia* (Tryon). A–B. Penis, USNM 1075362. Scale bar = 0.5 mm. A. Dorsal surface. B. Ventral surface. Pd = penial duct, Pf = penial filament, Pg = penial gland, Pl = penial lobe, Tg = terminal gland, Vg = ventral gland.

The new records detailed herein extend the range of *P. intermedia* about 65 km north of its type locality area into the lower Owyhee River basin. It may be appropriate to reconsider the conservation status of this species in the light of this significant range extension.

Acknowledgements

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