

# TRANSFER OF *COCHLIOPA TEXANA* TO *PYRGULOPSIS* (HYDROBIIDAE) AND DESCRIPTION OF A THIRD CONGENER FROM THE LOWER PECOS RIVER BASIN

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## ABSTRACT

The Phantom Cave snail (*Cochliopa texana*), a little-studied rissooidean gastropod that is locally endemic within the lower Pecos River basin (Texas) and currently a candidate for addition to the Federal list of threatened and endangered species, is redescribed and transferred to the hydrobiid genus *Pyrgulopsis*, based on shell and anatomical characters. Specimens from the type locality (Phantom Lake Spring) and San Solomon Spring are larger than those from East Sandia Spring and also differ somewhat in shell shape and shape of the central cusps of the lateral radular teeth. However genetic (mtCOI, NDI) variation within and among these geographically proximal (6–13 km) populations was slight, providing no basis for the recognition of distinct conservation units of this imperiled species. We also describe *Pyrgulopsis ignota* n. sp., which was recently discovered in a different part of the lower Pecos River basin and initially confused with the Phantom Cave snail. These two species differ in shell shape, operculum morphology, and form and glandular ornament of the penis. They are also strongly differentiated genetically from each other and from (13) other regional congeners (pairwise sequence divergence >6.3% for both genes). A Bayesian phylogenetic analysis of the COI and NDI dataset indicated that these two snails are not closely related and that *P. ignota* occupies a basal position relative to other regional congeners.

## INTRODUCTION

Although the systematics of North American cochliopid gastropods has been extensively studied and revised for more than four decades (e.g. Taylor, 1966, 1987; Thompson, 1968; Hershler & Thompson, 1992; Hershler, 2001; Liu, Hershler & Thompson, 2001; Hershler, Liu & Stockwell, 2002), various problems have yet to be resolved. One of these concerns the identity of the Phantom Cave snail, *Cochliopa texana* Pilsbry, 1935, a tiny, little studied species that is locally distributed in the lower Pecos River basin near Balmorhea (west Texas) and a candidate for addition to the Federal list of threatened and endangered wildlife (USFWS, 2001). Pilsbry's (1935) brief description of this species was bereft of anatomical details and he presumably assigned it to *Cochliopa* based on shell shape. Morrison (1946) later restricted *Cochliopa* to three species from Panama following Stimpson's (1865) original diagnosis (also see Thompson & Hershler, 1991). He allocated most of the other snails which had been previously assigned to this genus to *Cochliopina*, but did not treat *Cochliopa texana*. Taylor (1966: 179) commented that the Phantom Cave snail 'is not referable to *Cochliopa* in the strict sense according to Morrison (1946)', but was unable to make a definitive generic assignment for this species, which he classified in the Cochliopinae (*sensu* Taylor, 1966) based on its multispiral operculum with subcentral nucleus. The Phantom Cave snail was not treated in a subsequent review of the cochliopid genera (Hershler & Thompson, 1992) and in recent works is either tentatively retained in *Cochliopa* (e.g. Turgeon *et al.*, 1998) or listed as genus *incertae sedis* (e.g. Burch, 1989).

We recently studied the anatomy of the Phantom Cave snail and determined that it belongs to the North American hydrobiid genus *Pyrgulopsis*. Here we provide a new description and

taxonomic assignment for this species and also describe a new congener that was recently discovered in the lower Pecos River basin c. 150 km from Balmorhea and originally confused with the former. We also use mitochondrial DNA sequence data to further describe variation within these two species and examine their phylogenetic relationships.

## 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) are provided when available for a given sample. Types for the new species and other voucher material from this study were deposited in the National Museum of Natural History (USNM) collection. Types and other material of the Phantom Cave snail in the collections of the Academy of Natural Sciences of Philadelphia (ANSP) and University of Minnesota Bell Museum of Natural History (UMBMNH) were also examined during the course of this study.

Five females and five males (all adults) from each population of *Cochliopa texana* and the new species described herein were dissected. 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 those used in recent taxonomic investigations of *Pyrgulopsis* (Hershler, 1998; Hershler *et al.*, 2003). Shell data were analysed using Systat for Windows 11.00.01 (SSI, 2004).

The molecular phylogenetic analysis included the two species treated herein, *P. davisii* (Taylor, 1987), which is the only other congener found in the lower Pecos River basin, and 12 other

**Table 1.** Locality details and GenBank accession numbers for COI and NDI sequences.

Species (code)	Locality	GenBank accession number	
		COI	NDI
<i>Pyrgulopsis acarinata</i> (Hershler, 1985)	La Tecla Vieja, Cuatro Ciénegas basin, Coahuila, Mexico	AY627954*	AY628034*
<i>P. arizonae</i> (Taylor, 1987)	Medicine Spring, Bylas, Gila River drainage, Graham Co., AZ	AY627948*	AY628072*
<i>P. bernardina</i>	Spring, El Chorro, Rio Yaqui drainage, Sonora, Mexico	AY627951*	AY628075*
<i>P. chupaderae</i>	Willow Springs, Rio Grande drainage, Socorro Co, NM	GQ904209	GQ904223
<i>P. davisii</i>	Spring tributary to Limpia Creek, Pecos River drainage, Jeff Davis Co., TX	AY627950*	AY628074*
<i>P. gilae</i> (Taylor, 1987)	Spring tributary to East Fork Gila River, Grant Co., NM	AY627952*	AY628076*
<i>P. ignota</i> (Ct3, BKL2)	Caroline Spring, Pecos River drainage, Terrell Co., TX	GQ904203–GQ904206	GQ904215–GQ904218
<i>P. manantiali</i> (Hershler, 1985)	Santa Tecla canal at Puerto Salado, Rio Salado drainage, Coahuila, Mexico	AY627955*	AY628079*
<i>P. metcalfi</i>	Naegele Spring, Rio Grande drainage, Presidio Co., TX	GQ904210	GQ904224
<i>P. minckleyi</i> (Taylor, 1966)	East Cold Spring, Cuatro Ciénegas basin, Coahuila, Mexico	AY627917*	AY628034*
<i>P. pecosensis</i> (Taylor, 1987)	Blue Spring, Pecos River drainage, Eddy Co., NM	AF520909†	AY628081*
<i>P. roswellensis</i>	Sago Spring, Pecos River drainage, Chaves Co., NM	GQ904211	GQ904225
<i>P. texana</i> (Ct4, BKL4)	Phantom Lake Spring, Pecos River drainage, Jeff Davis Co., TX	GQ904207, GQ90428	GQ904219–GQ904222
<i>P. texana</i> (Ct1, BKL1)	San Solomon Spring, Pecos River drainage, Reeves Co., TX	GQ904200	GQ904212
<i>P. texana</i> (Ct2, BKL3)	East Sandia Spring, Pecos River drainage, Reeves Co., TX	GQ904201, GQ904202	GQ904213, GQ904214
<i>P. thermalis</i> (Taylor, 1987)	Hot Spring, Gila River drainage, Grant Co., NM	AY627953*	AY628077*
<i>P. trivialis</i> (Taylor, 1987)	Spring, Three Forks, Gila River drainage, Grant Co., NM	AY627941*	AY628065*
<i>Floridobia floridana</i>	Juniper Springs, St. Johns River drainage, Marion CO., FL	AF520916†	AY628035*
<i>F. winkleyi</i> (Pilsbry, 1905)	Salt marsh, Scarborough, Saco River drainage, Cumberland Co., ME	AY520917†	AY628036*

\*Liu &amp; Hershler (2005); †Liu et al. (2003).

regional members of the genus. Two species of *Floridobia* were used as outgroups based on the close relationship between this eastern North American genus and *Pyrgulopsis* (Liu & Hershler, 2005); the type species of the former, *F. floridana* (Frauenfeld, 1863), was used as the root. Prior to our final analysis we performed a comprehensive (unpublished) survey of mtDNA variation within *Pyrgulopsis* to confirm that close relatives of the Phantom Cave snail and the new species had not been omitted.

Genomic DNA was extracted from entire snails using a CTAB protocol (Bucklin, 1992). A 658 bp segment of cytochrome *c* oxidase subunit I (COI) (Folmer *et al.*, 1994) and a 530 bp segment of NADH dehydrogenase subunit I (NDI) (Liu, Hershler & Clift, 2003) were amplified and sequenced with primers LCO1490 and HCOI2198, and ND43F and RND592F, respectively, following protocols of Liu *et al.* (2003). Sequences were determined for both strands and then edited and aligned using Sequencher v. 4.8. *Pyrgulopsis chupaderae* (Taylor, 1987), *P. meicalfi* (Taylor, 1987), *P. roswellensis* (Taylor, 1987) and the two congeners treated herein were newly sequenced for this study; sequences for the other species were from our previously published investigations (Hershler, Liu & Thompson, 2003; Liu & Hershler, 2005). We sequenced eight specimens from each sample of the two species treated herein to assess variation. Sample information and GenBank accession numbers for the sequenced specimens utilized in this study are in Table 1. The 26 new sequences reported herein were deposited in GenBank under accession numbers GQ904200–GQ904225 (Table 1).

Sequence divergences (uncorrected *p* distance) were calculated using MEGA4 (Tamura *et al.*, 2007). Phylogenetic relationships were inferred using Bayesian inference in MrBayes v. 3.12 (Ronquist & Huelsenbeck, 2003). MrModeltest (Nylander, 2004) selected the General Time Reversible model (GTR + G), which best fit the data under the Akaike Information Criterion. In the initial Bayesian analysis the burn-in was set at 10% (10,000 generations) of the chain length (100,000 generations). Three runs were conducted in MrBayes using the General Time Reversible model (GTR + G) selected by MrModeltest 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 likelihoods started around -10,000 and quickly converged upon a stable value of about -5,670 after 8,000 generations. 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 tree with the first 5,000 trees (equal to 50,000 generations) removed to ensure that the chain sampled a stationary portion.

## SYSTEMATIC DESCRIPTIONS

### Family Hydrobiidae Troschel, 1857

### Subfamily Nymphophilinae Taylor, 1966

### Genus *Pyrgulopsis* Call & Pilsbry, 1886

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

*Diagnosis: Liu & Hershler* (2005: 296).

### *Pyrgulopsis texana* (Pilsbry, 1935) new combination (Figs 1–3)

*Cochliopa texana* Pilsbry, 1935: 91–92, fig. 5a–b (Phantom Lake near Toyahvale, Reeves Co., Texas). Baker, 1964: 177

(lectotype selection). Dundee & Dundee, 1969: 205–210, figs 1, 2, 3a (morphology). Williams *et al.*, 1985: 26. Taylor, 1987: 40–41 (new record from San Solomon Spring, notes on ecology). Turgeon *et al.*, 1998: 73, 217. Besse, 2002: x (new record from East Sandia Spring).

*‘Cochliopa’ texana*—Taylor, 1966: 179. Taylor, 1975: 188–189 (summary of literature citations). Burch & Tottenham, 1980: 130, fig. 317 (illustration from Pilsbry, 1935). Burch, 1989: 130, fig. 317.

*Types:* Figured lectotype, ANSP 163887; paralectotypes (from same lot), ANSP 420561.

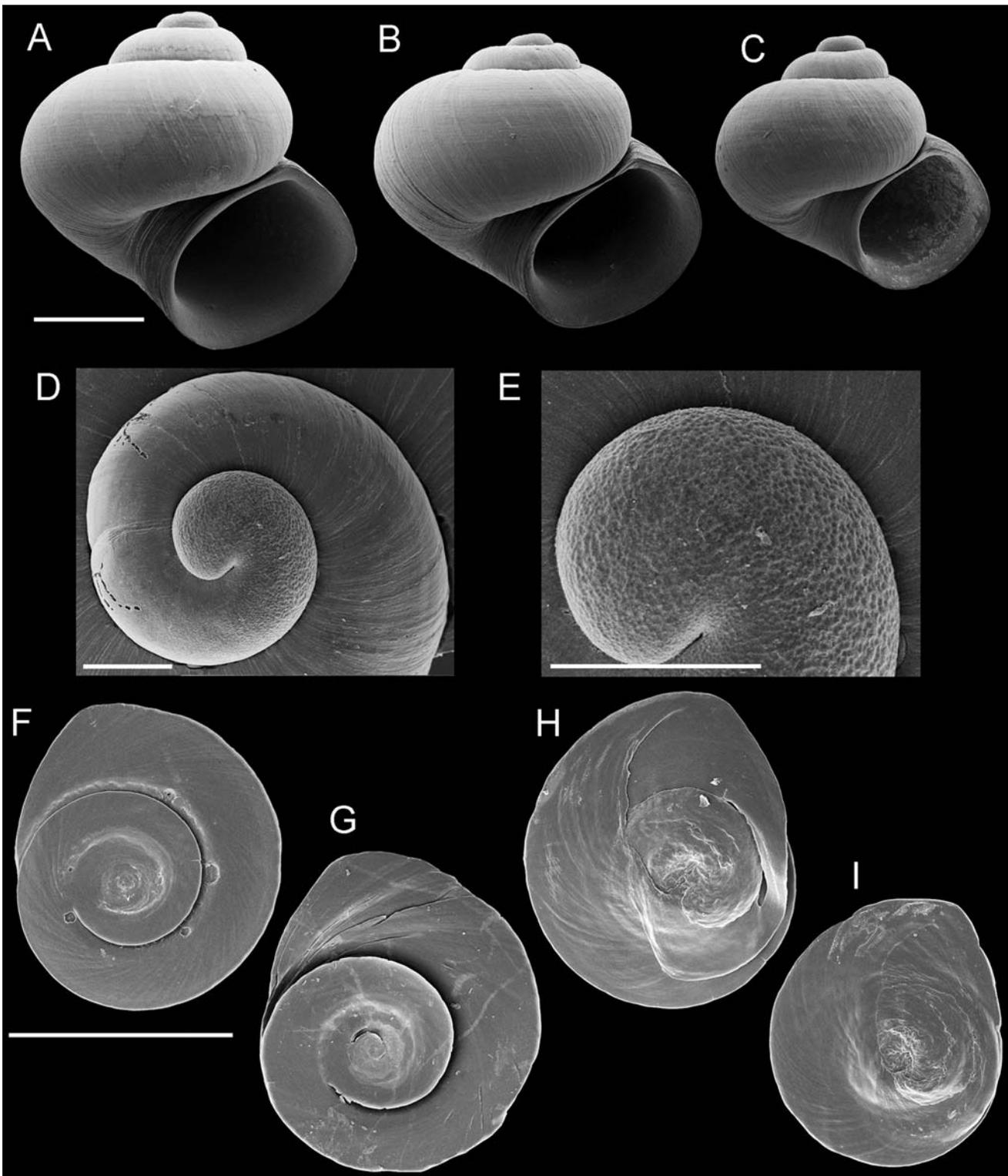
*Referred material:* Jeff Davis County, Texas: USNM 421498, 4.5 miles (7.2 km) southwest of Toyahvale, 9/1934. USNM 873212, USNM 894372, *c.* 160 feet (49 m) inside entrance to Phantom Cave, 6/8/1979, 25/6/1968. USNM 894371, *c.* 50 feet (15 m) inside entrance to Phantom Cave, 25/6/1968. UMBMNH uncat., Phantom Cave, 2 miles (3.2 km) west of Toyahvale, 5/8/1967. USNM 1068972, USNM 1121838, Phantom Lake Spring, at mouth of cave, 15/6/2000, 2/12/2008. UMBMNH uncat., Phantom Lake Spring, Kingston Ranch, 25/6/1968. UMBMNH uncat., Phantom Lake Spring outflow, Kingston Ranch house, 10/4/1965, 25/6/1968. USNM 884954, canal outflow just below Phantom Lake Spring, 3/7/1995. USNM 874850, USNM 874851, USNM 883951, USNM 883953, canal outflow *c.* 50 feet (15 m) below Phantom Cave, 14/7/1973, 14/7/1973, 3/4/1995, 3/4/1995. UMBMNH uncat., Phantom Spring outflow, first lateral north, 25/6/1968. UMBMNH uncat., Phantom Lake Spring outflow, second lateral south, 25/6/1968. Reeves County, Texas: UMBMNH uncat., Phantom Lake Spring outflow, Joe Kingston Ranch, 5/11/1981. UMBMNH uncat., spring-fed creek, Toyahvale (ex UMMZ 60851). USNM 874849, canal outflow 0.57 mile (0.92 km) below Phantom Cave, 18/11/1971. USNM 894373, canal outflow 1.45 miles (2.33 km) below Phantom Cave, 25/6/1968. UMBMNH uncat., San Solomon Spring, Balmorhea State Park, 5/11/1981. USNM 1116250, USNM 1123756, San Solomon Spring, main canal from pool, N 3424124, E 615904, Zone 13, 4/6/2008, 29/3/2009. USNM 1116591, East Sandia Spring, *c.* 30 feet (9 m) downflow from source, N 3429301, E 621404, Zone 13., 5/6/2008. USNM 1003865, East Sandia Spring, 15/6/2000.

*Diagnosis:* A small species of *Pyrgulopsis* having a depressed valviform–trochiform, perforate shell with highly convex whorls. Penis simple (lacking lobes and glands); having short, conical, well-demarcated filament that is densely pigmented (white-yellow) internally.

*Description:* Shell depressed valviform or trochiform (Fig. 1A–C), apex often eroded; height about 1.2–2.0 mm; whorls 3.0–4.0. Periostracum tan or dark brown. Protoconch near planispiral, about 1.2 whorls, diameter about 290 μm (Fig. 1D); initial 0.75–1.0 whorl strongly wrinkled (Fig. 1E), remaining portion smooth. Teleoconch whorls highly convex, rarely shouldered; sculpture of colabral growth lines. Aperture ovate, slightly angled above. Inner lip usually adnate, rarely slightly disjunct, usually slightly thickened internally, rarely thick; columellar shelf usually absent, rarely narrow; outer lip usually thin, rarely slightly thickened, strongly prosocline, often sinuate near base. Umbilicus well developed, perforate. Shell measurements and whorl counts for paratypes and specimens from East Sandia Spring are given in Table 2.

Operculum thin, light amber, broadly ovate, multispiral with subcentral nucleus (Fig. 1F, G); last 0.5–1.0 whorl strongly frilled on outer side, last 0.25 whorl sometimes frilled on inner side (Fig. 1H); attachment scar border variably thickened on inner side (Fig. 1H, I).

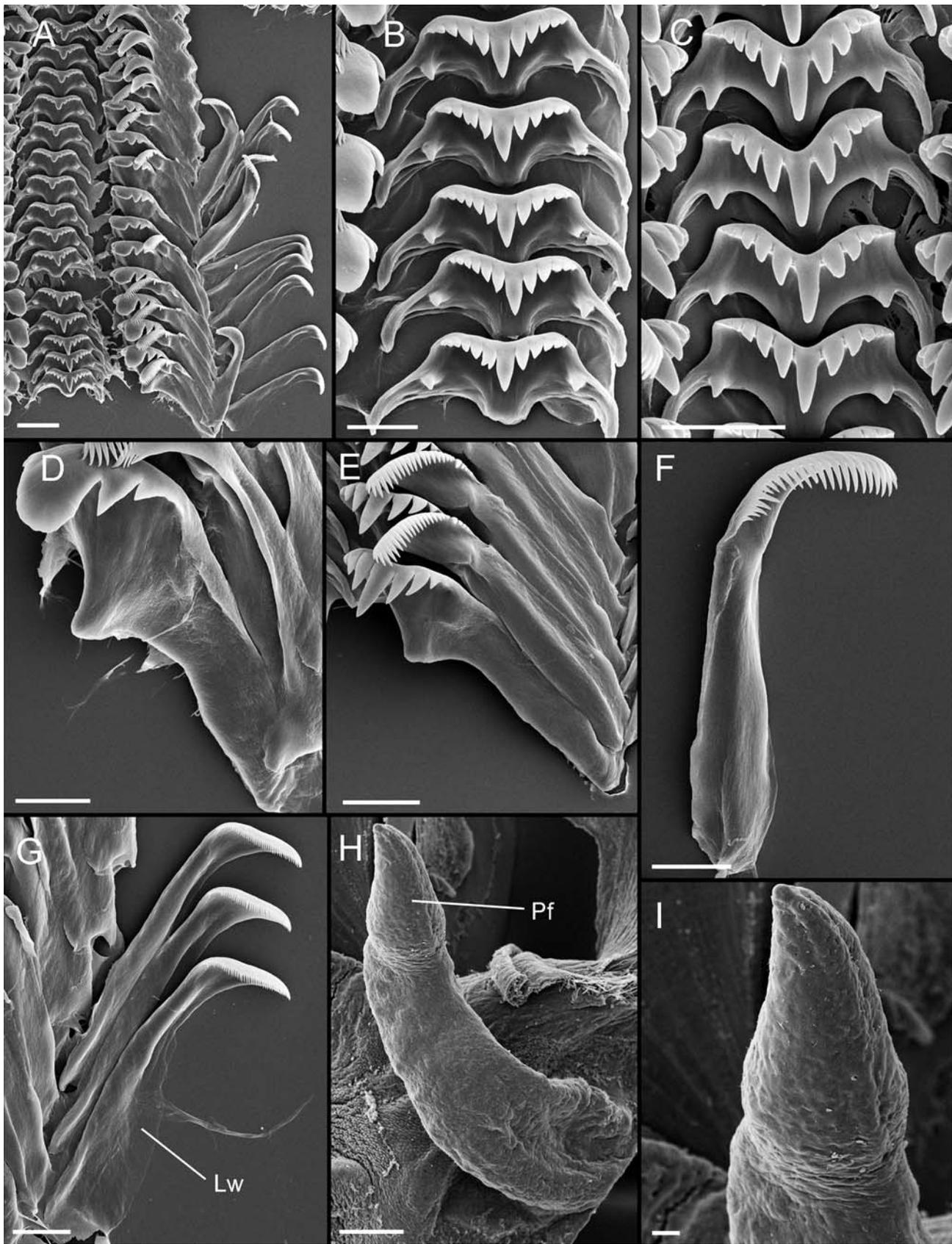
Radula taenioglossate (Fig. 2A), with about 62 well-formed rows of teeth. Central teeth about 40 μm wide, cutting edge



**Figure 1.** Scanning electron micrographs of shells and opercula of *Pyrgulopsis texana*. **A.** Shell (USNM 883954). **B.** Shell (USNM 1123756). **C.** Shell (USNM 1003865). **D.** Shell apex, showing protoconch sculpture (USNM 883954). **E.** Close up of protoconch sculpture (USNM 883954). **F, G.** Opercula, outer side (USNM 883953, UMBMNH uncat., respectively). **H, I.** Opercula, inner side (USNM 883953). Scale bars **A** = 0.5 mm; **D, E** = 100  $\mu$ m; **F** = 250  $\mu$ m. **B, C** to same scale as **A**; **G–I** to same scale as **F**.

weakly to strongly concave (Fig. 2B, C); lateral cusps 3–6; central cusp narrow, pointed, often parallel-sided proximally; basal cusp 1, very small; basal tongue U-shaped, slightly shorter than lateral margins. Lateral tooth face slightly taller than wide; central cusp hoe-like (Fig. 2D) or narrow-pointed

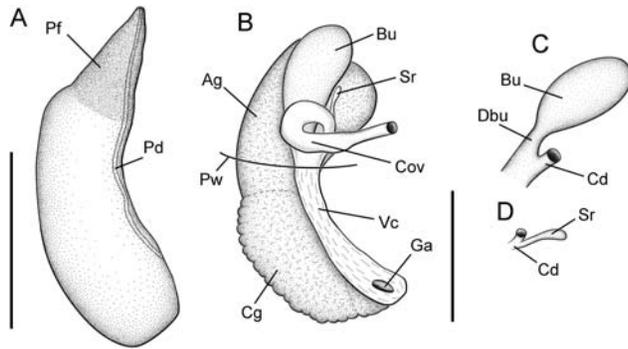
(Fig. 2E); lateral cusps 0–3 (inner), 1–4 (outer); outer wing broad, sometimes flexed, about 170% length of cutting edge; basal tongue well developed. Inner marginal teeth having 18–29 cusps (Fig. 2F); fourth to sixth cusp from outer edge enlarged. Outer marginal teeth having 33–47 cusps (Fig. 2G);



**Figure 2.** Scanning electron micrographs of radula and critical-point dried penis of *Pyrgulopsis texana*. **A.** Portion of radular ribbon (USNM 883953). **B, C.** Central radular teeth (USNM 883953, USNM 1003865, respectively). **D, E.** Lateral and inner marginal teeth (USNM 883953, USNM 1003865, respectively). **F.** Inner marginal tooth (USNM 883953). **G.** Outer marginal teeth (USNM 883953). **H.** Penis, dorsal surface (USNM 883954). **I.** Close up of distal penis, dorsal surface (USNM 883954). Abbreviations: Pf, penial filament; Lw, lateral wing. Scale bars **A** = 20  $\mu\text{m}$ ; **B–G** = 10  $\mu\text{m}$ ; **H** = 100  $\mu\text{m}$ ; **I** = 20  $\mu\text{m}$ .

inner edge sometimes having an elongate, weakly delineated wing. Radular count data were from USNM 883953, USNM 1003865 and UMBMNH uncat. (San Solomon Spring).

Cephalic tentacles dark brown dorsally except for pale patches surrounding eyes, ventral surfaces pale. Snout dark brown, distal lips pale. Foot dark brown, sole pale or pigmented with scattered brown granules. Pallial roof, visceral coil dark brown or black dorsally. Ctenidium well developed, positioned a little in front of pericardium; ctenidial filaments 13–19 ( $n = 15$ ), broadly triangular, lateral surfaces having prominent ridges. Osphradium narrow, positioned posterior to middle of ctenidium. Hypobranchial gland small, positioned between anterior portion of kidney and pallial genital duct. Prostate gland rather 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 pallial wall, section of duct on columellar muscle usually having a prominent bend, rarely straight. Penis medium-sized, base rectangular, inner edge smooth (Figs. 2H, 3A); filament short, strongly tapered, demarcated from base by slight constriction (Fig. 2H, I), nearly horizontal. Penis lacking a lobe and superficial glands. Penial



**Figure 3.** Reproductive anatomy of *Pyrgulopsis texana* (USNM 883954). **A.** Penis, dorsal surface (pigment in filament uniformly stippled). **B.** Female glandular oviduct and associated structures (viewed from left side). **C.** Bursa copulatrix. **D.** Seminal receptacle. Abbreviations: Ag, albumen gland; Bu, bursa copulatrix; Cd, common duct of seminal receptacle and coiled oviduct; Cg, capsule gland; Cov, coiled oviduct; Dbu, bursal duct; Ga, female genital aperture; Pd, penial duct; Pf, penial filament; Pw, posterior wall of pallial cavity; Sr, seminal receptacle; Vc, ventral channel of capsule gland. Scale bars **A** = 0.5 mm; **B** = 250  $\mu$ m. **C, D** to same scale as **B**.

duct very narrow, near outer edge, straight or having a few weak undulations. Filament densely pigmented internally (white-yellow). Female glandular oviduct and associated structures shown in Figure 3B–D. Coiled oviduct a simple, circular loop. Bursa copulatrix medium-sized, narrowly ovate, horizontal, largely overlapped by albumen gland. Bursal duct short, narrow or medium width, slightly broader distally, opening from distal edge, junction with common duct a little behind posterior wall of pallial cavity. Seminal receptacle very small, finger-shaped, positioned along ventral edge of bursa. Albumen gland having short pallial section. Capsule gland composed of two distinct tissue sections. Genital aperture a terminal pore.

*Distribution and habitat:* *Pyrgulopsis texana* is distributed in three springs in the vicinity of Balmorhea (Fig. 4). This species is concentrated near the sources of these springs and typically found on hard substrates where it is often extremely abundant (Dundee & Dundee, 1969; Taylor, 1987; R.H. & B.K.L., personal observations).

*Remarks:* The Phantom Cave snail is excluded from the Cochliopidae by the absence of the female sperm tube (confirmed in section) that is diagnostic of this family (Hershler & Thompson 1992). (Note that this group was recently elevated to separate family status by Wilke *et al.*, 2001.) We transfer this species to the hydrobiid genus *Pyrgulopsis* based on the combination of its wrinkled protoconch microsculpture, simple penis, superficial position of the bursa copulatrix and its duct on the albumen gland, single seminal receptacle opening to the distal arm of the coiled oviduct, and junction of the bursal duct and common duct of the oviduct and seminal receptacle behind the posterior wall of the pallial cavity (Liu & Hershler, 2005). Our molecular phylogenetic evidence also places this species unambiguously within the *Pyrgulopsis* clade (Fig. 5).

*Pyrgulopsis texana* is readily distinguished from geographically proximate *P. davisii* and *P. ignota* (described below) by its more depressed shell shape and simple penis. It is also differentiated from these congeners by its more convex shell whorls, larger umbilicus, subcentral operculum nucleus, strongly frilled operculum whorls and mtDNA sequences (COI, 11.6%, 9.7%; NDI, 13.4%, 11.7%, respectively). *Pyrgulopsis texana* is differentiated from other congeners included in this study by 9.1–12.8% (COI) and 11.6–17.4% sequence divergence (NDI). The simple penis of *P. texana* is shared with many congeners (Hershler & Sada, 2002). In the Bayesian analysis (Fig. 5) *P. texana* was

**Table 2.** Shell parameters for *Pyrgulopsis texana* and *P. ignota*.

	WH	SH	SW	HBW	WBW	AH	AW	SW/SH	HBW/SH	AH/SH
<i>P. texana</i> , paratypes, ANSP 420561 ( $n = 30$ )										
Mean	3.54	1.67	1.70	1.48	1.34	0.98	0.93	1.02	0.89	0.59
S.D.	0.22	0.15	0.10	0.12	0.09	0.06	0.05	0.06	0.02	0.04
Range	3.25–4.0	1.46–1.97	1.50–1.89	1.31–1.70	1.20–1.53	0.85–1.15	0.83–1.04	0.90–1.14	0.84–0.92	0.52–0.67
<i>P. texana</i> , USNM 1003865 ( $n = 30$ )										
Mean	3.46	1.16	1.19	0.98	0.96	0.64	0.61	1.03	0.84	0.56
S.D.	0.16	0.07	0.06	0.05	0.04	0.03	0.03	0.06	0.02	0.03
Range	3.25–3.75	1.02–1.36	1.09–1.32	0.89–1.08	0.88–1.04	0.60–0.73	0.57–0.66	0.88–1.11	0.76–0.88	0.49–0.60
<i>P. ignota</i> , holotype, USNM 1123757										
	3.75	1.41	1.23	1.23	1.02	0.82	0.71	0.87	0.88	0.58
<i>P. ignota</i> , paratypes, USNM 1133657 ( $n = 8$ )										
Mean	3.66	1.48	1.26	1.28	1.03	0.84	0.74	0.85	0.86	0.56
S.D.	0.13	0.08	0.07	0.08	0.06	0.05	0.04	0.02	0.02	0.01
Range	3.50–3.75	1.37–1.63	1.15–1.35	1.19–1.43	0.96–1.12	0.76–0.92	0.69–0.78	0.82–0.88	0.84–0.89	0.55–0.59

Abbreviations: WH, total shell whorls; SH, shell height; SW, shell width; HBW, height of body whorl; WBW, width of body whorl; AH, aperture height; AW, aperture width.

positioned as a terminal clade sister to one of these species, *P. bernardina* (Taylor, 1987), albeit without strong support.

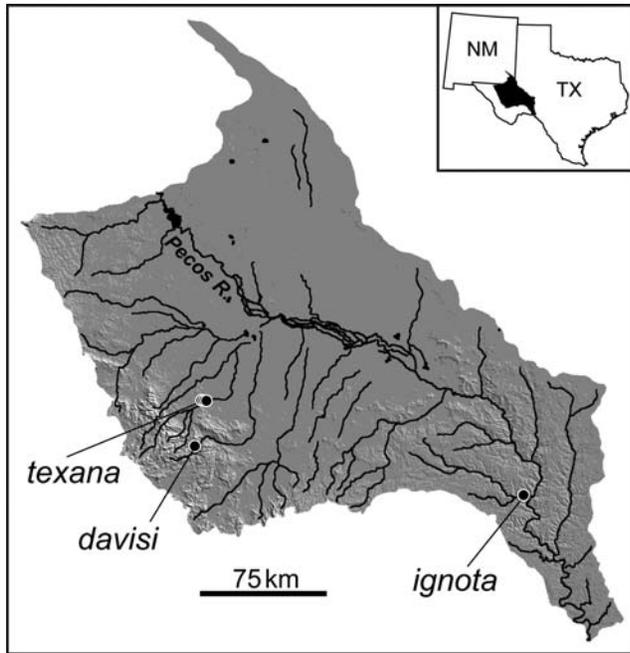
Shells from East Sandia Spring (Fig. 1C) are smaller and have a smaller (relative to shell height) aperture and taller than those

from the type locality (Fig. 1A) (*t*-test, separate variance,  $P < 0.001$ ,  $df = 40.5-57.4$ ; from data summarized above) and San Solomon Spring (Fig. 1B) (the latter could not be statistically analysed as few specimens in this population have a complete shell apex). The East Sandia Spring population also differed from the other two in having fewer gill filaments (13–15 compared to 15–18 in Phantom Lake Spring outflow and 17–19 in San Solomon Spring,  $n = 5$  in all cases;  $P < 0.005$ ,  $df = 2, 12$ ; ANOVA with Tukey pairwise *post hoc* testing of differences among means) and frequently narrow and pointed (Fig. 2E) rather than hoe-shaped (Fig. 2D) central cusps of the lateral radular teeth. We did not observe any other morphological differences among these populations. Mitochondrial DNA sequence variation within *P. texana* was minimal (Fig. 5, Table 3), both within (0–0.2%, COI; 0–0.6%, NDI) and among (0.1–0.2%, COI; 0.2–0.5%, NDI) populations. Seven of the eight sequenced specimens from the East Sandia Spring population had haplotypes that were shared with one (NDI, San Solomon Spring) or both (COI) of the other two populations. This finding does not support recognition of distinct conservation units of *P. texana*, which is not surprising given that the springs inhabited by this snail are separated from each other by only 6–13 km and may have been integrated (through Toyah Creek) prior to recent decreases and rerouting of surface flows (USFWS, 2007).

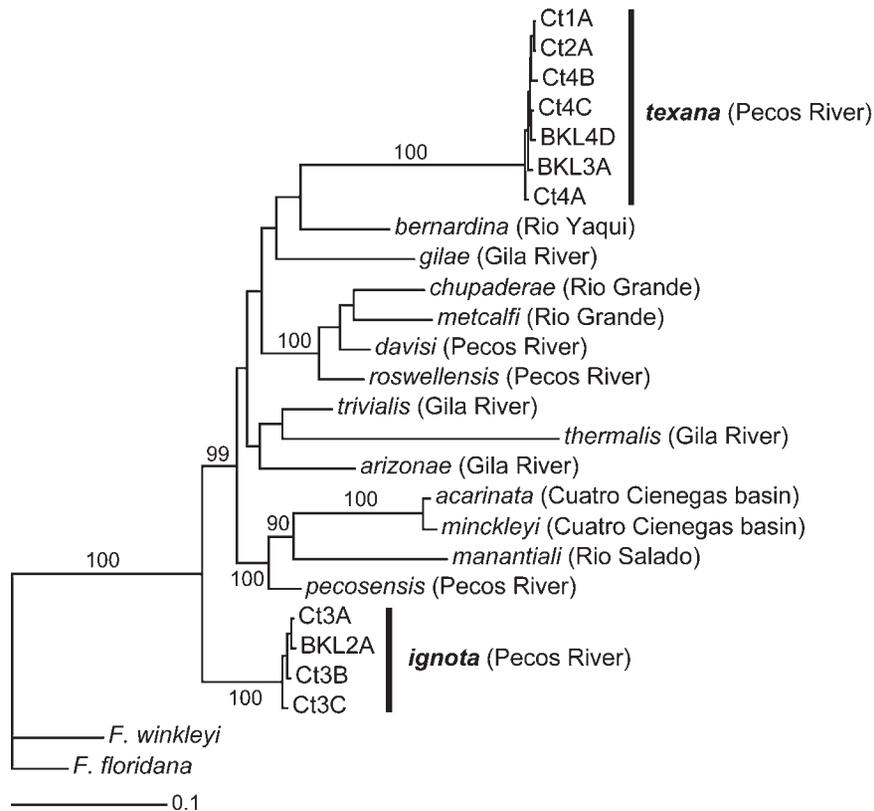
In an unpublished doctoral dissertation, Fullington (1978: 40) provided a synonymy for the Phantom Cave snail which mistakenly indicated this species had been transferred to *Cochliopina* by Taylor (1966).

***Pyrgulopsis ignota* new species  
(Figs 6–8)**

*Types:* Holotype, USNM 1123757, Caroline Springs, just below the first pond downflow from spring-fed lake, Terrell County,



**Figure 4.** Shaded relief map of the lower Pecos River basin (USGS hydrologic subregion 1307) showing the distributions of *Pyrgulopsis texana*, *P. ignota* and the third congener (*P. davisii*) found in this watershed.



**Figure 5.** Bayesian tree based on the combined COI and NDI dataset. Posterior probabilities for nodes are provided when  $\geq 90\%$ . The two species of *Pyrgulopsis* treated in this paper are highlighted with bold-face type. Terminals are labelled as in Table 1.

Texas, N 3373903, E 230992, Zone 14, coll. R.H. and J. Jerry Landye, 30/3/2009. Paratypes (from same lot), USNM 1133657.

**Etymology:** Based on the Greek *ignotus* in reference to the unusual penial morphology of this snail. We propose that ‘Caroline Springs pyrg’ be used as the vernacular name.

**Referred material:** *Terrell County, Texas:* USNM 1120337, USNM 1123945, USNM 116592, Caroline Springs, outflow from first pond below spring-fed lake, c. 200 m below pump house, N 231017, E 3373900, Zone 14, 17/9/2008, 11/3/2009, 9/6/2008.

**Diagnosis:** A small species of *Pyrgulopsis* having a trochoid shell with weakly to moderately convex whorls. Penis having a large lobe and short filament; penial ornament consisting of a large pad-like gland on the dorsal surface of the lobe.

**Description:** Shell trochoidal, often appearing decollate owing to erosion of apex (Fig. 6A–C); height about 1.3–1.5 mm; whorls about 3.5. Periostracum tan or orange. Protoconch near planispiral, about 1.3 whorls, diameter about 300 µm (Fig. 6D), initial portion weakly wrinkled and sometimes having a few short spirals (Fig. 6E). Teleoconch whorls weakly to moderately convex, last 0.25 whorl sometimes strongly shouldered; sculpture of colabral growth lines, later whorls having numerous weak spiral striae (crossed by growth lines) (Fig. 6C). Aperture ovate, strongly angled above. Inner lip usually adnate, rarely slightly disjunct, thickened internally; columellar shelf absent; outer lip usually thin, rarely slightly thickened, prosocline, weakly sinuate. Umbilicus chink-like or absent. Shell measurements and whorl counts for the holotype and paratypes are given in Table 2.

Operculum somewhat thickened, amber, multispiral with eccentric nucleus (Fig. 6F, G); last 0.25 whorl sometimes frilled on outer side (Fig. 6G), weak rim sometimes present along outer edge (Fig. 6H); attachment scar border variably thickened, sometimes prominently so almost all around.

*Radula taenioglossate* (Fig. 7A), with about 52 well-formed rows of teeth. Central teeth about 18 µm wide, cutting edge highly convex (Fig. 7B); lateral cusps 5–8; central cusp narrow, pointed, parallel-sided proximally, sometimes distally bifurcate; basal cusp 1, small; basal tongue V-shaped, about as long as lateral margins. Lateral tooth face rectangular, angled; central cusp pointed, parallel-sided proximally (Fig. 7C); lateral cusps 3–4 (inner), 5–8 (outer); outer wing narrow, flexed, about 240% length of cutting edge; basal tongue well developed. Inner marginal teeth having 27–32 cusps, fourth cusp from outer edge enlarged (Fig. 7D). Outer marginal teeth having 29–36 cusps; inner edge with short wing near mid-length (Fig. 7E). Radular count data were from USNM 1133657.

Cephalic tentacles light grey or dark brown except for pale distal tips and sometimes pale patches surrounding eyes. Snout similarly pigmented, distal lips pale. Foot dark brown along anterior and posterior edges, light grey centrally, sole pigmented with scattered grey granules. Pallial roof, visceral coil dark brown or black dorsally. Ctenidium positioned a little in front of pericardium; ctenidial filaments 13–15 ( $n = 5$ ), narrowly triangular, lateral surfaces smooth. Osphradium narrow, positioned well posterior to middle of ctenidium. Hypobranchial gland large, almost completely overlapping pallial genital duct and rectum. Prostate gland very 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 a slight bend. Penis large, base rectangular, folded along inner edge and basal portion of outer edge; filament short, narrow, tapering, slightly oblique; lobe large, rectangular (sometimes distally rounded), horizontal or slightly oblique (Fig. 8A). Dorsal side

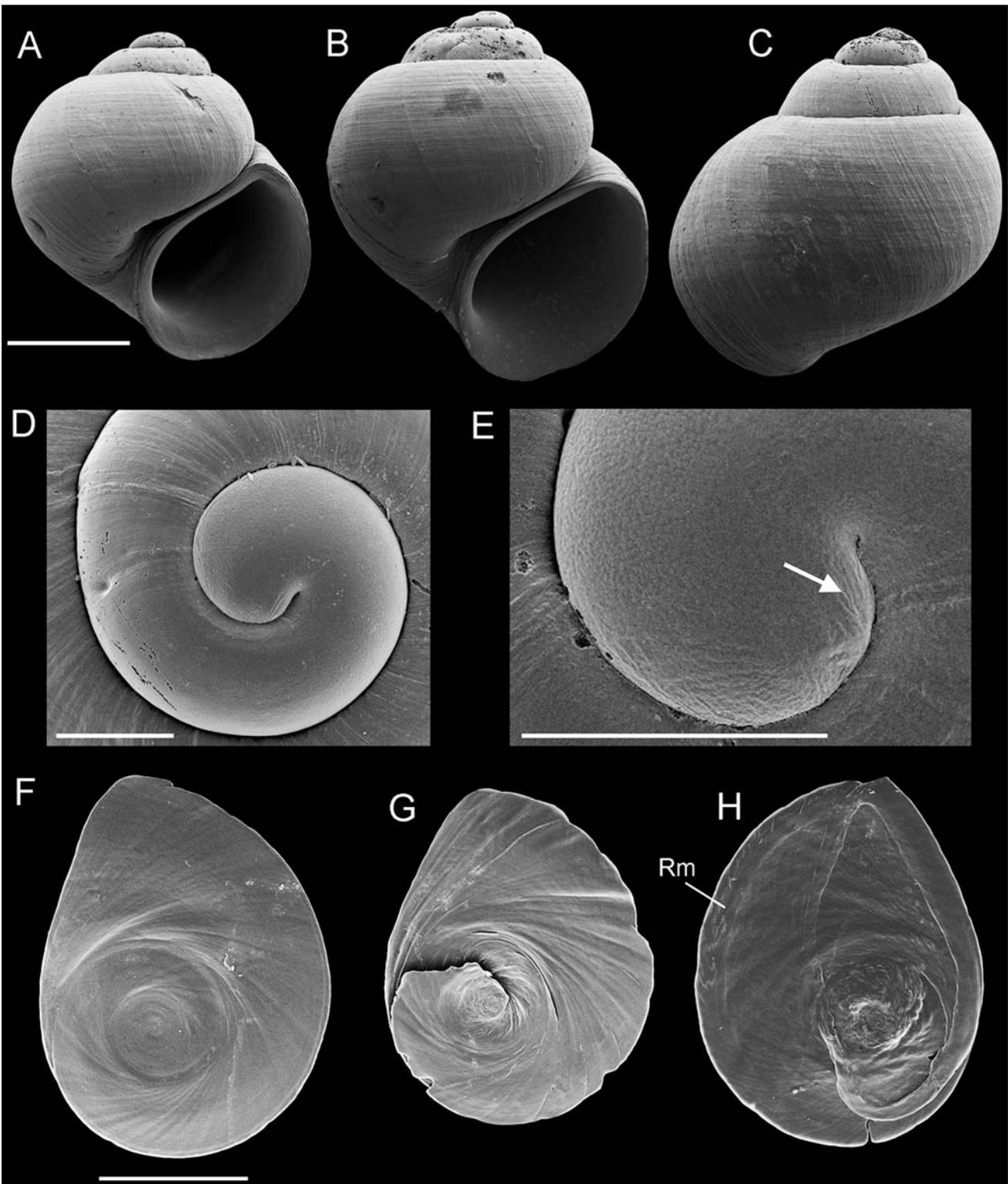
of lobe having large slightly raised, pad-like structure (Fig. 7F) containing numerous ovate, glandular units (Fig. 8A). Ventral surface of penis with distinct swelling centrally, but without gland (Fig. 8B). Penial duct narrow, near outer edge, almost straight. Penial filament pigmented with black granules proximally, mostly along inner edge. Female glandular oviduct and associated structures shown in Figure 8C–E. Coiled oviduct circular or posterior-oblique; proximal section sometimes pigmented with a few black granules. Bursa copulatrix small, elongate-ovate, horizontal, largely overlapped by albumen gland. Bursal duct as long or slightly longer than bursa, narrow, opening from distal edge, shallowly embedded in albumen gland distally, junction with common duct a little in front of posterior wall of pallial cavity. Seminal receptacle small, sac-like, positioned along antero-dorsal edge of bursa. Albumen gland almost entirely visceral. Capsule gland composed of three distinct tissue sections. Genital aperture a terminal slit.

**Distribution and habitat:** *Pyrgulopsis ignota* is endemic to Caroline Springs (also known as T5 Springs), which is located about 24 km south-southeast of Sheffield (Fig. 4). The outflow of this large spring (which discharges through several vents) courses through a large lake and several ponds (all man-made) before entering Independence Creek. *Pyrgulopsis ignota* was found abundantly on cobbles in the outflow of the first pond below the lake (Fig. 9). Caroline Springs is currently being managed by the Nature Conservancy, which acquired the property on which it is situated (Oasis Ranch) in 2000 (Karges, 2003).

**Table 3.** COI and NDI variation within *Pyrgulopsis texana*.

Specimen	Base pair position									
	COI		NDI							
	302	343	009	032	035	038	102	233	371	479
<b>Phantom Lake Spring</b>										
Ct4A	G	A	C	T	C	A	G	G	T	A
Ct4B	A	A	T	T	C	G	A	A	T	A
Ct4C	G	A	C	T	C	G	A	G	T	A
Ct4D	G	A	C	T	C	G	A	G	T	A
BKL4A	G	A	C	T	C	G	A	G	T	A
BKL4B	G	A	C	T	C	G	A	G	T	A
BKL4C	G	A	C	T	C	A	G	G	T	A
BKL4D	G	A	C	T	T	G	A	G	T	G
<b>San Solomon Spring</b>										
Ct1A	G	A	C	T	C	G	A	G	C	A
Ct1B	G	A	C	T	C	G	A	G	C	A
Ct1C	G	A	C	T	C	G	A	G	C	A
Ct1D	G	A	C	T	C	G	A	G	C	A
BKL1A	G	A	C	T	C	G	A	G	C	A
BKL1B	G	A	C	T	C	G	A	G	C	A
BKL1C	G	A	C	T	C	G	A	G	C	A
BKL1D	G	A	C	T	C	G	A	G	C	A
<b>East Sandia Spring</b>										
Ct2A	G	A	C	T	C	G	A	G	C	A
Ct2B	G	A	C	T	C	G	A	G	C	A
Ct2C	G	A	C	T	C	G	A	G	C	A
Ct2D	G	A	C	T	C	G	A	G	C	A
BKL3A	G	G	C	C	C	G	A	G	T	A
BKL3B	G	A	C	T	C	G	A	G	C	A
BKL3C	G	A	C	T	C	G	A	G	C	A
BKL3D	G	A	C	T	C	G	A	G	C	A

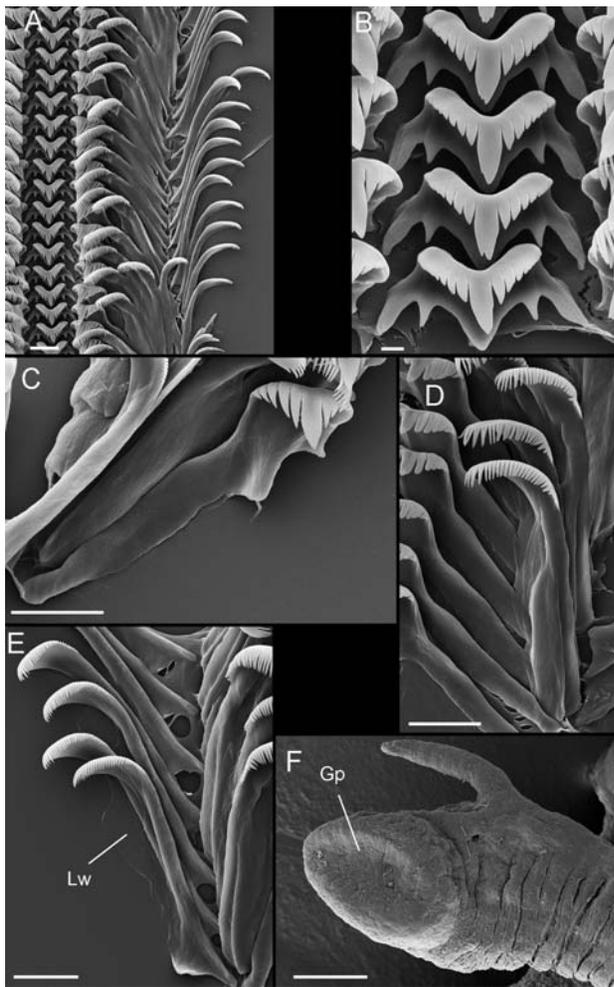
Base pairs differing from those of the common haplotypes are shaded.



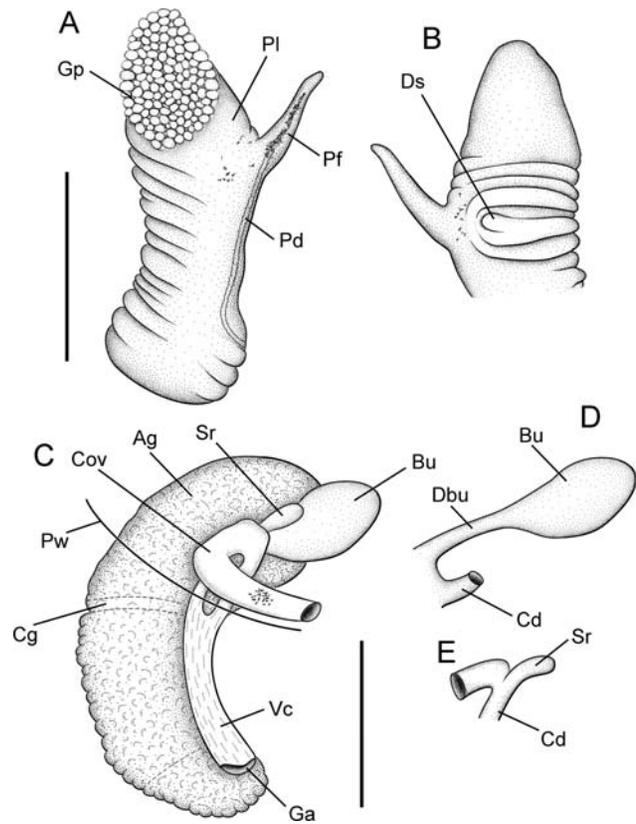
**Figure 6.** Scanning electron micrographs of shells and opercula of *Pyrgulopsis ignota*. **A.** Holotype (USNM 1123757). **B, C.** Shells (USNM 1133657). **D.** Shell apex, showing protoconch sculpture (USNM 1133657). **E.** Close up of protoconch sculpture (arrow indicates spiral elements) (USNM 1133657). **F.** Operculum, outer side (USNM 1133657). **G, H.** Opercula, inner side (USNM 1133657). Abbreviation: Rm, rim. Scale bars **A** = 0.5 mm; **D, E** = 100  $\mu$ m; **F** = 250  $\mu$ m. **B, C** to same scale as **A**; **G, H** to same scale as **F**.

*Remarks:* This species was initially confused (in the field) with *P. texana* when first discovered owing to their generally similar shells. Upon closer inspection *P. ignota* was readily differentiated from its congener by the features listed in the Remarks

above as well as by its strongly angled (adapically) shell aperture. *Pyrgulopsis ignota* also differs from *P. texana* in its smaller shell umbilicus, relatively longer outer wing of the lateral radular teeth, mid-length position of the lateral wing of the



**Figure 7.** Scanning electron micrographs of radula and critical-point dried penis of *Pyrgulopsis ignota* (USNM 1133657). **A.** Portion of radular ribbon. **B.** Central radular teeth. **C.** Lateral radular tooth. **D.** Inner marginal teeth. **E.** Inner and outer marginal teeth. **F.** Distal penis, dorsal surface. Abbreviations: Gp, pad-like gland; Lw, lateral wing. Scale bars **A** = 10  $\mu\text{m}$ ; **B** = 2  $\mu\text{m}$ ; **C** = 12  $\mu\text{m}$ ; **D, E** = 10  $\mu\text{m}$ ; **F** = 100  $\mu\text{m}$ .



**Figure 8.** Reproductive anatomy of *Pyrgulopsis ignota* (USNM 1133657). **A.** Penis, dorsal surface. **B.** Penis, ventral surface. **C.** Female glandular oviduct and associated structures (viewed from left side). **D.** Bursa copulatrix. **E.** Seminal receptacle. Abbreviations: Ag, albumen gland; Bu, bursa copulatrix; Cd, common duct of seminal receptacle and coiled oviduct; Cg, capsule gland; Cov, coiled oviduct; Dbu, bursal duct; Ds, distal swelling; Ga, female genital aperture; Gp, pad-like gland; Pd, penial duct; Pf, penial filament; Pl, penial lobe; Pw, posterior wall of pallial cavity; Sr, seminal receptacle; Vc, ventral channel of capsule gland. Scale bars **A, B** = 0.5 mm; **C** = 250  $\mu\text{m}$ . **D, E** to same scale as **C**.



**Figure 9.** Photographs of the type locality area of *Pyrgulopsis ignota*. **A.** First pond below the lake fed by Caroline Springs, with the outlet (where snails were found) to the right. **B.** Stream outlet about 5 m downflow from above pond (scale provided by J. Jerry Landye). Photograph taken by R.H. on 29/3/2009. (This figure appears in colour in the online version of *Journal of Molluscan Studies*.)

outer marginal teeth, insertion of the bursal duct into the common duct in front of the pallial wall, more posteriorly positioned osphradium, larger hypobranchial gland, pea-shaped prostate gland, weaker bend of the anterior vas deferens on the columellar muscle, larger penis, smaller bursa copulatrix and capsule gland composed of three glandular sections. The occurrence of three glandular sections of the capsule gland and a large glandular pad on the dorsal surface of the penis are unique to *P. ignota* within the genus. The junction of the bursal duct and common duct of the oviduct and seminal receptacle in front of the pallial wall (Fig. 8C) is also unique within *Pyrgulopsis*, but is more posteriorly positioned than in the eastern North American genus *Marstonia* (Hershler, 1994: fig. 5c). Placement in *Pyrgulopsis* nonetheless is supported by our molecular phylogenetic evidence (Fig. 5) and the relatively small genetic divergence between *P. ignota* and other members of the genus (see above). Note that *Pyrgulopsis*, as currently envisaged, contains other species which have unique anatomical features yet are unambiguously nested within the genus based on molecular evidence, e.g. *P. peculiaris* Hershler, 1998, which has a uniquely bifid bursal duct (Hershler, 1998: fig. 43E) (see Liu & Hershler, 2005: fig. 2, appendix B, for molecular evidence supporting placement in *Pyrgulopsis*).

The sequence divergence between *P. ignota* and other congeners included in this study ranged from 6.3% to 10.3% for COI and 8.3% to 13.5% for NDI. Variation among the eight specimens analysed for this species was minimal ( $0.4 \pm 0.2\%$  for both genes). In the Bayesian tree *P. ignota* occupied a well-supported basal position.

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