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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

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ABSTRACT

The large, predominantly western North American hydrobiid gastropod genus *Pyrgulopsis* includes a single species from the upper Missouri River basin (MRB), *P. bedfordensis*, which is thought to have originated through a late Neogene transfer of drainage from the eastern Snake River Plain across the northern continental divide. Here we describe a second, morphologically distinctive congener living in the MRB, *P. blainica* new species; investigate the phylogenetic relationships of these two snails relative to other regional congeners using cytochrome *c* oxidase subunit I (COI) sequences; and evaluate whether our findings are consistent with vicariance resulting from prior stream capture across the northern continental divide. A Bayesian analysis of 39 COI sequences delineated the two MRB species as a well supported, terminal clade that is most closely related to a congener from the southern Bonneville Basin (*P. anguina*). Application of an available molecular clock for *Pyrgulopsis* suggests that the MRB clade diverged from its sister taxon 3.64 − 2.53 Ma (late Pliocene). Although these results are not consistent with the hypothesized origin of this fauna through a late Miocene drainage transfer from the upper Snake River basin, they conform to a more recent stream capture event involving truncation of southward drainage from the MRB that was previously postulated on the basis of molluscan distributions. This study provides the first molecular phylogenetic evidence that bears upon the freshwater molluscan biogeography across the northern continental divide.

INTRODUCTION

The North American hydrobiid gastropod genus *Pyrgulopsis* is composed of 126 currently recognized species which live in springs, streams, rivers and other perennial waters (Liu & Hershler, 2005). *Pyrgulopsis* is divided into non-overlapping western and eastern subunits by the continental (Pacific-Gulf Coast/Atlantic) divide (Fig. 1). The western fauna consists of 113 congeners that are distributed within a huge area extending from the Snake-Columbia River basin south to the lower Colorado River basin and west to the Pacific margin (Fig. 1). The much smaller eastern fauna is composed of 12 congeners that range within internal drainages of northern Mexico and the Pecos-Rio Grande River basin; and *P. bedfordensis* Hershler & Gustafson, 2001, which lives more than 1,200 km to the north–northwest of these species in the upper Missouri River basin (MRB) (Fig. 1).

The molecular phylogenetic structure of this large, taxonomically difficult genus is only beginning to be teased apart and the biogeographic history of the group in relation to the major barrier defined by the continental divide (Bănărescu, 1991) has not been resolved. A recent phylogenetic study that included 59 western and five eastern species currently assigned to *Pyrgulopsis* divided the latter into two evolutionarily distinct and well differentiated (8.9 ± 0.9% COI sequence divergence, Liu & Hershler, unpublished) lineages – *P. davisi* (Davis, 1987) and a clade containing *P. acarinata* (Hershler, 1985), *P. manantiali* (Hershler, 1985), *P. minckleyi* (Taylor, 1966) and *P. pecosensis* (Taylor, 1987) (Liu & Hershler, 2005: fig. 2). Although the sister relationships of these two lineages were not resolved in this analysis, the extent of their differentiation relative to western congeners (7.5 ± 0.7, 8.4 ± 0.7% for COI, respectively; Liu & Hershler, unpublished) nonetheless suggests relatively ancient (i.e. pre-Pleistocene) divergence events across the southern continental divide based on the available COI clock for *Pyrgulopsis* (Liu & Hershler, 2007).

The isolated northern member of the group on the eastern side of the continental divide, *P. bedfordensis*, was not included in the analysis discussed above and its phylogenetic relationships within the genus are unknown. Hershler & Gustafson (2001: 302) indicated that *P. bedfordensis* does not closely resemble any congener, but noted that it shares a large, elongate-rectangular penial lobe with several western species that also live in thermal habitats. They suggested a close relationship between *P. bedfordensis* and upper Snake River basin congeners based on geographic proximity (see Hershler & Gustafson, 2001: fig. 7) and speculated that this single spring endemic is a vicariant product of a late Neogene drainage transfer across the northern continental divide.

A new, morphologically distinct species of *Pyrgulopsis* was recently discovered in the Missouri River headwater region (Madison River drainage), about 140 km south-southwest of the locality of *P. bedfordensis*. In this paper we describe this novelty and assess the divergence and phylogenetic relationships of the two upper MRB congeners based on analysis of sequence variation of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. We also discuss the biogeographic history of *Pyrgulopsis* across the northern continental divide based on our findings and other evidence that was not

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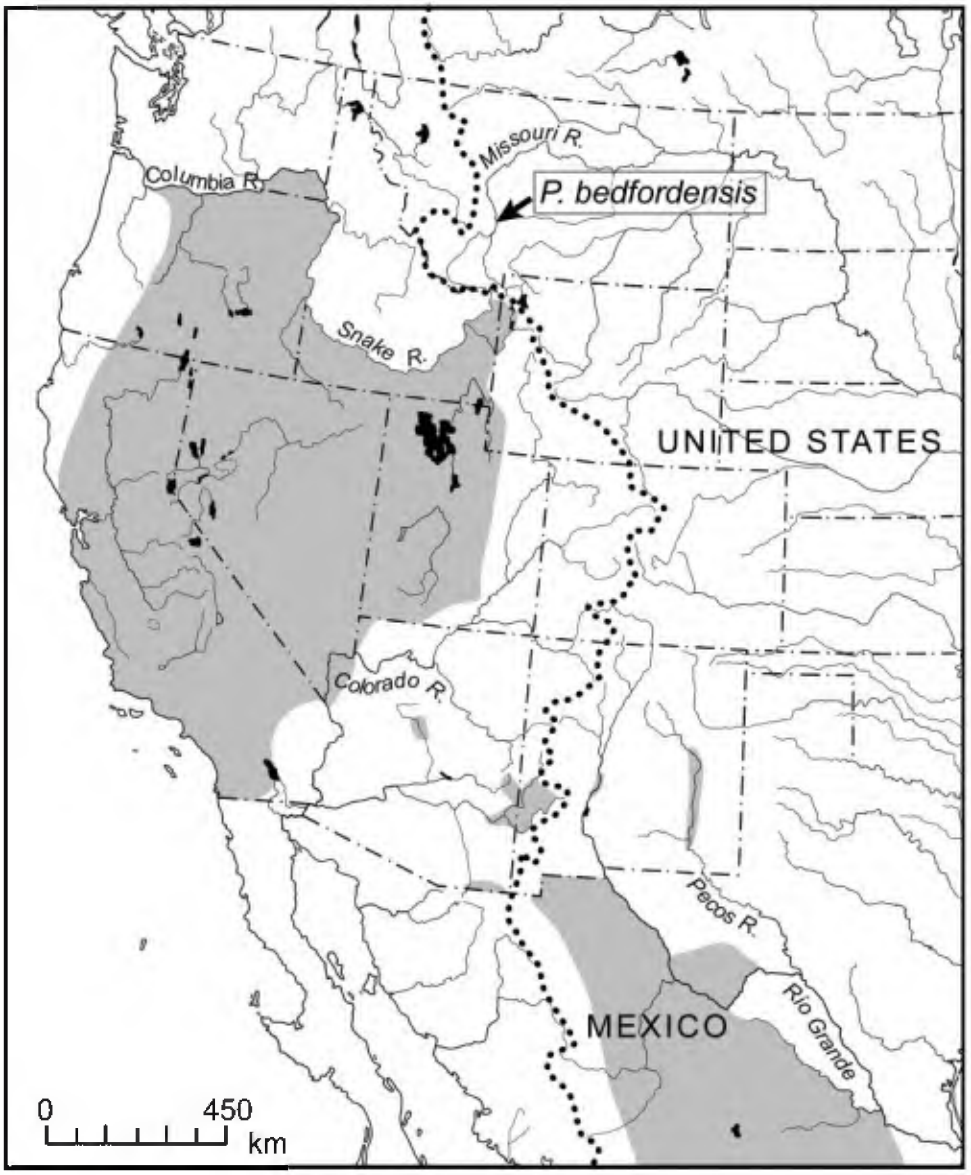

Figure 1. Map (modified from Hershler & Sada, 2002: fig. 1) showing distribution of *Pyrgulopsis* (shaded areas) in relation to the North American continental divide (dotted line). Note the disjunct occurrence of *P. bedfordensis*.

utilized in the previous treatment of this subject (Hershler & Gustafson, 2001).

MATERIAL AND METHODS

Anatomical study of the new species 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. Voucher material for samples utilized in this study was deposited in the National Museum of Natural History (USNM) collection.

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 follow those used in recent taxonomic investigations of *Pyrgulopsis* (Hershler, 1998; Hershler *et al.*, 2003b). Shell data were analysed using Systat for Windows 11.00.01 (SSI, 2004).

Our molecular phylogenetic analysis included the two MRB species, 22 species from proximal western drainages (Bonneville, Lahontan, Snake River basins and several isolated drainages in eastern Nevada) and eight taxonomically undescribed populations from the Snake River basin. Collection localities are shown in Figure 2. Locality details and GenBank accession numbers are given in Table 1. A species of *Floridobia*, *F. winkleyi* (Pilsbry, 1912), was used as the root based on the close relationship between this eastern North American genus and *Pyrgulopsis* (Liu & Hershler, 2005). Prior to our final analyses we performed a comprehensive (unpublished) survey of mtDNA variation within *Pyrgulopsis* to confirm that the set of species utilized included the closest relatives of the MRB congeners. Multiple specimens of the new species and its MRB congener (*P. bedfordensis*) were sequenced to assess variation; single exemplars were used for each of the other taxa except *P. pilsbryana*, for which we included both Bear River and Bear Lake Valley haplotypes. A total of 39 COI sequences were utilized; 23 of these were newly obtained for this study while the other 16 were from our previously published investigations (Hershler *et al.*, 2003a, b; Hershler & Liu, 2004a, b; Liu & Hershler, 2005).

Genomic DNA was extracted from entire snails using a CTAB protocol (Bucklin, 1992). A partial (658 bp) segment of

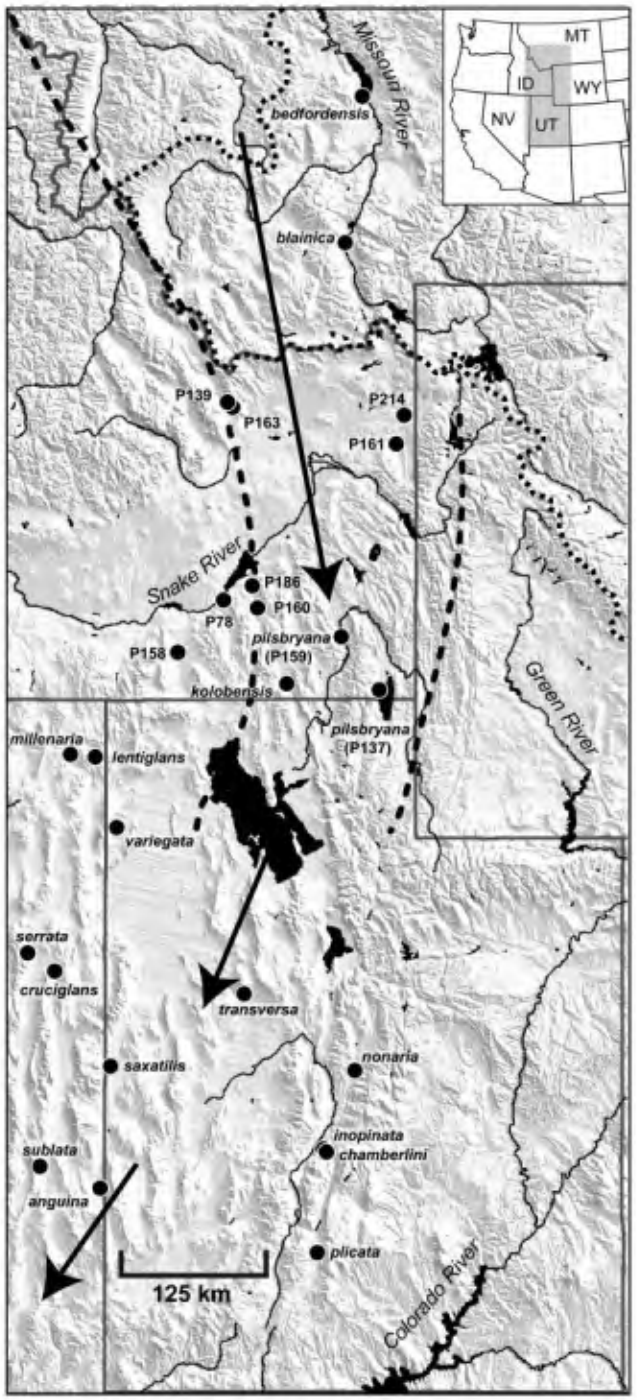

Figure 2. Map showing locations of samples used in the molecular phylogenetic analysis. Sample labels are from Table 1. Samples from the north-central and northeastern Great Basin (*P. aurata*, *P. bryantwalkeri*, *P. gibba*, *P. leporina*, *P. pictilis*, *P. vinyardi*) and middle Snake River basin (*P. bruneauensis*) are located to the west of the area shown in this map. The arrows and heavy dashed lines indicate the direction and boundaries of the Neogene drainage postulated by Taylor (1985: fig. 25). The dotted line delineates the modern continental divide.

mitochondrial COI (mtCOI) (Folmer *et al.*, 1994) was amplified and sequenced with primers COIL1490 and COIH2198 following the protocols of Liu, Hershler & Clift (2003). This region was used because of its proven utility for population and species level studies of *Pyrgulopsis* (Hershler *et al.*, 2003b; Liu *et al.*, 2003; Hurt, 2004; Liu & Hershler, 2005). Sequences were determined for both strands and then edited and aligned using Sequencherä version 4.8.

Phylogenetic relationships were inferred using Bayesian inference in MrBayes 3.12 (Ronquist & Huelsenbeck, 2003). MrModeltest (Nylander, 2004) was used to determine which evolutionary model best fits 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 + I + 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 −4,850 and quickly converged upon a stable value of about −2,950 after 10,000 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.

Sequence divergences (uncorrected p distance) within and between phylogenetic lineages were calculated using MEGA4 (Tamura *et al.*, 2007); standard errors were estimated by 1,000 bootstrap replications with pairwise deletion of missing data. A molecular clock hypothesis for the pertinent portion of the Bayesian topology was tested using Tajima's (1993) non-parametric relative rate test in MEGA4.

SYSTEMATIC DESCRIPTION

Family HYDROBIIDAE TROSCHEL, 1857

Subfamily NYMPHOPHILINAE TAYLOR, 1966

Pyrgulopsis Call & Pilsbry, 1886

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

Diagnosis: Liu & Hershler, 2005: 296.

Pyrgulopsis blainica new species
(Figs 3–8)

Types: Holotype (Fig. 3), USNM 1082064, Blaine Spring Creek at bridge crossing just below the Ennis National Fish Hatchery, Madison County, Montana (N 5007027, E 437937, Zone 12, elevation 1698 m), 18/viii/2005, DLG. Paratypes (from same lot), USNM 1112484.

Etymology: A geographic adjectival epithet referring to the type locality, Blaine Spring, which was named after an individual who operated a milk ranch at the site prior to its conversion to a federal fish hatchery (Cheney, 1984). We propose the vernacular name, 'Blaine pyrg'.

Referred material: USNM 1082069, topotypes, 8/28/2005, DLG; USNM 1094136, topotypes, 7/iv/2006, DLG; USNM 1094135, Blaine Spring Creek, bypass channel, Madison County, Montana (N 5007663, E 437715, elevation 1706 m), 7/iv/2006, DLG.

Diagnosis: A large species of *Pyrgulopsis* having an ovate- to elongate-conic shell with convex whorls and prominent spire.

Table 1. Locality details and GenBank accession numbers for COI sequences used in the phylogenetic analysis.

Species (code)	Drainage basin	Locality	GenBank accession number
P. anguina Hershler, 1998	Great Basin/Bonneville	Big Spring, Snake Valley, White Pine Co., NV	EU700466
P. aurata Hershler, 1998	Great Basin	Coyote Spring, Pleasant Valley, Pershing Co., NV	EU700473
P. bedfordensis Hershler & Gustafson, 2001	Missouri River	Bedford Warm Spring, Broadwater Co., MT	EU700483-486
P. blainica	Missouri River	Blaine Spring Creek, Madison River drainage, Madison Co., MT	EU700478-480
P. bruneauensis Hershler, 1990	Snake River	Bruneau Hot Springs, Bruneau River drainage, Owyhee Co., ID	AF520941†
P. bryantwalkeri Hershler, 1994	Great Basin/Lahontan	Warm Spring, Humboldt River drainage, Elko Co., NV	AY627942¶
P. chamberlini Hershler, 1998	Great Basin/Bonneville	Spring at Glenwood, Sevier River drainage, Sevier Co., UT	EU700468
P. cruciglans Hershler, 1998	Great Basin	Flat Spring, Steptoe Valley, White Pine Co., NV	AY627931¶
P. gibba Hershler, 1995	Great Basin/Lahontan	Springs west of Fee Reservoir, Surprise Valley, Lassen Co., CA	AY197603*
P. inopinata Hershler, 1998	Great Basin/Bonneville	Spring at Glenwood, Sevier River drainage, Sevier Co., UT	AY426360§
P. kolobensis (Taylor, 1987)	Great Basin/Bonneville	Big Malad Spring, Malad Valley, Oneida Co., ID	AY379448‡
P. lentiglans Hershler, 1998	Great Basin/Bonneville	Crittenden Springs, Thousand Springs Creek drainage, Elko Co., NV	AY627936¶
P. leporina Hershler, 1998	Great Basin/Lahontan	Springs along Rabbit Creek, Humboldt River drainage, Elko Co., NV	EU700471
P. millenaria Hershler, 1998	Great Basin/Bonneville	Spring below 21-Mile Dam, Thousand Springs Creek drainage, Elko Co., NV	EU700469
P. nonaria Hershler, 1998	Great Basin/Bonneville	Spring east of Ninemile Reservoir, Sevier River drainage, San Pete Co., UT	EU700467
P. pictilis Hershler, 1998	Great Basin/Lahontan	Cain Spring, Antelope Valley, Lander Co., NV	AY627944¶
P. pilsbryana (Baily & Baily, 1952) (P137)	Great Basin/Bonneville	Spring along St. Charles Creek, Bear Lake Valley, Bear Lake Co., ID	AY426363§
P. pilsbryana (P159)	Great Basin/Bonneville	Bear River, Black Canyon, Caribou Co., ID	EU700475
P. plicata Hershler, 1998	Great Basin/Bonneville	Spring, Black Canyon, Sevier River drainage, Garfield Co., UT	AY627935¶
P. saxatilis Hershler, 1998	Great Basin/Bonneville	Warm Springs, Snake Valley, Millard Co., UT	AY627934¶
P. serrata Hershler, 1998	Great Basin	Indian Ranch Spring, Steptoe Valley, White Pine Co., NV	EU700464
P. sublata Hershler, 1998	Great Basin	Wambolt Springs, Lake Valley, Lincoln. Co., NV	AY627938¶
P. transversa Hershler, 1998	Great Basin/Bonneville	Sixmile Springs, Old River Bed, Tooele Co., UT	EU700470
P. variegata Hershler, 1998	Great Basin/Bonneville	Spring, south of South Patterson Spring, Pilot Valley, Box Elder Co., UT	AY627937¶
P. vinyardi Hershler, 1998	Great Basin/Lahontan	Unnamed spring, Squaw Valley, Elko Co., NV	EU700482
P. sp. (P78)	Snake River	Indian Springs, Cold Creek drainage, Power Co., ID	EU700465
P. sp. (P139)	Snake River	Birch Creek, above Mud Creek, Birch Creek Valley, Lemni Co., ID	EU700472
P. sp. (P158)	Snake River	McClenden Spring, Raft River drainage, Cassia Co., ID	EU700474
P. sp. (P160)	Snake River	Upper Rock Spring, Bannock Creek drainage, Power Co., ID	EU700476
P. sp. (P161)	Snake River	Teton River at Buxton Bridge, Teton Co., ID	AY379446‡
P. sp. (P163)	Snake River	Kaufman Cabin Springs, Birch Creek Valley, Lemhi Co., ID	EU700477

Continued

Table 1. *Continued*

Species (code)	Drainage basin	Locality	GenBank accession number
P. sp. (P186)	Snake River	East Fork Rock Creek, Rockland Valley, Power Co., ID	AY426344[§]
P. sp. (P214)	Snake River	Spring, at Porcupine Ranger Station, Henry's Fork drainage, Fremont Co., ID	EU700481
F. winkleyi (Pilsbry, 1912)	Atlantic Coastal	Salt marsh, Scarborough, Saco River drainage, Cumberland Co., ME	AF520917[†]

*Hershler *et al.* (2003a); †Hershler *et al.* (2003b); ‡Hershler & Liu (2004a); §Hershler & Liu (2004b); ¶Liu & Hershler (2005).

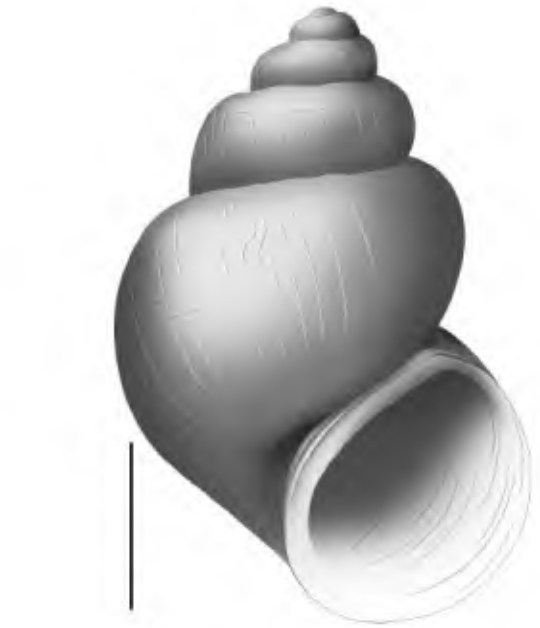

Figure 3. *Pyrgulopsis blainica*, holotype (USNM 1082064). Shell height, 3.56 mm; shell width, 2.28 mm, total number of whorls, 4.8. Scale bar = 1.0 mm.

Penis having a small lobe and large filament; penial ornament consisting of a small terminal gland, a gland along the outer edge of the penial lobe (Dg3; Hershler, 1994) and ventral gland. *Pyrgulopsis blainica* is readily distinguished from geographically proximate and closely related (see below) *P. bedfordensis* by its larger shell, more convex teleoconch whorls, less angular adapical end of aperture and narrower columellar shelf. It is further differentiated from this congener by its smaller penial lobe, larger penial filament, ventral penial gland (absent in *P. bedfordensis*), smaller terminal gland, broader central cusps on the central radular teeth and mitochondrial DNA sequences (see below).

Description: Shell usually ovate-conic (Fig. 4A–G), rarely narrow-conic; height about 3.3–4.6 mm; whorls 4.0–6.0. Periostracum dark brown, rather thick. Protoconch near planispiral, flattened above, about 1.4 whorls, diameter about 350 μm, surface weakly wrinkled near apex. Teleoconch whorls strongly convex, often distinctly shouldered (e.g. Fig. 4A), last few whorls often slightly loosened, sometimes scalariform in appearance (Fig. 4H, I); spire outline convex, sometimes markedly so (Fig. 4I); sculpture of collabral growth lines, later whorls also having numerous weak spiral lines. Aperture ovate, rounded or weakly angled adapically. Inner lip narrowly adnate or slightly disjunct, usually thickened internally, rarely thin; columellar shelf narrow or absent; outer lip thin or slightly thickened, orthocline or slightly prosocline. Umbilicus usually absent or rimate, rarely perforate. Operculum fairly thick, reddish, ovate, multispiral with eccentric nucleus (Fig. 5A); inner side having weak rim along outer edge, attachment scar border thickened almost all around (Fig. 5B, C). Radula taenioglossate (Fig. 5D), with about 60 well-formed rows of teeth. Central teeth (Fig. 5E) about 44 μm wide, cutting edge concave; lateral cusps 3–6; central cusp narrow, pointed, sometimes parallel-sided proximally; basal cusp 1, small; basal tongue rounded, a little shorter than lateral margins. Lateral tooth (Fig. 5F) face rectangular, angled; central cusp large, hoe-like; lateral cusps 2–3 (inner), 2–4 (outer); outer wing fairly broad, weakly flexed, about 125% length of cutting edge; basal tongue well developed. Inner marginal teeth (Fig. 5G) having 23–34 cusps, sixth cusp from outer edge enlarged. Outer marginal teeth (Fig. 5H) having 28–42 small cusps; inner edge with long, rectangular wing. Cephalic tentacles dark brown dorsally except for pale patches surrounding eyes, ventral surfaces lightly pigmented. Snout dark brown, distal lips nearly pale. Foot dark brown. Pallial roof, visceral coil dark brown dorsally. Ctenidium well developed, positioned a little in front of pericardium; ctenidial filaments about 22, broadly triangular, lateral surfaces having several prominent ridges. Osphradium narrow, positioned posterior to middle of ctenidium. Prostate gland small, bean-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 prominent bend. Penis (Fig. 6A, B) small or medium-sized, base rectangular, weakly folded along inner edge; filament elongate, tapering, horizontal; lobe medium length, narrow or tapering, horizontal or slightly oblique. Terminal gland small, circular, overlapping dorsal and ventral edges of lobe. Dg3 rather large, ovate, slightly raised, positioned proximally, overlapped by filament. Ventral gland similar in appearance to Dg3, positioned medially near outer edge. Penial duct narrow, nearly straight. Penial filament containing a dense core of black pigment, penis otherwise pigmented with scattered dark granules on dorsal and ventral surfaces. Female glandular oviduct and associated structures shown in Figure 6C, D. Coiled oviduct a small, vertical loop; posterior section sometimes pigmented with a few granules. Bursa copulatrix small, ovate, horizontal, largely overlapped by and partly embedded within albumen gland. Bursal duct about as long as bursa, rather broad, opening from distal edge. Seminal receptacle small, globular, sometimes lightly pigmented, positioned near anterior edge of bursa. Albumen gland almost entirely visceral. Capsule gland composed of two distinct tissue sections. Genital aperture a terminal slit, often slightly muscularized.

Habitat: Blaine Spring, located about 4.4 km west–southwest of the historic site of Varney, is composed of two cold water (12.0°C) spring sources that collectively discharge about 945 l/s (USFWS, 2008). The springs have been highly modified for use by the Ennis National Fish Hatchery (constructed in 1931) and are now enclosed in buildings to prevent the introduction of

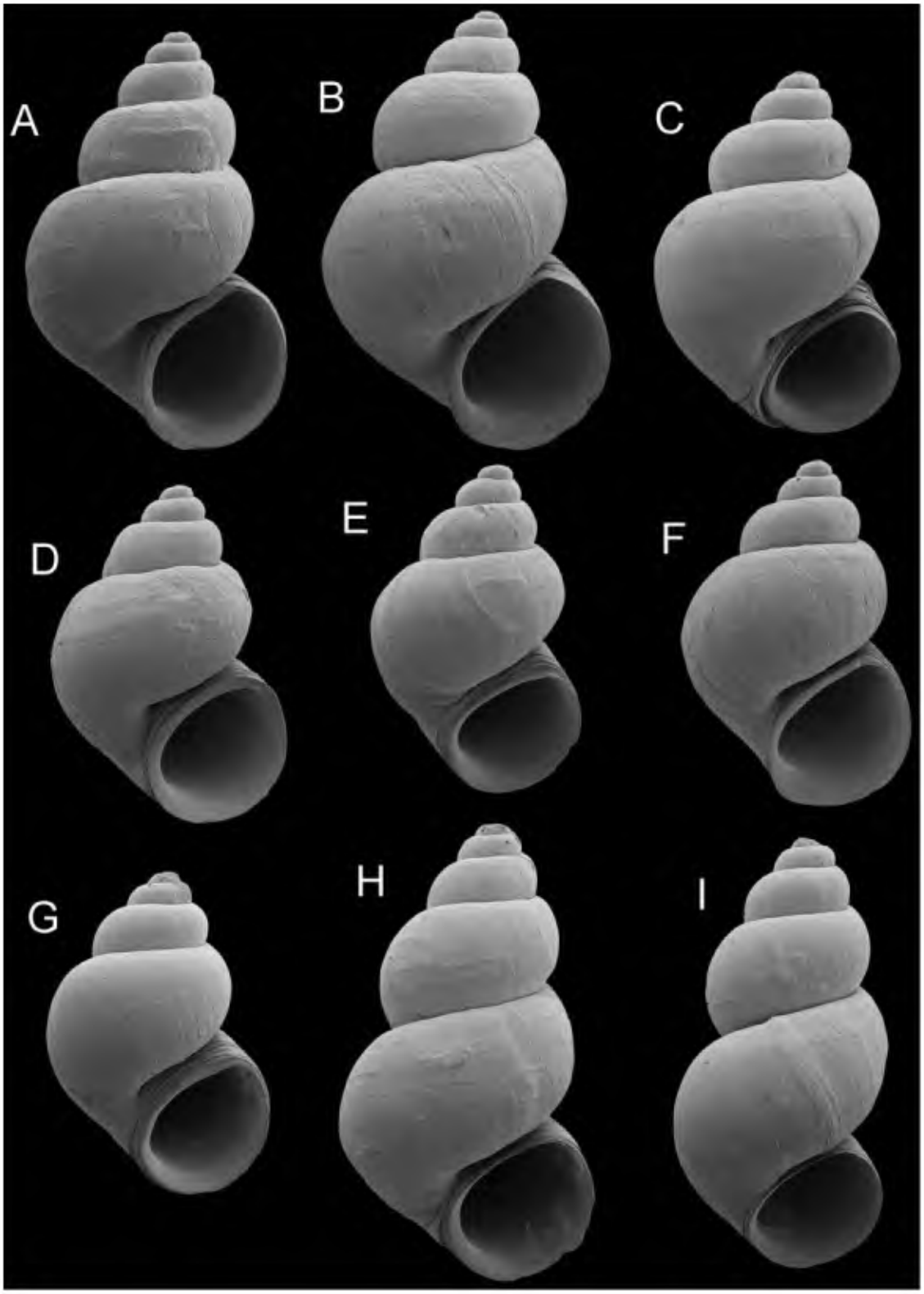

Figure 4. Scanning electron micrographs of shells of *P. blainica* (USNM 1112484). Scale bar = 1.0 mm.

whirling disease (USFWS, 2001), with most of their discharge piped underground to the hatchery (Fig. 7). However, a small fraction of the discharge runs for several hundred meters in a seemingly natural 'bypass channel' before entering a pipe. *Pyrgulopsis blainica* was extremely abundant in this channel and was also collected in the outflow from the hatchery at the 'high bridge' crossing, but was not found below this point in Blaine Spring Creek. Blaine Spring harbours a large, entirely native invertebrate fauna that includes two other molluscs, *Physella gyrina* (Say, 1821) and *Stagnicola elodes* (Say, 1821).

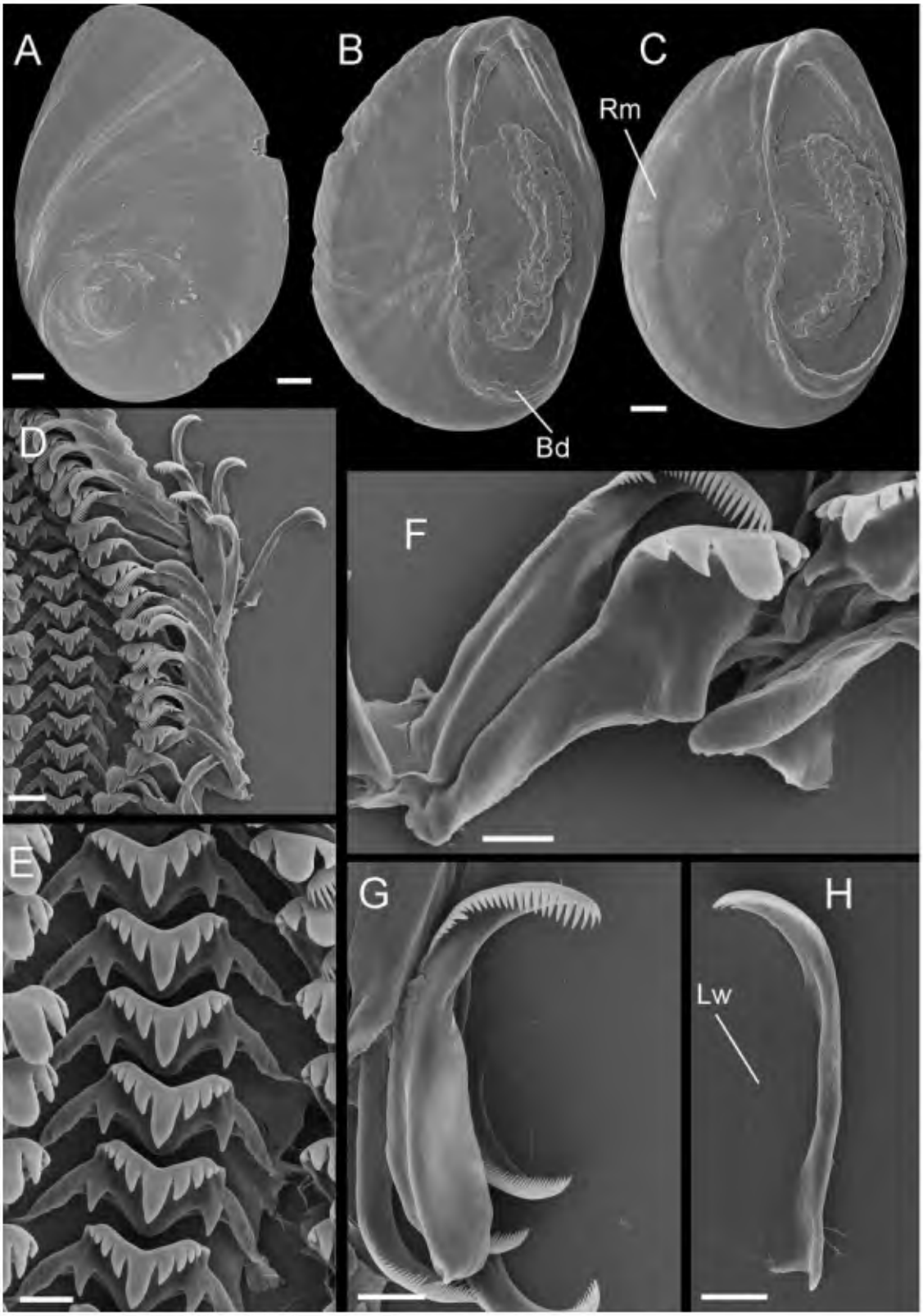

Figure 5. Scanning electron micrographs of opercula and radula of *P. blainica* (USNM 1112484). **A.** Opercula, outer side. **B**, **C.** Opercula, inner side. **D.** Portion of radular ribbon. **E.** Central radular teeth. **F.** Lateral radular tooth. **G.** Inner marginal tooth. **H.** Outer marginal tooth. Abbreviations: Bd, (thickened) border of operculum attachment scar; Lw, lateral wing of outer marginal tooth; Rm, rim on inner side of operculum. Scale bars **A–C** = 100 μm; **D** = 20 μm; **E–H** = 10 μm.

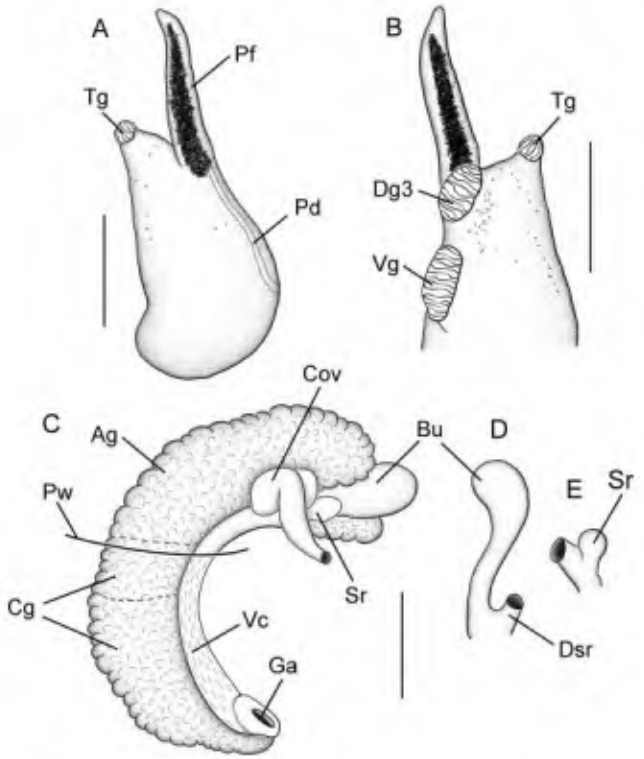

Figure 6. Reproductive anatomy of *P. blainica* (USNM 1112484). **A.** Penis, dorsal surface (pigment in penial filament darkly stippled). **B.** Penis, ventral surface. **C.** Female glandular oviduct and associated structures (viewed from left side). **D.** Bursa copulatrix. **E.** Seminal receptacle. Abbreviations: Ag, albumen gland; Bu, bursa copulatrix; Cg, capsule gland; Cov, coiled oviduct; Dg3, gland on outer edge of penial lobe; Dsr, seminal receptacle duct; Ga, female genital aperture; Pd, penial duct; Pf, penial filament; Pw, posterior wall of pallial cavity; Sr, seminal receptacle; Tg, terminal gland; Vc, ventral channel of capsule gland; Vg, ventral gland. Scale bars = 250 μm.

Remarks: Shell form diversity in *P. blainica* is considerable, not only in terms of overall shape, but also in spire shape, loosening of teleoconch whorls, size of aperture and thickness of the inner lip. We did not discern qualitative anatomical differences between shell morphotypes; but noted that highly elongate, thin-lipped specimens were frequently infected by digenean trematodes, which suggests that some of the shell variation may be parasite-induced.

Shell measurements of *P. blainica* and its closely similar congener, *P. bedfordensis*, are given in Table 2. Samples of these two species differed significantly ($P < 0.001$) in all parameters. In addition to the features mentioned in the Diagnosis, *P. blainica* can usually be distinguished from *P. bedfordensis* by the combination of its narrower shell and smaller aperture (Fig. 8).

Pyrgulopsis blainica is the new species of snail from the Ennis National Fish Hatchery mentioned by Beck (2006).

MOLECULAR ANALYSIS

New sequences were deposited in GenBank under accession numbers EU700464–EU700486 (Table 1). The two MRB species (*P. bedfordensis*, *P. blainica*) formed a well supported, terminally positioned clade that was sister to *P. anguina* (Fig. 9), which lives in the southeast part of the Bonneville Basin (Fig. 2). The sister relationship of this more inclusive monophyletic group was not well resolved.

Figure 7. Digital aerial photograph (DOQ) of a portion of southern Montana (taken in 1995) showing Blaine Spring and its outflow. The two collection localities for *P. blainica* are indicated by filled circles.

One variable site (141) was found among the four *P. bedfordensis* sequences, giving two haplotypes. The three sequences of *P. blainica* also contained but a single variable site (478), yielding two haplotypes. *Pyrgulopsis bedfordensis* and *P. blainica* can be distinguished by 14 mutations; the mean sequence divergence between them ($2.2 \pm 0.5\%$) exceeded the sequence divergence within both of these snails ($0.1 \pm 0.1\%$). The divergence between these two species and *P. anguina* ranged from 4.9 to $5.1 \pm 0.8\%$.

The relative rate test failed to reject clocklike behaviour within the MRB lineage ($\chi^2 = 0.08$, df = 1, $P = 0.78$; *P. anguina* used as outgroup) and between the members of this clade and *P. anguina* ($\chi^2 = 0.67–0.86$, df = 1, $P = 0.41–0.35$; *F. winkleyi* used as outgroup). Based on a previously derived COI clock rate for *Pyrgulopsis* (1.62% per million years; Liu &

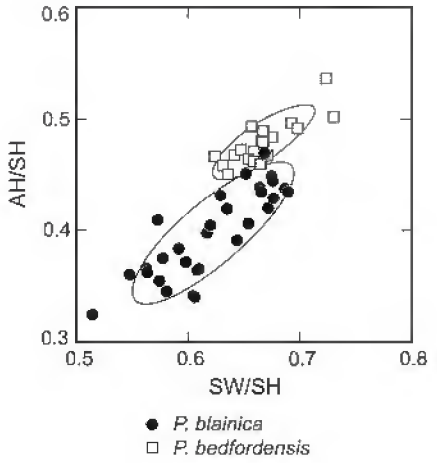

Figure 8. Scatterplot (SW/SH *vs* AH/SH) showing differentiation between *P. blainica* (USNM 1112484, $n = 30$) and *P. bedfordensis* (USNM 892152, $n = 19$). The sample size of the latter was constrained by the paucity of specimens with intact (uneroded) spires. Confidence ellipses centered on sample means ($P = 0.6827$).

Hershler, 2007), our results imply a 3.64–2.53 Ma (late Pliocene) split between *P. anguina* and the MRB clade (Fig. 9, node A) and 1.67–1.05 Ma (early to middle Pleistocene) divergence between *P. blainica* and *P. bedfordensis* (Fig. 9, node B).

DISCUSSION

The biogeography of freshwater molluscs across the northern continental divide is thought to have been shaped by a series of late Cenozoic drainage transfers involving the MRB (Taylor, 1985; Taylor & Bright, 1987; Gangloff & Gustafson, 2000; Hershler & Gustafson, 2001). Our study, which provides the first molecular phylogenetic evidence pertinent to this subject, suggests that the *Pyrgulopsis* fauna of the MRB diverged from western progenitors during the late Pliocene. This finding would appear to rule out the possibility that members of the genus were recently introduced to the MRB by anthropogenic activities, dispersal on birds or other mechanisms, but is consistent with a vicariant origin through a prior rearrangement of drainage across the northern continental divide.

Hershler & Gustafson (2001) speculated that *P. bedfordensis* was a product of a geologically-documented late Miocene (6.5 Ma) transfer of drainage from south-central Idaho (Snake River basin) to the MRB. Our results do not support this biogeographic hypothesis, because the MRB clade is not closely related to Idaho species and diverged well after this geomorphic event. However, our findings conform to, and add additional support for, a more recent stream capture event proposed on the basis of molluscan evidence (Taylor, 1985; Taylor & Bright, 1987). Taylor (1985) attributed the disjunct distributions of various fossil and recent freshwater taxa across the northern continental divide to the disruption of a postulated late Neogene drainage that was thought to have flowed southward from the Missouri River headwater region (southwestern Montana) through southeastern Idaho and western Utah into southeastern Nevada (Fig. 2). This drainage was thought to have pre-dated the development of the modern Snake River, Bonneville, and Colorado River basins (Taylor, 1985) and to have been truncated more than 2.0 Ma (Taylor & Bright, 1987). Although this scenario remains speculative, several of its key assumptions have been confirmed by geological studies, including a recent uplift of the east–west trending

Table 2. Shell parameters for *P. blainica* and *P. bedfordensis* and results of *t*-tests (separate variances) comparing samples of these species.

	WH	SH	SW	HBW	WBW	AH	AW	SW/SH	HBW/SH	AH/SH
P. blainica										
Paratypes ($n = 30$)	5.04	3.98	2.47	2.78	2.18	1.57	1.40	0.621	0.700	0.397
Mean range	4.50–5.50	3.54–4.62	2.19–2.93	2.51–3.13	1.93–2.43	1.31–1.94	1.21–1.61	0.515–0.690	0.604–0.759	0.324–0.468
SD	0.301	0.313	0.163	0.147	0.135	0.134	0.094	0.047	0.034	0.040
P. bedfordensis										
Paratypes ($n = 19$)	4.61	2.79	1.86	2.14	1.61	1.33	1.12	0.666	0.769	0.479
Mean range	4.25–5.00	2.29–3.30	1.53–2.06	1.72–2.44	1.29–1.81	1.12–1.54	0.953–1.23	0.623–0.730	0.703–0.817	0.451–0.537
SD	0.192	0.236	0.139	0.190	0.132	0.108	0.084	0.028	0.0291	0.020
T	−6.189	−15.214	−13.986	−12.466	−14.604	−6.881	−10.860	4.204	7.634	9.455
df	47.0	45.4	42.9	31.4	39.1	44.2	41.5	47.0	43.1	45.3
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

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; *T* = *t* value; df, degrees of freedom.

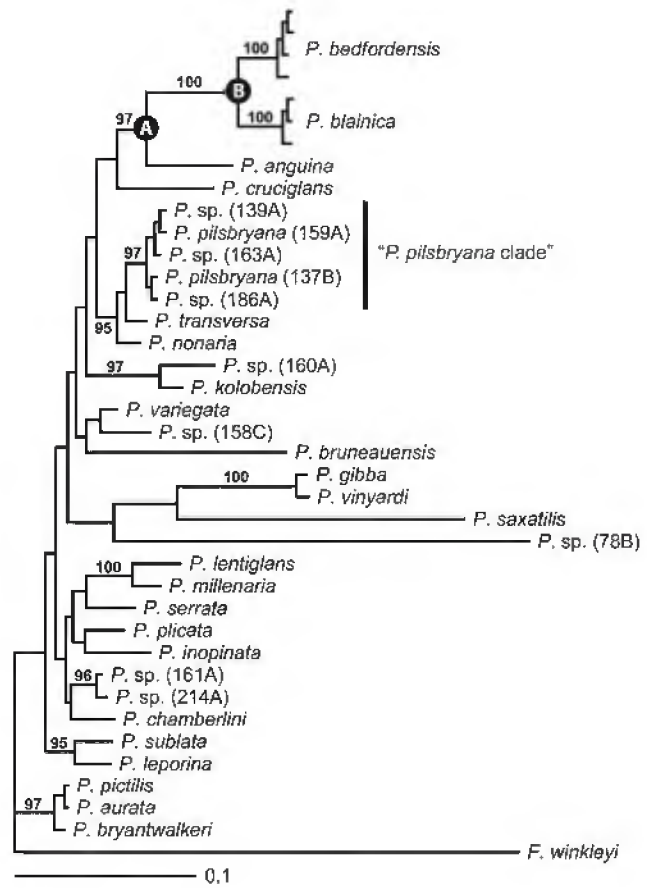

Figure 9. Bayesian tree based on the COI dataset. Posterior probabilities for nodes are provided when >95%. The two nodes discussed in the text are highlighted with filled circles. Terminals are labelled as in Table 1.

Centennial Mountains, which currently separate the upper Missouri and upper Snake River drainages (Sonderegger *et al.*, 1982; Garson *et al.*, 1992; Anders *et al.*, 1993); and development of a temporary topographic divide in the modern Snake River Plain (resulting from passage of this area over the Yellowstone plume or hotspot) during the late Neogene, which forced drainage of southeastern Idaho southward into what is now the Bonneville Basin (Pierce & Morgan, 1992; Beranek, Link & Fanning, 2006).

Taylor's (1985) hypothesis was constrained by the fact that none of the molluscs that he used to infer palaeodrainage are (or were) distributed in the MRB, although he assumed that they had been at one time. Our findings thus provide more direct evidence of prior southward drainage from the MRB as well as an estimated date for the transfer of the headwaters of this watershed to the eastern side of the modern continental divide (3.64–2.53 Ma). Furthermore, our study provides the first evidence that this palaeodrainage facilitated west to east faunal movement across the continental divide; all of the taxa discussed by Taylor (1985) were thought to have crossed the site of this barrier in the other direction based on their predominantly eastern distributions.

The large disjunction (750 km) between *P. anguina* and the MRB lineage is puzzling as relatives of the latter would be expected to be distributed in closer proximity to the northern continental divide under a stream-capture hypothesis. Some of the taxa originally used to infer this palaeodrainage also have large gaps in the western portion of their geographic ranges (e.g. Taylor, 1985: figs 12, 13), suggesting a possible common cause. We do not have a confident explanation for this pattern, but consider it possible that members of these lineages went extinct in the intervening region as a consequence of geologically recent perturbations such as the extensive volcanism in the eastern Snake River Plain and the development of Lake Bonneville. We note in this regard that the very shallow structuring delineated within the '*P. pilsbryana* clade' (Fig. 9; mean sequence divergence, 0.1 ± 0.1%) suggests recent colonization of habitats within the region consistent with this hypothesis.

Our finding that the MRB clade is most closely related to *P. anguina*, which has an entirely different pattern of penial ornament (see Hershler, 1998: fig. 44B–E), also contributes to a growing body of evidence suggesting that morphological characters currently utilized in taxonomic studies of this genus are prone to homoplasy and therefore poor indicators of phylogenetic relationships (Liu & Hershler, 2005).

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