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Research article

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Integrative taxonomy confirms the synonymy of three springsnail species (Hydrobiidae: *Pyrgulopsis*)

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Abstract. The small, spring-dwelling snails, *Pyrgulopsis* Call & Pilsbry, 1886, form a diverse radiation in Western North America. In this study, we examine the validity of three species of *Pyrgulopsis*, *Pyrgulopsis pilsbryana* (Baily & Baily, 1952), *P. nonaria* Hershler, 1998, and *P. transversa* Hershler, 1998. These three species of *Pyrgulopsis* are found primarily in Utah, with ranges extending into Idaho, Wyoming, and Nevada. They were originally diagnosed primarily on the number and position of penial glands, but subsequent examination with mitochondrial DNA data suggested they should be synonymized. Here, we incorporate additional mitochondrial and nuclear DNA data, increased geographic sampling, and quantify some stated differences in shell and radula morphology. We test the utility of penial ornamentation and other anatomical features used to distinguish these species. We found that the shell shape and radula features measured tend to be unique to each local population, not species-specific distinguishing features. Further, we found the diagnostic features used to erect the three focal species are not associated with phylogenetic relationships. Therefore, we present a synonymy of the three focal species under the name with priority, *P. pilsbryana*.

Keywords. Mollusk phylogenetics, integrative taxonomy, CO1, LSU, springsnails.

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Introduction

In desert environments, unique communities of animals, plants, and microbes depend on groundwater at springs and ciénegas (freshwater ground-water dependent wetlands). There is a diverse radiation of small (<5 mm) snails, called “springsnails” found across southwest North America. This radiation of springsnails includes 200+ species of *Pyrgulopsis* Call & Pilsbry, 1886, with most species endemic to a single spring. As such, springsnail species are considered critically imperiled with their existence depending on maintenance of spring-flows in regions of declining water availability (Hershler *et al.* 2014). These springsnails have been the focus of considerable conservation attention as most species are considered Species of Greatest Conservation Need (defined in state wildlife action plans, U.S. Fish & Wildlife Service) and numerous species have been petitioned for listing under the U.S. Endangered Species Act. This has led to the coalition of state and federal agencies, non-governmental organizations, and conservation organizations cooperating to share information to assist in conservation of these organisms (Stevens *et al.* 2022).

Effective conservation efforts rely on accurate taxonomy of these critically imperiled animals (Lysne *et al.* 2008; Perez & Minton 2008; Delicado *et al.* 2025). For example, if we propose a hypothesis of a single species of *Pyrgulopsis* with many populations and a wide range, that species might not be at a high conservation risk. However, if we propose that this species should be split into several single-site or narrow-range endemic species, each of them could be considered imperiled. In one case, the severity of threat of a species could be underestimated and irreplaceable biodiversity could be lost. But in the other case, scarce conservation resources could be misallocated. In either case, the scientifically justifiable approach is to use the best evidence available to formulate species’ hypotheses, as well as critically examine the performance of each of those pieces of evidence. In this study, we are applying an integrative taxonomic approach (Dayrat 2005; Schlick-Steiner *et al.* 2010), defining a species as distinct once it has become a mostly independent evolutionary unit (*sensu* Nekola & Horsák 2022). This requires examination of the congruence of data across multiple sources, which could include mitochondrial and nuclear DNA, genitalia, radula, shell, behavior, geography, etc., and designation of species boundaries as a statistically-supported, evidence-based hypothesis (Nekola & Horsák 2022).

The ecology of springsnails poses a challenge to some of these forms of evidence. *Pyrgulopsis* populations are often highly abundant in spring headwaters (Brown *et al.* 2008) but usually do not extend far downstream (Hershler *et al.* 2014). Therefore, they are found in isolated freshwater springs with no apparent corridors for gene flow. These allopatric populations show a tendency for isolation in mitochondrial lineages with a meta-analysis finding intraspecific variation in cytochrome oxidase 1 (CO1) typically <3.2% and interspecific variation ranging from 3.2% to 4.4% (Liu *et al.* 2018). A challenge for accurate species-level taxonomy of these isolated, single-site endemic species is the tendency for mitochondrial lineages and even some aspects of morphology to differentiate rapidly in each spring, making it difficult to discern population versus species-level differentiation (for review see Avise 2009).

The level of differentiation (barcode gap) in mitochondrial DNA data (CO1) has been used recently to distinguish species in *Pyrgulopsis* (Hershler *et al.* 2015, 2017; Liu *et al.* 2018), along with aspects of the radula, reproductive anatomy, and shell morphology. However, the taxonomic utility of these anatomical features has not received scrutiny. The radiation of *Pyrgulopsis* could be considered morphostatic, with very few species having diagnostic shell morphologies. In addition, springsnail shells are so nondescript that *Pyrgulopsis* are sometimes indistinguishable from members of other genera such as *Juturnia* Hershler, Liu & Stockwell, 2002 and *Colligyryus* Hershler, 1999 without dissection (Morningstar *et al.* 2014). While features of the radula and penial morphology have been described and used to diagnose species (Hershler 1994; Perez *et al.* 2022), they are usually figured as composites from a single or small

(usually not reported) number of individuals examined and the reliability of these features to delineate species has received little deliberate study.

To study the utility of these anatomical features in a group of organisms fitting the conservation scenario described above, we consider three closely related species of *Pyrgulopsis*. *Pyrgulopsis pilsbryana* (Baily & Baily, 1952) was described (as *Amnicola pilsbryi*) from beach drift on the shores of Bear Lake which straddles the border of Utah and Idaho. This description was based on the morphology of dry shells which were briefly described (Baily & Baily 1952). *Pyrgulopsis nonaria* Hershler, 1998 and *Pyrgulopsis transversa* Hershler, 1998 were both described from springs in Utah, Ninemile Spring and Sixmile Springs, UT, respectively. These species were diagnosed based on their isolated localities and penial ornamentation. In that same study, the anatomy of samples representing *P. pilsbryana* were also figured and described (Hershler 1998) for the first time. Subsequently, these species were included in a mitochondrial DNA study attempting to delineate species among the *P. kolobensis* (Taylor, 1987) species complex. Liu *et al.* (2018) found *P. pilsbryana*, *P. transversa*, and *P. nonaria* all part of a single clade, with minimal genetic divergence within the clade. They called this group “Lineage A” and recommended they be considered a single species; however, they did not publish a formal synonymy and considered only mitochondrial DNA and penial gland data. They recommended that further work incorporating nuclear data, more extensive sampling, and testing the utility of penial morphological features be conducted to illuminate the status of these taxa.

In this study, we incorporate new and existing mitochondrial and nuclear sequence data, with the addition of material from type localities and newly discovered populations to test the species hypotheses for these three nominal taxa. We also examine new anatomical evidence to determine the specific status of *P. pilsbryana*, *P. transversa*, and *P. nonaria*. We evaluate the utility of several of the standard anatomical characteristics used to diagnose *Pyrgulopsis*, including features of the shell shape, central radular tooth, and penial ornamentation.

Material and methods

Samples from 41 localities were collected, preserved for DNA and morphological analyses (Fig. 1). Thirty springsnails per locality were flash boiled and preserved in 70–95% non-denatured ethanol for genetic analysis (Perez *et al.* 2021). Approximately 25 springsnails from each locality were relaxed in a saturated solution of menthol and then preserved in 70% nondenatured ethanol for morphological analysis (Perez *et al.* 2021). These included samples from (or near) the type localities of the focal species and from sites that had previously been indicated (Liu *et al.* 2018) as part of the lineage that includes *P. pilsbryana*, *P. nonaria*, and *P. transversa*. Also included were samples collected from 16 populations that had not been previously characterized or identified referred to as “unknown” or “non-type locality populations” (see Appendix 1).

Sequence data

DNA extraction was conducted with the DNeasy Blood and Tissue Kit (#69506, Qiagen®). Following the quick-start protocol, each snail was placed in 180 µL extraction buffer and 20 µL proteinase K. The whole animal including the shell was incubated at 56°C for 48 hours, and vortexed once mid-way. The rest of the extraction followed the manufacturer protocol, with elution into 50 µL of elution buffer. PCR was conducted to amplify mitochondrial locus cytochrome oxidase subunit 1 (COI or *cox1*) using primers COIH2198 and COIL1490 (Folmer *et al.* 1994) and the nuclear Large Ribosomal Subunit (LSU), using primers LSU1 & LSU3 (Wade *et al.* 2001). Details of primers and PCR reaction conditions followed recent work on *Pyrgulopsis* (Perez *et al.* 2021). These genes were targeted as there is existing data on COI for *Pyrgulopsis* and recent work has shown the nuclear LSU has value for distinguishing *Pyrgulopsis* species (e.g., Perez *et al.* 2021). Amplified PCR products were confirmed via agarose electrophoresis

and cleaned using Gel/PCR DNA Fragments Extraction Kit (IBI Scientific, Peosta), followed by elution into 50 μ L of elution buffer. Quantification was conducted using a Qubit 3.0 Fluorometer and Qubit dsDNA HS Assay Kit (Invitrogen-Life Technologies, Eugene). DNA sequencing was performed by Eton Bioscience, Inc. All sequences were deposited to GenBank (COI: OR363185–OR363208; LSU: OR365616–OR365639; <https://www.ncbi.nlm.nih.gov/genbank/>) so they are available for future use (Appendix 1).

For COI data analysis, contigs were assembled in Geneious ver. 10.2.3 (Kearse *et al.* 2012) then aligned using Multiple Sequence Comparison by Log-Expectation (MUSCLE, also implemented in Geneious). For LSU data which includes many indels, contigs were assembled in Geneious, then aligned using the Multiple alignment program for amino acid or nucleotide sequences (MAFFT) server online (Kato *et al.* 2019) applying most default settings, with the L-INS-I iterative refinement method and attempt to align gappy regions (Kato *et al.* 2002). All *Pyrgulopsis* sequences, ~1000 COI sequences, were downloaded from GenBank and used to create an initial alignment with our new sequences. This initial alignment had 1070 COI sequences. All LSU sequences from GenBank were also included; however, there are very few as previous work on this group focused on COI. Some species distantly related to our target taxa had 10+ sequences represented in this alignment and therefore some were removed, retaining fewer sequences to ease visualization. Sequences requiring gaps for alignment were also removed. In the protein-coding COI gene, we presume these to be low quality or suspected pseudogenes. Tree inference was conducted on COI and LSU alignments separately. This resulted in a 387-terminal COI alignment which was analyzed using a partitioned maximum likelihood analysis considering the 1st, 2nd, and 3rd

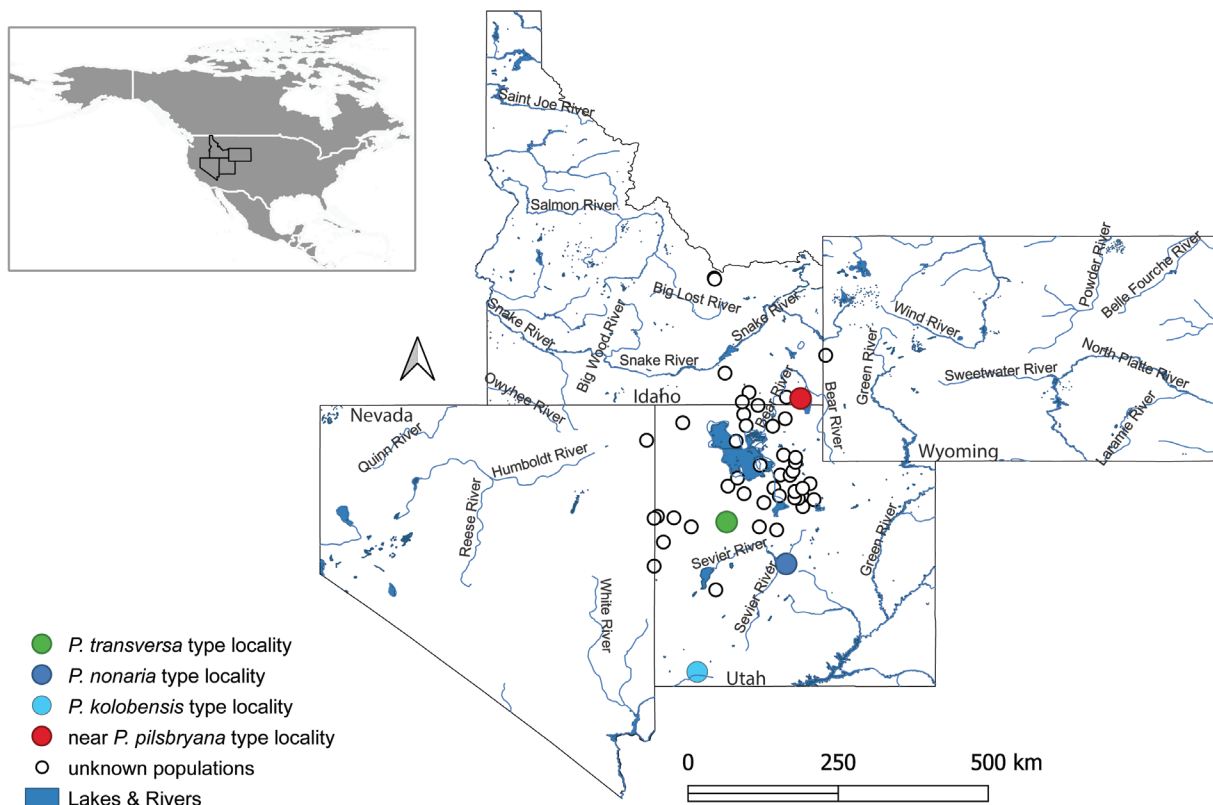


Fig. 1. Map of states in the Western United States with samples included in this study. Showing confirmed type localities and localities for unknown (non-type locality) populations now assigned to *Pyrgulopsis pilsbryana* (Baily & Baily, 1952). Shown in blue are major rivers and lakes (USGS 2004). Inset map shows position of the western states in North America.

codon positions separately. To aid analysis and visualization of the haplotype network, another dataset with 97 terminals, just comprising Lineage A, *P. sterilis* Hershler, 1998, *P. plicata* Hershler, 1998, *P. inopinata* Hershler, 1998, and *P. kolobensis* was examined.

Model selection followed by maximum likelihood analyses were conducted in three separate runs in IQ-TREE ver. 1.6.12 with 1000 ultra-fast bootstrap replicates. The model chosen by BIC for the COI 387-terminal analysis was: GTR+F+R4: 1st position, TIM3e+I+G4: 2nd position, F81+F+I+G4: 3rd position. The same method was used for model selection for LSU selecting JC+I. Identical sequences from the same locality were removed retaining haplotypes unique to each locality. Kishino-Hasegawa and Shimodaira-Hasegawa tests were conducted to determine if there were significant differences in highest likelihood trees among runs. Initially *Pyrgulopsis texana* (Pilsbry, 1935) was used to root the tree; however, this dataset found *P. texana* to be 17–26% divergent from the other *Pyrgulopsis* examined, which was too distant to serve as a reasonable outgroup. Following initial analysis, *P. texana* was omitted and *P. ignota* Hershler, H.-P. Liu & B.K. Lang, 2010 was used for further analyses of COI. The resulting tree was examined, and visuals designed in FigTree ver. 1.4.4.

MEGA was used to select an appropriate model for COI barcoding analyses. To determine if there is a barcoding gap, we compared the within- and between-species pairwise distances for all *Pyrgulopsis* data available (1070 terminals), the reduced dataset (387 terminals), and the focal taxa (97 terminals). For the 1070-terminal dataset and the 387 terminal dataset the T92+G+I model had the highest value of the Bayesian Information Criterion (BIC) and was used to calculate pairwise distances. For the 97-terminal dataset the model with the highest value of BIC was T92+G which was used to calculate pairwise distances.

PopART ver. 1.7 was used to create a median joining network from the 97-terminal COI alignment of Lineage A and outgroups *P. plicata*, *P. inopinata*, *P. sterilis*, and *P. kolobensis*. *Pyrgulopsis kolobensis* was included as this is the species the others were split from. Less data is available for LSU, the final alignment had 55 terminals and 711 characters. Outgroups include *P. texana*, *P. ignota*, and other species of *Pyrgulopsis* from Texas.

Shell morphometrics

For geometric morphometric comparison of shell shapes, we included mature individuals from type localities or near them for *P. nonaria* (Ninemile Spring, UT, type locality, n = 20 and one image of holotype from original description), *P. transversa* (Sixmile Springs, UT, type locality, n = 20 and one image of holotype from original description), and *P. pilsbryana* (near-type locality, St Charles, Idaho, n = 20 and one image of holotype), and four additional populations selected for the greatest geographical separation among the sites that have been confirmed to have “Lineage A” haplotypes present. These were Vernon Reservoir (n = 10), Dove Creek Hills (n = 10), North Beck (n = 10), and Red Barn WMA (n = 10). To examine the shape disparity among additional closely related species, topotypical samples of *P. inopinata* Hershler, 1998, *P. sterilis* Hershler, 1998, and *P. plicata* Hershler, 1998 were also examined. Complete locality details in Appendix 1.

Each of the shells were photographed following the apertural view (APT) listed in the standard views for imaging shells (Callomon 2019). The photos were captured using a Leica S9i under 20× magnification with an AmScope (MU1003) camera and software (ver. x64) by focusing on different points of the shell. A single focused image was produced using focus stacking in Helicon Focus ver. 6.7.1 (Heliconsoft.com, Kharkiv, Ukraine). Images were cropped and brightened (if necessary) in Adobe Photoshop 2023 (Adobe Systems Incorporated). Images of types from original descriptions that were oriented in the apertural view were cropped from the published plates and landmarked.

Shell images, captured as described above, were landmarked ($n = 20$ landmarks, $n = 103$ individuals) around the perimeter of their shells and aperture using tpsDig ver. 2.32 (Rohlf 2017). Landmarks were placed and numbered as seen in Fig. 2. Twenty landmarks were placed on the shell and aperture. Landmarks 1, 2, 4, 5, 6, 10, 12, 16, and 18 were positioned on discrete points such as the spire and sutures. The remaining (3, 7, 8, 9, 11, 13, 14, 15, 17, 19, 20) were semilandmarks positioned in-between fixed landmarks to capture the curves of the shell. Landmarks were converted to Procrustes coordinates using least squares superimposition in CoordGen ver. 8 (Sheets 2014). The Procrustes coordinates were imported to PAST ver. 3.16 (Hammer *et al.* 2001) where shell shapes were compared with Principal Components Analysis (PCA), Canonical Variate Analysis (CVA), MANOVA, and post-hoc tests using Bonferroni-corrected p -values. The results of PCA and CVA were visualized in JMP Pro ver. 15.1.0 (1989–2019) and two analyses were performed. The first including just Lineage A populations, and the second comparing all Lineage A populations to other named species. The PCA was examined using the Variance-covariance matrix disregarding groups.

Internal anatomy

Our initial aim was to characterize the penial morphology of each individual that we intended to sequence. Dissections and examination of shells and anatomical features were carried out using a Leica S9i, and the Leica LAS X software. Because removing the animal from the shell after preservation in alcohol destroys the shell, photos were taken for photovouchers of each individual (Appendices 2–3) following the procedures detailed above.

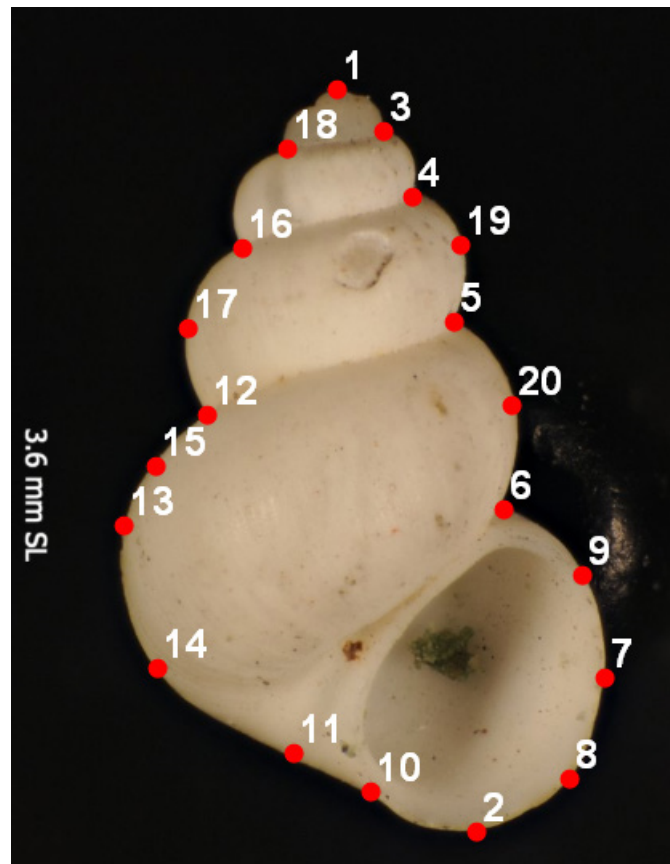


Fig. 2. Shell of *Pyrgulopsis pilsbryana* (Baily & Baily, 1952), holotype, ANSP 187691 with landmarks (1–20) used for shell morphometric analyses indicated. Photo courtesy of ANSP.

Dissections conducted to examine the anatomical features (not DNA specimens) began by dissolving the shell by immersion in 50% HCL for one minute. This removed the calcareous shell leaving the periostracum. The periostracum was then removed with a fine forceps. Soft-tissue dissections were conducted in water and/or with the addition of Bouin's solution to allow visual contrast and hardening of tissues. Penial morphology was characterized as conforming to the description of *P. pilsbryana*, *P. nonaria*, or *P. transversa* and indicated in Figs 3–4. The specific features of individuals identified as *P. pilsbryana* included a short penial filament and lobe, with large terminal and ventral glands. The diagnostic features of *P. transversa* were a medium length filament and lobe, with a small terminal gland and possibly a small ventral gland. Finally, the diagnostic feature of *P. nonaria* was a small penial filament and lobe with two terminal glands and absent a ventral gland. Prior to digestion for DNA work, when male reproductive parts were present, they were diagnosed as belonging to one of the nominal species. This approach to testing the utility of features of penial ornamentation was suggested in an unpublished comparison of other species of *Pyrgulopsis* (Liu & Dillon 2021). Other aspects of the internal anatomy, i.e., ctenidium, bursa, etc. were observed during dissection, compared to the original descriptions, and a composite description was written encompassing the features of all three nominal species.

Individuals intended for DNA extraction were not immersed in HCL or Bouin's. Instead, these snails were dissected in alcohol with the following protocol. First, the body whorl was broken away and the left edge of the mantle cavity was dissected (following Hershler & Liu 2017). Dissecting the edge of the mantle allowed it to be reflected and visible examination determined if the individual was male or female. For male individuals, the penis was photographed and preserved. We aimed to characterize the penis and DNA of five individuals per population; however, that was not usually possible because in some populations no males were encountered in >20 individuals dissected.

Radula data

During dissection, opercula and radula were set aside for scanning electron microscopy (SEM). In addition, snails were photographed, and radula were extracted using the DNA digestion buffers from the DNeasy Blood & Tissue Kit. As with DNA extractions, they were incubated for 48 hours, and vortexed once after 24 hours. The radula were removed from the solution and placed in a tube with 70% nondenatured ethanol prior to mounting. Ultra-Smooth Carbon Adhesive Tabs (Electron Microscopy Sciences, Hatfield) were placed directly onto an aluminum mount (Electron Microscopy Sciences). The radula was transferred by micropipettor with ~4 μ L water. Once placed on the adhesive tab, the radula was broken using insect pins from the center teeth outward to spread the teeth and determine the orientation and allowed to air dry. After drying, radula were coated with 75 angstroms of gold palladium alloy using a Quorum Sputter coater. Scanning electron micrograph (SEM) images were taken using a Zeiss EVO LS10, and high vacuum from 100–20k magnification. The usual working distance was 4.5–5 mm, spot size of 242, accelerating voltage of 10.94 kilovolts (kV).

Several radulae were imaged from individuals from Ninemile Springs (*P. nonaria* type locality, six individuals, 195 teeth), Sixmile Springs (*P. transversa* type locality, six individuals, 133 teeth), Vernon Reservoir (six individuals, 239 teeth), and Big Springs (seven individuals, 422 teeth). An individual representing *P. pilsbryana* from a spring NW of Lakota, Bear Lake Basin (USNM 858279) is figured in Hershler (1994: fig. 39a) with three central radular teeth visible. Limited material for radula data collection was available for *P. pilsbryana*. We repeatedly sampled but were unable to re-collect snails resembling *P. pilsbryana* from the region for radula measurements. Four features of the central radular tooth that had been described to differ between the target taxa were measured: central cusp length (μ m), and width (μ m) at the widest point, and the number of lateral cusps in each direction. For some teeth, the number of cusps were obscured so there are fewer measurements for those features (Table 2). ImageJ ver. 1.54f (Schindelin *et al.* 2012) was used to measure radula features and statistical tests were run in JMP Pro ver. 16.2.0 (SAS Institute Inc., Cary, NC). The measurements were each tested for normality

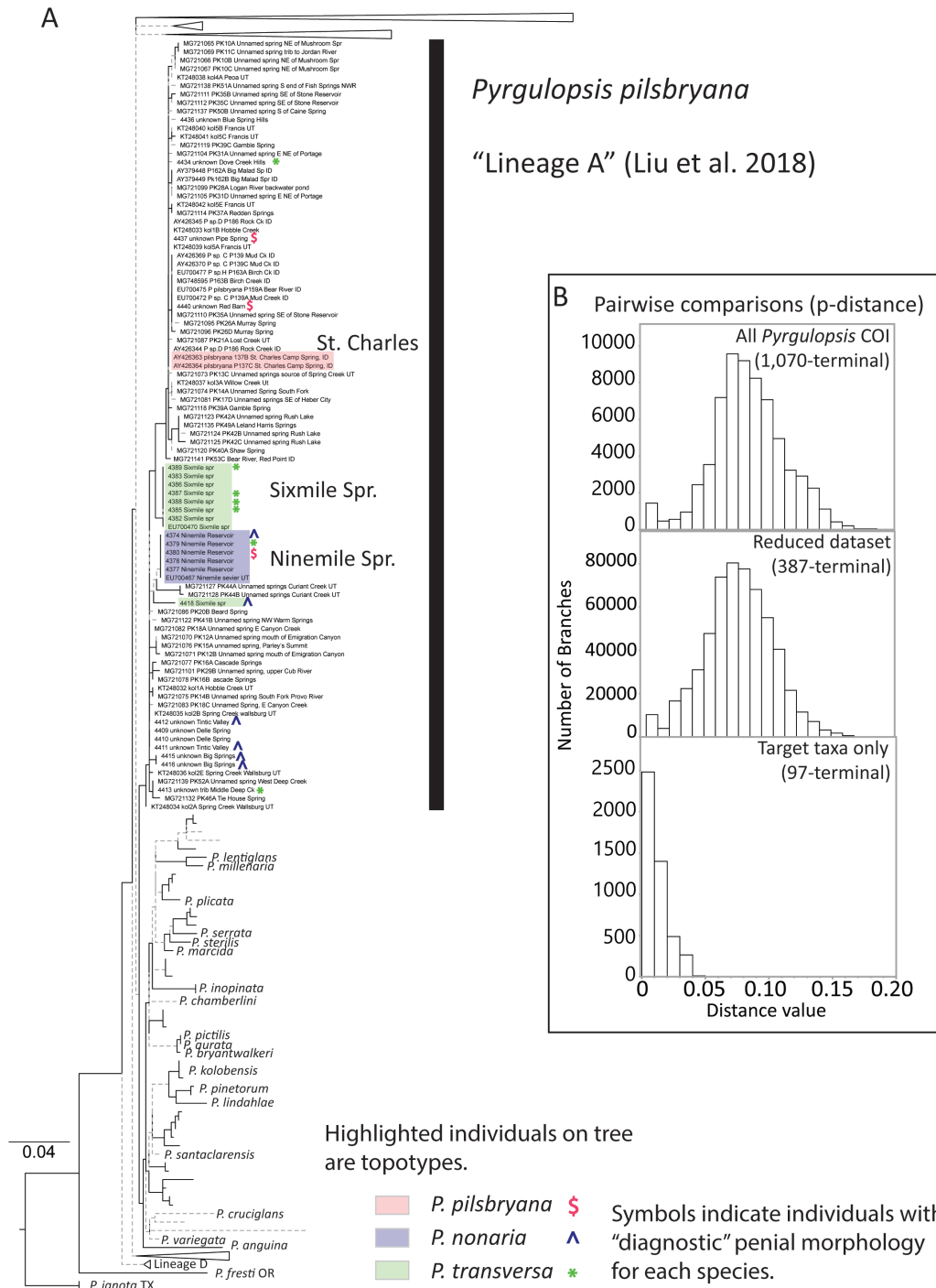


Fig. 3. A. Maximum likelihood phylogeny from 657 bp alignment of 387 COI sequences presenting only the focal taxa and the most closely related clade, more distant clades shown collapsed. Taxa examined in nuclear DNA or morphological analyses are also labeled. Ultra-fast bootstrap values > 95% indicated using black lines, < 95% indicated with dashed gray lines. Terminals from representative type or near-type localities indicated with colored boxes. The penial morphology of male individuals as described in their descriptions are indicated with colored symbols: \$ = similar to *Pyrgulopsis pilsbryana* (Baily & Baily, 1952); * = similar to *P. transversa* Hershler, 1998; ^ = similar to *P. nonaria* Hershler, 1998. **B.** Histograms illustrating pairwise distances for all comparisons shown for all *Pyrgulopsis* Call & Pilsbry, 1886, the reduced dataset, and the focal taxa only.

using a Shapiro-Wilks Goodness of Fit test and were found to be significantly non-normal ($p < 0.001$), except *P. pilsbryana*, which had too few values to conduct this test. Wilcoxon / Kruskal-Wallis (Rank Sums) tests were therefore used to compare these measurements. Multiple comparisons were conducted using the Steel-Dwass method to protect the error rate for $n = 10$ comparisons.

Museum collection

ANSP = The Academy of Natural Sciences of Philadelphia of Drexel University
 USNM = National Museum of Natural History, Smithsonian Institution

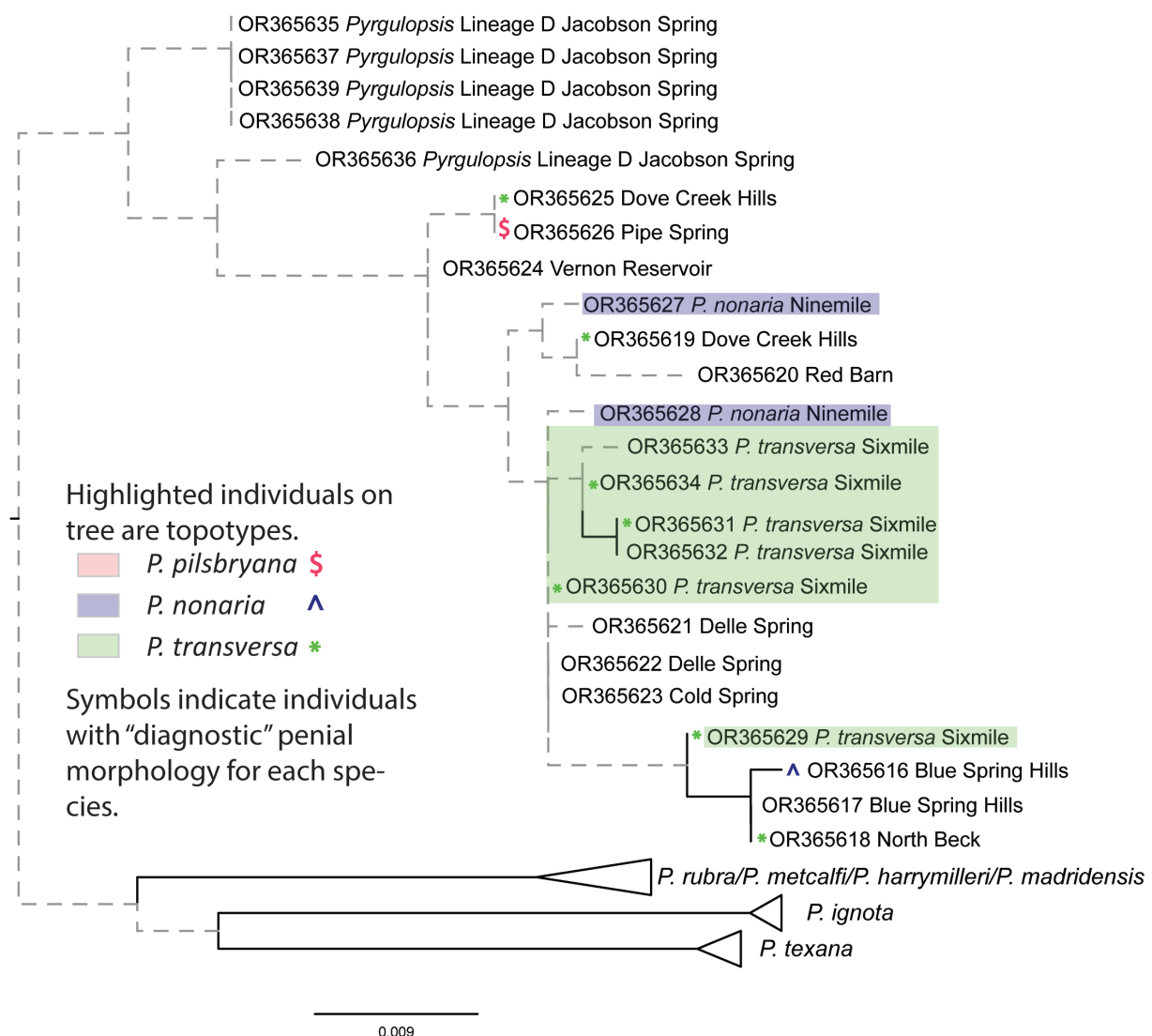


Fig. 4. Maximum likelihood phylogeny from 702 bp alignment of 55 LSU sequences presenting only the focal taxa and localities. Ultra-fast bootstrap values $> 95\%$ indicated using black lines, $< 95\text{--}50\%$ indicated with dashed gray lines. Terminals from representative type or near-type localities or identified by penial morphology indicated with colored boxes. The penial morphology of male individuals as described in their descriptions are indicated with colored symbols: \$ = similar to *Pyrgulopsis pilsbryana* (Bailey & Bailey, 1952); * = similar to *P. transversa* Hershler, 1998; ^ = similar to *P. nonaria* Hershler, 1998.

Results

Phylogenetic analyses

The 387-terminal alignment was found to have 256 parsimony informative sites. The log-likelihood of trees from each ML run differed slightly but Kishino-Hasegawa ($p = 0.399, 0.448, 0.552$) and Shimodaira-Hasegawa tests ($p = 0.503, 0.671, 1$) did not find them significantly different. The tree with the highest log-likelihood score is presented -13157.768 (Fig. 3). A single highest likelihood tree from the Maximum likelihood analysis of the 55-terminal LSU dataset had a log likelihood score of -1572.586. The 55-terminal LSU alignment was found to have 55 parsimony informative sites (Fig. 4).

Pairwise distance between Lineage A and the outgroup species *P. plicata*, *P. sterilis*, *P. inopinata*, and *P. kolobensis* averaged 0.03 (3%). Within Lineage A the average p-distance was 0.0087 (0.08%). Figure 3B illustrates histograms of pairwise distances for all three datasets. There is not a discrete barcode gap, although there is a drop around 0.04 (4%) where there are lower numbers of pairwise comparisons.

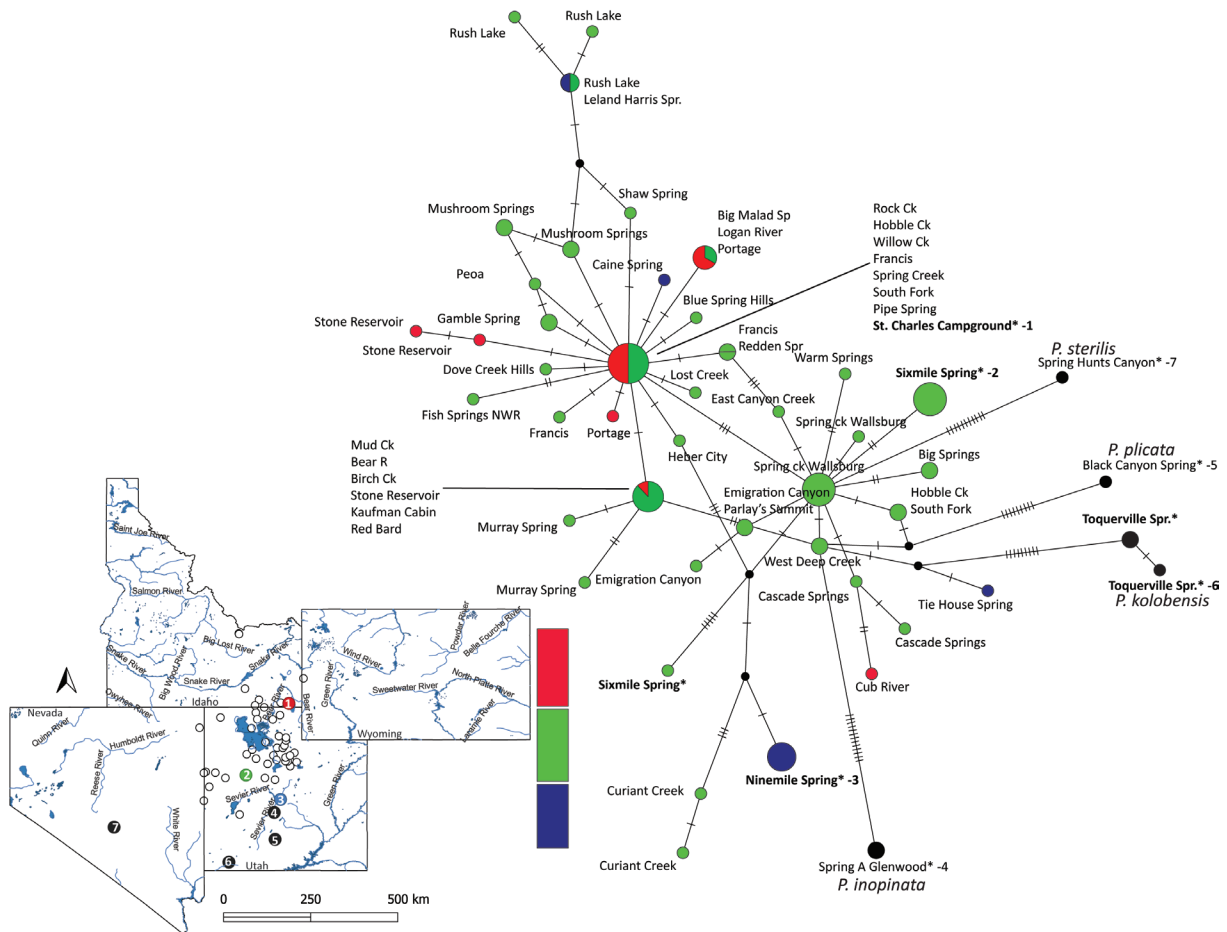


Fig. 5. Haplotype network of the 93-terminal dataset of “Lineage A”, *Pyrgulopsis plicata* Hershler, 1998, *P. inopinata* Hershler, 1998, *P. sterilis* Hershler, 1998, and *P. kolobensis* (Taylor, 1987). Nodes of “Lineage A” haplotypes are color coded according to latitude as indicated on the inset map. Nodes of outgroup taxa are colored black. Type localities are indicated with an *. Map as described in Fig. 1 with key localities indicated by numbers 1–7, also labeled on the haplotype network. The location of all localities can be seen in Appendix 4.

PopART ver. 1.7 was used to create a median joining network from the reduced, 97-terminal COI alignment of Lineage A, *P. sterilis*, *P. plicata*, *P. inopinata*, and *P. kolobensis*. The two haplotypes from near the type locality of *P. pilsbryana* differed by two bases, there was a single haplotype present at the *P. nonaria* type locality, and the haplotypes present at the *P. transversa* type locality differed by 10 bases (1.8% maximum value). Among all the “Lineage A” sequences there was an average of <1% p-distance, with a maximum value of 2.9%. The population at Curiant Creek, UT had the greatest divergence from other Lineage A populations with 1–2.9% divergence from some other haplotypes. The median joining network from PopART ver. 1.7 had 63 segregating sites of which 40 were parsimony-informative (Fig. 5). Individuals of *P. kolobensis*, *P. sterilis*, *P. plicata*, and *P. inopinata* are separated from the Lineage A individuals by 10–13 mutations. All individuals from Ninemile Springs (*P. nonaria*) are closest to those from Curiant Creek, UT. Individuals from Sixmile Springs (*P. transversa*) are found in different clusters of haplotypes among unknown (non-type locality) populations. There is not a consistent pattern of haplotype distribution geographically on the North to South axis examined.

Shell morphometrics

A PCA of the Lineage A populations finds that PC1 explains 38.98% of the variance, PC2 = 21.83%, and PC3 = 8.37% (Fig. 6). This analysis finds the populations representing the three Lineage A nominal species, *P. nonaria*, *P. transversa*, and *P. pilsbryana* are mostly separated but with overlap among them. The four geographically dispersed (non-type locality) populations overlap with the samples representing *P. nonaria* and *P. transversa* as well as images of their types. It is notable that the type of *P. pilsbryana* falls within the 95% convex hull of the St Charles population and the type of *P. transversa* is found among *P. transversa* as well as overlapping several of the unknown populations. The type of *P. nonaria* does not fall within other Ninemile Springs representatives, instead it is within the cloud of the non-type locality populations. A MANOVA finds significant differences in shape among groups (Wilks’ lambda = 0.001933, df1 = 120, df2 = 10.61, $p \leq 0.001$). Bonferroni-corrected pairwise comparisons among groups finds significant differences ($p \leq 0.001$) between the unknown populations and *P. pilsbryana* and *P. transversa* which was also observable from Fig. 6A with those populations mostly not overlapping the other groups. Pairwise comparisons did not find significant differences in shape among the three nominal species. LDA (Fig. 7) separates *P. pilsbryana* and *P. transversa* from the others and has the four populations overlapping with each other and *P. nonaria*. The confusion matrix did not misidentify any individuals based on LD1 and LD2. A jackknifed confusion matrix correctly classified 84.47% of individuals with misclassification among the unknowns and *P. nonaria* (one *P. transversa* individual incorrectly classified as *P. nonaria*).

In the analysis incorporating three closely related species for context, PC1 = 50.51 %, PC2 = 14.6 %, and PC3 = 7.64 % of the variance. MANOVA found a significant result with pairwise comparisons detailing that Lineage A altogether were significantly different from *P. sterilis*, *P. plicata*, and *P. inopinata* (Wilks’ lambda = 0.008597, df1 = 120, df2 = 360.4, $p \leq 0.001$). The unknown populations were significantly different from *P. sterilis*, *P. plicata*, *P. inopinata*, and *P. pilsbyana*; however, there was no difference from *P. nonaria* and *P. transversa* (Wilks’ lambda = 0.000107, df1 = 240, df2 = 704, $p \leq 0.001$). The LDA found separation between Lineage A and the other species with no individuals misclassified. The jackknifed confusion matrix found 94.48% of individuals correctly classified. The jackknifed confusion matrix shows two individuals of Lineage A classified as other species, one as *P. sterilis* and one as *P. plicata*. Several individuals of *P. sterilis* and *P. plicata* were misclassified for each other and only *P. inopinata* was never misclassified.

Radula measurements

Summary measurements for features of the central radular tooth are presented in Tables 1–2 and Fig. 9. Individuals from Big Springs and Vernon Reservoir had the largest central radula cusp measurements

with *P. pilsbryana* measuring the smallest. In comparisons of length, a Wilcoxon/Kruskal-Wallis test found a significant difference among the populations ($\chi^2 = 399.0728$, $df = 4$, $p < 0.0001$). Multiple comparisons using the Steel-Dwass method found most pairs significantly different except for two pairs: *P. transversa* compared to *P. pilsbryana* and Vernon Reservoir compared to Big Springs ($p = 0.07$).

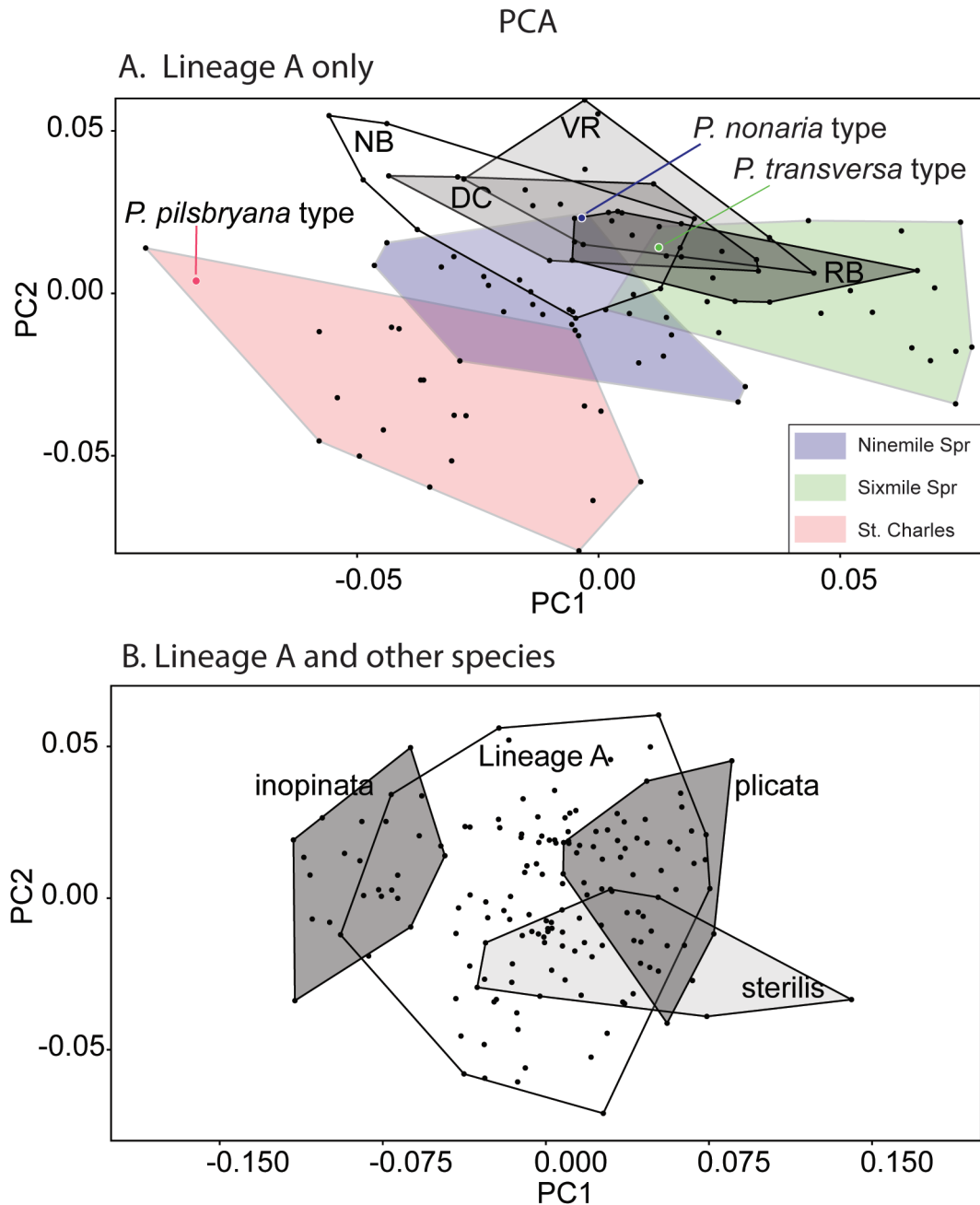


Fig. 6. A. Plot of principal component analysis on *Pyrgulopsis pilsbryana* (Bailey & Bailey, 1952) ($n = 20$), *P. nonaria* Hershler, 1998 ($n = 20$), and *P. transversa* Hershler, 1998 ($n = 20$) shells collected from the type localities as well as four additional localities. The individuals in colored convex hulls are from the type localities of each nominal species. **B.** All Lineage A specimens compared to *P. inopinata* Hershler, 1998 ($n = 20$), *P. plicata* Hershler, 1998 ($n = 20$), and *P. sterilis* Hershler, 1998 ($n = 20$). Abbreviations: DC = Dove Creek Hills; NB = North Beck; RB = Red Barn; VR = Vernon Reservoir.

In comparisons of width, a Wilcoxon/Kruskal-Wallis test found a significant difference among the populations ($\chi^2 = 715.6020$, $df = 4$, $p < 0.0001$). Multiple comparisons using Steel-Dwass method found all pairs significantly different, most pairs $p \leq 0.001$, except pairs with *P. pilsbryana* where $p = 0.02$ – 0.03 . The comparison of the number of cusps in direction one found significant differences overall and among a few pairs: *P. pilsbryana* vs *P. nonaria* ($p = 0.03$), *P. transversa* vs *P. nonaria* ($p \leq 0.001$),

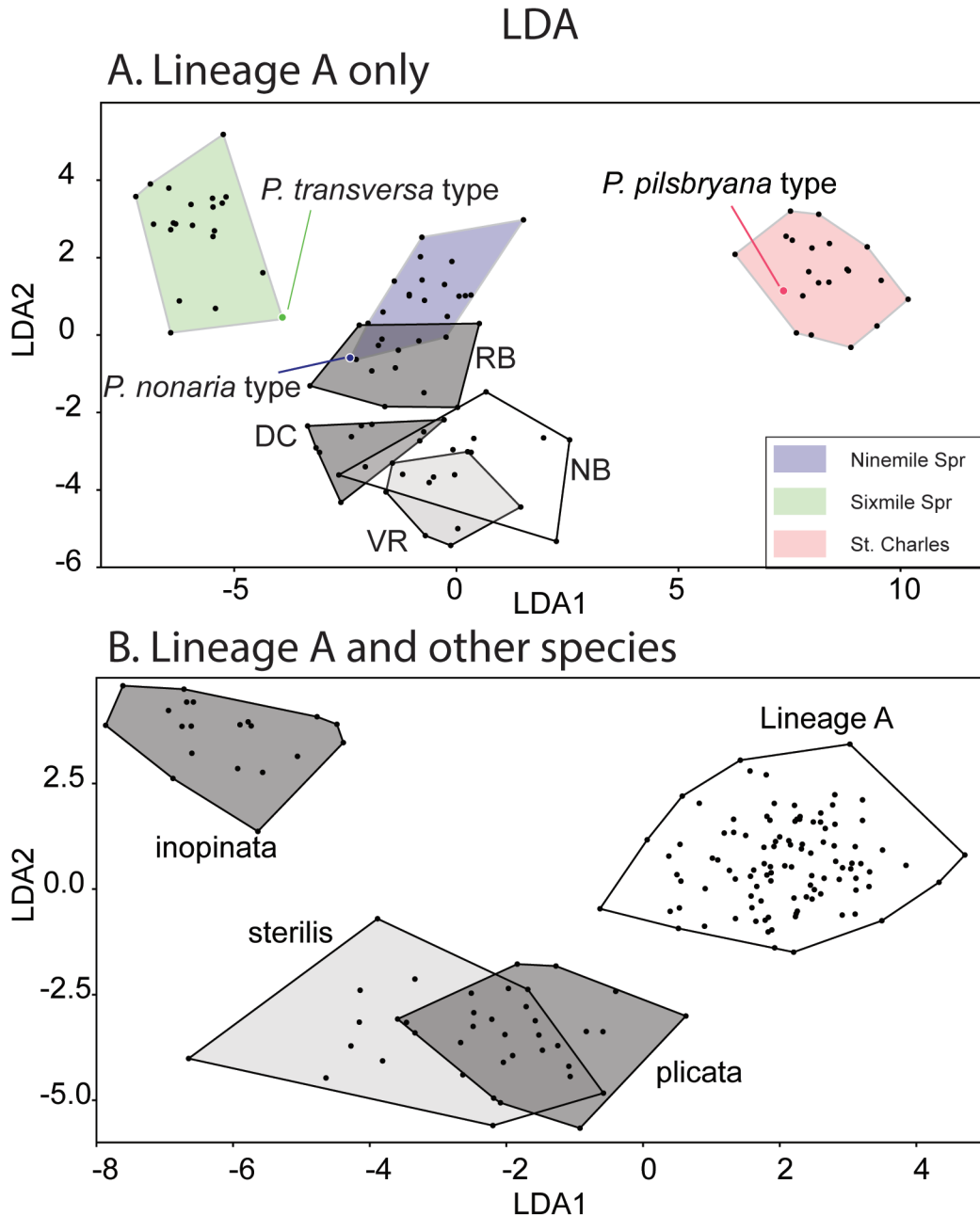


Fig. 7. **A.** Plot of least discriminant analysis on *Pyrgulopsis pilsbryana* (Bailey & Bailey, 1952) ($n = 20$), *P. nonaria* Hershler, 1998 ($n = 20$), and *P. transversa* Hershler, 1998 ($n = 20$) shells collected from the type localities as well as additional localities. The individuals in colored convex hulls are from the type localities of each nominal species. **B.** All Lineage A specimens compared to *P. inopinata* Hershler, 1998 ($n = 20$), *P. plicata* Hershler, 1998 ($n = 20$), and *P. sterilis* Hershler, 1998 ($n = 20$). Abbreviations: DC = Dove Creek Hills, NB = North Beck; RB = Red Barn; VR = Vernon Reservoir.

Table 1. Measurements of central radular tooth (N individuals = number of different individuals examined, N teeth = number of teeth measured) in μm , mean and standard deviation (SD) of central cusp length and width.

Population	N individuals	N teeth	Length mean	Length SD	Width mean	Width SD
NW Lakota (<i>P. pilsbryana</i>)	1	3	2.00	1.00	9.83	0.72
Ninemile (<i>P. nonaria</i>)	6	195	4.95	0.89	2.73	0.64
Sixmile (<i>P. transversa</i>)	6	133	3.62	0.61	1.56	0.27
Vernon	6	228	6.91	1.65	3.32	0.66
Big Springs	7	415	7.10	2.21	4.71	0.88

Table 2. Lateral cusp counts of central radular teeth (number of teeth with cusps visible for counting = N of teeth with cusps counted) in each direction (Lat1 and Lat2). Mean and standard deviation (SD) given.

Population	N of teeth with cusps counted	Mean Lat1	SD Lat1	Mean Lat2	SD Lat2
NW Lakota (<i>P. pilsbryana</i>)	3	6.08	0.07	3.67	0.58
Ninemile (<i>P. nonaria</i>)	195	4.49	1.39	3.80	1.28
Sixmile (<i>P. transversa</i>)	133	5.48	0.81	5.26	1.11
Vernon	8	4.88	1.64	4.88	1.25
Big Springs	6	5.33	0.82	5.83	0.98

and *P. transversa* vs *P. pilsbryana* ($p = 0.03$). The comparison of number of cusps in direction two found significant differences overall and among two pairs: *P. pilsbryana* vs *P. nonaria* ($p \leq 0.001$) and *P. nonaria* vs Big Springs ($p = 0.003$). In summary, populations were significantly different in their central radula cusps length and width, and populations were mostly similar in the number of cusps on the central radular tooth.

Systematic account

Phylum Mollusca Linnaeus, 1758
 Class Gastropoda Cuvier, 1795
 Subclass Caenogastropoda L.R. Cox, 1960
 Order Littorinimorpha A.N. Golikov & Starobogatov, 1975
 Family Hydrobiidae Stimpson, 1865

Genus *Pyrgulopsis* Call & Pilsbry, 1886

Pyrgulopsis Call & Pilsbry, 1886: 9.

Type species

Pyrgulopsis nevadensis (Stearns, 1883).

Remarks

Distinguished anatomically from other hydrobioid genera by small size, ovate to ovate-conic shell and presence of penial glands.

Pyrgulopsis pilsbryana (Baily & Baily, 1952)

Figs 8–11

Paludestrina longinqua Pilsbry, 1899: 122 (in part).

Amnicola pilsbryana Baily & Baily, 1952: 144 [new name for above].

Pyrgulopsis nonaria Hershler, 1998: 125–127, figs 10i, 25g, 48a–c. **Syn. nov.**

Pyrgulopsis transversa Hershler, 1998: 129–130, figs 10j, 25h, 48d–f. **Syn. nov.**

Paludestrina longinqua – Stearns 1901: 285 (in part). — Henderson & Daniels 1917: 58–59. — Henderson 1924: 190; 1931: 110. — Chamberlain & Jones 1929: 177 (in part).

Amnicola pilsbryi – Baily & Baily 1952: 50–51, pl. 4 fig. 3. — Baker 1964: 175.

Amnicola pilsbryana – Taylor 1965: 599.

Fontelicella pilsbryana – Gregg & Taylor 1965: 108. — Taylor 1975: 152. — Burch 1982: 26. — Turgeon *et al.* 1998: 61.

Pyrgulopsis pilsbryi – Hershler & Thompson 1987: 30.

Pyrgulopsis pilsbryana – Hershler 1994: 60, figs 23g–i, 39a, 50b.

Pyrgulopsis pilsbryana “Lineage A” – Liu *et al.* 2018: 107.

Original description

“This species is narrowly umbilicate, conic, smooth, with about four and a half very strongly convex whorls. The suture is deeply impressed. The aperture occupies about 40% of the length and is of a broadly oval shape, noticeably oblique. The columellar margin is slightly expanded. Length 3.0 mm, diameter 1.8 mm.”

Material examined

UNITED STATES OF AMERICA – **Idaho** • Spring, Saint Charles Canyon, Saint Charles Campground; 42.11° N, 111.45° W; 12 May 1990; R. Hershler leg.; USNM 858281. – **Utah** • Vernon Reservoir, Tintic Valley, 0.38 km upstream of reservoir; 39.98356° N, 112.38211° W; 1 Nov. 2022; K. Purington leg.; ANSP 509761 • Big Springs, Tooele Co., UT, 0.35 air km W of Hwy 73 and W Big Springs Street intersection; 40.26159° N, 112.09927° W; 29 Sep. 2022; K. Purington leg.; ANSP 509765 • Blue Spring Hills area, Blue Creek Spring; 41.83215° N, 112.458297° W; 20 Nov. 2022; C. Lundskog leg.; ANSP 509740 • Cold Spring; 39.45514° N, 113.956962° W; 3 Aug. 2022; Kevin Wheeler and Luke Matscheck leg.; ANSP 509745 • Delle Spring, 40.55572° N, 112.73913° W; 8 Feb. 2022; K. Purington leg.; ANSP 509762 • Dove Creek Hills, 41.68214° N, 113.543057° W; 22 Sep. 2022; C. Lundskog leg.; ANSP 509739 • Ninemile Reservoir 1 (Spring along east side of Reservoir), 0.3 air km N of Hwy 89 and Hwy 137 junction, 39.17333° N, 111.70344° W; 7 Nov. 2022; K. Purington leg.; ANSP 509753 • Ninemile Reservoir 2 (south end of reservoir on north side of Hwy 89); 39.16877° N, 111.71576° W; 7 Nov. 2022; K. Purington leg.; ANSP 509754 • North Beck, 39.30384° N, 114.008049° W; 2 Aug. 2022; Kevin Wheeler, Luke Matscheck legs.; ANSP 509743 • Pipe Spring, (near unnamed spring SE Lampo junction), 41.62948° N, 112.415231° W; 20 Nov. 2022; C. Lundskog leg.; ANSP 509741 • Red Barn WMA, 41.10818° N, 111.750344° W; 20 Nov. 2022; C. Lundskog leg.; ANSP 509742 • Sixmile Spring (near Mantai WMA), 0.09 air km SE of Hwy 89; 39.24485° N, 111.64901° W; 7 Nov. 2022;

K. Purington leg.; USNM 509766 • Tintic Valley, McIntyre, 1.0 air km SW of McIntyre Rd and Union Pacific Rd Intersection; 39.83149° N, 112.18005° W; 1 Nov. 2022; K. Purington leg. ANSP 509763 • unnamed spring near Sixmile Springs, 1.65 air km E of Simpson Springs Rd, ~ 500 m NW of Sixmile; 39.91864° N, 112.76211° W; 3 Nov 2022; K. Purington leg.; ANSP 509767 • unnamed spring tributary to Middle Deep Creek at South Ibapah CSF monitoring site; 40.01749° N, 113.99415° W; 10 Mar. 2022; K. Purington leg.; ANSP 509764 • Murray Spring, Cache Valley; 41.6208° N, 111.9419° W; 22 Sep. 2022; C. Lundskog leg.; ANSP 509737 • unnamed spring, northeast of Mushroom Springs, Antelope Island; 40.9256° N, 112.1658° W; 22 Sep. 2022; C. Lundskog leg.; USNM 509738 • Gandy Marsh; 39.48507° N, 113.91688° W; 2 Aug. 2022; Kevin Wheeler and Luke Matscheck legs.; ANSP 509744 • Foote Spring (Bishop); 39.41553, 113.870355° W; 2 Aug. 2022; Kevin Wheeler, Luke Matscheck leg.; ANSP 509746 • Cascade Springs, Provo River drainage; 40.45842° N, 111.54962° W; 21 Sep. 2022; K. Purington leg.; ANSP 509747 • unnamed spring, City Creek Canyon; 40.8255° N, 111.79621° W; 20 Sep. 2022; K. Purington leg.; ANSP 509748 • unnamed spring, north of Heber City, Provo River drainage, 0.25 air km SE of E 1200 N and Hwy 189 intersection, E side of Hwy 189; 40.51965° N, 111.4105° W; 10 Nov. 2022; K. Purington leg.; ANSP 509749 • unnamed spring, South Fork, Provo River drainage (near River); 40.3474° N, 111.55202° W; 21 Sep. 2022; K. Purington leg.; ANSP 509750 • unnamed spring, South Fork, Provo River drainage, (near Spring); 40.34701° N, 111.55191° W; 21 Sep. 2022; K. Purington leg.; ANSP 509751 • unnamed springs, source of Spring Creek, Utah Lake drainage; 40.38348° N, 111.82372° W; 20 Sep. 2022; K. Purington leg.; ANSP 509752 • Clover Creek, at Clover Springs Campground, 0.11 air km W of S Hwy 199; 40.34613° N, 112.55098° W; 24 Oct.

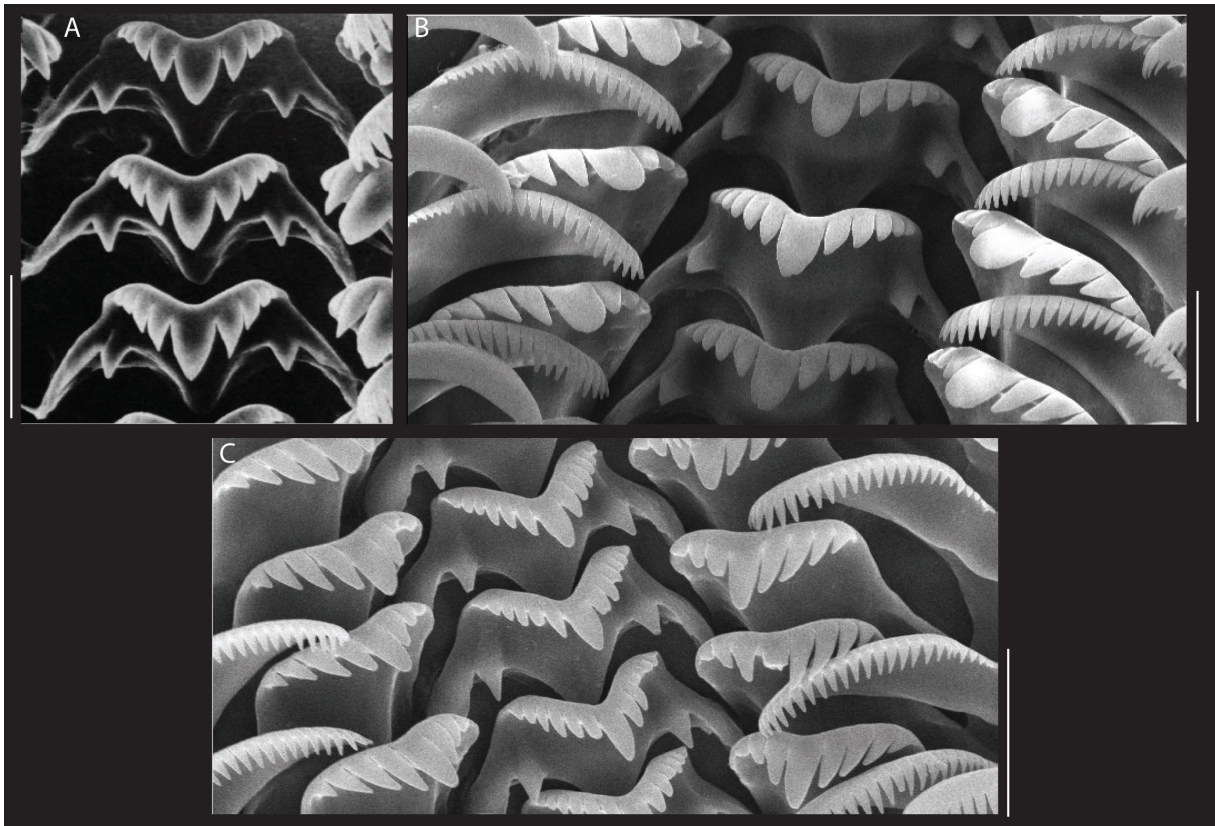


Fig. 8. Radula of individuals from: **A.** Unnamed spring about 0.6 km northwest of Lakota, Rich Co. UT, from redescription of *Pyrgulopsis pilsbryana* (Baily & Baily, 1952) USNM 858279 (Hershler 1994: fig. 29a). **B.** Ninemile Springs (*P. nonaria* Hershler, 1998 type locality). **C.** Sixmile Springs (*P. transversa* Hershler, 1998, type locality). Scale bars = 10 μ m.

2022; K. Purington leg.; ANSP 509755 • Big Springs at Fountain Green Fish Hatchery; 39.63629° N, 111.67309° W; 7 Nov. 2022; K. Purington leg.; ANSP 509756 • Indian Springs, 4.75 km E of Simpson Springs Rd; 39.98514° N, 112.73612° W; 3 Nov. 2022; K. Purington leg.; ANSP 509757 • Lee Creek, 1.75 air km SW of E Lee Creek Rd; 39.98786° N, 112.69083° W; 3 Nov. 2022; K. Purington leg.; ANSP 509758 • Thistle Creek, Peaceful Creek Ranch; 39.87572° N, 111.53957° W; 4 Oct. 2022; K. Purington leg.; ANSP 509759 • unnamed spring ~500 m E of Mud Spring, 2.4 air km WNW of Black Crook Peak; 39.98438° N, 112.57024° W; 1 Nov. 2022; K. Purington leg.; ANSP 509760.

Redescription

Shell small to medium-sized, ovate to narrowly conical (Fig. 10); height, 2.7–5 mm; width, 1.3–2.2 mm, whorls, 4.25–5.25. Protoconch mostly smooth with weak wrinkles at apex or slight spiral lines. Teleoconch whorls convex, shoulders vary from weak to strong; body whorl often separated from the aperture in the largest shells. Aperture ovate, inner lip slightly thickened to thick, with or without

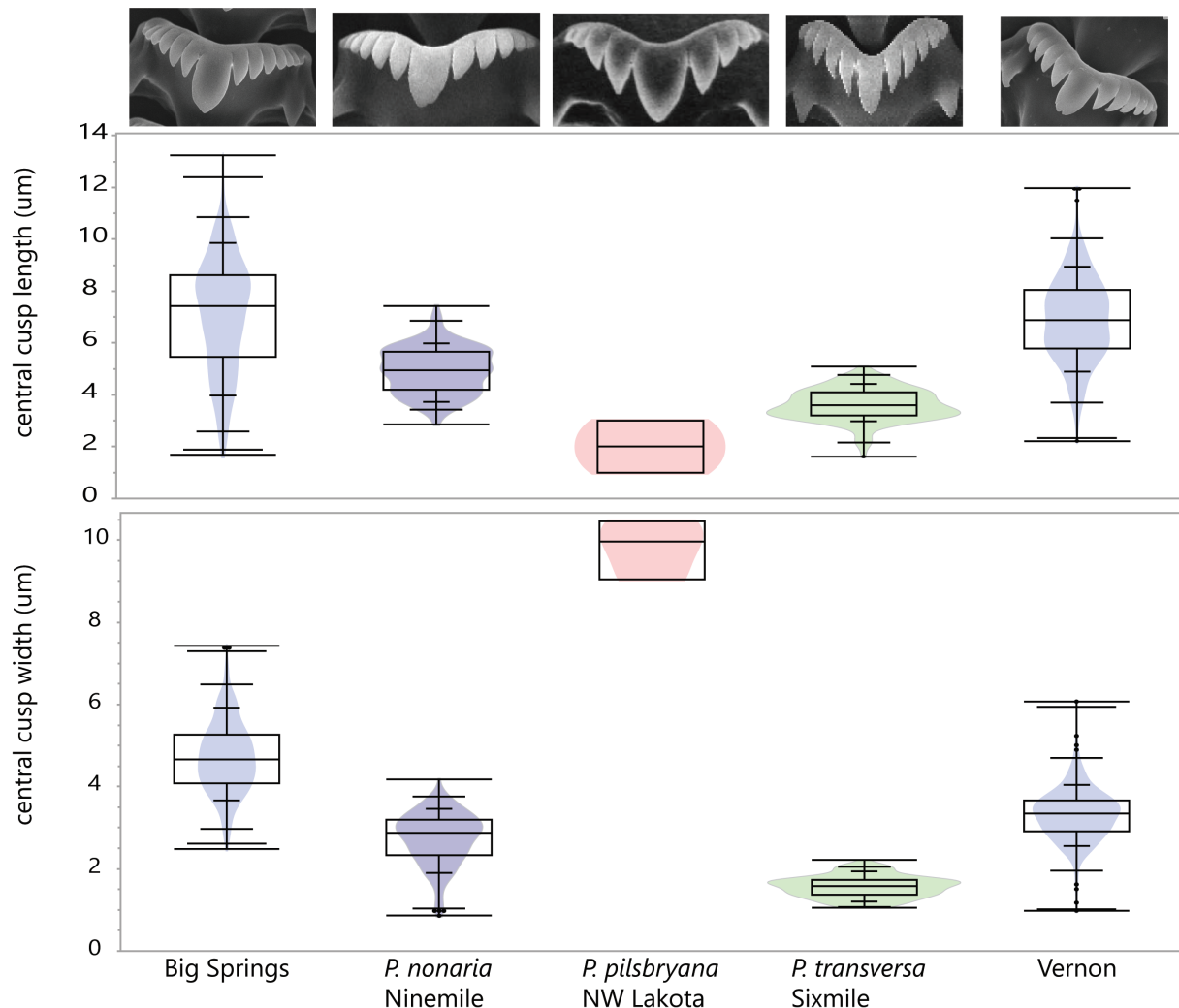


Fig. 9. Violin plot with box plot (quantiles) showing distribution of measurements of the length and width of the central radula cusp for populations from Ninemile Springs (six individuals, 195 teeth), Sixmile Springs (six individuals, 133 teeth), Vernon Reservoir (six individuals, 239 teeth), and Big Springs (seven individuals, 422 teeth). A Wilcoxon rank sum test found these populations were significantly different ($p \leq 0.001$) in this measurement.

columellar shelf, columellar lip slightly reflected, outer lip thin and slightly prosocline. Umbilicus rimate to shallowly perforate. Periostracum brown to light brown, or tan. Often periostracum is missing and spire is variously pitted and eroded.

Operculum amber to dark amber, ovate, eccentric nucleus sometimes reddish in color, transparent surface; dorsal surface frilled. Attachment scar margin sometimes thickened along inner edge or all around, callus weak. Central tooth 26–32 μm wide, with indented to highly indented dorsal edge, medium to largely; lateral cusps, 4–7; central cusp wide or narrow, long to short, longer than laterals; sometimes notched at the distal end, shovel to dagger-like; basal cusps small to medium-sized (Figs 8–9). Basal tongue broadly V-shaped, basal sockets are medium depth. Lateral margins thickened, neck weak. Inner marginal teeth with 20–33 cusps; outer marginal teeth with 28–39 cusps.

Cephalic tentacles, snout, foot unpigmented, light brown, to brown-gray. Opercular lobe dark all around. Neck pigmented with dark granules. Pallial roof and visceral coil brown to black, sometimes extending to the entire animal. Penial filament unpigmented to lightly on proximal half to completely pigmented. Ctenidium of medium height and width, filaments 16–23, overlapping pericardium; osphradium narrow, small, positioned from posterior to central portion of the ctenidium.

Ovary with 0.5–1.0 whorl, overlapping posterior stomach chamber. Albumen gland extent into pallial cavity variable from none to long (0–25%). Capsule gland equal in length to the albumen gland or shorter and narrow; ovate to subglobose in section, with a weak to medium depth rectal furrow. Ventral channel overlaps the capsule gland, well-developed longitudinal fold. Genital aperture subterminal or

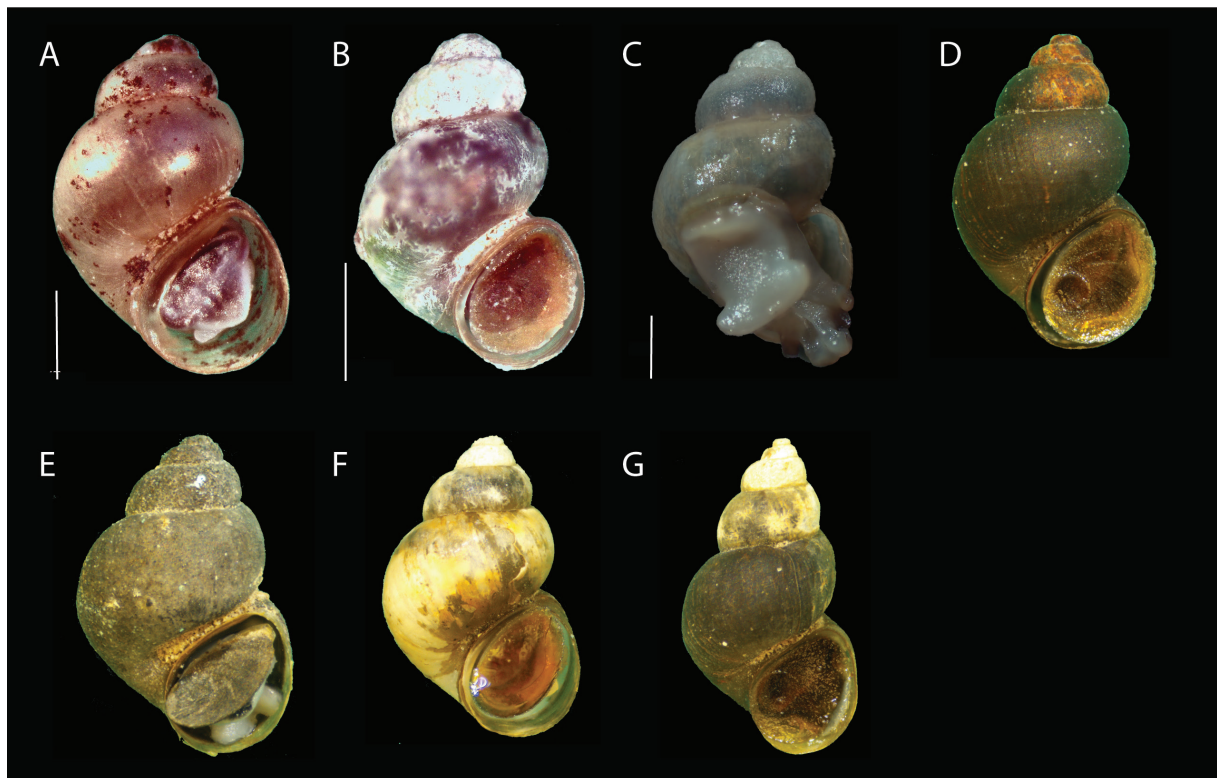


Fig. 10. Shells of *Pyrgulopsis pilsbryana* (Baily & Baily, 1952). **A.** OR363192, DNA 4383, Sixmile Springs. **B.** OR363185, OR365628, DNA 4374, Ninemile Spring. **C.** St Charles Campground. **D.** Dove Creek Hills. **E.** Vernon Reservoir. **F.** Red Barn WMA. **G.** North Beck. For complete locality details and USNM numbers see Appendix 1. Scale bars = 0.5 mm.

terminal pore or slit, sometimes weakly raised with or without a short anterior extension. Coiled oviduct a posterior loop sometimes with a weak twist, small coil, or horizontal loop slightly behind pallial wall. Oviduct and bursa duct merge just behind wall of pallial cavity. Bursa copulatrix medium in length and width, ovate-elongate to subglobular, longitudinal with 33–80% of the length posterior to the gland, anterior portion slightly overlapped by gland. Bursa duct originating from anterior edge or slightly lateral to the midline, narrow to medium width and medium length, embedded shallowly in albumen gland, the same length as bursa copulatrix. Seminal receptacle pouch or sac-like, small to medium-sized, narrow, short, overlapping proximal bursal duct.

Testis 1.5–2 whorls, filling half to nearly all of digestive gland behind the stomach, overlapping both chambers of stomach to the edge of the style sac. Prostate gland small, bean-shaped, ovate in section, entirely visceral or with short section in pallial cavity; pallial portion of vas deferens undulating or with distinctive bend or loop at proximal end. Penis large (Fig. 11); with an elongate-rectangular base, smooth; filament tapered and slightly to darkly pigmented, filament varies from short (Fig. 11D) and narrow to medium length and elongate (Fig. 11A); penial lobe short to medium and distally tapered

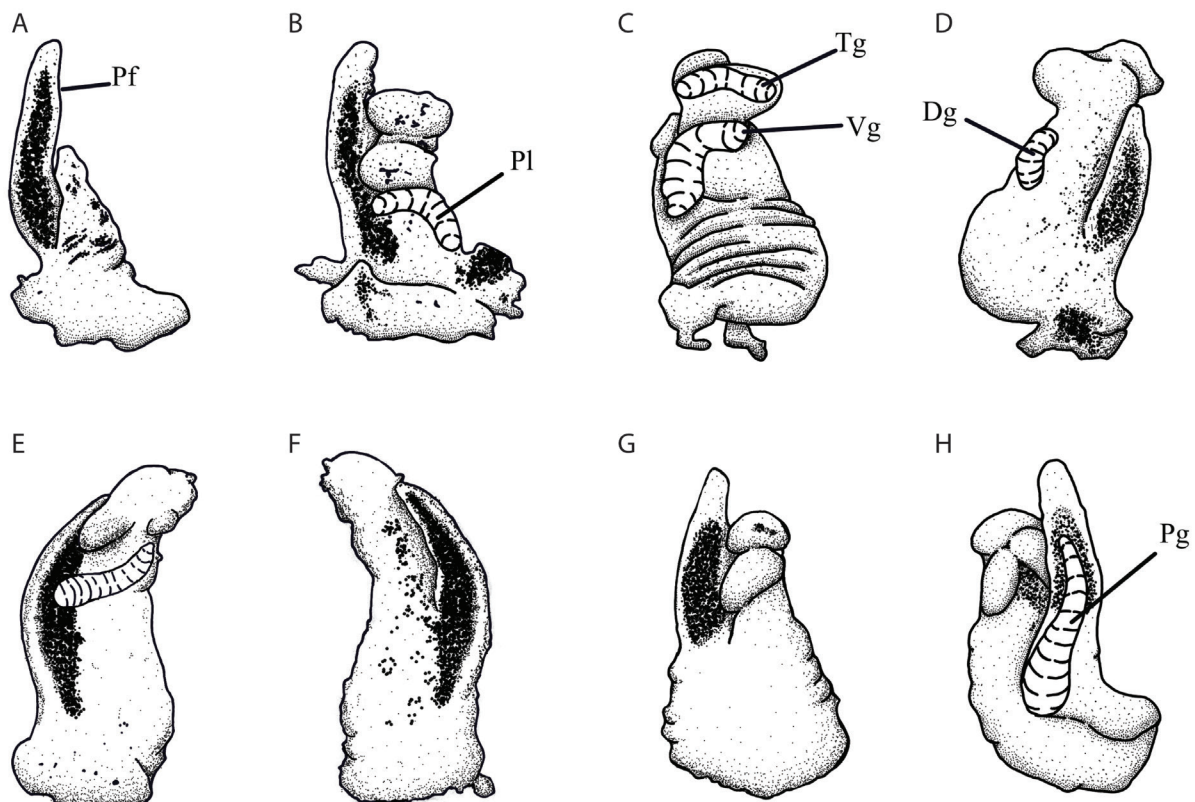


Fig. 11. Variation in penial morphology in *Pyrgulopsis pilsbryana* (Bailey & Bailey, 1952). **A.** Ventral aspect of individual resembling description of *P. transversa* Hershler, 1998, OR363193, OR365631, DNA 4385, Sixmile Spring. **B.** Ventral aspect of individual resembling description of *P. nonaria* Hershler, 1998 morphology, OR363185, OR365628, DNA 4374, Ninemile Spring. **C–D.** Same individual, ventral and dorsal view, OR363198, OR365616, DNA 4436, Blue Spring Hills. **E–F.** Same individual, ventral and dorsal view, OR365619, DNA 4433, Dove Creek Hills. **G–H.** Same individual, ventral and dorsal view, DNA 4414, Middle Deep Creek, morphology with features of both *P. transversa* and *P. pilsbryana*. Abbreviations: Dg = dorsal gland; Pf = penial filament; Pg = penial gland; Pl = penial lobe; Tg = terminal gland; Vg = ventral gland. Illustrations by Kevin Alanis.

or only slightly narrower than base. Penial glands vary in their presence and position. Terminal gland, if present, may be small to large, transverse, slightly curved, and largely borne on the ventral surface. Terminal gland sometimes split into two (Fig. 11D). Ventral gland (if present) small to large, narrow or circular-ovate, transverse, positioned near base of lobe on low swelling. In some individuals, additional glands at the base of the filament have been observed and on the dorsal surface of the penial lobe. Penial duct straight, near outer edge.

Distribution

Distribution confirmed here with DNA sequence data includes USA: Utah, Idaho, Wyoming, and Nevada. The Material examined (above and Appendix 1) lists the localities where *P. pilsbryana* has been confirmed present.

Taxonomic remarks

The source population of the holotype and paratypes described as *P. pilsbryana* is uncertain. This species was described from dry shells found in beach drift (Baily & Baily 1952) as *Amnicola pilsbryi* from Lifton, ID. The description of the travels on which the types were collected includes “on the shores of Bear Lake, Lifton Idaho at the North End...” and two sites were listed as “Lifton; Ideal Beach”. In the description of their collecting trip (Baily 1950), the author described collecting drift shells at these two beach sites in the north and south ends of the lake. The description of spring-dwelling snails from beach drift (dry shells) makes it impossible to precisely identify the spring that is the source of the shells found by Baily & Baily (1952). There are numerous springs in the area that drain into Bear Lake and there are several named species and unnamed lineages of *Pyrgulopsis* found near the lake. In our attempts to sample representatives of *P. pilsbryana*, we collected these specimens at Swan Springs and Jacobson Springs, both within 20 km of the type locality, resulting in collection of *Colligyrus greggi* (Pilsbry, 1935) and *Pyrgulopsis* Lineage D (Liu *et al.* 2018), making selection of material to represent *P. pilsbryana* challenging but an adequate substitute must be used to consider the identity of this species.

The first description and figures of the internal anatomy of *P. pilsbryana* (Hershler 1994), used snails sampled from “an unnamed spring about 0.6 km NW of Lakota”. This spring is about 18 km from the type locality on the west side of the lake and based on the coordinates, was likely called “Little Spring Stream” (41.985174° N, 111.413132° W). Unfortunately, we did not find springsnails at this site and material could not be incorporated into this study. For the phylogenetic study that recommended synonymy of *P. pilsbryana* (Liu *et al.* 2018), the samples representing this species were collected from “Spring, Saint Charles Campground, Bear Lake Co., ID”, approximately 13 km from the type locality and on the north end of the lake. As this locality is the closest to the type locality and could result in shells being washed to the beach at Lifton, we took the snails from St Charles Campground as a better likely representative of *P. pilsbryana*. In addition, we examined this lot (USNM 858281) and some individuals from this lot strongly resemble the shell morphology of the holotype and paratypes of this species. Finally, in the morphometric analysis individuals from this population fall within the 95% ellipse with the type of *P. pilsbryana*. It remains possible that another spring in the vicinity hosts “true *pilsbryana*” but we consider these samples to be the best representative of *P. pilsbryana* and the likely source of beach drift shells described by Baily & Baily (1952).

Pyrgulopsis nonaria and *P. transversa* were described (Hershler 1998) and distinguished from *P. pilsbryana* largely on the basis of penial ornamentation, a feature that the same author (Liu *et al.* 2018) later came to realize had greater variability across individuals and populations than initially understood. In Table 3, we summarize the lines of evidence we have assembled to determine if there is one or multiple distinct biological units in this group. We conclude from the majority of this evidence that there is one unit of diversity and by priority it should retain the name *P. pilsbryana*.

Discussion

We have examined a variety of sources of evidence to determine if *P. pilsbryana*, *P. nonaria*, and *P. transversa* represent three distinct biological entities. While there are unique features of each population, the weight of this evidence leads us to conclude that there is a single, widespread, biological entity which should retain the name with priority, *P. pilsbryana*. Below, we elaborate on the evidence and reasoning leading to this conclusion.

In both mitochondrial and nuclear phylogenies, individuals from the type (or near type) localities of *P. pilsbryana*, *P. nonaria*, *P. transversa*, and additional unknown (non-type locality) populations form a single well-supported (100% bootstrap support) shallow clade, supporting the synonymy of these three species. Individuals from the type localities of *P. pilsbryana* and *P. transversa* were distributed in different parts of the COI tree, and individuals from the type locality of *P. nonaria* were in different clades in the LSU tree. The individuals from Ninemile Springs (*P. nonaria* type locality) were very similar or identical in their mitochondrial COI sequence. The widespread distribution of this species, compared to most *Pyrgulopsis*, indicates a relatively high potential for gene flow among populations and this seems supported by the lack of deep geographic structure across the phylogeny (Figs 3–4) and haplotype network (Fig. 5). Some populations are nearly identical in sequence, but haplotypes belonging to this species are scattered across its' range.

In addition to examination of phylogenetic relationships, we can also consider the divergence in mitochondrial sequence in comparison with other *Pyrgulopsis* species. In a previous meta-analysis (666 COI sequences, 122 recognized species), Liu *et al.* (2018) determined that in 95% of comparisons of *Pyrgulopsis* the intraspecific divergence was less than 3.2% and interspecific divergence was greater than 4.4%. If we compare our taxa of interest to these general guidelines, we found that in COI, topotypic (or near) individuals of *P. pilsbryana*, *P. nonaria*, and *P. transversa* all differed from each other by an average of 0.9%, which is well within the range of intraspecific not species-level divergence. The most divergent lineages in this clade (Rush Lake compared to Curiant Creek) had a maximum p-distance of 3% which is within the range of intraspecific variation but approaching the divergence between Lineage A and the outgroups. We note that the Rush Lake population harbored several haplotypes including one typical of the type locality of *P. nonaria*.

To summarize the DNA data, with wider geographical sampling and adding nuclear data, we support the conclusion of Liu *et al.* (2018) that the three target taxa represent a single species. However, we note that other *Pyrgulopsis* taxa that are closely related to this clade are also not highly divergent, averaging 3% genetic distance, but with haplotypes that are several mutational steps different from the others sampled. The outgroup taxa (*P. kolobensis*, *P. sterilis*, *P. inopinata*, and *P. plicata*) are all currently represented by single populations in our dataset. It is possible that intensive sampling, like that conducted to examine these three focal species would find intermediate haplotypes among the outgroup taxa. This suggests that additional exploration of the support for species-level status of other related species of *Pyrgulopsis* is warranted.

The shells of the target taxa were not described as having distinctive or diagnostic features and are hardly distinguishable from other species of *Pyrgulopsis* by shell features. This overall impression of similar shell shapes was borne out by geometric morphometric analysis that included the three target taxa and four geographically separated populations. This analysis found that individuals from some populations such as St Charles and Sixmile Springs are somewhat distinctive in shape, which is partially what led to their initial delineation as species. In contrast, we also found that they are not hugely different in shape, either with some overlap or very similar shapes in the PCA (but separable by LDA). For example, we found that the individuals from St Charles (representing *P. pilsbryana*) had a more elongate spire than individuals from Sixmile Springs (representing *P. transversa*), in which the spire is shorter relative to

the body whorl. If you select a few individuals from some populations, they appear distinctive, but the apparent difference does not hold up when you examine a larger number of individuals.

To evaluate the utility of overall shell shape for taxonomic work among *Pyrgulopsis* we compared all “Lineage A” morphometric samples with more distantly related species of *Pyrgulopsis*. This analysis found that Lineage A is more variable in shape than the species of *Pyrgulopsis* that were compared. This result is not surprising because the other species of *Pyrgulopsis* are all single-site endemics compared to seven geographically separated populations we examined from Lineage A. We also found that Lineage A had a great deal of overlap in shape (PCA) with *P. plicata*, *P. sterilis*, and even *P. inopinata*, the most morphologically distinct, although they were separable in the LDA. We conclude from the examination of shell morphometrics that while there are some populations that are more distinct in shell shape, they seem part of a continuum of variation in shell shape, rather than diagnosably different in shell shape, when we include additional populations from a wider geographic range.

Species descriptions of *Pyrgulopsis* typically figure and describe the radula (e.g., Hershler 1994, 1998); however, radula features are rarely included in the differential diagnosis. Moreover, the diagnostic value of radular features as diagnostic species-level characteristics have not been carefully examined in the group. In this study, we compared several aspects of the central radular tooth across five populations including multiple individuals and numerous teeth. We found that nearly every population had significantly different measurements and counts. Nearly all populations were significantly different in central cusp length and width (of the central radular tooth), the least distinguishable was *P. pilsbryana*. We found that individuals representing *P. pilsbryana* had a central cusp of the central radular tooth that was short and wide compared to Ninemile Springs (*P. nonaria*) and Big Springs, which were long and wide, or *P. transversa* which were short and narrow.

While radula features were not included in the differential diagnoses for the focal species (*P. pilsbryana*, *P. transversa*, and *P. nonaria*), they were noted to differ in central cusp length and width (Hershler 1998). The qualitative observation that these populations differ in central cusp length and width was supported by our quantitative analysis with expanded sampling. Indeed, nearly every individual differs in central cusp length and width. When we compare the radula of the focal taxa with other related species (e.g., *P. inopinata*, *P. sterilis*, and *P. plicata*), the descriptions (these were not figured when described) do not allow us to distinguish among species. Our result that each population of nearly genetically identical individuals significantly differed in these teeth measurements, casts extreme doubt on the utility of this feature as a species-level diagnostic character.

The three target taxa in this study were originally diagnosed as distinct by the size, position, and number of penial glands. We set out to test the utility of these features by dissecting and characterizing the penial morphology prior to DNA sequencing. They were characterized as conforming to the description of *P. pilsbryana*, *P. nonaria*, or *P. transversa*. We were not able to reach our ideal of characterizing every individual prior to sequencing because some populations were predominantly female (>20:1) and we did not have sufficient material to sequence all males. After dissection of 20+ individuals we proceeded with DNA work on as many males as we had encountered. The penial morphology of males encountered (Fig. 11) was characterized by the diagnostic criteria used in the original species descriptions or first description of their anatomy (Hershler 1994, 1998) and what was observed casts doubt on the diagnostic utility of these subtle features of penial ornamentation. The features of long, medium, and small penial filament and one vs. two terminal glands do not appear to be aligned with evolutionary lineages (Figs 3–4). On the phylogenies (Figs 3–4) the penial characterization is indicated by symbols which are scattered across the Lineage A clade, not forming monophyletic groups by penial morphology. As an example, Ninemile Spring, the type locality of *P. nonaria*, included individuals identified by penial morphology as diagnostic of *P. nonaria*, *P. transversa*, and *P. pilsbryana*, all with nearly identical

mitochondrial DNA sequences. In both mitochondrial and nuclear DNA phylogenies we found closely related haplotypes with the “diagnostic” penial morphologies of more than one of the three target taxa. While other features of penial morphology may be useful taxonomically, we conclude that the length of the penial filament and number of terminal glands do not serve as accurate species-level diagnostic features in this species.

In this study, we have examined various lines of evidence for their application to the species status of *P. pilsbryana*, *P. nonaria*, and *P. transversa* and considered their taxonomic utility more broadly in the delineation of species of *Pyrgulopsis*. The newly delineated *P. pilsbryana* is one of the few species of *Pyrgulopsis* that is relatively widespread (>20 populations up to 600 km distant) rather than restricted to a single population. This relatively widespread species when formally re-evaluated will likely have a much lower conservation priority. We also found that some populations have a significantly different shell or central radular tooth shape. The fact that either mitochondrial or some anatomical features, when examined in isolation, can distinguish populations leads us to urge caution in the taxonomy in this specific group. A single “diagnostic” feature could potentially be misleading about species-level status, especially if approaching from the assumption that species are likely to be single-site endemics. Examination of multiple populations of a species expanded the scope of what should be considered a single species of *Pyrgulopsis*. Re-consideration of the species-level status of many species in the “*P. kolobensis*” species complex will be required to accurately discern the diversity of this group in the region.

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Appendices

See the next pages.

Appendix 1 (continued on next five pages). Material examined.

Source of Data	Species	Code	COI GenBank No.	LSU GenBank No.	State	County	Locality	Vouchers (USNM)
Liu <i>et al.</i> 2018	<i>P. kolobensis</i>	Tq1, 2, 3	AY485532–33, AY627939		UT	Washington	*Toquerville Springs, Washington Co., UT	USNM 894877
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	P159A	EU700475		ID	Caribou	Bear River, Black Canyon, Caribou Co., ID	USNM 904951
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	PK53A, C	MG721140–41		ID	Franklin	Bear River, Red Point, Franklin Co., ID (42.2341° N, 111.7599° W)	USNM 905383
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	P162A	AY379448		ID	Oneida	Big Malad Spring, Malad Valley, Oneida Co., ID	USNM 1003673
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	P139A, B, E	EU700472, AY426369–70		ID	Lemhi	Birch Creek, Mud Creek, Lemhi Co., ID (44.2533° N, 112.9827° W)	USNM 905287
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	P186A, B, D	AY426344–45, MG748594		ID	Power	East Fork Rock Creek, Power Co., ID (42.5627° N, 112.7912° W)	USNM 905313
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	PK30A	MG721102		ID	Bannock	Heart Mountain Spring, Bannock Co., ID (42.3294° N, 111.9314° W)	USNM 1193688
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	P137B, C	AY426363–64		ID	Bear Lake	Spring, Saint Charles Campground, Bear Lake Co., ID	USNM 905389
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	P163A, B	EU700477, MG748595		ID	Lemhi	Springs at Kaufman Cabin, Lemhi Co., ID (44.2333° N, 112.9747° W)	USNM 1003727
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	PK34A	MG721109		ID	Oneida	Twin Springs, Curlew Valley, Oneida Co., ID (42.2631° N, 112.765° W)	USNM 1193694
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	PK35A, B, C, D	MG721110–13		ID	Oneida	Unnamed springs, southeast of Stone Reservoir, Curlew Valley, Oneida Co., ID (42.0522° N, 112.4839° W)	USNM 1193695
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	PK39A, C	MG721118–19		NV	Elko	Gamble Spring, Thousand Springs drainage, Elko Co., NV (41.3692° N, 114.1858° W)	USNM 1207928
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	PK50A, B	MG721136–37		NV	White Pine	Unnamed spring, south of Caine Spring, Snake Valley, White Pine Co., NV (39.13336° N, 114.05244° W)	USNM 1265757
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	PK52A	MG721139		NV	White Pine	Unnamed springs, West Deep Creek, White Pine Co., NV (39.98342° N, 114.05158° W)	USNM 1265763
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>		EU700470		UT	Tooele	*Sixmile Springs, Old River Bed, Tooele Co., UT	USNM 894885
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>		EU700467		UT	San Pete	*Spring east of Ninemile Reservoir, Sevier River drainage, San Pete Co., UT	USNM 894846
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	PK20A, B	MG721085–86		UT	Summit	Beard Spring, Weber River drainage, Summit Co., UT (40.9797° N, 111.5381° W)	USNM 1193674

Appendix 1 (continued). Material examined.

Source of Data	Species	Code	COI GenBank No.	LSU GenBank No.	State	County	Locality	Vouchers (USNM)
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk16A, B	MG721077–78		UT	Wasatch	Cascade Springs, Provo River drainage, Wasatch Co., UT (40.4586° N, 111.5478° W)	USNM 1193669
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk43A	MG721126		UT	Juab	Cherry Creek, north of road at stream crossing, Juab Co., UT (39.7706° N, 112.4192° W)	USNM 1235860
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk22C	MG721089		UT	Weber	Chicken Springs, Ogden River drainage, Weber Co., UT (41.3275° N, 111.8794° W)	USNM 1193677
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk19A	MG721084		UT	Morgan	Dixie Spring (outflow), Weber River drainage, Morgan Co., UT (40.9381° N, 111.5608° W)	USNM 1193673
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk49A	MG721135		UT	Juab	Leland Harris Springs, Snake Valley, Juab Co., UT (39.5611° N, 113.8869° W)	USNM 1265755
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk28A, D	MG721099–1100		UT	Cache	Logan River, backwater pond, Cache Co., UT (41.7536° N, 111.72° W)	USNM 1193685
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk21A, C	MG721087–88		UT	Morgan	Lost Creek, Weber River drainage, Morgan Co., UT (41.0656° N, 111.5353° W)	USNM 1193675
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk26A, D	MG721095–96		UT	Cache	Murray Spring, Cache Valley, Cache Co., UT (41.6208° N, 111.9419° W)	USNM 1193683
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk48A	MG721134		UT	Millard	Painter Spring, Tule Valley, Millard Co., UT (39.18969° N, 113.44231° W)	USNM 1265751
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk37A	MG721114		UT	Tooele	Redden Springs, Tooele Co., UT (39.9925° N, 113.7003° W)	USNM 1207924
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk40A	MG721120		UT	Box Elder	Shaw Spring, Box Elder Co., UT (41.3568° N, 112.4269° W)	USNM 1207938
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Kol4A	KT248038		UT	Summit	Spring at Peoa, Summit Co., UT	USNM 1193671
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Kol2A, B, E	KT248034–36		UT	Wasatch	Spring Creek, Wallsburg, Wasatch Co., UT	USNM 1193666
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Kol5A, B, C, E	KT248039–42		UT	Summit	Spring southwest of Francis, Summit Co., UT	USNM 1193681
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Kol1A, B	KT248032–33		UT	Utah	Spring, Right Fork Hobbble Creek, Utah Co., UT	USNM 1193663
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Kol3A	KT248037		UT	Wasatch	Spring, Willow Creek, Wasatch Co., UT	USNM 1193666
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk46A	MG721132		UT	Millard	Tie House Spring, Beaver River drainage, Millard Co., UT (38.7119° N, 112.955° W)	USNM 1235868

Appendix 1 (continued). Material examined.

Source of Data	Species	Code	COI GenBank No.	LSU GenBank No.	State	County	Locality	Vouchers (USNM)
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk24A	MG721092		UT	Salt Lake	Unnamed spring, City Creek Canyon, Salt Lake Co., UT (40.8261° N, 111.7958° W)	USNM 1193679
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk18A, C	MG721082–83		UT	Morgan	Unnamed spring, East Canyon Creek, Weber River drainage, Morgan Co., UT (40.8211° N, 111.5828° W)	USNM 1193672
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk23A	MG721091		UT	Rich	Unnamed spring, Home Canyon, Bear River drainage, Rich Co., UT (41.4161° N, 111.2219° W)	USNM 1193678
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk47A	MG721133		UT	Juab	Unnamed spring, Mount Laird, Juab Co., UT (39.57422° N, 113.05239° W)	USNM 1265748
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk12A, B	MG721070–71		UT	Salt Lake	Unnamed spring, mouth of Emigration Canyon, Salt Lake Co., UT (40.7533° N, 111.8064° W)	USNM 1193661
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk17A, C, D	MG721079–81		UT	Wasatch	Unnamed spring, north of Heber City, Provo River drainage, Wasatch Co., UT (40.5169° N, 111.4108° W)	USNM 1193670
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk10A, B, C	MG721065–67		UT	Davis	Unnamed spring, northeast of Mushroom Springs, Antelope Island, Davis Co., UT (40.9256° N, 112.1658° W)	USNM 1193659
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk41A, B	MG721121–22		UT	Tooele	Unnamed spring, northwest of Warm Springs, at mineral development, Tooele Co., UT (40.6978° N, 112.5686° W)	USNM 1235850
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk15A	MG721076		UT	Salt Lake	Unnamed spring, Parleys Summit, Salt Lake Co., UT (40.7486° N, 111.6331° W)	USNM 1193667
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk42A, B, C	MG721123–25		UT	Tooele	Unnamed spring, Rush Valley, Tooele Co., UT (40.4214° N, 112.4519° W)	USNM 1235857
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk51A	MG721138		UT	Juab	Unnamed spring, south end of Fish Springs National Wildlife Refuge, Juab Co., UT (39.83208° N, 113.392° W)	USNM 1265758
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk14A, B	MG721074–75		UT	Utah	Unnamed spring, South Fork, Provo River drainage, Utah Co., UT (40.3469° N, 111.5514° W)	USNM 1193664
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk11A, C	MG721068–69		UT	Salt Lake	Unnamed spring, tributary to Jordan River, Riverton, Salt Lake Co., UT (40.5208° N, 111.9222° W)	USNM 1193660
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk29B	MG721101		UT	Cache	Unnamed spring, upper Cub River, Cache Co., UT (42.1372° N, 111.6983° W)	USNM 1193686
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	Pk44A, B	MG721127–28		UT	Juab	Unnamed spring, Curiant Creek, Juab Valley, Juab Co., UT (39.7772° N, 111.8719° W)	USNM 1235864

Appendix 1 (continued). Material examined.

Source of Data	Species	Code	COI GenBank No.	LSU GenBank No.	State	County	Locality	Vouchers (USNM)
This study	<i>P. pilsbryana</i>	4378	OR363188		UT	Emery	Ninemile Reservoir 1 (Spring along east side of Reservoir), .3 air km N of Hwy 89 and Hwy 137 junction	ANSP 509753
This study	<i>P. pilsbryana</i>	4379	OR363189		UT	Emery	Ninemile Reservoir 1 (Spring along east side of Reservoir), .3 air km N of Hwy 89 and Hwy 137 junction	ANSP 509753
This study	<i>P. pilsbryana</i>	4380	OR363187		UT	Emery	Ninemile Reservoir 1 (Spring along east side of Reservoir), .3 air km N of Hwy 89 and Hwy 137 junction	ANSP 509753
This study	<i>P. pilsbryana</i>				UT	Emery	Ninemile Reservoir 1 (Spring along east side of Reservoir), .3 air km N of Hwy 89 and Hwy 137 junction	ANSP 509753
This study	<i>P. pilsbryana</i>	4452		OR365618	UT	Millard	North Beck	ANSP 509743
This study	<i>P. pilsbryana</i>				UT	Millard	North Beck	ANSP 509743
This study	<i>P. pilsbryana</i>	4437	OR363201		UT	Box Elder	Pipe Spring: 146875: 121407818 (near unnamed spring SE lampo junction)	ANSP 509741
This study	<i>P. pilsbryana</i>	4438		OR365626	UT	Box Elder	Pipe Spring: 146875: 121407818 (near unnamed spring SE lampo junction)	ANSP 509741
This study	<i>P. pilsbryana</i>	4440	OR363199		UT	Morgan	Red Barn WMA	ANSP 509742
This study	<i>P. pilsbryana</i>				UT	Morgan	Red Barn WMA	ANSP 509742
This study	<i>P. pilsbryana</i>	4418	OR363197		UT	Sanpete	Sixmile Spring (near Mantai WMA), .09 air km SE of Hwy 89	ANSP 509766
This study	<i>P. pilsbryana</i>	4411	OR363207		UT	Juab	Tintic Valley, McIntyre, 1.0 air km SW of McIntyre Rd and Union Pacific Rd Intersection	ANSP 509763
This study	<i>P. pilsbryana</i>	4412	OR363208		UT	Juab	Tintic Valley, McIntyre, 1.0 air km SW of McIntyre Rd and Union Pacific Rd Intersection	ANSP 509763
This study	<i>P. pilsbryana</i>	4382	OR363191		UT	Tooele	unnamed spring near Sixmile Springs, 1.65 air km E of Simpson Springs Rd, ~ 500 m NW of sixmile	ANSP 509767
This study	<i>P. pilsbryana</i>	4383	OR363192		UT	Tooele	unnamed spring near Sixmile Springs, 1.65 air km E of Simpson Springs Rd, ~ 500 m NW of sixmile	ANSP 509767
This study	<i>P. pilsbryana</i>	4385	OR363193		UT	Tooele	unnamed spring near Sixmile Springs, 1.65 air km E of Simpson Springs Rd, ~ 500 m NW of sixmile	ANSP 509767

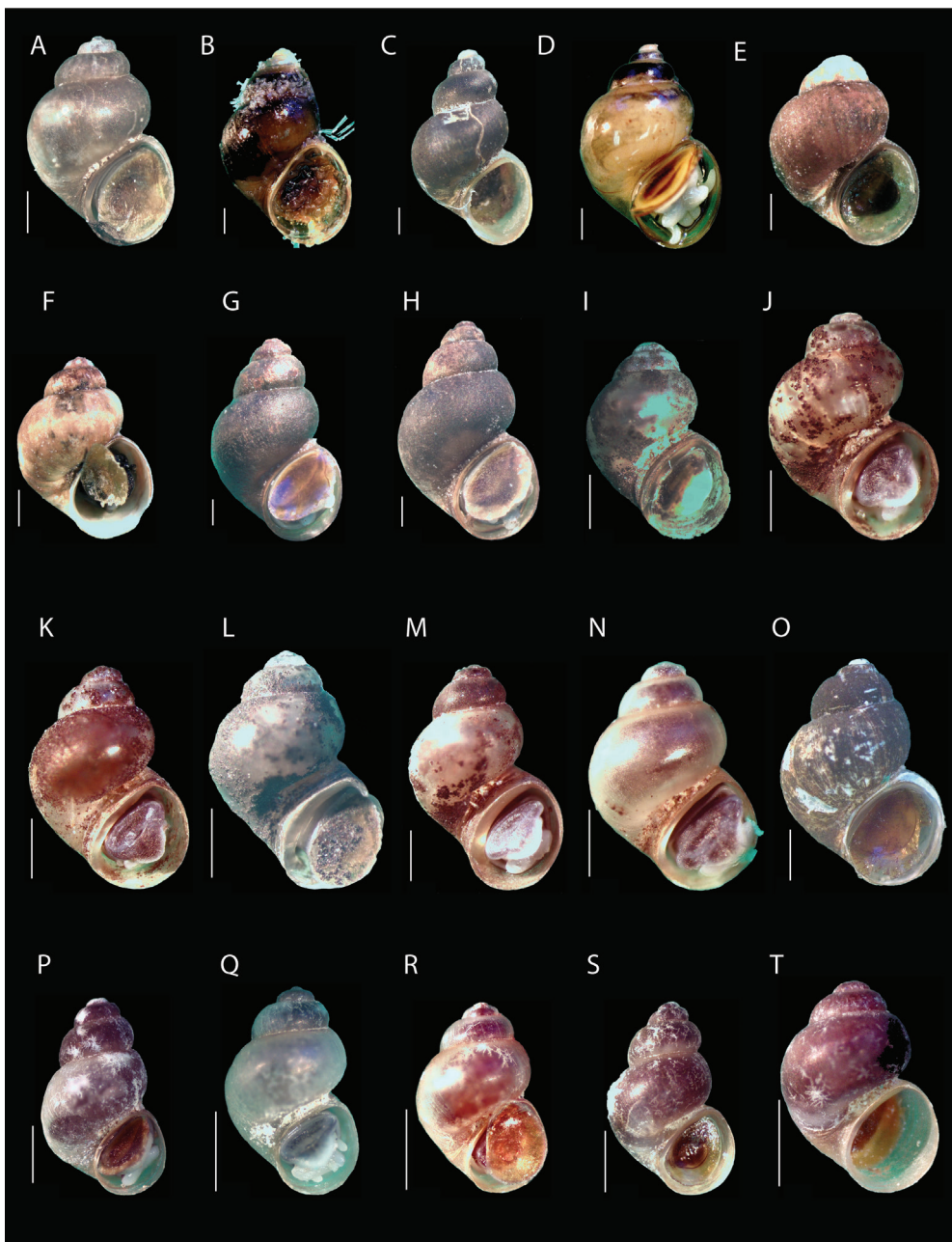
Appendix 1 (continued). Material examined.

Source of Data	Species	Code	COI GenBank No.	LSU GenBank No.	State	County	Locality	Vouchers (USNM)
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	PK31A, D	MG721104-05		UT	Box Elder	Unnamed springs, east-northeast of Portage, Malad Valley, Box Elder Co., UT (41.99° N, 112.2031° W)	USNM 1193690
Liu <i>et al.</i> 2018	<i>P. pilsbryana</i>	PK13B, C	MG721072-73		UT	Utah	Unnamed springs, source of Spring Creek, Utah Lake drainage, Utah Co., UT (40.3836° N, 111.8253° W)	USNM 1193662
This study	<i>P. inopinata</i>				UT	Sevier	Spring (A), Glenwood, Sevier River drainage	
This study	<i>P. pilsbryana</i>				ID	Bear Lake	Spring, St. Charles Canyon, Saint Charles Campground	USNM 858281
This study	<i>P. pilsbryana</i>	4408		OR365624	UT	Tooele	Vernon Reservoir (Tintic Valley), .38 km upstream of reservoir	ANSP 509761
This study	<i>P. pilsbryana</i>				UT	Tooele	Vernon Reservoir (Tintic Valley), .38 km upstream of reservoir	ANSP 509761
This study	<i>P. pilsbryana</i>	4415	OR363204		UT	Tooele	Big Springs, Tooele Co., UT, .35 air km W of Hwy 73 and W Big Springs St intersection	ANSP 509765
This study	<i>P. pilsbryana</i>	4416	OR363205		UT	Tooele	Big Springs, Tooele Co., UT, .35 air km W of Hwy 73 and W Big Springs St intersection	ANSP 509765
This study	<i>P. pilsbryana</i>	4435		OR365617	UT	Box Elder	Blue Spring Hills area, Blue Cr. Spring	ANSP 509740
This study	<i>P. pilsbryana</i>	4436	OR363198	OR365616	UT	Box Elder	Blue Spring Hills area, Blue Cr. Spring	ANSP 509740
This study	<i>P. pilsbryana</i>	4451		OR365623	UT	Millard	Cold Spring	ANSP 509745
This study	<i>P. pilsbryana</i>	4409	OR363202	OR365622	UT	Tooele	Delle Spring	ANSP 509762
This study	<i>P. pilsbryana</i>	4410	OR363203	OR365621	UT	Tooele	Delle Spring	ANSP 509762
This study	<i>P. pilsbryana</i>	4433	OR365619		UT	Box Elder	Dove Creek Hills	ANSP 509739
This study	<i>P. pilsbryana</i>	4434	OR363200	OR365625	UT	Box Elder	Dove Creek Hills	ANSP 509739
This study	<i>P. pilsbryana</i>				UT	Box Elder	Dove Creek Hills	ANSP 509739
This study	<i>P. pilsbryana</i>	4374, 4376	OR363185	OR365628	UT	Emery	Ninemile Reservoir 1 (Spring along east side of Reservoir), .3 air km N of Hwy 89 and Hwy 137 junction	ANSP 509753
This study	<i>P. pilsbryana</i>	4377	OR363186	OR365627	UT	Emery	Ninemile Reservoir 1 (Spring along east side of Reservoir), .3 air km N of Hwy 89 and Hwy 137 junction	ANSP 509753

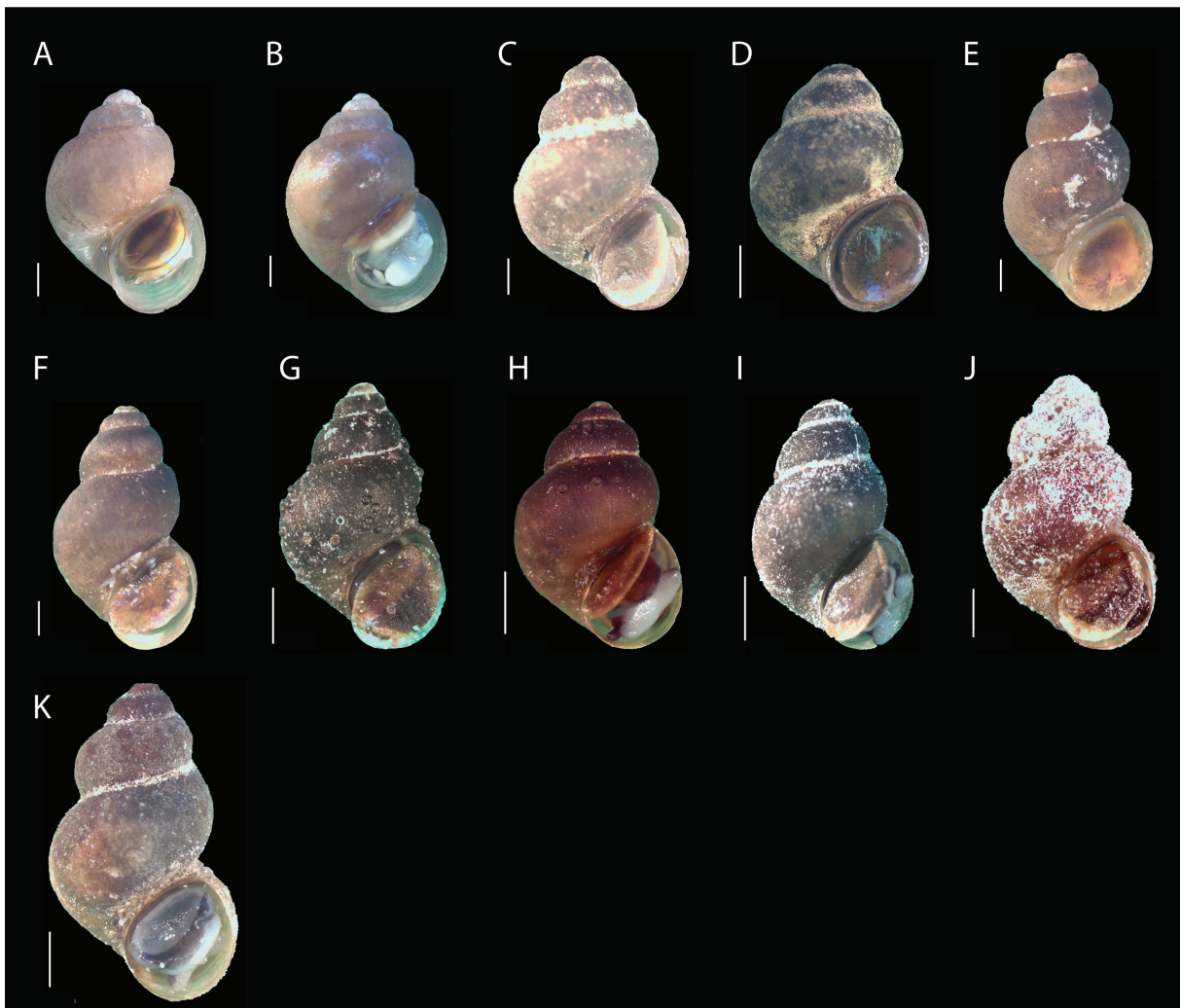
Appendix 1 (continued). Material examined.

Source of Data	Species	Code	COI GenBank No.	LSU GenBank No.	State	County	Locality	Vouchers (USNM)
This study	<i>P. pilsbryana</i>	4386	OR363194	OR365632	UT	Tooele	unnamed spring near Sixmile Springs, 1.65 air km E of Simpson Springs Rd, ~ 500 m NW of sixmile	ANSP 509767
This study	<i>P. pilsbryana</i>	4387		OR365629	UT	Tooele	unnamed spring near Sixmile Springs, 1.65 air km E of Simpson Springs Rd, ~ 500 m NW of sixmile	ANSP 509767
This study	<i>P. pilsbryana</i>	4388	OR363196	OR365630	UT	Tooele	unnamed spring near Sixmile Springs, 1.65 air km E of Simpson Springs Rd, ~ 500 m NW of sixmile	ANSP 509767
This study	<i>P. pilsbryana</i>	4389	OR363190	OR365634	UT	Tooele	unnamed spring near Sixmile Springs, 1.65 air km E of Simpson Springs Rd, ~ 500 m NW of sixmile	ANSP 509767
This study	<i>P. pilsbryana</i>				UT	Tooele	unnamed spring near Sixmile Springs, 1.65 air km E of Simpson Springs Rd, ~ 500 m NW of sixmile	ANSP 509767
This study	<i>P. pilsbryana</i>	4413	OR363206		UT	Tooele	Unnamed Spring tributary to Middle Deep Creek at South Ibapah CSF monitoring site	ANSP 509764
This study	<i>P. plicata</i>				UT	Garfield	Spring, Black Canyon, Sevier River drainage	
This study	<i>P. sterilis</i>				NV	Nye	Hunts Canyon Ranch Springs, Ralston Valley	
This study	<i>Pyrgulopsis</i> sp. "Lineage D"	4347		OR365637	UT	Rich	Jacobson Spring	
This study	<i>Pyrgulopsis</i> sp. "Lineage D"	4348		OR365638	UT	Rich	Jacobson Spring	
This study	<i>Pyrgulopsis</i> sp. "Lineage D"	4349		OR365636	UT	Rich	Jacobson Spring	
This study	<i>Pyrgulopsis</i> sp. "Lineage D"	4350		OR365639	UT	Rich	Jacobson Spring	
This study	<i>Pyrgulopsis</i> sp. "Lineage D"	4352		OR365635	UT	Rich	Jacobson Spring	

Appendix 2. Photovouchers of shells damaged for examination of penial morphology and DNA extraction. For complete locality details and USNM numbers see Appendix 1. **A.** OR363199, OR365620, 4440, Red Barn WMA. **B.** OR365617, 4435, Blue Spring Hills. **C.** OR365635, 4452 North Beck. **D.** OR363198, OR365616, 4436, Blue Spring Hills. **E.** OR363201, 4437, Pipe Spring. **F.** OR365626, 4438, Pipe Spring. **G.** OR365619, 4433, Dove Creek. **H.** OR363200, OR365625, 4434, Dove Creek. **I.** OR363190, OR365634, 4389, Sixmile Springs. **J.** OR363193, OR365631, 4385, Sixmile Springs. **K.** OR363194, OR365632, 4386, Sixmile Springs. **L.** OR363196, OR365630, 4388, Sixmile Springs. **M.** OR363195, OR365629, 4387, Sixmile Springs. **N.** OR363191, OR365633, 4382, Sixmile Springs. **O.** OR363197, 4418, Sixmile Springs. **P.** OR363185, OR365628, 4376, Ninemile Spring. **Q.** OR363189, 4379, Ninemile Spring. **R.** OR363187, 4380, Ninemile Spring. **S.** OR363188, 4378, Ninemile Spring. **T.** OR363186, OR365627, 4377, Ninemile Spring. Any green background color visible on the shells is the clay used to support them for photography. Scale bars = 0.5 mm.



Appendix 3. Photovouchers continued. For complete locality details and USNM numbers see Appendix 1. **A.** OR363202, OR365622, 4409, Delle Spring. **B.** OR363203, OR365621, 4410, Delle Spring. **C.** OR363207, 4411, Tintic Valley. **D.** OR363208, 4412, Tintic Valley. **E.** OR363204, 4415, Big Springs. **F.** OR363205, 4416, Big Springs. **G.** OR365635, 4352, *Pyrgulopsis* Call & Pilsbry, 1886, Lineage D Jacobson Spring. **H.** OR365637, 4347, *Pyrgulopsis* Lineage D Jacobson Spring. **I.** OR365639, 4350 *Pyrgulopsis* Lineage D, Jacobson Spring. **J.** OR365638, 4348, *Pyrgulopsis* Lineage D, Jacobson Spring. **K.** OR363206, 4413, Middle Deep Creek. Photo not available: 4318 Sixmile Springs. Included in Fig. 10: OR363185, OR365628, 4374, Ninemile Spring. OR363192, 4383 Sixmile Springs. Any green background color visible on the shells is the clay used to support them for photography. Scale bars = 0.5 mm.



Appendix 4. Map of all sites sampled with sites matching tip labels.

