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An inventory of springsnails (*Pyrgulopsis* spp.) in and adjacent to the Spring Mountains, Nevada

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Abstract.—Springsnails (genus *Pyrgulopsis,* hereafter pyrgs) are small freshwater aquatic gastropods that occur in isolated springs in western North America. Pyrgs are species of conservation concern, but patterns of occupancy and speciation are complex. We investigated patterns of occurrence for pyrgs in the Spring Mountains, Clark County, Nevada. We were primarily concerned with identifying springs containing the species *P. deaconi*, the Spring Mountains pyrg, and *P. turbatrix*, the southeast Nevada pyrg. We identified species through genetic analysis of the COI-1 mitochondrial region and examined patterns of genetic structure. We located aquatic gastropods in 26 springs and analyzed 420 aquatic gastropods, of which 392 were pyrgs, the remainder representing an unknown species of *Physa*. Of the 26 springs, 25 contained pyrgs and 5 contained *Physa* sp. For pyrgs, at COI-1 we identified a total of 29 haplotypes that formed 6 distinct monophyletic groups. Five of the 6 groups were consistent with pyrgs previously identified: *P. bacchus*, *P. deaconi*, *P. fausta*, *P. turbatrix*, and an unknown species which had been identified previously in the Grapevine Springs. The sixth group, found in 2 springs, does not match any reference specimen and is genetically divergent from the other 5 groups. It is most closely related to *P. micrococcus*. Prior to this study, *P. bacchus* had not been located in the Spring Mountains. Both *P. deaconi* and *P. turbatrix* were located in multiple springs on both the east and west sides of the Spring Mountains, even though the Las Vegas Valley (east) and Pahrump Valley (west) are hydrologically distinct. At the scale of the hydrologic basin, genetic structure was not discernable; haplotype divergence did not align with basin boundaries and the most common haplotype for *P. turbatrix* occurred on both the east and west sides of the Spring Mountains. While there was little evidence for genetic structuring at the hydrologic-basin level, there was good evidence for structuring at the level of the individual spring. All told, 79% (23/29) of pyrg haplotypes were unique to specific springs, suggesting that pyrg diversity primarily occurs at the level of the individual spring.

Resumen.—Los caracoles del género *Pyrgulopsis* (en adelante pyrg) son pequeños gasterópodos acuáticos de agua dulce que habitan en manantiales aislados del oeste de América del Norte. Las especies de esté género, se encuentran en peligro de extinción. Sin embargo, sus patrones de ocupación y especiación son complejos. En el presente trabajo investigamos los patrones de incidencia de los caracoles pyrgs en Spring Mountains, condado de Clark, Nevada. Nuestro objetivo principal fue identificar los manantiales que albergan las especies *P. deaconi* (el pyrg de Spring Mountains) y *P. turbatrix*, (el pyrg del sureste de Nevada). Identificamos las especies mediante un análisis genético de la región mitocondrial COI-1 y examinamos los patrones de estructura genética. Localizamos gasterópodos acuáticos en 26 manantiales y analizamos 420 gasterópodos acuáticos, de los cuales 392 fueron pyrgs y el resto perteneciente a una especie desconocida de *Physa*. En 25 de los 26 manantiales, hallamos pyrgs y en cinco hallamos *Physa* sp. En la región mitocondrial COI-1 de los caracoles del género pyrgs identificamos un total de 29 haplotipos, que formaron seis grupos monofiléticos distintos. Cinco de los seis grupos fueron consistentes con pyrgs previamente identificados: *P. bacchus*, *P. deaconi*, *P. fausta*, *P. turbatrix* y una especie desconocida, previamente identificada en Grapevine Springs. El sexto grupo (hallado en dos manantiales) no coincide con ningún espécimen de referencia, diverge genéticamente de los otros cinco grupos y está más estrechamente relacionado con *P. micrococcus.* Previo a este estudio, no había registros del *P. bacchus* en Spring Mountains. Tanto los *P. deaconi* como los *P. turbatrix* fueron hallados en múltiples manantiales en los lados este y oeste de Spring Mountains. A pesar de que, el valle de Las Vegas (este) y el Valle de Pahrump (oeste) son hidrológicamente distintos. En cuanto a la cuenca hidrológica, la estructura genética no fue discernible; la divergencia del haplotipo no se alineó con los límites de la cuenca y el haplotipo más común del *P. turbatrix* ocurrió a ambos lados este y oeste de Spring Mountains. Aunque, existe poca evidencia de estructuración genética en la cuenca hidrológica, hubo indicios de estructuración a nivel del manantial individual. En total, el 79% (23/29) de los haplotipos de los pyrg fueron exclusivos de manantiales específicos, indicando que la mayor diversidad de caracoles del género pyrgs se encuentra a nivel de manantiales individuales.

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Springsnails (*Pyrgulopsis* spp., hereafter pyrgs) are small benthic freshwater gastropods that most commonly inhabit small areas immediately adjacent to spring outflows (Hershler and Sada 2002). Many species are limited to specific springs or small hydrographic areas. These kinds of habitats are often subject to development or disturbance in the relatively arid portions of the western United States, hence pyrg species have received significant attention under the 1973 Endangered Species Act (ESA; Hershler et al. 2014). A search of the U.S. Fish and Wildlife Service's (USFWS) database on "*Pyrgulopsis*" identified 76 species or putative species; 8 are listed as "Endangered," 1 as "Threatened," and 21 as "Under Review in the Candidate or Petition Process" (USFWS 2017a). All told, over half of the described pyrgs (up to 140 in 2016; see Hershler et al. 2016) were receiving or have received special attention from the USFWS.

The Spring Mountains in southern Nevada, USA, constitute one of the highest ranges in the Mojave Desert; the highest point, Charleston Peak, is 3632 m. The Spring Mountains have been isolated since the early Holocene (Quade et al. 1998) and are one of the most isolated "sky islands" in the Mojave Desert. The closest areas that feature elevations similar to those in the Spring Mountains lie in the southern Sierra Nevada, ~220 km from Charleston Peak. Because of their isolation, the Spring Mountains contain many endemic species (Billings 1978, Harper et al. 1978, Austin 1981, Nachlinger and Reese 1996). In the early Holocene, the Spring Mountains were surrounded by valley wetlands, but these disappeared circa 6000 years BP (Quade et al. 1998). Thus, current springs have likely been hydrologically isolated for several thousand years. The highest elevations receive significant snow, which provides groundwater recharge (Winograd et al. 1998), and the range contains ~140 known springs (Coles-Ritchie et al. 2014). While many of these springs lie within the Spring Mountains National Recreation Area (SMNRA), disturbance is common. Sada et al. (2005) classified 56% (25/45) of the springs they studied in this area as either "moderately disturbed" or "highly disturbed."

The Spring Mountains are part of 3 separate hydrologic basins. On the east, the Las Vegas Valley drains into the Colorado River. On the west, the Pahrump Valley is largely bounded, though there is biological evidence of historical connections to the Amargosa River (Hubbs and Miller 1948). The northern tip of the Spring Mountains lies in the Amargosa River basin, which flows into Death Valley. The Pahrump and Amargosa basins are not hydrologically linked to the Las Vegas Valley.

Previous studies have identified 3 species of pyrgs in the Spring Mountains: *P. deaconi*, *P. turbatrix*, and an undescribed species (hereafter *Pyrgulopsis* sp. following Hershler et al. 2013) from Grapevine Springs in the NW corner of the Spring Mountains (see supplementary data *in* Hershler et al. 2013). *Pyrgulopsis deaconi* is thought to be limited to the Spring Mountains (Hershler 1998, Hershler and Sada 2002), whereas *P. turbatrix* is more broadly distributed (Hershler and Sada 2002). Recently, specimens collected from the central Death Valley region and San Bernardino Mountains, California, and previously assigned to *P. micrococcus* were reclassified as *P. turbatrix* (Hershler et al. 2013), greatly expanding its range.

Pyrgulopsis deaconi was determined not to be warranted for listing under the ESA in 2017 (USFWS 2017b), and *P. turbatrix* is presently classified by the USFWS as "Under Review." Part of the ESA review process is to assess the status and trend of the taxon under evaluation. For springsnails, this assessment includes estimating the number of occupied springs and their characteristics, as well as assessing threats to individual populations and their habitats. In this study we surveyed a large number of springs in and adjacent to the Spring Mountains to assess habitat occupancy by pyrgs, which were identified to species using genetic analyses (Folmer et al. 1994, Liu et al. 2003, Hershler and Liu 2008, Hershler et al. 2013). The use of genetic analyses also allowed examination of patterns of within-species genetic variability across the sample area.

Methods

Data Collection

In 2008, the SMNRA developed a "Comprehensive Inventory and Monitoring Strategy for Conserving Biological Resources of the Spring Mountains National Recreation Area" (USDA Forest Service 2008). Part of this strategy focused on the inventory of springs using national "Level II" protocols (USDA Forest Service 2012). This survey was designed to catalog multiple attributes of each spring, and though it did not specifically target pyrgs, the

survey reported them when they were found. Of 137 identified springs in the Spring Mountains, 77 were surveyed. The survey design was spatially balanced and the entire SMNRA received uniform survey effort (see Fig. 2.2 *in* Solem et al. 2013). To more fully complete the cataloging of pyrgs, additional springs thought likely to contain pyrgs were surveyed in subsequent years. In addition to previously unsampled springs within the SMNRA, pyrg surveys were also completed on adjacent Bureau of Land Management lands (Fig. 1). Two springs, Cane Spring and an Unnamed Spring ~0.5 km SE of Corn Creek Field Station, were not within the Spring Mountains. Cane Spring is on the Nevada National Security Site in the area of Skull Mountain ~25 km north of the Spring Mountains. Corn Creek Field Station is on the east side of the Las Vegas Valley within the Desert National Wildlife Range, ~25 km west of the Spring Mountains.

Aquatic gastropods (hereafter snails), when located, were collected for genetic identification to species. Because we were working with crews with highly divergent knowledge and experience levels concerning gastropods, inexperienced collectors made no attempt to field-identify snails. We chose to identify snails using genetic analyses for reasons similar to the challenges and characteristics described by Morningstar et al. (2014).

If snails were found and conditions at the spring permitted, samplers would determine the approximate boundaries of the snail population and collect samples evenly across the identified area to provide a representative sample. If more than one population was located, samples were distributed across all discovered populations (see Supplementary Material 1 for within-spring location data). Snails were collected with forceps, placed in coin envelopes, and put into larger sealed containers containing silica desiccant. Spring names and coordinates were recorded. If snails were plentiful, a sample of 30 was collected; assuming snails were collected randomly, this method provides a >95% chance of finding any species or haplotype making up more than 10% of the total population. Because sampling was destructive, smaller samples were taken in the places where snails were less common; the samplers were directed not to take samples that would, in their judgement, have a detrimental effect on the local population. After collection, the desiccant-filled containers were shipped to the USFS National Genomics Center for Wildlife and Fish Conservation in Missoula, Montana, for analysis.

Genetic Analyses

Following methods of Liu et al. (2003), we amplified 710 bp of the cytochrome c oxidase 1 (COI-1) region of mitochondrial DNA (mtDNA) using primers LCO1490 and HCO2198 (Folmer et al. 1994). Reaction volumes of 50 µL contained 50–100 ng DNA, 1× reaction buffer (Applied Biosystems), 2.5 mM $MgCl_2$, 200 µM each dNTP, 1 µM each primer, and 1 U Taq polymerase (Applied Biosystems). The PCR program was 94 °C/5 min, [94 °C/1 min, 55 °C/1 min, 72 °C/1 min 30 s] × 34 cycles, 72 °C/5 min. The quality and quantity of template DNA were determined by 1.6% agarose gel electrophoresis prior to downstream analyses. PCR products were purified using ExoSap-IT (Affymetrix-USB Corporation, OH) according to manufacturer's instructions.

DNA sequence data were obtained using the Big Dye kit and the 3700 DNA Analyzer (ABI; High Throughput Genomics Unit, Seattle, WA). DNA sequence data for COI-1 was generated using the PCR primers provided above. Sequences were determined for both strands and aligned using Sequencher (Gene Codes Corp., Ann Arbor, MI). Neighbor-joining phylogenetic trees (Saitou and Nei 1987) were produced using MEGA 6. We chose the evolutionary model that minimized AICc, and we built trees based on this model using 1000 bootstrap replicates. Haplotype statistical parsimony networks (Joly et al. 2007) were made using TCS (Clement et al. 2002) as implemented in PopART (http://popart.otago.ac.nz). We designated a sequence of *Marstonia hershleri* (GenBank AF520946) as the outgroup.

Results

We analyzed 420 snails from 26 springs (Table 1), of which 392 were pyrgs, the remainder being from an unknown species of the genus *Physa* (Supplementary Material 2); 25 springs contained pyrgs (Fig. 1, Table 1). Some of these springs form geographically proximal groups; when speaking of these springs as a group, we use the plural (e.g., Grapevine Springs), whereas when referring to an individual spring, we follow the naming conventions in Table 1. For pyrgs, we identified a total of 29 haplotypes (Labeled A

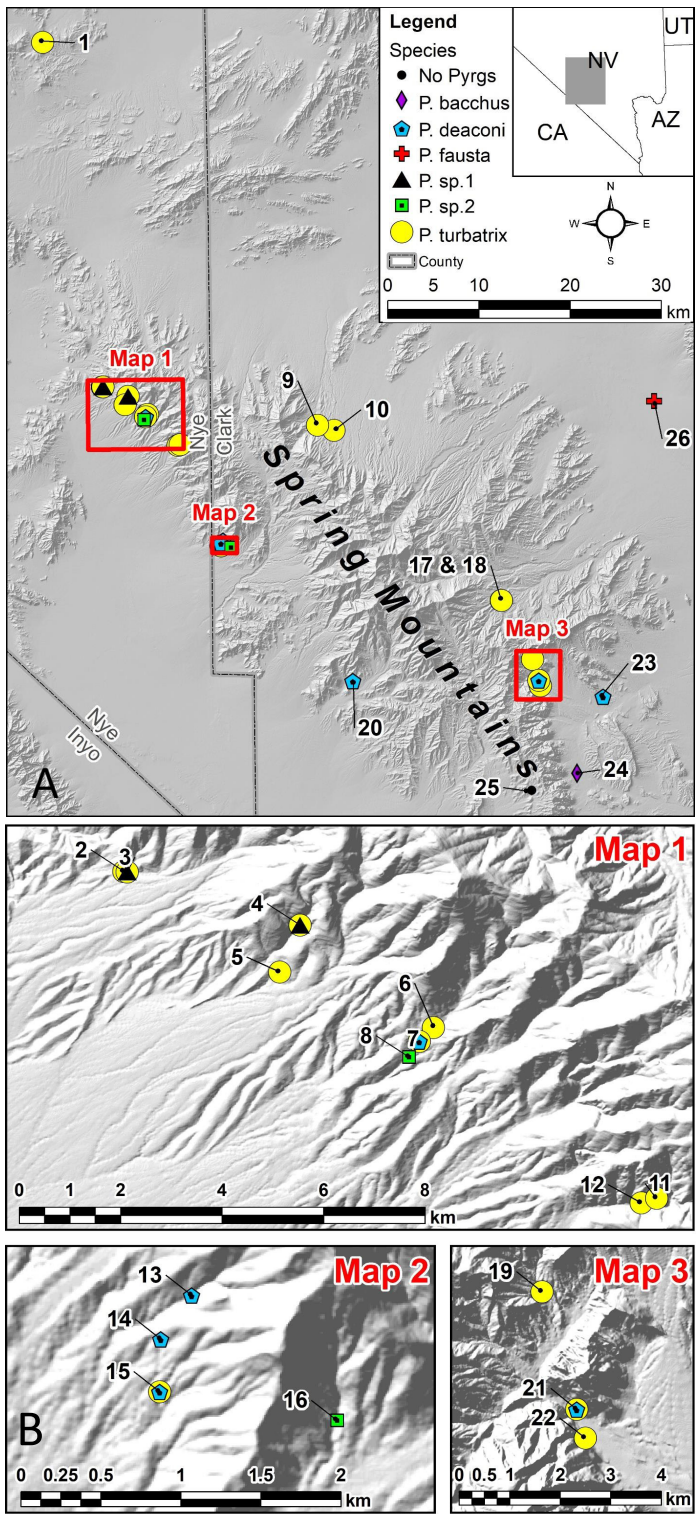

Fig. 1. Gastropod sample locations and species-level results in and around the Spring Mountains, Nevada. Rectangular red insets in 1A are enlarged in 1B to show additional detail.

Table 1. Springsnail localities and geographic distribution of mitochondrial haplotypes. Haplotypes associated with *Pyrgulopsis* spp. were labeled A through AC based on the order in which they were identified. See Fig. 2 for information concerning identification of haplotypes to species. Some springs have various names that have been associated with them over time. Names following slashes are alternative names for springs. Numbers in parentheses are the number of snail samples having a particular haplotype. Genetic variability in *Physa* spp. is shown in Supplementary Materials 2 and 3.

Spring name	Map #	Latitude	Longitude	*P. deaconi*	*P. turbatrix*	*P. fausta*	*P. bacchus*	*P.* sp.	*P.* sp2	*Physa* sp.
Cane Spring	1	36.79875	−116.09586		I(30)					
Cold Creek Spring	10	36.41162	−115.74418		C(2)					
Crystal Spring A/ Crystal Springs	7	36.42715	−115.97446	A(1)*	C(4), I(11)*					
Crystal Spring B/Unnamed 24	6	36.42960	−115.97194		I(22)*					
Crystal Spring (Re-emergence)	8	36.42437	−115.97627						H(1)*	
Grapevine Spring (Bench)	2	36.45730	−116.02682		I(4)					
Grapevine Spring (Tunnel)/ Grapevine Springs	3	36.45718	−116.02625		I(16)			M(1), N(11)		
Green Spot Spring	24	36.06899	−115.45360				AA(22), AB(4), AC(2)*			10
Harris Spring A	17	36.24096	−115.54267		C(15)					
Harris Spring B	18	36.24096	−115.54292		C(15)					
Horse Spring A/Unnamed 49	15	36.29860	−115.88407	A(28), E(1), F(1)	B(3), G(1)*					
Horse Spring B/South of Horse Spring	14	36.30156	−115.88394	A(9)						
Horse Spring C/Upper Horse Spring	13	36.30401	−115.88176	A(17)						
Horseshutem Spring (Lower)	5	36.43946	−115.99919		I(6), J(1)					
Horseshutem Spring (Upper)/ Unnamed 35	4	36.44776	−115.99560		C(1), I(6), K(1)*			L(1)*		
Kiup Spring	20	36.16356	−115.72523	O(1), P(2), Q(2), R(23)						
La Madre Spring	19	36.18241	−115.50568		C(8), S(2)					
Lost Creek Spring/ Lost Canyon Spring	22	36.15616	−115.49667		S(2)					8
Red Spring	23	36.14435	−115.42054	T(1), U(2), V(26)						1
South Rainbow Spring	25	36.05281	−115.50921							1
Unnamed 50 Spring	16	36.29687	−115.87173						H(13)*	
Unnamed Spring SE of Corn Creek Station	26	36.43579	−115.35204			Z(9)*				
Willow Spring (BLM)	21	36.16134	−115.49846	W(1), X(1)	‡					8
Willow Spring (USFS)	9	36.41692	−115.76420		C(27), D(1)					
Wood Canyon Spring A	12	36.39852	−115.93511		C(18), Y(1)					
Wood Canyon Spring B	11	36.39931	−115.93238		C(5), G(10)*					

*Indicates a new *Pyrgulopsis* species location verified by this study.

‡Indicates a location where the species was historically located but not detected during sampling in this study.

through AC in the order identified). For building a phylogeny, evolutionary model T92+G (Tamura 3-parameter; Tamura et al. 2013) yielded the lowest AICc and the highest log-likelihood values for these data. The neighbor-joining phylogenetic tree based on these rules grouped *Pyrgulopsis* haplotypes from the Spring Mountains region into 6 monophyletic groups with moderate to high bootstrap support (Fig. 2) and low levels of internal variation (Supplementary Material 4). Based on relationships to previously published genotypes from GenBank (accession numbers provided in Fig. 2), 12 haplotypes (A, E, F, O–R, and T–X) form a monophyletic group that includes a single GenBank sample labeled as *P. deaconi*. Nine haplotypes (B–D, G, I–K, and Y) form a monophyletic group containing 3 reference specimens labeled as *P. turbatrix*. Samples of *P. turbatrix* collected in Death Valley and San Bernardino (Hershler et al. 2013) form a closely related but distinct monophyletic group (Fig. 2). Haplotype Z was sequence-identical to a specimen identified as *P. fausta*, which was previously located within the same Corn Spring complex (Liu et al. 2003; Table 2). Haplotypes AA–AC formed a monophyletic group containing 4 samples identified as *P. bacchus*. Haplotype AA is identical to one of the 4 reference specimens (Fig. 2, Table 2). The *P. bacchus* reference specimens were collected at Tassie Spring (Hershler et al. 2013), ~130 km east of the Spring Mountains. Haplotypes L–N group with *Pyrgulopsis* sp., identified as an unknown species in Hershler et al. (2013). This species was found in the same spring where it was previously observed (Hershler et al. 2013; Grapevine Springs, Table 1), but also in an additional spring ~2.8 km from Grapevine Springs (Fig. 1, Table 2). Lastly, we identified what may constitute an unknown species, hereafter *Pyrgulopsis* sp2, which was found in 2 springs: Unnamed 50 Spring and Crystal Spring (Re-emergence) (Fig. 1, Table 1). This haplotype (H) is not genetically similar to any available reference sample (Fig. 2) and is divergent from the other 5 species located in the Spring Mountains (Fig. 2). Haplotype H is most closely aligned with a reference sample of *P. micrococcus* from the headwaters of the Amargosa River (Fig. 2; Hershler et al. 2013).

For those species that had previously been identified within the area we surveyed, all reference haplotypes were relocated in the springs where they had previously been located, and most were also located in additional springs (Table 2). Overall, *P. deaconi* was located at 7 springs and *P. turbatrix* at 16. Five springs contained *Physa* spp. (Table 1), with South Rainbow Spring containing only *Physa.* Four springs contained more than one species of pyrg. Grapevine Spring (Bench) and Horseshutem (Upper) contained both *P. turbatrix* and *Pyrgulopsis* sp1. Crystal Spring A and Horse Spring A contained both *P. turbatrix* and *P. deaconi* (Table 1).

Five of the haplotypes were distributed across multiple springs. Haplotype A, associated with *P. deaconi*, was found in 4 springs, though 3 of the 4 were in Horse Springs; all other *P. deaconi* haplotypes were unique to individual springs. *Pyrgulopsis turbatrix* haplotypes C and I were located in 9 and 7 springs, respectively. Additionally, haplotypes G and S, associated with *P. turbatrix*, were found in 2 springs; the remainder of *P. turbatrix* haplotypes were found in single springs. All told, 23 haplotypes (79%) were unique to individual springs. It was common to locate more than one haplotype in a spring; 4 of the 7 springs where *P. deaconi* was found contained multiple haplotypes unique to that spring (Table 1). These haplotype groups formed star phylogenies (Gillespie 1984; Fig. 3) with a common haplotype and multiple haplotypes separated by single nucleotide substitutions, a pattern consistent with population isolation. Kiup Spring contained a star phylogeny consisting of haplotypes O, P, and R, as well as more distantly

Fig. 2. (See facing page.) Phylogenetic relationships between gastropods are based on the COI region of the mitochondrial genome amplified by primers described in Folmer et al. (1994). Species labels on reference samples follow Hershler et al. (2013) and in many cases differ from identifications in GenBank. Specifically, gastropods labeled as *Pyrgulopsis perforata*, *P. licina*, and *P. sanchezi*, and accession numbers AY367441, AY367459, and AY367448, labeled as *P. turbatrix*, are all labeled as *P. micrococcus* in GenBank. Accession number DQ363999, labeled as *Pyrgulopsis* sp., was labeled as *P. turbatrix* in GenBank. Relationships are inferred using the neighbor-joining method (Saitou and Nei 1987). The evolutionary distances were computed using the Tamura 3-parameter method (Tamura et al. 2013) and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 0.2). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

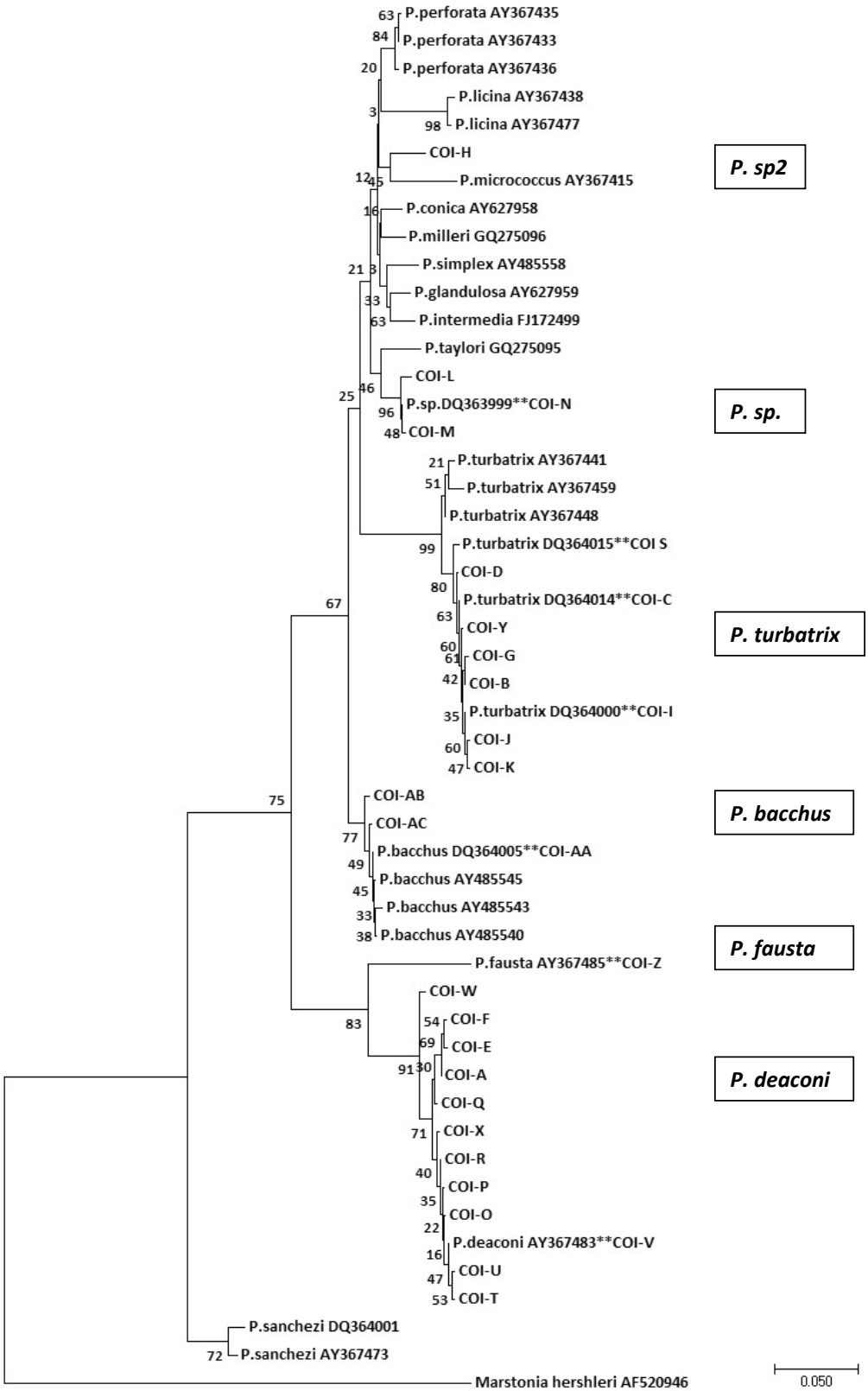
P.perforata AY367435
P.perforata AY367433
P.perforata AY367436
P.licina AY367438
P.licina AY367477
COI-H
P.micrococcus AY367415
P.conica AY627958
P.milleri GQ275096
P.simplex AY485558
P.glandulosa AY627959
P.intermedia FJ172499
P.taylori GQ275095
COI-L
P.sp.DQ363999**COI-N
COI-M
P. sp2
P. sp.
P.turbatrix AY367441
P.turbatrix AY367459
P.turbatrix AY367448
P.turbatrix DQ364015**COI S
COI-D
P.turbatrix DQ364014**COI-C
COI-Y
COI-G
COI-B
P.turbatrix DQ364000**COI-I
COI-J
COI-K
P. turbatrix
COI-AB
COI-AC
P.bacchus DQ364005**COI-AA
P.bacchus AY485545
P.bacchus AY485543
P.bacchus AY485540
P. bacchus
P.fausta AY367485**COI-Z
P. fausta
COI-W
COI-F
COI-E
COI-A
COI-Q
COI-X
COI-R
COI-P
COI-O
P.deaconi AY367483**COI-V
COI-U
COI-T
P. deaconi
P.sanchezi DQ364001
P.sanchezi AY367473
Marstonia hershleri AF520946
0.050

Table 2. Samples and spring locations that matched reference specimens found in GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Sequence-identical haplotypes for all reference specimens within or adjacent to the Spring Mountains were relocated in the same springs.

Species	Haplotype	Spring name	Accession number	New location
Pyrgulopsis turbatrix	C	Multiple localities[a]	DQ364014	Yes
	I	Multiple localities[b]	DQ364000	Yes
	S	La Madre Spring, Lost Creek Spring[c]	DQ364015	Yes
Pyrgulopsis deaconi	V	Red Spring	AY367483	No
Pyrgulopsis fausta	Z	Unnamed Spring, SE of Corn Creek Station	AY367485	Yes
Pyrgulopsis bacchus	AA	Green Spot Spring[d]	DQ364005	Yes
Pyrgulopsis sp.	N	Grapevine Spring (Tunnel)	DQ363999	No[e]

[a]Haplotype C was found in Cold Creek Spring, Crystal Spring A, Harris Spring A and B, Horseshutem Spring (Upper), La Madre Spring, Willow Spring (USFS), and Wood Canyon Spring A and B. The reference specimen was found in Cold Creek Spring.
[b]Haplotype I was found in Cane Spring, Crystal Springs, Grapevine Springs, and Horseshutem Springs. The reference specimen was found in Grapevine Springs.
[c]The reference specimen was found in Lost Creek Spring.
[d]The reference specimen was found in Tassi Springs.
[e]*Pyrgulopsis* sp. was relocated within the same spring complexes as the reference specimens. In GenBank it is identified as *P. turbatrix*.

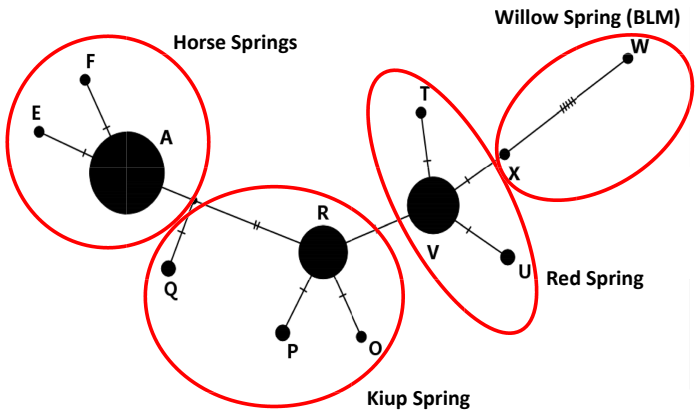

Fig. 3. A haplotype statistical parsimony network (Joly et al. 2007) for those haplotypes identified as *Pyrgulopsis deaconi*. Ellipse size is proportional to the total number of samples with the identified haplotype. Red ellipses enclose groups that occurred at springs containing multiple haplotypes. With the exception of haplotype A, all *P. deaconi* haplotypes were only located in a single spring. Springsnails were recently transplanted from Red Spring to Willow Spring (BLM).

related haplotype Q; Willow Spring (BLM) contained haplotype X, which is similar to haplotypes found in Red Spring, and haplotype W, which is the most divergent *P. deaconi* haplotype (Figs. 2, 3, Table 1). Haplotype A was not unique to Horse Springs—it also was located in Crystal Spring A—but Horse Springs also contains a star phylogeny of haplotypes A, E, and F (Fig. 3).

Both *P. deaconi* and *P. turbatrix* are found in both the Pahrump and Las Vegas valleys (Fig. 1). Additionally, *P. turbatrix* is found in the Amargosa River basin and closely related pyrgs are found across a much broader area that includes the central Death Valley and the San Bernardino Mountains (Hershler et al. 2013). Haplotypes C and I, associated with *P. turbatrix*, are located in more than one basin. Haplotype C is located in several springs both in the Pahrump and Las Vegas basins. Haplotype I is found in the Amargosa and Pahrump basins.

Discussion

Both *P. deaconi* and *P. turbatrix* were widespread in the Spring Mountains and adjacent areas, and both showed fairly high levels of genetic diversity. *Pyrgulopsis turbatrix* was both found in more springs and contained more broadly distributed haplotypes than *P. deaconi* did. Genetic structure in *P. turbatrix* appears to be largely regional in nature: *P. turbatrix* found in the vicinity of the Spring Mountains forms a shallow but well-supported monophyletic group compared to *P. turbatrix*

from California (Fig. 2; Hershler et al. 2013). *Pyrgulopsis deaconi*, genetically very divergent from other identified pyrgs (Fig. 2), also exihibited little phylogenetic structure at the basin level. Even during the Pleistocene, when conditions were periodically much wetter and within-basin hydrologic connectivity presumed higher, the Amargosa and Pahrump basins had no direct connectivity to the Las Vegas basin (Blackwelder 1933). Thus, transfers of pyrgs between these basins probably involved some form of nonaquatic transport. For *P. turbatrix*, the fact that the same haplotypes occurred within the Pahrump and Las Vegas basins suggests that such transfers may have occurred quite recently, perhaps after the end of the Pleistocene. Haplotypes associated with *P. deaconi* tended to be unique to specific springs and displayed patterns of star phylogeny consistent with evolution in situ. As noted above, haplotype X, found only in Willow Spring (BLM), was closely related to haplotypes in Red Spring that are associated with a star phylogeny. *Pyrgulopsis deaconi* was introduced into Willow Spring (BLM) from Red Spring in 2001 (Sada 2002). It is certainly conceivable that haplotype X was associated with this transfer, whereas haplotype W, which was also present in Willow Spring (BLM) but is only distantly related to haplotype X, was native to the spring. Red Spring and Willow Spring (BLM), however, are geographically proximal and could easily contain related haplotypes, whereas haplotype W, only found at Willow Spring (BLM), is distantly related to all other *P. deaconi* haplotypes (Table 2, Fig. 2).

While both *P. deaconi* and *P. turbatrix* are located in multiple springs that are widely distributed across the Spring Mountains, the 2 unknown species at this point appear to be very limited in their distributions (Table 1, Fig. 1). Not only have they not been found exterior to the Spring Mountains, but each was only located in 2 springs within the Spring Mountains. *Pyrgulopsis* sp. had been previously located in Grapevine Spring (Hershler et al. 2013); we relocated it there and in one additional spring. *Pyrgulopsis* sp2 is novel. Similarly, *P. fausta* has only been located within a single springs complex. *Pyrgulopsis bacchus* had previously only been identified in springs within the Grand Wash area of Mojave County, Arizona (Sada 2005). Thus, locating it in the Spring Mountains, at least 100 km west of its previously known range, was novel.

The large number of spring-specific haplotypes, coupled with weak phylogenetic structure within basins, suggests the prominence of local effects in the evolution of springsnails, particularly supported here for *P. deaconi*. Additionally, the presence of 2 undescribed species, as well as the extralimital discovery of *P. bacchus* in a single spring, further indicates a high level of genetic uniqueness at the level of the individual spring. That multiple haplotypes occur in many springs also indicates that large populations of pyrgs historically existed to support this level of diversity (Kimura and Crow 1964).

The genetic sample collected in this study represents by far the most thorough sample of its kind within a specific mountain range. Along with identifying many previously undescribed haplotypes, we sampled specimens that were sequence-identical to all of the reference haplotypes associated with the Spring Mountains (Table 2). However, our sampling was not exhaustive. The maximum sample size for collected snails was limited; at most, 30 snails were collected from any spring and, in some springs where snails were scarce, only a few samples were collected (Table 1). Further, while we attempted to sample all springs where pyrgs were thought likely to exist, we did not census all springs. Therefore, although the genetic variability is significant in terms of both haplotypes and species, it has to be considered an underestimate of the true diversity contained across all populations in all springs within the Spring Mountains and surrounding areas. A sample of 30 will find common species and haplotypes with high reliability. However, a species or haplotype representing 1% of the population will only be located ~25% of the time. Given our intentionally small samples due to snail rarity, most springs were characterized by fewer than 30 pyrgs (Table 1). Because we documented multiple *Pyrgulopsis* haplotypes in the majority of springs (52%; Table 1), and because many of the haplotypes were represented by a single individual (41%), we can state with near certainty that additional undocumented haplotypes exist within the Spring Mountains. Further, we cannot rule out the possibility that more species and/or cryptic diversity remain to be documented.

While multiple springs contained pyrgs, the proportion of springs containing pyrgs was low. Assuming ~140 springs in the study area

(Coles-Richie et al. 2014), less than 18% are known to contain pyrgs. The springs where pyrgs were located in this survey were all lower-elevation springs (median elevation = 1566 m) and therefore exist as water holes in otherwise arid landscapes. These springs were also generally larger springs, with average minimum flows of 20.5 L/m (range 0–300 L/m; Supplementary Material 5). For these reasons, these springs have been the focus of both human and animal activities for millennia. Introduced elk (*Cervus canadensis*), horses (*Equus ferus caballus*), and donkeys (*Equus africanus asinus*) utilize these springs, and in some cases the physical properties of springs have been altered for irrigation and other anthropogenic uses.

Postscript.—At the time this manuscript was in final draft, a population of pyrgs was located in Cottonwood Spring (36.04545, −115.40597) on private land located approximately 5 km southeast of Green Spot Spring. Sampling occurred in December 2019 and 33 snail samples were analyzed; 29 were *P. bacchus* and one was *Physa*. In our review of spring data, we found one record for the spring dated December 1991 (Sada 2016 *in* Supplementary Material 5) indicating that pyrgs were absent. In 1991, the spring was highly disturbed by recreation and water diversion, and all the water was being captured for domestic use. The discovered 2019 population doubled the number of springs occupied by *P. bacchus* in the Spring Mountains and showed that this degraded spring system had retained its population of pyrgs. Additionally, on the same day, we analyzed 29 snail samples collected at Willow Spring (BLM) (Table 1), all of which were *P. deaconi*. We identified haplotypes A and X (Fig. 2). Haplotype A had not been found at Willow Spring (BLM) (Table 1) or in the Las Vegas basin previously.

Supplementary Material

Six online-only supplementary files accompany this article (https://scholarsarchive.byu.edu/wnan/vol80/iss2/6).

Supplementary Material 1. Coordinates and associated haplotypes for all springs with more than one sampling location.

Supplementary Material 2. A neighbor-joining tree at COI-1 for *Physa* samples located in and adjacent to the Spring Mountains, Nevada.

Supplementary Material 3. A haplotype statistical parsimony network for those haplotypes identified as Physa1 to Physa7.

Supplementary Material 4. Pairwise haplotype differences among species of pyrgs. Within-species differences are low, with only 1 haplotype differing from other within-species samples by more than 10 base pairs (bp). Conversely, between-species differences are large, generally more than 30 bp.

Supplementary Material 5. Four water quality parameters characterizing the spring water conditions occupied by the springsnails and *Physa* entities discussed in this paper: water temperature (°C), specific conductance (µS/cm, typically standardized to 25 °C), pH, and stream flow (L/m). The values listed are the minimum and maximum at each spring followed by the total number of daily observations. Period of record for the data is between 16 October 1912 and 31 December 2017. Source citations are provided along with further explanation of data collection methods.

Supplementary Material 6. GenBank accession numbers for identified springsnail haplotypes.

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