Applications in Plant Sciences 2016 4(3): 1500105

Primer Note

Identification and characterization of microsatellite markers in *Penstemon scariosus* (Plantaginaceae)[1]

Chris D. Anderson[2], Nathan J. Ricks[2], Kevin M. Farley[2], Peter J. Maughan[2], and Mikel R. Stevens[2,3]

[2]Department of Plant and Wildlife Sciences, Brigham Young University, Provo, Utah 84602 USA

- *Premise of the study: Penstemon scariosus* var. *albifluvis* (Plantaginaceae) has been proposed to be federally listed as threatened due to its unique, geologically oil-rich habitat. Developing simple sequence repeat (SSR) markers to study its genetic diversity would be most useful.
- *Methods and Results:* Using genomic reduction in combination with next-generation sequencing, we identified SSR motifs with five to 15 perfect repeats in 1067 *P. scariosus* contigs. After multiple qualifying tests, 16 SSRs were selected for their robust polymorphic reliability across 12 taxa with as high as 21 alleles in a given taxon. With the exception of two monomorphic loci, the observed and expected heterozygosity values ranged from 0.083 to 1.000 and 0.398 to 0.920, respectively.
- *Conclusions:* These microsatellite markers will directly aid in studies of the genetic diversity and relatedness of *P. scariosus*, *P. comarrhenus*, *P. compactus*, *P. cyananthus* var. *cyananthus*, *P. fremontii* var. *fremontii*, *P. fremontii* var. *glabrescens*, *P. gibbensii*, *P. strictus*, and *P. subglaber*.

Key words: 454 sequencing; cross-amplification; *Penstemon scariosus* var. *albifluvis*; Plantaginaceae; simple sequence repeat (SSR) markers; White River penstemon.

Penstemon scariosus Pennell (Plantaginaceae) exhibits a broad and complex range of morphological variability (Holmgren, 1984; Neese and Atwood, 2008). Of the four penstemon varieties recognized by Neese and Atwood (2008), *P. scariosus* var. *albifluvis* (England) N. H. Holmgren (White River penstemon), native to the Green River Formation of the western United States, is considered the most distinct (England, 1982; Holmgren, 1984; Neese and Atwood, 2008). Because of increasing efforts to recover hydrocarbon deposits found in this geological formation, *P. scariosus* var. *albifluvis* is being considered for listing under the Endangered Species Act of 1973 (Ashe, 2013). Thus, there is an urgent need to understand genetic diversity within *P. scariosus* and especially within variety *albifluvis*. Identifying robust and reliable *P. scariosus* simple sequence repeats (SSRs, i.e., microsatellites) would prove useful in such diversity studies.

Two previous reports of SSR markers for *Penstemon* Schmidel have been reported (Kramer and Fant, 2007; Dockter et al., 2013) in the literature. However, only two of the reported SSR markers from Kramer and Fant (2007) proved to be robust and produce reliable results, while the others were less dependable, without modifications, in our expanded survey of *P. scariosus*. Thus, the objective of this study was to develop additional extensively tested SSR markers for *P. scariosus*.

[1]Manuscript received 15 September 2015; revision accepted 21 October 2015.

The authors gratefully acknowledge Dr. Robert L. Johnson for assisting in the taxonomic identification of the samples used in this study. Financial support for this study was provided by Brigham Young University and a Bureau of Land Management Vernal Field Office conservation agreement (no. L14AC00346).

[3]Author for correspondence: mikel_stevens@byu.edu

doi:10.3732/apps.1500105

METHODS AND RESULTS

DNA was extracted from lyophilized leaf tissue collected, in situ, from up to four individual plants from each population described in Appendix 1, using the method detailed by Todd and Vodkin (1996). To identify the SSRs, we used the genomic reduction protocol described by Maughan et al. (2009), which has also been successfully used to develop SSRs for Utah agave (*Agave utahensis* Engelm.) and post oak (*Quercus stellata* Wangenh.) (Byers et al., 2014; Chatwin et al., 2014). The genomic reduction procedure used DNA samples from two *P. scariosus* var. *albifluvis*, six var. *cyanomontanus* Neese, six var. *garrettii* (Pennell) N. H. Holmgren, and eight var. *scariosus*. The 454 pyrosequencing of those samples provided us with a total of 1,579,847 reads, representing over 877 Mb, equaling approximately 60,763 reads per sample or about 1,336,786 reads across the 22 *P. scariosus* samples. The average read and mode lengths were 556 bp and 594 bp, respectively. Using default parameters of the Roche Newbler assembler program (version 2.3; 454 Life Sciences, Branford, Connecticut, USA), we obtained a total of 46,628 contigs from those reads. Using the computer program MISA (Thiel et al., 2003), we identified 1067 *P. scariosus* contigs with perfect di-, tri-, tetra-, and pentanucleotide motifs with five to 15 repeat units. There were 45 sequences with two repeating motifs in a single contig meeting the above criteria, while 433, 357, 107, and 144 had single di-, tri-, tetra-, and pentanucleotide repeats within the sequences, respectively. The most common repeat motifs were AT/AT (258), AAT/ATT (150), AAAT/ATTT (38), and AAAAT/ATTTT (20).

We randomly selected 240 putative SSRs and designed flanking primers using Primer3 version 2.0 (Rozen and Skaletsky, 2000), with default parameters except for: product size = 120–250 bp, maximum melting temperature (T_m) difference = 1°C, and maximum poly X = 3. These synthesized primer pairs (Integrated DNA Technologies, Iowa City, Iowa, USA) were first screened for PCR amplification and polymorphism efficacy on 3% Apex SFR Agarose Super Fine Resolution (Genesee Scientific, San Diego, California, USA) gels electrophoresed at 45 V for 20–24 h. PCR amplifications were performed in 12-µL reactions consisting of 3.0 µL (30 ng/µL) DNA, 0.5 µL of each 10 µM forward and reverse primer, 6.0 µL MyTaq HS Red Master Mix (Bioline, Taunton, Massachusetts, USA), and 2.0 µL ddH_2O. PCR reactions were performed using a C1000 or a T100 thermal cycler (Bio-Rad, Applied Biosystems, Foster City, California, USA) with the following parameters: 95°C for 60 s; 35 cycles of 95°C for 15 s, 60°C for 15 s, and 72°C for 10 s; and a single final extension cycle of 72°C for 60 s.

From the 240 primer pairs tested, we selected 17 for fluorescent labeling of one member of each pair with one of three dyes: NED (yellow [Life Technologies, Grand Island, New York, USA]), 6-FAM (blue [Integrated DNA Technologies]), or HEX (green [Integrated DNA Technologies]). These primers were selected based on robust repeatability and allele diversity across 30 samples. The samples included the broad geographic range of *P. scariosus*, *P. comarrhenus* A. Gray, *P. compactus* (D. D. Keck) Crosswh., *P. cyananthus* Hook. var. *cyananthus*, *P. fremontii* Torr. & Gray var. *fremontii*, *P. fremontii* var. *glabrescens* Dorn & Lichvar, *P. gibbensii* Dorn, *P. strictus* Benth., and *P. subglaber* Rydb. Labeled markers were amplified in 6-μL reactions containing 1.5 μL (30 ng/μL) DNA, 0.25 μL of each primer (10 μM each fluorescently labeled forward and unlabeled reverse), 3.0 μL MyTaq HS Red Master Mix (Bioline), and 1.0 μL ddH_2O. PCR reactions were performed using the following parameters: 95°C for 60 s; 25 cycles of 95°C for 15 s, 57°C for 15 s, and 72°C for 10 s; and a single final extension cycle of 72°C for 60 s. PCR products were diluted 1 : 20, and 1 μL of each sample was vacuum dried at 45°C for approximately 30 min using an SPD1010 SpeedVac (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Samples were analyzed at the Brigham Young University DNA Sequencing Center (Provo, Utah, USA), utilizing the ABI 3730xl (Applied Biosystems) with GeneScan 500 ROX Size Standard (Applied Biosystems). Fragment length analysis was accomplished using Geneious version 8.0.5 (Kearse et al., 2012). Hardy–Weinberg equilibrium (HWE) for loci within a population was calculated with GenAlEx version 6.501 (Peakall and Smouse, 2012).

Sixteen of the 17 SSRs provided reliable products across the 95 samples listed in Appendix 1, while one marker could not be scored with precision (Table 1). Ten of these 16 markers have not been previously reported (PS077–PS086), and four of the previously reported (Dockter et al., 2013) markers (PS014, PS016, PS048, PS064) were redesigned for this study to optimize for reliability across the range of *P. scariosus* varieties. The two remaining markers, Pen04 and Pen23, were SSR markers found to be viable in this study using the same PCR primers reported by Kramer and Fant (2007). These 16 SSR markers produced 360 unique alleles, ranging from one to 21 per taxon, or an average of 22.5 alleles per locus across *P. scariosus* and *P. fremontii* var. *glabrescens* (Table 2). The mean observed and expected heterozygosity values for each taxon tested were as follows: *P. scariosus* var. *albifluvis* (0.549 and 0.705), *P. scariosus* var. *cyanomontanus* (0.586 and 0.686), *P. scariosus* var. *garrettii* (0.496 and 0.808), *P. scariosus* var. *scariosus* (0.394 and 0.655), and *P. fremontii* var. *glabrescens* (0.430 and 0.646) (Table 2). Within populations of varieties *albifluvis* and *cyanomontanus*, none of the loci deviated from HWE, while two of the loci significantly deviated from HWE in the var. *scariosus* population. Interestingly, 12 of the 16 loci in the var. *garrettii* population demonstrated significant deviation from HWE, which may reflect that our sampling of this variety was from across a large geographic range representing multiple populations (Appendix 1). Markers PS078 and PS048 were monomorphic in *P. scariosus* var. *cyanomontanus* and *P. fremontii* var. *glabrescens*, respectively (Table 2). We note, however, the limited numbers of samples tested for these two taxa, which may account for the lack of polymorphisms (Appendix 1). Fifteen of the 16 primer combinations produced robust, usually polymorphic, markers across *P. comarrhenus*, *P. compactus*, *P. cyananthus* var. *cyananthus*, *P. fremontii* var. *fremontii*, *P. gibbensii*, *P. strictus*, and *P. subglaber*. However, PS078 poorly amplified with multiple weak bands in *P. compactus* and *P. gibbensii* (Table 3).

CONCLUSIONS

The 16 markers presented here consistently produced robust data sets across the four *P. scariosus* varieties tested, and 15 were reliable across eight additional related taxa. The SSRs identified in this study will provide a reliable set of markers needed to conduct studies of the genetic diversity of *P. scariosus*.

TABLE 1. Characteristics of 16 microsatellite markers developed for *Penstemon scariosus*.

Locus[a]	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	T_a (°C)	Fluorescent label	GenBank accession no.
Pen04	F: GATGGAAAATGTGCCAGGAC	$(TC)_{22}$	207–255	57	NED	DQ917425
	R: CTCTGCGGTGCATGAAAGTA					
Pen23	F: TGGTCTGATTTCAGGAAAAGC	$(GA)_{21}$	149–195	57	FAM	DQ917430
	R: TGCTCAAGACGATAATAAAAGTGC					
PS014	F: ATGTGCGCTCCTAACTGAAG	$(TGA)_6$	173–239	57	NED	JQ951616
	R: ACTGTGGTACATGTTCTTTCTGA					
PS016	F: GAAAGAGTCAAATGCGGACAG	$(CT)_8$	136–188	57	FAM	JQ967002
	R: GACGCGGTTGGCTATACAGT					
PS048	F: GGGCATACAAACAGGACTTCTC	$(CA)_9$	211–252	57	NED	JQ951621
	R: TGCCTGTAGGTTGATTTCCTTT					
PS064	F: CGGTGGATGAAGGAAACGGA	$(AG)_8$	133–204	57	FAM	JQ967029
	R: ACACTGACTACCAGCTTAACCG					
PS077	F: TAGCCGTTAGCCAAAGCAAT	$(GTGTT)_7$	112–150	57	HEX	KT271761
	R: ATTAATTGGACCTCCCTCCG					
PS078	F: CAAAAGGGGACTTCAAACCA	$(AGT)_{10}$	131–179	57	HEX	KT277090
	R: TCCCAGCCTGAAGAGATACC					
PS079	F: TGGAAAGAGAACCCATTTCG	$(CTT)_9$	139–225	57	FAM	KT277091
	R: AACAGCATGGAGGTTTGGAT					
PS080	F: ACCCCTACCAGTACCCACCT	$(AG)_8$	206–253	57	NED	KT277092
	R: CTCAATGCCAGACCACCTCT					
PS081	F: TCCTTTGGCCAATCAAGAGT	$(AAAG)_5$	144–190	57	FAM	KT277093
	R: GATGGGACCACAAAATGACA					
PS082	F: CGTACAAATCCAGGTATCCGA	$(TTCA)_5$	164–220	57	FAM	KT277094
	R: ACCTTTACAGGCTTCCTCCG					
PS083	F: GGGTTGAGATCCTTAGGGGA\	$(AT)_{10}$	148–204	57	NED	KT277095
	R: TCCTGTTTTGCACTTAGCCC					
PS084	F: TACTACGTCCAGGGTAGGGG	$(CAGGT)_6$	114–143	57	HEX	KT277096
	R: TAAGTCAAAGCCCAAAAGGC					
PS085	F: AGAGCTATCCTCCTCCTCCG	$(TCC)_7$	126–176	57	HEX	KT277097
	R: GCACTGAAGGCGGTGATTAT					
PS086	F: ACAGCCCAACCTTGAACAAC	$(ATAAA)_4…(TA)_9$	104–238	57	NED	KT277098
	R: GCTGATGATTCCACCTACACG					

Note: T_a = annealing temperature.

[a] Pen04 and Pen23 were SSR markers found to be viable in this study using the same PCR primers reported by Kramer and Fant (2007). We completely redesigned primers for PS014, PS016, PS048, and PS064, published in Dockter et al. (2013), to make smaller, more robust, and reliable PCR products.

TABLE 2. Observed and expected heterozygosity values using 16 SSR markers of the four named varieties of *Penstemon scariosus* and *P. fremontii* var. *glabrescens*.[a]

	Penstemon scariosus var.												*P. fremontii* var.			Total	
	albifluvis (N = 12)			*cyanomontanus* (N = 8)			*garrettii* (N = 36)			*scariosus* (N = 12)			*glabrescens* (N = 8)				
Locus[b]	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A_T	A_C
Pen04	5	0.583	0.569	9	0.500	0.867	14	**0.444**	**0.747**	4	0.583	0.632	6	0.286	0.745	25	11
Pen23	9	0.636	0.835	10	0.750	0.844	17	0.778	0.892	9	0.667	0.883	4	0.500	0.555	22	13
PS014	8	0.917	0.819	7	0.625	0.789	12	0.667	0.794	7	0.583	0.622	4	0.375	0.680	17	10
PS016	5	0.500	0.625	10	0.500	0.833	19	**0.667**	**0.892**	5	0.182	0.748	7	0.714	0.816	28	9
PS048	4	0.273	0.715	3	0.500	0.398	9	**0.167**	**0.663**	3	0.083	0.226	1[c]	0.000	0.000	12	4
PS064	9	0.583	0.854	5	0.250	0.641	12	**0.500**	**0.851**	6	0.667	0.691	6	0.500	0.758	16	9
PS077	5	0.583	0.524	5	0.625	0.625	6	0.444	0.711	3	0.083	0.469	3	0.250	0.586	8	6
PS078	3	0.545	0.566	1[c]	0.000	0.000	5	**0.083**	**0.649**	5	**0.182**	**0.744**	5	0.500	0.766	11	5
PS079	6	0.583	0.767	6	1.000	0.734	16	**0.611**	**0.837**	5	0.333	0.701	5	0.500	0.656	16	12
PS080	7	0.500	0.806	9	0.750	0.836	17	**0.472**	**0.875**	3	0.250	0.642	6	0.500	0.641	22	10
PS081	7	0.667	0.688	5	0.750	0.758	13	**0.667**	**0.847**	5	0.333	0.681	6	0.625	0.688	17	8
PS082	6	0.500	0.684	5	0.875	0.656	17	0.583	0.849	8	0.583	0.799	9	0.875	0.875	24	10
PS083	7	0.417	0.802	6	0.250	0.781	21	**0.294**	**0.920**	8	0.417	0.740	7	0.375	0.781	30	13
PS084	5	0.583	0.705	4	0.750	0.680	6	0.706	0.784	4	0.273	0.442	4	0.250	0.414	9	6
PS085	4	0.500	0.566	4	0.750	0.688	9	0.611	0.789	7	**0.500**	**0.785**	6	0.500	0.758	12	7
PS086	8	0.417	0.757	7	0.500	0.797	15	**0.235**	**0.826**	8	0.583	0.726	4	0.125	0.617	22	11
Mean	6.1	0.549	0.705	6.0	0.586	0.686	13.0	0.496	0.808	5.6	0.394	0.655	5.2	0.430	0.646	22.5	9.0

Note: A = number of alleles observed; A_C = number of common alleles observed; A_T = total number of unique observed alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; N = number of samples for each variety.

[a] Bold values indicate a significant deviation ($P < 0.001$) from Hardy–Weinberg equilibrium.

[b] Pen04 and Pen23 were SSR markers found to be viable in this study using the same PCR primers reported by Kramer and Fant (2007). We completely redesigned primers for PS014, PS016, PS048, and PS064, published in Dockter et al. (2013), to make smaller, more robust, and reliable PCR products.

[c] Locus was monomorphic.

TABLE 3. Cross-amplification of the 16 microsatellite markers developed for *Penstemon scariosus* in each of eight related *Penstemon* taxa.[a]

Species name	*N*	Pen04	Pen23	PS014	PS016	PS048	PS064	PS077	PS078	PS079	PS080	PS081	PS082	PS083	PS084	PS085	PS086
P. comarrhenus	6	8	8	4	5	2	7	4	1	7	3	4	5	4	2	6	6
P. compactus	2	4	1	3	2	1	1	3	+	3	2	3	3	2	2	4	2
P. cyananthus var. *cyananthus*	2	3	2	2	4	2	1	4	1	3	2	4	3	3	2	2	3
P. fremontii var. *fremontii*	2	4	3	2	4	1	2	2	2	4	2	3	2	2	1	2	2
P. fremontii var. *glabrescens*	8	6	4	4	7	1	6	3	5	5	6	6	9	7	4	6	4
P. gibbensii	2	3	4	1	2	2	2	2	+	2	2	4	2	1	2	3	4
P. strictus	2	2	2	2	1	1	2	3	2	2	3	3	3	2	2	2	2
P. subglaber	3	5	2	3	3	5	4	3	1	3	3	4	4	3	2	5	5

Note: + = only one individual amplified with an unknown number of alleles; *N* = number of individuals.
[a]Numbers presented for each locus represent number of alleles observed.

LITERATURE CITED

ASHE, D. M. 2013. Endangered and threatened wildlife and plants; threatened species status for Graham's beardtongue (*Penstemon grahamii*) and White River beardtongue (*Penstemon scariosus* var. *albifluvis*), Department of the Interior, Fish and Wildlife Service. *Federal Register* 78: 47590–47611.

BYERS, C., P. J. MAUGHAN, J. CLOUSE, AND J. R. STEWART. 2014. Microsatellite primers in *Agave utahensis* (Asparagaceae), a keystone species in the Mojave Desert and Colorado Plateau. *Applications in Plant Sciences* 2: 1400047.

CHATWIN, W. B., K. K. CARPENTER, F. R. JIMENEZ, D. B. ELZINGA, L. A. JOHNSON, AND P. J. MAUGHAN. 2014. Microsatellite primer development for post oak, *Quercus stellata* (Fagaceae). *Applications in Plant Sciences* 2: 1400070.

DOCKTER, R. B., D. B. ELZINGA, B. GEARY, P. J. MAUGHAN, L. A. JOHNSON, D. TUMBLESON, J. FRANKE, ET AL. 2013. Developing molecular tools and insights into the *Penstemon* genome using genomic reduction and next-generation sequencing. *BMC Genetics* 14: 66.

ENGLAND, J. L. 1982. A new species of *Penstemon* (Scrophulariaceae) from the Uinta Basin of Utah and Colorado. *Great Basin Naturalist* 42: 367–368.

HOLMGREN, N. H. 1984. *Penstemon. In* A. Cronquist, A. H. Holmgren, N. H. Holmgren, J. L. Reveal, and P. K. Holmgren [eds.], Intermountain flora: Vascular plants of the Intermountain West, vol. 4, 370–457. New York Botanical Garden, Bronx, New York, USA.

KEARSE, M., R. MOIR, A. WILSON, S. STONES-HAVAS, M. CHEUNG, S. STURROCK, S. BUXTON, ET AL. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics (Oxford, England)* 28: 1647–1649.

KRAMER, A. T., AND J. B. FANT. 2007. Isolation and characterization of microsatellite loci in *Penstemon rostriflorus* (Plantaginaceae) and cross-species amplification. *Molecular Ecology Notes* 7: 998–1001.

MAUGHAN, P. J., S. M. YOURSTONE, E. N. JELLEN, AND J. A. UDALL. 2009. SNP discovery via genomic reduction, barcoding and 454-pyrosequencing in amaranth. *Plant Genome* 2: 260–270.

NEESE, E. C., AND N. D. ATWOOD. 2008. *Penstemon* Mitchell. *In* S. L. Welsh, N. D. Atwood, S. Goodrich, and L. C. Higgins [eds.], A Utah flora, 702–733. Printing Services, Brigham Young University, Provo, Utah, USA.

PEAKALL, R., AND P. E. SMOUSE. 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research–An update. *Bioinformatics (Oxford, England)* 28: 2537–2539.

ROZEN, S., AND H. J. SKALETSKY. 2000. Primer3 on the WWW for general users and for biologist programmers. *In* S. Krawetz and S. Misener [eds.], Bioinformatics methods and protocols: Methods in molecular biology, 365–386. Humana Press, Totowa, New Jersey, USA.

THIEL, T., W. MICHALEK, R. K. VARSHNEY, AND A. GRANER. 2003. Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics* 106: 411–422.

TODD, J. J., AND L. O. VODKIN. 1996. Duplications that suppress and deletions that restore expression from a chalcone synthase multigene family. *Plant Cell* 8: 687–699.

Appendix 1. Geographic origin of 95 accessions of *Penstemon scariosus* and eight additional related taxa included in this study. Vouchers for each accession were deposited in the Stanley L. Welsh Herbarium (BRY), Brigham Young University, Provo, Utah, USA.

Species	*N*	Voucher no.	Population location	GPS coordinates[a]	
				Latitude	Longitude
Penstemon scariosus Pennell					
var. *albifluvis* (England) N. H. Holmgren	4	BRY109463	Bayless Pad Site, Rio Blanco Co., CO, USA	39°56′52.9″N	109°02′13.3″W
var. *albifluvis*	4	BRY121019	Upper Agency Draw, Uintah Co., UT, USA	39°44′23.2″N	109°39′08.8″W
var. *albifluvis*	4	BRY121017	Book Cliff Ridge, Uintah Co., UT, USA	39°26′22.5″N	109°17′45.4″W
var. *cyanomontanus* Neese	4	BRY133610	Blue Mountain, Uintah Co., UT, USA	40°26′18.0″N	109°03′39.8″W
var. *cyanomontanus*	4	BRY121024	Diamond Peak, Moffat Co., CO, USA	40°56′44.8″N	108°52′06.8″W
var. *garrettii* (Pennell) N. H. Holmgren	4	BRY117079	Price Canyon, Utah Co., UT, USA	39°49′43.2″N	110°57′28.0″W
var. *garrettii*	4	BRY133591	East of Fruitland, Duchesne Co., UT, USA	40°12′15.7″N	110°47′57.1″W
var. *garrettii*	4	BRY117064	Midway, Wasatch Co., UT, USA	40°32′03.2″N	111°28′57.7″W
var. *garrettii*	4	BRY121016	East of Tabiona, Duchesne Co., UT, USA	40°19′17.6″N	110°41′10.1″W
var. *garrettii*	4	BRY121021	Argyle Canyon, Duchesne Co., UT, USA	39°53′44.3″N	110°38′18.7″W
var. *garrettii*	4	BRY121020	Pine Mountain, Sweetwater Co., WY, USA	41°03′42.5″N	108°57′45.0″W
var. *garrettii*	4	BRY121028	Goslin Mountain, Daggett Co., UT, USA	40°56′44.5″N	109°15′35.1″W
var. *garrettii*	4	BRY121027	North of Lone Tree, Uinta Co., WY, USA	41°05′10.1″N	110°11′19.3″W
var. *garrettii*	4	BRY109209	Cat Peak, Utah Co., UT, USA	39°53′56.8″N	110°57′34.0″W
var. *scariosus*	4	BRY34543	Top of Ferron Canyon, Sanpete Co., UT, USA	39°06′54.1″N	111°18′13.0″W
var. *scariosus*	4	BRY121025	North of Scipio, Juab Co., UT, USA	39°25′33.5″N	112°04′33.3″W
var. *scariosus*	4	BRY106700	Northeast of Antimony Piute Co., UT, USA	38°09′16.5″N	111°55′39.0″W
P. comarrhenus A. Gray	2	BRY47030	Near Spring Canyon, Sevier Co., UT, USA	38°51′39.1″N	111°32′55.6″W
P. comarrhenus	4	BRY119516	Geyser Peak, Sevier Co., UT, USA	38°30′43.0″N	111°27′43.7″W
P. compactus (D. D. Keck) Crosswh.	2	BRY130986	Tony Grove, Cache Co., UT, USA	41°54′14.6″N	111°38′50.6″W
P. cyananthus Hook. var. *cyananthus*	2	BRY130991	Tony Grove, Cache Co., UT, USA	41°54′15.5″N	111°38′58.8″W
P. fremontii Torr. & Gray var. *fremontii*	2	BRY121022	Near Meeker, Rio Blanco Co., CO, USA	39°58′59.1″N	107°58′02.6″W
P. fremontii var. *glabrescens* Dorn & Lichvar	4	BRY126454	Piceance Canyon, Rio Blanco Co., CO, USA	39°45′42.4″N	108°00′46.4″W
P. fremontii var. *glabrescens*	4	BRY126453	Piceance Canyon, Rio Blanco Co., CO, USA	40°03′51.4″N	108°15′06.7″W
P. gibbensii Dorn	2	BRY28472	Browns Park, Daggett Co., UT, USA	40°50′49.1″N	109°02′59.3″W
P. strictus Benth.	2	BRY35430	Diamond Peak, Moffat Co., CO, USA	40°56′30.6″N	108°51′47.3″W
P. subglaber Rydb.	3	BRY50072	Top of Ferron Canyon, Sanpete Co., UT, USA	39°08′12.7″N	111°22′31.1″W

Note: *N* = number of individuals.

[a]Datum WGS 84 was used for the GPS latitude and longitude coordinates.