



Figure S1. Chromatograms showing the difference in the nucleotide sequence of the three *psbA-trnH* spacer haplotypes. In each panel, the top two rows represent forward and reverse reads from the “*albicaulis*” haplotype and the bottom two rows the forward and reverse reads from the “*flexilis*” or “*flexilis-i*” haplotype. A. The deleted C in the “*flexilis*” and “*flexilis-i*” haplotypes at position 116 in Figure 3. B. The derived T in the “*flexilis*” haplotype at position 250 in Figure 3, which represents the PstI restriction sites encompassed by sites 250-255 in Figure 3. C. The derived T in the “*flexilis*” and “*flexilis-i*” haplotypes at position 377 in Figure 2, which represents the PstI restriction site encompassed by sites 376-381 in Figure 3.

Table S1. Metadata for the 111 five-needle pine samples from which we comprehensively DNA sequenced and PsiI-restriction-site analyzed the *psbA-trnH* spacer (Appendix). The “Species” column represents the visual identification of 43 *Pinus albicaulis* and 68 *P. flexilis* trees using the morphology of the seed cones attached to the sampled tree. The “isolate” column is the DNA isolation number reported with the GenBank accession number(s) of DNA sequences generated during our study. GenBank accessions include samples analyzed for variation at the *trnG* intron, *matK*, and *trnL* region using both DNA sequencing and restriction site analysis, and these represented geographical extremes of the three different the *psbA-trnH* haplotypes. The *psbA-trnH* spacer haplotypes “albicaulis” and “flexilis” identify the only or most common haplotype, respectively, detected for that species. The “flexilis-i” haplotype (“i” for intermediate) indicates the five *P. flexilis* samples that had the nucleotide C occupying position #250 from the 5’ end of the *psbA-trnH* spacer (Figure 3), which is a similarity shared with the “albicaulis” haplotype. The “flexilis” haplotype has the nucleotide T occupying that position (Figure 3). The “flexilis-i” haplotype is found in all GenBank accessions of *Pinus flexilis* and close North American relatives that report the DNA sequence of the *psbA-trnH* spacer. The common “flexilis” haplotype is newly detected in this study and is apparently unique to *P. flexilis*.

Species	isolate	GenBank # (<i>psbA-trnH</i> , ¹ <i>trnG</i> , ² <i>matK</i> , ³ <i>trnL</i>)	<i>psbA-trnH</i> haplotype	Locality in the U.S.A. (State. County. Local site)	longitude	latitude	collector
<i>P. albicaulis</i>	2639	MN603507, ³ MN537000	albicaulis	MT. Gallatin. Spanish Peaks. Beehive Basin	-111.38598	45.31128	Hansen
<i>P. albicaulis</i>	2640	MN603508, ³ MN537001	albicaulis	MT. Gallatin. Spanish Peaks. Beehive Basin	-111.38678	45.31517	Hansen
<i>P. albicaulis</i>	2641	MN603509, ³ MN537002	albicaulis	MT. Gallatin. Spanish Peaks. Beehive Basin	-111.38863	45.3189	Hansen
<i>P. albicaulis</i>	2642	MN603510, ³ MN537003	albicaulis	MT. Gallatin. Spanish Peaks. Beehive Basin	-111.39215	45.33048	Hansen
<i>P. albicaulis</i>	2643	MN603511, ³ MN537004	albicaulis	MT. Gallatin. Spanish Peaks. Beehive Basin	-111.39751	45.34145	Hansen
<i>P. albicaulis</i>	2646	MN603512	albicaulis	WY. Park. S of Cooke City. Republic Ridge cc #1	- 109.94327	44.992844	Hansen
<i>P. albicaulis</i>	2647	MN603513, ³ MN537005	albicaulis	WY. Park. S of Cooke City. Republic Ridge cc #2	- 109.94327	44.992844	Hansen
<i>P. albicaulis</i>	2648	MN603514, ³ MN537006	albicaulis	WY. Park. S of Cooke City. Republic Ridge cc #3	- 109.94327	44.992844	Hansen
<i>P. albicaulis</i>	2670	MN603515, ¹ MN542795, ² MN542807	albicaulis	WY. Fremont. Wind River Mts. SE of Togwotee Pass	- 109.95303	43.600859	Laufenberg

Species	isolate	GenBank # (<i>psbA-trnH</i> , ¹ <i>trnG</i> , ² <i>matK</i> , ³ <i>trnL</i>)	<i>psbA-trnH</i> haplotype	Locality in the U.S.A. (State. County. Local site)	longitude	latitude	collector
<i>P. albicaulis</i>	2671	MN603516	albicaulis	WY. Fremont. Wind River Mts. SE of Togwotee Pass	- 109.95303 4	43.600859	Laufenberg
<i>P. albicaulis</i>	2672	MN603517	albicaulis	WY. Fremont. Wind River Mts. SE of Togwotee Pass	- 109.95303 5	43.600859	Laufenberg
<i>P. albicaulis</i>	2674	MN603518	albicaulis	MT. Madison. Madison Range. Bear Creek 2	-111.53633	45.20631	Hansen
<i>P. albicaulis</i>	2675	MN603519	albicaulis	MT. Madison. Madison Range. Bear Creek 3	-111.53634	45.20632	Hansen
<i>P. albicaulis</i>	2685	MN603520	albicaulis	MT. Gallatin. N of Big Sky. Dudley 1	-111.27479	45.29523	Hansen
<i>P. albicaulis</i>	2686	MN603521	albicaulis	MT. Gallatin. N of Big Sky. Dudley 2	-111.27478	45.29523	Hansen
<i>P. albicaulis</i>	2687	MN603522	albicaulis	MT. Gallatin. N of Big Sky. Dudley 3	-111.27061	45.29543	Hansen
<i>P. albicaulis</i>	2688	MN603523	albicaulis	MT. Gallatin. N of Big Sky. Dudley 4	-111.27062	45.29543	Hansen
<i>P. albicaulis</i>	2695	MN603524	albicaulis	WY. Park. Beartooth Hwy. Clay Butte. Scenic overlook	- 109.63011 3	44.934329	Vukelich
<i>P. albicaulis</i>	2697	MN603525, ¹ MN542796, ² MN542808	albicaulis	NV. Elko. East Humboldt Range. Slope S of Angel Lake	- 115.08878 7	41.024427	Lavin
<i>P. albicaulis</i>	2857	MN603526, ¹ MN542797, ² MN542809	albicaulis	NV. Washoe. Carson Range. Mount Rose. Trail saddle	- 119.93084 9	39.340415	Lavin
<i>P. albicaulis</i>	2858	MN603527	albicaulis	NV. Washoe. Carson Range. Mount Rose. Radio Tower Rd.	- 119.91977 8	39.307774	Lavin
<i>P. albicaulis</i>	2860	MN603528	albicaulis	NV. Washoe. Carson Range. Tahoe Meadows	- 119.91016 5	39.303362	Lavin
<i>P. albicaulis</i>	2861	MN603529	albicaulis	NV. Washoe. Carson Range. Mount Rose. Trailhead	- 119.89995 6	39.311964	Lavin

Species	isolate	GenBank # (<i>psbA-trnH</i> , ¹ <i>trnG</i> , ² <i>matK</i> , ³ <i>trnL</i>)	<i>psbA-trnH</i> haplotype	Locality in the U.S.A. (State. County. Local site)	longitude	latitude	collector
<i>P. albicaulis</i>	2863	MN603530, ¹ MN542798, ² MN542810	albicaulis	ID. Blaine. N of Baker Lake along Norton Lakes trail	- 114.67855 7	43.714624	Lavin
<i>P. albicaulis</i>	2864	MN603531	albicaulis	ID. Blaine. Boulder Mountains. Left Fork Boulder Creek	- 114.53024 7	43.835175	Lavin
<i>P. albicaulis</i>	2866	MN603532	albicaulis	MT. Gallatin. Bridger Range. Bridger Bowl. Midway Station	- 110.91943 4	45.817300	Lavin
<i>P. albicaulis</i>	2867	MN603533, ¹ MN542799, ² MN542811	albicaulis	MT. Gallatin. Gallatin Range. Mount Blackmore	- 110.99436 2	45.437896	Lavin
<i>P. albicaulis</i>	P41	MN603534	albicaulis	MT. Gallatin. Dudley Creek, sample3 zone4	- 111.30093 48	45.324162 54	Becker
<i>P. albicaulis</i>	P44	MN603535	albicaulis	MT. Gallatin. Dudley Creek, sample6 zone4	- 111.30498 45	45.323254 29	Becker
<i>P. albicaulis</i>	P65	MN603536	albicaulis	MT. Madison. Mile Creek, sample2 zone4	- 111.40725 19	44.728155 86	Becker
<i>P. albicaulis</i>	P67	MN603537	albicaulis	MT. Madison. Mile Creek, sample4 zone4	- 111.40656 98	44.728992 95	Becker
<i>P. albicaulis</i>	P76	MN603538	albicaulis	ID. Fremont. Sawtell, sample7 zone1	- 111.39109 57	44.533864 13	Flanary
<i>P. albicaulis</i>	P88	MN603539	albicaulis	ID. Fremont. Sawtell, sample8 zone3	- 111.41353 89	44.542585 39	Flanary
<i>P. albicaulis</i>	P95	MN603540	albicaulis	ID. Fremont. Sawtell, sample7 zone4	- 111.43991 76	44.557573 66	Flanary
<i>P. albicaulis</i>	P96	MN603541	albicaulis	ID. Fremont. Sawtell, sample8 zone4	- 111.43522 04	44.551432 04	Flanary

Species	isolate	GenBank # (<i>psbA-trnH</i> , ¹ <i>trnG</i> , ² <i>matK</i> , ³ <i>trnL</i>)	<i>psbA-trnH</i> haplotype	Locality in the U.S.A. (State. County. Local site)	longitude	latitude	collector
<i>P. albicaulis</i>	P109	MN603542	albicaulis	WY. Lincoln. Cottonwood Lake, sample5 zone4	- 110.80216 86	42.643575 03	Stalling
<i>P. albicaulis</i>	P123	MN603543	albicaulis	WY. Sublette. Deadline Ridge, sample5 zone3	- 110.42212 9	42.355879 53	Flanary
<i>P. albicaulis</i>	P125	MN603544	albicaulis	WY. Sublette. Deadline Ridge, sample7 zone3	- 110.42384 75	42.355961 94	Stalling
<i>P. albicaulis</i>	P127	MN603545	albicaulis	WY. Sublette. Deadline Ridge, sample2 zone4	- 110.43634 34	42.361869 56	Stalling
<i>P. albicaulis</i>	P130	MN603546	albicaulis	WY. Sublette. Deadline Ridge, sample5 zone4	- 110.45399 35	42.367516 6	Stalling
<i>P. albicaulis</i>	P139	MN603547	albicaulis	WY. Teton. Teton Pass, sample2 zone2	- 110.95281 77	43.501183 81	Stalling
<i>P. albicaulis</i>	P150	MN603548	albicaulis	WY. Teton. Teton Pass, sample1 zone4	- 110.95052 13	43.504661 27	Stalling
<i>P. albicaulis</i>	P154	MN603549	albicaulis	WY. Teton. Teton Pass, sample5 zone4	- 110.94773 84	43.514277 21	Stalling
<i>P. flexilis</i>	2649	MN603550, ¹ MN542804, ² MN542816, ³ MN537009	flexilis-i	MT. Park. S of Gardiner gt #4	- 110.69400 4	45.011769	Hansen
<i>P. flexilis</i>	2650	MN603551, ³ MN537010	flexilis-i	MT. Park. S of Gardiner gt #5	- 110.69400 4	45.011769	Hansen
<i>P. flexilis</i>	2658	MN603552, ¹ MN542805, ² MN542817, ³ MN537018	flexilis-i	MT. Gallatin. Story Hills road	- 111.00664 5	45.689148	Lavin
<i>P. flexilis</i>	2667	MN603553, ¹ MN542806, ² MN542818	flexilis-i	MT. Fergus. Big Snowy Mts. West Peak	- 109.53897 8	46.784294	Lavin

Species	isolate	GenBank # (<i>psbA-trnH</i> , ¹ <i>trnG</i> , ² <i>matK</i> , ³ <i>trnL</i>)	<i>psbA-trnH</i> haplotype	Locality in the U.S.A. (State. County. Local site)	longitude	latitude	collector
<i>P. flexilis</i>	2682	MN603554	flexilis-i	MT. Gallatin. Just NW of Gardiner 2	-110.75556	45.04893	Hansen
<i>P. flexilis</i>	2644	MN603555, ³ MN537007	flexilis	MT. Gallatin. Spanish Peaks. Dudley Basin road	-111.24918	45.26981	Hansen
<i>P. flexilis</i>	2645	MN603556, ³ MN537008	flexilis	MT. Gallatin. Bozeman. MSU Campus	111.05193	45.669049	Lavin
<i>P. flexilis</i>	2651	MN603557, ³ MN537011	flexilis	MT. Park. S of Gardiner gt #6	110.69400	45.011769	Hansen
<i>P. flexilis</i>	2652	MN603558, ³ MN537012	flexilis	MT. Park. S of Gardiner gt #7	110.69400	45.011769	Hansen
<i>P. flexilis</i>	2653	MN603559, ³ MN537013	flexilis	MT. Park. S of Gardiner gt #8	110.69400	45.011769	Hansen
<i>P. flexilis</i>	2654	MN603560, ³ MN537014	flexilis	MT. Park. S of Gardiner gt #9	110.69400	45.011769	Hansen
<i>P. flexilis</i>	2656	MN603561, ³ MN537016	flexilis	WY. Teton County. Teton Pass. South jp-s #2	110.95105	43.481417	Laufenberg
<i>P. flexilis</i>	2657	MN603562, ³ MN537017	flexilis	WY. Teton County. Teton Pass. North jp-n #3	110.95298	43.499687	Laufenberg
<i>P. flexilis</i>	2659	MN603563	flexilis	MT. Gallatin. Bridger Range. Near summit of Baldy	110.95320	45.751795	Lavin
<i>P. flexilis</i>	2660	MN603564	flexilis	MT. Gallatin. Bridger Range. Knob below of Baldy	110.96430	45.731213	Lavin
<i>P. flexilis</i>	2665	MN603565	flexilis	MT. Carbon. Rock Creek Vista Point above Red Lodge	109.40763	45.045124	Johnson
<i>P. flexilis</i>	2666	MN603566	flexilis	MT. Fergus. Big Snowy Mts. Ice Cave Trail	109.49631	46.790225	Lavin

Species	isolate	GenBank # (<i>psbA-trnH</i> , ¹ <i>trnG</i> , ² <i>matK</i> , ³ <i>trnL</i>)	<i>psbA-trnH</i> haplotype	Locality in the U.S.A. (State. County. Local site)	longitude	latitude	collector
<i>P. flexilis</i>	2668	MN603567	flexilis	MT. Wheatland. along Hwy 191 N of Harlowton	-109.894247	46.241068	Lavin
<i>P. flexilis</i>	2669	MN603568	flexilis	MT. Gallatin. Bridger Mts. Middle Cottonwood	-110.966628	45.762456	Laufenberg
<i>P. flexilis</i>	2673	MN603569	flexilis	MT. Madison. Madison Range. Bear Creek 1	-111.55602	45.18259	Hansen
<i>P. flexilis</i>	2676	MN603570	flexilis	MT. Gallatin. Near Jardine. Eagle 1	-110.67027	45.06703	Hansen
<i>P. flexilis</i>	2677	MN603571	flexilis	MT. Gallatin. Near Jardine. Eagle 2	-110.68494	45.05033	Hansen
<i>P. flexilis</i>	2679	MN603572	flexilis	MT. Gallatin. Near Jardine. Eagle 4	-110.69485	45.0358	Hansen
<i>P. flexilis</i>	2680	MN603573	flexilis	MT. Gallatin. Near Jardine. Eagle 5	-110.69485	45.0358	Hansen
<i>P. flexilis</i>	2681	MN603574	flexilis	MT. Gallatin. Gardiner 1	-110.71008	45.03016	Hansen
<i>P. flexilis</i>	2683	MN603575	flexilis	MT. Gallatin. N end of Paradise Valley. S of Livingston 1	-110.58476	45.57951	Hansen
<i>P. flexilis</i>	2684	MN603576	flexilis	MT. Gallatin. N end of Paradise Valley. S of Livingston 2	-110.57745	45.58345	Hansen
<i>P. flexilis</i>	2689	MN603577	flexilis	MT. Gallatin. Hwy 191 and road to Big Sky 1	-111.24832	45.26995	Hansen
<i>P. flexilis</i>	2690	MN603578	flexilis	MT. Gallatin. Hwy 191 and road to Big Sky 2	-111.24832	45.26995	Hansen
<i>P. flexilis</i>	2691	MN603579	flexilis	MT. Gallatin. Bozeman. Triple Tree. The middle tree	-110.990742	45.597791	Lavin
<i>P. flexilis</i>	2692	MN603580	flexilis	MT. Gallatin. Bridger Bowl. North Bowl	-110.925957	45.820136	Laufenberg
<i>P. flexilis</i>	2693	MN603581, ¹ MN542800, ² MN542812	flexilis	MT. Gallatin. Bridger Bowl. North Bowl	-110.925958	45.820136	Laufenberg
<i>P. flexilis</i>	2694	MN603582	flexilis	MT. Madison. Gravelly Range. Call Rd. S of Ennis	-111.802041	45.156031	Bergstrom
<i>P. flexilis</i>	2696	MN603583, ¹ MN542801, ² MN542813	flexilis	WY. Park. Jct Chief Joseph Hwy and Sunlight Rd.	-109.425149	44.764216	Vukelich
<i>P. flexilis</i>	2698	MN603584	flexilis	NV. Elko. East Humboldt Range. Angel Lake. S margin	-115.087035	41.024895	Lavin

Species	isolate	GenBank # (<i>psbA-trnH</i> , ¹ <i>trnG</i> , ² <i>matK</i> , ³ <i>trnL</i>)	<i>psbA-trnH</i> haplotype	Locality in the U.S.A. (State. County. Local site)	longitude	latitude	collector
<i>P. flexilis</i>	2699	MN603585	flexilis	NV. Elko. East Humboldt Range. Angel Lake outlet	- 115.08194 8	41.026717	Lavin
<i>P. flexilis</i>	2700	MN603586, ¹ MN542802, ² MN542814	flexilis	NV. Elko. East Humboldt Range. Angel Lake outlet	- 115.08140 0	41.026733	Lavin
<i>P. flexilis</i>	2865	MN603587, ¹ MN542803, ² MN542815	flexilis	MT. Gallatin. Bridger Bowl. NE of Midway Station	- 110.91771 9	45.817430	Lavin
<i>P. flexilis</i>	2907	MN603588	Flexilis	NM. Sandoval. Sandia Mts. Junction 10K and Crest trail	- 106.44971 3	35.237818	A. Lavin
<i>P. flexilis</i>	P5	MN603589	flexilis	MT. Beaverhead. Bean Creek, sample5 zone1	- 112.02459 5	44.575875 26	Flanary
<i>P. flexilis</i>	P7	MN603590	flexilis	MT. Beaverhead. Bean Creek, sample7 zone1	- 112.01936 78	44.568967 45	Flanary
<i>P. flexilis</i>	P15	MN603591	flexilis	MT. Beaverhead. Bean Creek, sample5 zone3	- 112.03609 23	44.553647 95	Flanary
<i>P. flexilis</i>	P48	MN603592	flexilis	MT. Madison. Mile Creek, sample4 zone1	- 111.43247 2	44.743640 68	Becker
<i>P. flexilis</i>	P54	MN603593	flexilis	MT. Madison. Mile Creek, sample4 zone2	- 111.42162 69	44.732954 71	Becker
<i>P. flexilis</i>	P57	MN603594	flexilis	MT. Madison. Mile Creek, sample7 zone2	- 111.41775 46	44.730365 54	Becker
<i>P. flexilis</i>	P59	MN603595	flexilis	MT. Madison. Mile Creek, sample2 zone3	- 111.41666 14	44.730356 43	Becker
<i>P. flexilis</i>	P115	MN603596	flexilis	WY. Sublette. Deadline Ridge, sample2 zone2	- 110.40096 37	42.352177 48	Flanary

Species	isolate	GenBank # (<i>psbA-trnH</i> , ¹ <i>trnG</i> , ² <i>matK</i> , ³ <i>trnL</i>)	<i>psbA-trnH</i> haplotype	Locality in the U.S.A. (State. County. Local site)	longitude	latitude	collector
<i>P. flexilis</i>	P132	MN603597	flexilis	WY. Teton. Teton Pass, sample1 zone1	- 110.95318 6	43.499402 4	Stalling
<i>P. flexilis</i>	P133	MN603598	flexilis	WY. Teton. Teton Pass, sample2 zone1	- 110.95305 1	43.499616 12	Stalling
<i>P. flexilis</i>	P140	MN603599	flexilis	WY. Teton. Teton Pass, sample3 zone2	- 110.95303 29	43.501924 59	Stalling
<i>P. flexilis</i>	P146	MN603600	flexilis	WY. Teton. Teton Pass, sample3 zone3	- 110.95176 76	43.503390 96	Stalling
<i>P. flexilis</i>	P185	MN603601	flexilis	WY. Park. Russel Peak, sample6 zone1	- 109.51327 85	44.836257 04	Shane
<i>P. flexilis</i>	P229	MN603602	flexilis	WY. Fremont. Louis Lake, sample21 zone1	- 108.79217 7	42.525832 44	Durglo
<i>P. flexilis</i>	P232	MN603603	flexilis	WY. Fremont. Louis Lake, sample24 zone1	- 108.77963 6	42.515098 73	Durglo
<i>P. flexilis</i>	P234	MN603604	flexilis	WY. Fremont. Louis Lake, sample14 zone2	- 108.80081 23	42.536046 28	Durglo
<i>P. flexilis</i>	P236	MN603605	flexilis	WY. Fremont. Louis Lake, sample16 zone2	- 108.79895 15	42.533278 84	Durglo
<i>P. flexilis</i>	P238	MN603606	flexilis	WY. Fremont. Louis Lake, sample18 zone2	- 108.79563 21	42.530099 41	Durglo
<i>P. flexilis</i>	P243	MN603607	flexilis	WY. Fremont. Louis Lake, sample11 zone3	- 108.80044 77	42.539506	Durglo
<i>P. flexilis</i>	P248	MN603608	flexilis	WY. Fremont. Louis Lake, sample4 zone4	- 108.81167 75	42.547933 92	Durglo

Species	isolate	GenBank # (<i>psbA-trnH</i> , ¹ <i>trnG</i> , ² <i>matK</i> , ³ <i>trnL</i>)	<i>psbA-trnH</i> haplotype	Locality in the U.S.A. (State. County. Local site)	longitude	latitude	collector
<i>P. flexilis</i>	P249	MN603609	flexilis	WY. Fremont. Louis Lake, sample5 zone4	- 108.81050 15	42.548176 94	Durglo
<i>P. flexilis</i>	P250	MN603610	flexilis	WY. Fremont. Louis Lake, sample6 zone4	- 108.80863 77	42.547215 37	Durglo
<i>P. flexilis</i>	P251	MN603611	flexilis	WY. Sublette. Scab Creek, sample19 zone1	- 109.55655 31	42.822862 2	Shane
<i>P. flexilis</i>	P280	MN603612	flexilis	WY. Fremont. Shoshone Lake, sample7 zone1	- 108.91578 48	42.820958 2	Durglo
<i>P. flexilis</i>	P281	MN603613	flexilis	WY. Fremont. Shoshone Lake, sample8 zone1	- 108.91119 1	42.825027 22	Durglo
<i>P. flexilis</i>	P288	MN603614	flexilis	WY. Fremont. Shoshone Lake, sample7 zone2	- 108.92299 98	42.812131 64	Durglo
<i>P. flexilis</i>	P289	MN603615	flexilis	WY. Fremont. Shoshone Lake, sample8 zone2	- 108.92123 95	42.813287 46	Durglo
<i>P. flexilis</i>	P302	MN603616	flexilis	WY. Fremont. Shoshone Lake, sample7 zone4	- 108.94901 63	42.804589 62	Durglo
<i>P. flexilis</i>	P303	MN603617	flexilis	WY. Fremont. Shoshone Lake, sample8 zone4	- 108.94433 71	42.805770 29	Durglo

