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PRIMER NOTE

IDENTIFICATION AND CHARACTERIZATION OF MICROSATELLITES FROM CALAFATE (*BERBERIS MICROPHYLLA*, BERBERIDACEAE)[1]

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- *Premise of the study:* Southern barberry or calafate (*Berberis microphylla*) is a shrub species endemic to the Patagonian region of South America that is used for human consumption. The fruit is very rich in vitamin C and anthocyanins and has a very high antioxidant capacity. There have been only a few genetic studies of this and other closely related species.
- *Methods and Results:* Here we present the first 18 microsatellite markers of *B. microphylla* that were characterized using 66 accessions of calafate from Patagonia. On average, they had 7.6 alleles per marker, with an expected heterozygosity of 0.688. The informativeness of these markers was also evaluated in another 15 *Berberis* species, including most of the native and endemic Chilean species.
- *Conclusions:* The results confirm that these new simple sequence repeat markers are very polymorphic and potentially useful in genetic studies in any species of the genus *Berberis*.

Key words: Berberidaceae; *Berberis microphylla*; genetic diversity; microsatellite; simple sequence repeat.

The southern barberry or calafate (*Berberis microphylla* G. Forst.) is a shrub native to the Southern Cone of South America (Chilean and Argentinean Patagonia). It belongs to the mostly temperate family Berberidaceae, composed of 15 genera and approximately 650 species (Landrum, 1999). There are 60 described species in South America, 20 of which are found in Argentina and Chile and half of these are endemic to Chile (Landrum, 1999). Orsi (1984) recognized 17 *Berberis* L. species in Patagonia, while Landrum (1999) grouped a number of species into a single taxon. Such is the case of *B. heterophylla* Juss. ex Poir. and *B. buxifolia* Lam., which are recognized as synonyms of *B. microphylla* (Landrum, 1999). The main problems for the taxonomy of this genus are phenotypic similarity and plasticity, in addition to the hypothetical presence of hybrids exhibiting intermediate phenotypes and genotypes between different species (Bottini et al., 2007).

Calafate fruits are considered one of the richest in vitamin C and anthocyanins among native Chilean species (Ruiz et al., 2010). The genus *Berberis* has been reported as tolerant to low temperature, drought, and wind, and consequently *B. microphylla* is a candidate species for domestication and cultivation in marginal Patagonian soils. To date, there have been only a few genetic studies of this species, mainly based on amplified fragment length polymorphism–type markers and ribosomal intergenic sequences (Bottini et al., 2002, 2007). In this work, we present the development of the first microsatellite or simple sequence repeat (SSR) markers known for this species, obtained through massive sequencing of genomic DNA and enriched libraries. SSRs are powerful tools to evaluate the genetic diversity and genetic structure of populations, to identify specific genotypes (varieties), and for paternity tests. This is because they are widely dispersed in the genome, highly polymorphic, codominant, and reproducible (Kalia et al., 2011). In this context, the main interest for developing these markers is to study the genetic diversity of this and related species, to explore their reproductive and propagative form, and eventually to identify single specimens in the framework of domestication programs.

METHODS AND RESULTS

To isolate microsatellites, genomic DNA was pooled from three samples of *B. microphylla* (accession no. 59, 64, and 66; see Appendix 1), from Magallanes, Chile. These plants were collected from the same locations as described previously (Dominguez and Aravena, 2012) and deposited at the Herbarium of Universidad de Concepción, Concepción, Chile (CONC 948). Samples belonging to the other *Berberis* species were identified by comparison to specimens deposited in the same herbarium (CONC). Total DNA was extracted from young stems, green fruits, and seeds following the protocol described by Lodhi et al. (1994), scaled down to a microtube. In brief, 0.1 g of tissue was milled in an automatic grinder with steel balls plus 700 µL of extraction buffer (20 mM EDTA, 100 mM Tris-HCl, 1.4 M NaCl, 2% [w/v] cetyltrimethylammonium bromide [CTAB], 10 mg PVP 40,000, 0.2% [v/v] 2-mercaptoethanol; pH 8.0) and incubated at 60°C for 30 min, followed by extraction with chloroform : isoamyl alcohol (24 : 1). Total DNA was precipitated using absolute ethanol precooled at −20°C. The precipitate was washed twice with 70% ethanol, and the pellet was dissolved in 50 µL of nuclease-free water containing RNase A at 0.1 µg/mL. A final incubation at 37°C for 15 min was done before storing the DNA solution at −20°C. The purified total DNA was quantified by spectrophotometric

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absorbance (NanoDrop ND-1000; Thermo Fisher Scientific, Wilmington, Delaware, USA) and its quality verified by agarose gel electrophoresis.

For the isolation of microsatellite-containing DNA sequences, the pooled DNA sample from Patagonian accessions of *B. microphylla* (40 µL, 250 ng/µL) was sent to Ecogenics GmbH (Zürich-Schlieren, Switzerland). Size-selected fragments from genomic DNA were directly analyzed on a Roche 454 platform using the GS FLX Titanium reagents (454 Life Sciences, a Roche Company, Branford, Connecticut, USA). Forty-six out of 13,230 reads (average length of 323 bp) harbored a microsatellite insert with a tetra- or a trinucleotide of at least six repeat units or a dinucleotide of at least 10 repeat units. Suitable primer design was possible in 33 reads. In a second approach, also done at Ecogenics GmbH, size-selected fragments from mechanically sheared genomic DNA were enriched for SSR content by using magnetic streptavidin beads and biotin-labeled CT and GT repeat oligonucleotides. The SSR-enriched library was analyzed on a Roche 454 platform using the GS FLX Titanium reagents (454 Life Sciences, a Roche Company). The total of 11,238 reads had an average length of 324 bp. Of these, 413 contained a microsatellite insert with a tetra- or a trinucleotide of at least six repeat units or a dinucleotide of at least 10 repeat units. Suitable primer design was possible in 128 reads (Rozen and Skaletsky, 2000). Primers were synthesized at Integrated DNA Technologies (Coralville, Iowa, USA). Their ability to amplify and produce variably sized fragments was evaluated on three DNA samples of *B. microphylla* (accession no. 108, 111, and 116; Appendix 1) representative of the geographical distribution of the species. PCR contained 30 ng of DNA, 1× Colorless GoTaq Flexi Buffer (Promega Corporation, Madison, Wisconsin, USA), 2 mM $MgCl_2$, 250 µM dNTPs, primers (5 µM each), and 0.5 U of *Taq* polymerase (GoTaq Flexi DNA, Promega Corporation). PCR cycling, after an initial denaturation of 3 min at 94°C, was as follows: 35 cycles of 30 s at 94°C, 30 s at 56°C, and 60 s at 72°C. Reactions were completed by incubating at 72°C for 4 min. PCR product separation and silver-staining was done as described by Narváez et al. (2001). The PCR protocol was the same for every sample considered in this study.

Of the 161 SSR-containing fragments identified, 88 were repeated twice or more. Of the remaining 73 unique sequences, 18 primer pairs generated easily scorable allelic patterns and were evaluated on 66 *B. microphylla* accessions collected from Chilean Patagonia (Appendix 1). Allele numbers ranged from two to 19, with an average of 7.6 alleles per marker. Table 1 summarizes the statistics for these markers, including heterozygosity (observed heterozygosity ranging from 0.164 to 1.0, with an average of 0.701; expected heterozygosity from 0.448 to 0.854, average of 0.668), repeat motifs, allele sizes, probability of confusion (i.e., the probability that two randomly chosen individuals have the same allelic pattern [Tessier et al., 1999]), and GenBank accession numbers. Finally, the markers developed in this work exhibited a very high level of interspecific transferability, evaluated with 15 other *Berberis* species, the majority of them from Chile (Table 2, Appendix 1). Another set of SSR markers developed in the related species *Mahonia aquifolium* (Pursh) Nutt. (Ross and Durka, 2006) was less informative when evaluated in *B. microphylla* from Chilean Patagonia (results not shown).

CONCLUSIONS

We have identified and characterized 18 new SSR markers from *B. microphylla*, all of them highly polymorphic and informative in every *Berberis* species tested, including most of the endemic and native Chilean species and two Old World

Table 1. Microsatellites of *Berberis microphylla* characterized in 66 accessions from Chilean Patagonia.[a]

Locus	Primer sequences (5′–3′)	Repeat motif	Size range (bp)	*A*	H_o	H_e	C_j	GenBank accession no.
BmLP-05	F: AACACCTGGTTCAACTTGCG	$(TTC)_9$	110–130	7	0.84	0.79	0.19	JX481194
	R: TGCTGCTACTGACTCTTCCG							
BmLP-07	F: CGAAAATCTCGGGAATGGGC	$(TGA)_7$	110–140	7	0.85	0.67	0.32	JX481196
	R: TGCCTGAAAAGTGTTGGCAC							
BmLP-09	F: CATCCATCTCTTGGGAATTCAAC	$(CAT)_8$	110–130	7	0.93	0.71	0.28	JX481198
	R: CGAAAATCTCGGGAATGGGC							
BmLP-11	F: GGAAGGAGAGCGAAAATCGAC	$(ATC)_{10}$	90–120	6	0.85	0.73	0.26	JX481200
	R: TGAGATGAAGGCATACATGAGC							
BmLP-19.2	F: TCACTTCAACCCCCTCTTCG	$(ACA)_9$	150–180	10	0.63	0.71	0.27	JX481207
	R: ACAGTGGAAGGTCTGCTCTG							
BmLP-26	F: TGTAAGCCTTCATGGGCTCC	$(AC)_{12}$	120–180	6	0.57	0.75	0.23	JX481214
	R: GGGGCGAACCAGATCAGC							
BmLP-30	F: ACTTCTCATACCCTGACGGC	$(CA)_{11}$	220–250	6	0.76	0.71	0.28	JX481218
	R: GAGTGAACCTTGACAATAAGTTGG							
BmLP-36	F: TCAAAATCTGGTGGCTCTGC	$(TG)_{13}$	190–230	8	0.80	0.76	0.23	JX481224
	R: AAAGGGCTCCCCTGAAAATG							
BmLP-38	F: CGAGGAAAATGGCCTAGAAGAC	$(TG)_{11}$	270–300	7	0.79	0.72	0.26	JX481226
	R: AGCTAAGGCTGTGTAGGGTG							
BmLP-39	F: GTTTGTGGGGGTGGTGAAAG	$(TC)_{11}$	130–170	6	0.25	0.47	0.53	JX481227
	R: ACTTGCAGAAGAGAATTGTGTG							
BmLP-46	F: TGCCTGAATTCTTGCTCGAC	$(CA)_{12}$	210–240	6	0.23	0.51	0.48	JX481234
	R: GGCAAAGGACTCTTGTGTCTG							
BmLP-49	F: TTTGTCTACGGATCCCACCC	$(AC)_{12}$	140–180	6	0.67	0.71	0.28	JX481238
	R: TGCTTAGCTCATGTGTCCTTG							
BmLP-53	F: GGACAACCCTGTTTCCTCAC	$(GT)_{13}$	230–260	2	0.16	0.45	0.43	JX481241
	R: AACGACACCAAAATACCGGC							
BmLP-54	F: CCAAGGCTGTCGGAAATGTG	$(AGA)_7$	200–270	7	0.97	0.80	0.19	JX481243
	R: GAGCTAGTCTCCTTCCGTCC							
BmLP-58.2	F: AAACCCTTTAGTGGGGCGAC	$(CTT)_8$	100–150	5	0.58	0.50	0.50	JX481248
	R: AGGATCGCTGGTGTACTTGG							
BmLP-59	F: CTAACGCATGCCATTGTTCAC	$(GT)_{11}$	110–130	6	0.78	0.71	0.28	JX481249
	R: CGATGATGACTTCTTAAATGTTCCG							
BmLP-65	F: TTGCAATGAACCTGGCTCTG	$(AC)_{12}$	200–260	16	1.00	0.85	0.14	JX481255
	R: TTGGTGCAAATCAGCTCAAC							
BmLP-71	F: TCGTGGTGGTAACAGAGAGG	$(CA)_{13}$	120–160	19	0.95	0.85	0.13	JX481261
	R: AAGATGCAAGTGGTGTTGTG							

Note: *A* = number of alleles; C_j = probability of confusion (Tessier et al., 1999); H_e = expected heterozygosity; H_o = observed heterozygosity.
[a]Annealing temperature was the same for all markers (56°C).

TABLE 2. Interspecific transferability of *Berberis microphylla* microsatellite markers.

Species	BmLP-05	BmLP-07	BmLP-09	BmLP-11	BmLP-19.2	BmLP-26	BmLP-30	BmLP-36	BmLP-38	BmLP-39	BmLP-46	BmLP-49	BmLP-53	BmLP-54	BmLP-58.2	BmLP-59	BmLP-65	BmLP-71	GA- 33[a]	GA-04[a]
B. thunbergii DC.	+	+	+	++	+/–	+	+	—	++	+	+	++	+	+	++	+	+	+	+	+
B. vulgaris L.	+	+	+	++	—	+	+	+	++	+	+	+	+	+	+	+	+	+	+	—
B. montana Gay	+	+	+	++	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B. corymbosa Hook. & Arn.	—	+/–	+	+/–	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B. serratodentata Lechl.	—	+	+	+/–	+	—	+	—	+	—	+	+	+	+	+	+	+	+	+	—
B. negeriana Tischler	+	+	+	+	++	—	++	+	+	+	+	—	—	+	+	+	+	+	+	+
B. sp. 1[b]	+	++	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B. sp. 2[b]	+	+	+	+	++	—	+	+	+	+	+	+	—	+	++	+	+	+	+	+
B. ilicifolia L. f.	+	+	+	+	+	—	+	'	+	+	+	+/–	+/+	+	+	+	+	+	+	+
B. litoralis Phil.	+	+	+	+/–	+	—	++	+	+	+	+	+	+	+	+	—	+	+	+	+
B. trigona Kunze ex Poepp. & Endl.	+		+	+/–	+	—	+	—	+	+	+	+	+	+	+	+				
B. horrida Gay	+	+	+/–	+	+	+	+	+/–	+	+	+	+	+	+	+	+	+	+	+	+
B. chilensis Gillies ex Hook. & Arn.	+	+	+	+	—	+	++	+	++	+	+	+	+	+	+	+	+	+	+	+
B. empetrifolia Lam.	+	+	+/–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	++	+	+
B. microphylla G. Forst.[c]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B. darwinii Hook.	+	—	+/–	+	+	+	+	+	+	+	+	+	—	+	+	+	+	++	+	+

Note: ++ = clear, strong PCR signal; + = fairly good PCR signal; +/– = weak PCR signal; — = no amplicon detected.

[a]GA markers are from *Mahonia aquifolium*.

[b]Unidentified species.

[c]Sample no. 76 (laboratory code 287), see Appendix 1.

species. This set of 18 markers appears more polymorphic than the ones recently developed for *B. thunbergii* DC. (Allen et al., 2012) that were evaluated with 24 accessions of that species and had an average of 4.4 alleles per marker. The markers described in this work can be used for genetic diversity and related studies among species of Berberidaceae.

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Appendix 1. List of accessions used in this work and their region of origin and GPS location.[a]

No. of sample	Laboratory code	Species	Region of collection	GPS location	Notes
1	4	*B. microphylla*	Región de Magallanes	52°42′10.02″S, 70°55′34.77″W	
2	12	*B. microphylla*	Región de Magallanes	52°41′58.89″S, 70°55′32.00″W	
3	34	*B. microphylla*	Región de Aysén	45°58′31.6″S, 71°52′25.03″W	
4	42	*B. microphylla*	Región de Aysén	45°39′59.70″S, 72°12′20.29″W	
5	44	*B. microphylla*	Región de Aysén	45°43′14.21″S, 72°02′26.14″W	
6	45	*B. microphylla*	Región de Aysén	45°39′59.92″S, 72°12′19.46″W	
7	47	*B. microphylla*	Región de Aysén	45°40′00.11″S, 72°12′19.58″W	
8	51	*B. microphylla*	Región de Magallanes	52°42′08.03″S, 70°55′15.29″W	
9	52	*B. microphylla*	Región de Magallanes	52°42′06.90″S, 70°55′11.79″W	
10	53	*B. microphylla*	Región de Magallanes	52°42′10.50″S, 70°55′12.36″W	
11	54	*B. microphylla*	Región de Magallanes	52°42′11.18″S, 70°55′16.60″W	
12	59	*B. microphylla*	Región de Magallanes	49°15′29.48″S, 74°05′51.84″W	
13	60	*B. microphylla*	Región de Magallanes	52°04′07.45″S, 69°46′59.27″W	
14	63	*B. microphylla*	Región de Magallanes	52°04′16.15″S, 69°47′48.63″W	
15	64	*B. microphylla*	Región de Magallanes	52°03′46.73″S, 69°48′11.96″W	
16	66	*B. microphylla*	Región de Magallanes	52°31′25.33″S, 69°56′20.55″W	
17	69	*B. microphylla*	Región de Magallanes	52°49′34.08″S, 70°59′18.47″W	
18	78	*B. microphylla*	Región del Biobío	36°54′25.78″S, 71°25′32.96″W	
19	79	*B. microphylla*	Región del Biobío	36°55′17.32″S, 71°26′34.91″W	
20	80	*B. microphylla*	Región del Biobío	36°35′40.46″S, 71°45′15.66″W	
21	82	*B. microphylla*	Región de Magallanes	53°10′15.90″S, 70°23′32.47″W	
22	84	*B. microphylla*	Región de Magallanes	53°03′01.46″S, 70°12′55.53″W	
23	85	*B. microphylla*	Región de Magallanes	52°59′42.72″S, 70°06′56.37″W	
24	86	*B. microphylla*	Región de Magallanes	52°54′47.08″S, 70°02′12.39″W	
25	87	*B. microphylla*	Región de Magallanes	52°51′37.61″S, 69°55′06.05″W	
26	88	*B. microphylla*	Región de Magallanes	52°49′38.68″S, 69°47′13.26″W	
27	89	*B. microphylla*	Región de Magallanes	52°46′12.90″S, 69°41′26.37″W	
28	90	*B. microphylla*	Región de Magallanes	52°41′10.26″S, 69°34′46.61″W	
29	91	*B. microphylla*	Región de Magallanes	52°38′04.22″S, 69°30′40.60″W	
30	92	*B. microphylla*	Región de Magallanes	52°32′04.96″S, 69°26′05.49″W	
31	93	*B. microphylla*	Región de Magallanes	52°29′40.57″S, 69°31′28.74″W	
32	107	*B. microphylla*	Región de Aysén	46°32′38.19″S, 71°41′28.73″W	

Appendix 1. Continued.

No. of sample	Laboratory code	Species	Region of collection	GPS location	Notes
33	108	*B. microphylla*	Región de Aysén	45°42′50.38″S, 72°02′49.83″W	
34	109	*B. microphylla*	Región de Aysén	46°18′16.18″S, 71°54′21.08″W	
35	111	*B. microphylla*	Región de Aysén	46°29′59.30″S, 71°50′55.81″W	
36	113	*B. microphylla*	Región de Aysén	46°15′25.79″S, 71°55′47.71″W	
37	114	*B. microphylla*	Región de Aysén	46°09′42.34″S, 72°10′33.85″W	
38	116	*B. microphylla*	Región de Magallanes	53°39′15.25″S, 70°57′10.20″W	
39	128	*B. microphylla*	Región de Magallanes	53°17′17.78″S, 70°56′54.25″W	
40	131	*B. microphylla*	Región de Magallanes	53°16′12.66″S, 70°57′05.63″W	
41	134	*B. microphylla*	Región de Magallanes	53°48′23.15″S, 71°12′31.76″W	
42	137	*B. microphylla*	Región de Magallanes	53°24′32.57″S, 71°15′16.68″W	
43	152	*B. microphylla*	Región de Magallanes	53°10′59.80″S, 71°01′35.85″W	
44	156	*B. empetrifolia*	Región de Magallanes	52°42′18.35″S, 70°55′36.26″W	
45	159	*B. microphylla*	Región de Magallanes	52°41′45.05″S, 70°54′01.37″W	
46	163	*B. microphylla*	Región de Magallanes	52°41′42.72″S, 70°54′52.76″W	
47	165	*B. microphylla*	Región de Magallanes	52°41′44.29″S, 70°54′42.94″W	
48	171	*B. microphylla*	Región de Magallanes	52°57′52.03″S, 71°08′57.80″W	
49	183	*B. microphylla*	Región de Magallanes	52°59′25.22″S, 70°52′48.97″W	
50	184	*B. microphylla*	Región de Magallanes	52°59′22.89″S, 70°49′07.81″W	
51	185	*B. microphylla*	Región de Magallanes	53°00′32.40″S, 70°49′17.01″W	
52	188	*B. microphylla*	Región de Magallanes	52°54′11.28″S, 71°06′26.57″W	
53	193	*B. microphylla*	Región de Magallanes	52°48′36.20″S, 71°12′20.15″W	
54	237	*B. microphylla*	Región de Aysén	46°36′59.06″S, 72°40′45.57″W	
55	239	*B. microphylla*	Región de Aysén	46°17′08.99″S, 71°56′08.26″W	
56	241	*B. microphylla*	Región de Aysén	46°17′46.52″S, 71°54′39.26″W	
57	243	*B. microphylla*	Región de Aysén	46°18′19.32″S, 71°53′05.28″W	
58	245	*B. microphylla*	Región de Aysén	45°42′15.37″S, 72°04′08.60″W	
59	247	*B. microphylla*	Región de Aysén	45°42′32.22″S, 72°04′25.87″W	
60	249	*B. microphylla*	Región de Aysén	45°42′27.08″S, 72°04′53.02″W	
61	251	*B. microphylla*	Región de Aysén	45°40′06.26″S, 72°11′51.46″W	
62	253	*B. microphylla*	Región de Aysén	46°30′54.73″S, 71°51′14.25″W	
63	255	*B. microphylla*	Región de Aysén	47°31′25.55″S, 71°51′15.01″W	
64	257	*B. microphylla*	Región de Aysén	46°33′02.66″S, 71°41′49.37″W	
65	259	*B. microphylla*	Región de Aysén	46°37′56.89″S, 72°40′00.44″W	
66	266	*B. microphylla*	Región de Magallanes	53°49′11.64″S, 70°10′24.27″W	
67	39	*B. darwinii*	Región de Aysén	45°46′55.35″S, 72°11′00.37″W	
68	72	*B. vulgaris*	Región del Biobío	36°49′40.79″S, 73°02′13.11″W	European origin, collected at the gardens of Universidad de Concepción, Concepción, Chile
69	155	*B. ilicifolia*	Región de Magallanes	53°09′30.03″S, 71°01′18.23″W	
70	262	*B. thunbergii*	Región Metropolitana	33°34′09.05″S, 70°37′52.97″W	European origin, obtained from the Plant Nursery of the Agronomy Faculty, Universidad de Chile, Santiago, Chile
71	271	*B.* sp. 1	Región Metropolitana	33°26′07.98″S, 70°30′40.77″W	
72	273	*B. litoralis*	Región de Antofagasta	23°40′30.06″S, 70°18′15.42″W	
73	278	*B.* sp. 2	Región Metropolitana	33°26′06.69″S, 70°30′04.96″W	
74	284	*B. chilensis*	Región del Maule	35°36′56.84″S, 71°00′41.56″W	
75	286	*B. microphylla*	Región de Magallanes	52°46′38.40″S, 71°51′26.91″W	
76	287	*B. microphylla*	Región de Aysén	46°36′29.93″S, 72°40′53.23″W	
77	288	*B. microphylla*	Región de los Lagos	42°16′34.36″S, 73°42′58.81″W	
78	289	*B. microphylla*	Región del Biobío	36°54′32.39″S, 71°24′33.51″W	
79	318	*B. trigona*	Región de la Araucanía	39°24′52.59″S, 72°02′50.34″W	
80	323	*B. serratodentata*	Región de la Araucanía	39°24′14.07″S, 72°03′32.76″W	
81	367	*B. negeriana*	Región del Biobío	36°52′16.12″S, 72°59′36.10″W	
82	386	*B. montana*	Región del Biobío	36°54′45.32″S, 71°24′41.48″W	
83	400	*B. horrida*	Región Metropolitana	33°29′33.30″S, 70°28′22.82″W	
84	406	*B. corymbosa*	Juan Fernández Archipelago, Robinson Crusoe Island	33°38′48.45″S, 78°50′16.88″W	Preserved and collected from the Jardín Botanico Nacional, Viña del Mar, Chile

[a] Samples 1–66 are *B. microphylla* accessions used in the isolation and characterization of the markers described in this work. Samples 67–84 are the accessions used to evaluate the interspecific transferability of these markers, plus the three accessions of *B. microphylla* (no. 286, 288, and 289) used for the preliminary evaluation of microsatellite informativeness.