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New polymorphic nuclear microsatellites from *Aristotelia chilensis* (Mol.) Stuntz (Elaeocarpaceae)

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ABSTRACT

Maqui (*Aristotelia chilensis* [Molina] Stuntz) is a dioecious small tree native to Chile and southwestern Argentina. This species has gained attention due to its high polyphenol content and anti-oxidant capacity. Nevertheless, genetics studies and information about *A. chilensis* population genetics are scarce and even contradictory. In fact, the available species-specific simple sequence repeat (SSR) markers are not informative at all, and so we decided to identify and characterize new ones able to trace individual genotypes, a basic tool intended for different genetic studies. We identified and characterized 15 new polymorphic SSR markers for *A. chilensis*. These markers were evaluated in three populations distributed along 1000 km of Central Chile, exhibiting up to 10 alleles per *locus* and a combined expected heterozygosity of 0.858. Markers were also informative in two related species, *Aristotelia peduncularis* (Labill.) Hook. f. and *Crinodendron patagua* Molina (Elaeocarpaceae), with 13 and six SSRs showing clear amplification patterns, respectively. This new set of SSR markers are highly polymorphic and informative, being the first ones available for the effective fingerprinting of maqui genotypes. A proof of concept of that was the differentiation of six maqui accessions that are under domestication for productive purposes, based on a subset of the polymorphic SSR markers.

Key words: Fingerprinting, maqui, maqui berry, molecular markers, Patagonian endemics, SSR.

INTRODUCTION

Aristotelia chilensis [Molina] Stuntz (Elaeocarpaceae), better known as maqui, is a dioecious evergreen shrub or tree (up to 6 m tall) endemic to Patagonia, distributed in Chile from Limarí (32° S lat) to Aysén (42° S lat) and from Coastal range to Andean slopes (2000 m a.s.l.) It is also ubiquitous in the adjacent Andean regions in Argentina. *Aristotelia chilensis* has been described as part of the structure of sclerophyllous heathlands and deciduous *Nothofagus* forests. Also, it is considered a pioneering species due to its capacity to firstly colonize disturbed soils, forming monospecific associations called "macales". Its small spherical fruits (2-5 mm diameter containing up to four seeds) are fully purple black when ripe and have been historically used as a source of food, dye and medicine mostly by Mapuche people (Misle et al., 2011). Many studies have reported high antioxidants content in its fruit, revealing maqui as one of highest antioxidant capacity among marketed fruits worldwide. Theses antioxidants include anthocyanins, tannins, flavonoids and other polyphenols: compounds that plays protective roles in oxidative stress (e.g. oxidized low-density lipoprotein [LDL]) and chronic noncommunicable diseases such as cardiovascular diseases and diabetes (Miranda-Rottmann et al., 2002; Céspedes et al., 2008; Rojo et al., 2012; Fernández et al., 2019).

In recent years, *A. chilensis* has been under domestication in order to create new lines of productive interest; up to now, there are five registered varieties ('Huiña', 'Pudú', 'Vicuña', 'Puyuhuapi', and 'Taitao') in the official site of Servicio Agrícola y Ganadero (SAG) (Agriculture and Livestock Service; www.sag.gob.cl), varieties authority in Chile (Vogel et al., 2016), and new domestication initiatives are in progress. However, little is known about *A. chilensis* genetics although there is some information describing genetic diversity of the species based on amplified fragment length polymorphism (AFLP) (Salgado et al., 2017), inter-simple sequence repeat (ISSR) (Fredes et al., 2014) and microsatellite simple sequence repeat (SSR) (Bastías et al., 2016) markers. Nevertheless, the available SSRs were unable to genetically differentiate *A. chilensis* accessions from different localities, including a group of genotypes belonging to a private domestication initiative, which exhibited clear phenotypic differences. Based on the aforementioned studies, the species appears as not having clearly structured populations, and even its genetic diversity is still an open question. Considering these antecedents, and that new cultivars of vegetative propagation are expected to be released during the next years, the aim of this study was to asses an effective differentiation and traceability protocol for *A. chilensis* genotypes based on a new set of highly polymorphic, informative SSR markers. At the same time, these new markers could become an effective tool to re-evaluate the genetic diversity and population structure of the species.

MATERIALS AND METHODS

For the analysis of the designed primers, leaves from 60 individuals of *A. chilensis* were sampled in three different geographic locations in Chile (Metropolitan, Maule, and Los Lagos Regions), with 20 individuals each. These geographic locations were considered as a representative range of the species distribution. Also, diverse sampling sites were selected prioritizing wild forest populations (Table 1). Samples of the related species *Crinodendron patagua* Molina and *Aristotelia peduncularis* (Labill.) Hook. f. were obtained from sclerophyll hills close to Santiago and wet sclerophyll forests in Ferntree, Tasmania, respectively.

Sequencing and identification of new primers is described in Bastías et al. (2019). Briefly, genomic DNA was obtained from fresh leaves of maqui (*Aristotelia chilensis* [Molina] Stuntz). Using a library with 300 bp insert size and paired-end-tag DNA sequencing using NextSeq 550 platform (Illumina, San Diego, California, USA) around 187 million 2 × 151 bp reads were generated. After a process of quality trimming and filtering of data using FastQC v0.11.5 (Babraham Institute, Cambridge, UK), which allow to remove reads containing more than 5% unknown nucleotides, low-quality reads (reads containing more than 50% bases with Q-value ≤ 20), all unpaired reads and short reads (< 35 bp), 95.87% from the total reads were suitable for genome assembling. Then, de novo assembly of the clean reads was performed to generate contigs and scaffolds. For de novo assembly, MaSuRCA 3.3.1 software (Zimin et al., 2013) was used, with optimized k-mer length of 85, calculated by KmerGenie software (Chikhi and Medvedev, 2014). The contig sequences obtained in FASTA files were screened with a repeat motif size range of 2 to 8 bp and a length of > 12 bp. This included dinucleotide repeats ≥ 6, trinucleotide repeats ≥ 4, and tetra-, penta-, hexa-, hepta- and octanucleotide repeats ≥ 3, using MIcroSAtellite identification software (Thiel et al., 2003). Then, primers were designed in the flanking regions of the 28 575 found SSRs using PRIMER 3 (Rozen and Skaletsky, 2000). From this large set, we filtered each set of di, tri, tetra, penta, hepta and octanucleotide SSRs by predicted amplicon size (range of 130-280 bp) and selecting the largest motif repetitions found in each SSR type. Sixty-nine SSRs were selected (Table 2) and subjected to screening for amplification quality using a set of 10 randomly chosen *A. chilensis* genotypes.

Genomic DNA (gDNA) extraction was carried out using modified cetyltrimethylammonium bromide (CTAB) method with 100 mg fresh young leaves (Lodhi et al., 1994). Yield and integrity of DNA was evaluated by electrophoresis on 1% agarose stained with ethidium bromide. Every PCR amplification contained, in a total volume of 12 µL, 3 µL gDNA (10 ng), 2.4 µL 5 × colorless GoTaq Flexi buffer (Promega, Madison, Wisconsin, USA), 1.5 mM $MgCl_2$, 0.5 mM dNTPs (0.125 mM each), 0.3 µM primers, 0.5 U (0.1 µL) of GoTaq DNA Polymerase Flexi (Promega) and completed with dH_2O. PCR conditions were the following: initially denaturation of 7 min at 94 °C; then 40 cycles of 1 min at 95 °C, 45 s at 58 °C and 1 min 30 s at 72 °C; finally, an incubation of 7 min at 72 °C was used for templates filling. PCR products were separated in polyacrylamide gels (6%) and visualized using a silver-staining protocol. A known-size ladder was used to estimate allele sizes. Number of alleles and heterozygosity was calculated using GenAlEx program (Peakall and Smouse, 2012).

Table 1. Global positioning system (GPS) for accessions of *Aristotelia chilensis* considered in this study.

Metropolitan Region		Maule Region		Los Lagos Region	
Sample number	GPS location	Sample number	GPS location	Sample number	GPS location
1	33°29'53.9" S 70°30'02.2" W	21	34°59'01.5" S 71°13'58.2" W	41	41°16'17.3" S 72°49'31.2" W
2	33°29'53.3" S 70°30'00.8" W	22	34°59'01.6" S 71°13'55.8" W	42	41°15'50.6" S 72°48'55.0" W
3	33°29'51.7" S 70°29'59.5" W	23	34°58'53.5" S 71°13'46.4" W	43	41°11'44.9" S 72°32'22.9" W
4	33°29'51.5" S 70°29'53.5" W	24	34°58'53.2" S 71°13'47.6" W	44	41°11'44.7" S 72°32'23.1" W
5	33°29'52.4" S 70°29'51.1" W	25	34°52'40.8" S 71°20'42.7" W	45	41°07'33.0" S 72°36'47.1" W
6	33°29'54.5" S 70°29'46.8" W	26	34°52'27.7" S 71°20'52.3" W	46	41°07'28.0" S 72°36'52.2" W
7	33°29'55.8" S 70°29'10.8" W	27	34°52'27.1" S 71°20'53.5" W	47	41°04'25.1" S 72°36'59.1" W
8	33°29'52.1" S 70°29'05.1" W	28	34°51'22.7" S 71°21'11.2" W	48	41°04'31.8" S 72°37'15.9" W
9	33°29'48.2" S 70°29'01.8" W	29	34°51'12.6" S 71°21'08.9" W	49	40°59'46.8" S 72°45'33.3" W
10	33°29'45.3" S 70°28'57.0" W	30	34°50'21.1" S 71°21'10.9" W	50	41°40'14.6" S 73°03'58.9" W
11	33°29'43.3" S 70°28'51.1" W	31	34°50'21.4" S 71°21'12.8" W	51	41°40'13.1" S 73°03'59.8" W
12	33°29'42.7" S 70°28'45.6" W	32	34°50'19.7" S 71°21'12.9" W	52	41°40'12.4" S 73°04'04.4" W
13	33°29'38.1" S 70°28'40.1" W	33	34°50'19.3" S 71°21'13.8" W	53	41°40'12.8" S 73°03'57.7" W
14	33°29'35.5" S 70°28'34.8" W	34	34°50'19.6" S 71°21'15.0" W	54	41°40'08.0" S 73°03'50.9" W
15	33°29'36.4" S 70°31'05.0" W	35	34°50'19.1" S 71°21'15.3" W	55	41°40'09.6" S 73°03'50.7" W
16	33°29'49.3" S 70°54'31.0" W	36	34°50'18.4" S 71°21'15.6" W	56	41°29'11.2" S 72°56'57.4" W
17	33°29'47.4" S 70°54'34.2" W	37	34°50'18.5" S 71°21'17.3" W	57	41°29'11.4" S 72°56'57.8" W
18	33°29'50.4" S 70°54'41.0" W	38	34°50'13.3" S 71°21'13.1" W	58	41°29'12.2" S 72°57'00.6" W
19	33°29'50.8" S 70°54'45.7" W	39	34°50'11.3" S 71°21'17.1" W	59	41°29'08.0" S 72°57'14.7" W
20	33°29'52.2" S 70°54'46.7" W	40	34°50'09.0" S 71°21'20.0" W	60	41°29'14.4" S 72°57'25.7" W

RESULTS AND DISCUSSION

Of the 69 SSRs initially screened (Table 3) 15 were dinucleotide, 13 trinucleotide, 23 tetranucleotide, four pentanucleotide, nine hexanucleotide and five heptanucleotide; eight of these SSRs showed monomorphic profiles. From the 61 remaining SSRs, 46 showed no amplification or complex patterns and 15 exhibited highly polymorphic profiles as well as clear and strong PCR signals (Table 2). These 15 SSRs were analyzed in three populations of *A. chilensis* (n = 60). After this, number of alleles, as well as expected and observed heterozygosity were determined for each population (Table 4). Alleles number ranged from 1 to 10 and expected heterozygosity varied from 0 to 0.858 per locus. It was interesting to note that the monomorphic profiles in two loci (Ach45 and Ach54) were evidenced in the southernmost population sampled in this study (Los Lagos Region). Based on the last glacial maximum (LGM) Patagonian ice sheet range (Moreno et al., 2018), we hypothesize that colonization event of Los Lagos population took place more recently than those populations northward, leading to a loss of alleles and decreased genetic diversity southward this region, explained by genetic drift

considering bottleneck or founder effects (Hewitt, 2004; Gugerli et al., 2009; Tóth et al., 2019). However, this genetic pattern remains to be studied, and new northern- and southernmost populations of *A. chilensis* should be considered in order to define possible refugia of this species during LGM period.

Table 2. General characteristics of 15 polymorphic SSRs markers for *Aristotelia chilensis*.

Locus	Primer sequences (5'-3')	Repeat motif	Size range (bp)	T_a (°C)
Ach3	F: CTGTCCTCAGCTGGTATTTA R: CCTTCTTTGGTTTCTTGCTTTA	$(AG)_{27}$	252-266	58
Ach4	F: TGCACATGAAACACAGAATTA R: GCAACATGCGAGTGAAATTA	$(AG)_{28}$	238-270	58
Ach5	F: TGATGGGTTTTTGTGCAAATT R: TGATCCTGCATTTCTTTCAATAG	$(AG)_{68}$	194-214	58
Ach11	F: AGGATGCCAACCTTTTCTAAT R: CCTTTCTCTGCATTCTCTTTTT	$(GA)_{22}$	248-276	58
Ach14	F: ACTTGTTACAAACCAAACAATTT R: TGCTTCCCATTTTCTTCTTTTT	$(GA)_{25}$	234-264	58
Ach28	F: TGCGGAGAAGTAAGTGTATT R: TGAACCACAGCTGAATGATAT	$(TTG)_{10}$	200-230	58
Ach29	F: TGGAATCATGGCCTCTACTA R: AATCTTCCCCAACGGTATTC	$(AAAG)_{6}$	200-230	58
Ach38	F: TTCCTTCTCGGCTTTCTTAA R: GCAAACCCTAGTTCCAATTT	$(ATCT)_{7}$	170-194	58
Ach41	F: ACTCACTGTGGAGATTTCAATA R: GCCTTTTGCCAGACTTAGTA	$(GAGT)_{7}$	196-218	58
Ach45	F: GGAGCCGATCACGTAGTA R: GCTGATTCGATGGGGATAAT	$(TACA)_{8}$	190-202	58
Ach47	F: AGTGGCTCAGGTTTGTAATT R: TGGTGGATTCCAGATTGTTAT	$(TGTA)_{8}$	156-164	58
Ach54	F: CTGAGAAGCACCCAAAGATA R: GGGCCAATCAGAGCTAATC	$(TAAAA)_{6}$	222-230	58
Ach57	F: CTCCCACAACAAGACCATAA R: AACTCCGAGTTGAGAAAATT	$(ACCAGA)^{5}$	258-270	58
Ach61	F: CCTATTGCACTCACCTGATAT R: AGAGCTGGACAGACTGAATA	$(GAAGTT)_{6}$	212-230	58
Ach68	F: GGTTGAGTGGGTTGACTATT R: AGAGCAAATCAAACCAACATTA	$(TATCATC)_{5}$	184-206	58

T_a: Annealing temperature.

Table 3. List of 69 dinucleotidic to heptanucleotidic microsatellite repeats (SSRs) from *Aristotelia chilensis* considered in this work.

SSR ID	Scaffold number	Forward primer (5'-3')	Reverse primer (5'-3')	SSR motif
Ach1	27 262	CATCTTGTCGCATGTGTATAC	ACATCCAAAAGCCCCATTAA	(AG)22
Ach2	12 158	TGGTTGTGGAGGAATGAATTA	GGATGGAGATGGTATGGTTAAT	(AG)25
Ach3	7 421	CTGTCCTCAGCTGGTATTTA	CCTTCTTTGGTTTCTTGCTTTA	(AG)27
Ach4	672	TGCACATGAAACACAGAATTA	GCAACATGCGAGTGAAATTA	(AG)28
Ach5	38 123	TGATGGGTTTTTGTGCAAATT	TGATCCTGCATTTCTTTCAATAG	(AG)68
Ach6	677	CAGCCGTCAGTTTGCTATAT	TCTCTTCACGCAACCATTAA	(CT)21
Ach7	678	GTGGAAGTGGTGGTCATAAT	AGCTTCCAAAGACAAGTTAGTA	(CT)22
Ach8	22 137	TGGAAACTTCAACAACACTAAA	GCTACAGGGAGAGAGATAGA	(GA)20
Ach9	1 006	AACTCAGCAGACACTGTTAT	GAAATCCTTGCACCCAAAAT	(GA)21
Ach10	32 056	ACAGTCCCAAATGGCATTTT	CATTCCATTCCTCCACATTTT	(GA)22
Ach11	1 079	AGGATGCCAACCTTTTCTAAT	CCTTTCTCTGCATTCTCTTTTT	(GA)22
Ach12	23 751	CTCACTCTTGCTCCTCATTA	CATCCTTGGCCTTTGAAATT	(GA)24
Ach13	7 717	TCAGCATGGTCTAAGGAAAAA	AACGAGCTTTGTCACTATTG	(GA)25
Ach14	25 455	ACTTGTTACAAACCAAACAATTT	TGCTTCCCATTTTCTTCTTTTT	(GA)25
Ach15	9 396	TGTGCTACAACTGTGCTATT	GTGGCTATGCAGAACCTAAT	(GA)28
Ach16	2 152	CTGATGCCGCGTTTAATATC	CAATTGCTTCACCGCTAATC	(AGT)14
Ach17	40 659	GGAGGGAACAAATTCATCAAAAT	GATGTTGATAGCGAGGATTTT	(ATC)10

Continuation Table 3.

SSR ID	Scaffold number	Forward primer (5'-3')	Reverse primer (5'-3')	SSR motif
Ach18	7 124	TCACAAAAGGATGCCCATAT	TTTCTGCCACAGGGATAGAT	(ATG)11
Ach19	12 255	ACACCCAAACAACACAAAAA	GTGGTGATGAAGGCAATAAG	(CAA)10
Ach20	19 103	GTTGCCATGATCCCTCTAAT	GCTATCTGCCCAAAGGTAAT	(CTT)10
Ach21	3 676	GCCACCTCTTCTTCCATAAA	GTGGAAGAGAAGAGCTAGTA	(CTT)12
Ach22	56	CTGGATCTTGAGGAGCTTAA	CAAGTGCAACCCCTTTTTAG	(GTA)11
Ach23	36 471	AAGCACTGGTTAGGCTTTAA	GCAAACAAACACCACAAAAA	(TCT)10
Ach24	3 236	ATTGCCTCAAAACCCATCTA	GGGTGCAAAGATGGTGATAT	(TCT)11
Ach25	9 487	TCAACTTTCTCAGATGCTTTTT	AGAAACATTGCCCCTCAATA	(TGA)10
Ach26	5 437	TGGGTTTGCTACTTGTCATAT	CACCCATTCACCATGTAAAA	(TGA)13
Ach27	18 876	GGAGACTTGAGAGGGTTTTT	CTGACCCCACATCTTCTAAA	(TGT)10
Ach28	578	TGCGGAGAAGTAAGTGTATT	TGAACCACAGCTGAATGATAT	(TTG)10
Ach29	32 305	TGGAATCATGGCCTCTACTA	AATCTTCCCCAACGGTATTC	(AAAG)6
Ach30	18 127	ACATGCGCATTTGATTGATAA	TGTGGGCGGTACTAATACTA	(AACT)6
Ach31	2 911	TGTAGCAAACGGTTGGATTA	CTCTTGACCAAAGCTCTTTTT	(AAGA)6
Ach32	24 448	TGCCGTCAATGAAAGAAAAA	TGAAGGGAATTGGGGTTTTT	(ACAA)6
Ach33	6 006	CCGCCCACGCTATTTTAA	TTGGCTTGCTTGAGGTTAAT	(AGTT)6
Ach34	1 795	TGTTTTTGCCTCAGCTTAAG	GTGCACCAAACTAGCATTTT	(ATAA)6
Ach35	36 883	ACTTCCTACGAATCGCATTA	TTGTATGTGGGTCTCAATTT	(ATAA)6
Ach36	2 369	AACGTGTAAGCTACCATCTA	CGGTACCGCTTTTACTAAAA	(ATAC)6
Ach37	19 251	GGTGGTTAAGCTGTCAATCTA	GGAGACAAGAAAAGGCAAAAT	(ATCA)7
Ach38	17 612	TTCCTTCTCGGCTTTCTTAA	GCAAACCCTAGTTCCAATTT	(ATCT)7
Ach39	13 652	TAATTCTGGCCGCAACTTAA	TGGTCGAAACCCTGAAAAAT	(CATA)6
Ach40	19 425	GGAAGGCACACATCAGTTAT	CTGTAGCCTCATGCATCTAT	(CATA)9
Ach41	12 025	ACTCACTGTGGAGATTTCAATA	GCCTTTTGCCAGACTTAGTA	(GAGT)7
Ach42	1 563	CCATGGCCACATATGTAGAT	TGTCATGCCCACCTTTAAAA	(GTAA)8
Ach43	31 317	ATTCTCTTCTGCGCCATTAT	GGCAGATCGTCGTCAATATA	(GTCT)6
Ach44	12 669	AAGAATGGGAGGCAGTATTC	ACGCTACTCGAATCGTAATT	(GTTA)6
Ach45	20 543	GGAGCCGATCACGTAGTA	GCTGATTCGATGGGGATAAT	(TACA)8
Ach46	2 678	CCCAGTTCCCCTTCAAATAA	AAAGTGCCACTTGACAAAAA	(TATC)7
Ach47	17 109	AGTGGCTCAGGTTTGTAATT	TGGTGGATTCCAGATTGTTAT	(TGTA)8
Ach48	10 951	CACGTGAAGCGAATACATAA	CAAAAACTGGACCCGTTTTA	(TTAG)7
Ach49	40 246	CCATACCACGCCTCTTTTAT	GTTGGGTCTGGGATACTAAA	(TTAT)7
Ach50	9 790	ACCCCAAATTGTTGCAAAAA	CTGTCCATGCTAAGATCAATTT	(TTTC)6
Ach51	24 781	CCCTTTTTACAGGCACAATTT	CAATGAACTTGCGCACATAT	(TTTC)6
Ach52	12 162	TTGTGTGCACGTACCATTAT	GCATTTGCTAAACGTGTAGAT	(AAAAG)6
Ach53	36 032	TCTGAAATGAGGAAAGCATTTT	ACCTGTAGCCCTAGTCATAT	(AAAAG)6
Ach54	15 539	CTGAGAAGCACCCAAAGATA	GGGCCAATCAGAGCTAATC	(TAAAA)6
Ach55	15 669	CTTGGGTGGGGGAATAAAG	CTGCCTAGGACTTGACATAA	(TCAAC)5
Ach56	38 303	GCAGTTTGAACGTGGTAATC	AAAGAGTTGGGGGTGATATG	(AAAAAG)5
Ach57	2 031	CTCCCACAACAAGACCATAA	AACTCCGAGTTGAGAAAATT	(ACCAGA)5
Ach58	710	ACGCATGACCCAAACTAAAT	GAACACACAACAACAGTTTTT	(ATATAC)5
Ach59	8 794	TGTTCCCATCTCATGCTAAT	CTGACTTGTGCCTCCTTTTA	(CAATCA)5
Ach60	13 927	TGCAGTGAAACAAGTGATTTT	GTGAAAAGGTTAGGTAGCTAAA	(CTAAAT)6
Ach61	14 205	CCTATTGCACTCACCTGATAT	AGAGCTGGACAGACTGAATA	(GAAGTT)6
Ach62	874	CTGGCAGGGAGAGTTAAAAA	TGGCACAAAACTGGATAATG	(TTGGTA)6
Ach63	7 478	TCCACAACCAAAGATTCTTATG	TGTGTCATCGGAGGATATTG	(TTTGAA)7
Ach64	39 884	AGCGAGTGACAGAACTATTT	AGAAGAGGTGTGGAGAAATT	(TTTTTG)6
Ach65	6 883	TTTCATGACCCTCCCAATAG	CCAGGCATCCAGTACTTAAG	(AAAAATT)6
Ach66	491	CAGGAGTCAGCACAGAAATA	TTACCTGCCACTGCTATTAC	(ATTCATC)5
Ach67	18 925	TGTCCTGGGGTAAGAAGATA	TGTGTTCCTCCTTCCAAAAT	(GAGTCTA)5
Ach68	12 652	GGTTGAGTGGGTTGACTATT	AGAGCAAATCAAACCAACATTA	(TATCATC)5
Ach69	18 164	ACTTGTTTCGAGCCTGATAA	TGAGTGACGTTGCAGAAATA	(TTTTTTC)5

Table 4. *Locus* diversity of 15 SSRs analyzed in three populations of *Aristotelia chilensis*.

	Pop. 1. Metropolitan Region (n = 20)			Pop. 2. Maule Region (n = 20)			Pop. 3. Los Lagos Region (n = 20)		
Locus	A	He	Ho	A	He	Ho	A	He	Ho
Ach3	6	0.725	0.389	7	0.758	0.700	4	0.675	0.650
Ach4	7	0.725	0.385	10	0.858	0.722	4	0.589	0.263
Ach5	5	0.681	0.714	8	0.799	0.529	3	0.526	0.611
Ach11	7	0.796	0.471	8	0.856	0.647	7	0.755	0.474
Ach14	5	0.691	0.583	7	0.831	0.733	7	0.741	0.421
Ach28	4	0.584	0.250	8	0.629	0.200	4	0.625	0.200
Ach29	4	0.722	0.421	4	0.600	0.611	2	0.499	0.350
Ach38	3	0.493	0.176	4	0.578	0.650	2	0.480	0.600
Ach41	4	0.719	0.625	5	0.716	0.556	4	0.606	0.500
Ach45	5	0.520	0.450	4	0.694	0.700	1	0.000	0.000
Ach47	3	0.486	0.400	3	0.329	0.389	2	0.375	0.200
Ach54	3	0.486	0.300	2	0.188	0.211	1	0.000	0.000
Ach57	5	0.683	0.765	6	0.565	0.400	3	0.421	0.250
Ach61	5	0.719	0.684	3	0.204	0.222	3	0.405	0.500
Ach68	5	0.669	0.800	4	0.477	0.444	3	0.586	0.950

A: Number of alleles; He: expected heterozygosity; Ho: observed heterozygosity; n: number of individuals considered in each population.

These microsatellite markers are not the first to be described in *A. chilensis* species, but the 11 available ones (Bastías et al., 2016) were unable to differentiate a group of seven *A. chilensis* accessions under domestication collected from different valleys in the South of Chile, which exhibited evident phenotypic differences; the new set of 15 SSRs presented here differentiated all but one pair of these domesticated accessions, partially fulfilling breeder's hypothesis, whom initially believed to have seven different accessions, turning out to be six -well defined- different genotypes (Figure 1).

The transferability of these markers in a wider genetic background was evaluated. For that purpose, representative samples of two related species belonging to Elaeocarpaceae family, *Crinodendron patagua* Molina and *Aristotelia peduncularis* (Labill.) Hook. f. were analyzed with the 15 polymorphic SSRs (Table 5). *Crinodendron patagua* is an endemic species of central Chile and results showed good amplification signal only in six out of 15 SSRs (Table 6).

Figure 1. Electrophoretic separation of alleles for seven *Aristotelia chilensis* domestication lines using two SSR markers. A) SSR marker Ach14; B) Ach28. Numbers and bars correspond to the different genotypes, with one to three replicates (different plants) each. Results revealed that samples #5 and #6 were non-differentiable based on 14 polymorphic markers, suggesting they are clones of the same material. Electrophoresis lasted 2 h at 75 W and 45 mA; 3 μL PCR product was loaded on each lane. DNA bands were silver-stained.

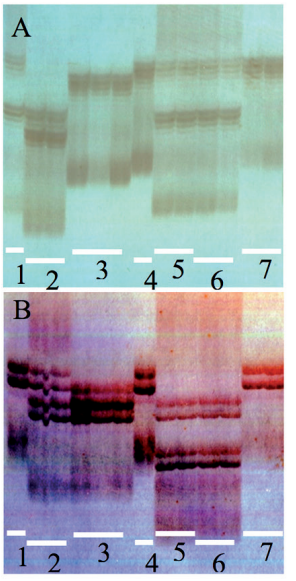

Table 5. Herbarium data for *Aristotelia chilensis*, *A. peduncularis* and *Crinodendron patagua* accessions.

Species	Population code	Voucher nr	Geographical coordinates
Aristotelia chilensis (Molina) Stuntz	Population 1	SGO 169 860	33°29'45" S, 70°28'57" W 900 m a.s.l
Aristotelia chilensis (Molina) Stuntz	Population 2	SGO 169 859	34°50'18" S, 71°21'17" W 250 m a.s.l
Aristotelia chilensis (Molina) Stuntz	Population 3	SGO 169 861	41°40'08" S, 73°03'50" W 70 m a.s.l
Aristotelia peduncularis (Labill.) Hook. f.	ND	HO 569 841	42°55'20" S, 147°15'11" W 420 m a.s.l
Crinodendron patagua Molina	ND	SGO 169 858	33°24'27" S, 70°36'20" W 650 m a.s.l

Table 6. Transferability of *Aristotelia chilensis* SSR markers to related species, *A. peduncularis* and *Crinodendron patagua* (Elaeocarpaceae).

	PCR signal quality										
	A. peduncularis					*C. patagua*					*A. chilensis*
SSR markers	1	2	3	4	5	1	2	3	4	5	
Ach3	210-230	++	++	++	++	+	+	+	+	+	++
Ach4	246-254	++	++	++	++	--	--	--	--	--	++
Ach5	246-260	++	++	++	++	+/-	+/-	+/-	+/-	+/-	++
Ach11	246-250	++	++	++	++	+	+	+	+	+	++
Ach14	+	+	+	+	+	+/-	+/-	+/-	+/-	+/-	++
Ach28	190-210	++	++	++	++	+	+	+	+	+	++
Ach29	224-246	++	++	++	++	--	--	--	--	--	++
Ach38	--	--	--	--	--	--	--	--	--	+/-	++
Ach41	226-230	++	++	++	++	--	--	--	--	--	++
Ach45	160-180	++	++	++	++	--	--	--	+	+	++
Ach47	170-190	++	++	++	++	+	+	+	+	+	++
Ach54	--	--	--	--	--	+	+	+	+	+	++
Ach57	252-256	++	++	++	++	+	+	+	+	+	+
Ach61	210-224	++	++	++	++	--	--	--	--	--	++
Ach68	196-206	++	++	++	++	--	--	--	--	+/-	++

++: Clear and strong PCR signal; +: good signal; +/-: weak signal; --: no amplification.
The estimated allele sizes (bp) for each primers/species combination is indicated for sample #1 of *A. peduncularis*.

Results of banding pattern showed an outstanding performance for *A. peduncularis*, native to Tasmania in Australia, with clear and strong PCR signals in most of the SSR analyzed (13 out of 15 SSRs amplified) (Table 6). These results are not unexpected, considering that the transferability of markers tend to be lower when phylogenetic distance is higher, such as relatives at the family level, compared to higher transferability usually observed in the framework of species of the same genus (in this case, *Aristotelia* sp.) This is commonly observed in many fruit crops and other taxonomical groups, a good example being the inter-specific hybrids used as rootstocks, as has been described in the genus *Prunus* spp. (Arismendi et al., 2012) and *Vitis* spp. (Lin and Walker, 1998; Gizella et al., 2011), among others. In these cases, a small set of SSR markers were able to tag the corresponding genomic regions and identify hybrids derived from different species.

CONCLUSIONS

A new set of 15 SSRs for *Aristotelia chilensis* species giving highly polymorphic allelic patterns in 60 individuals belonging to three populations from different geographical regions in Chile was obtained. It was possible to efficiently differentiate between many genotypes of maqui, including some specimens under domestication, making this set of markers the first useful tool for maqui genotypes traceability. Additionally, 13 out of 15 SSR markers showed clear and polymorphic amplification signals when analyzed in five specimens of the related species *A. peduncularis*, suggesting a high transferability of this new set of markers into new species of the same genus.

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