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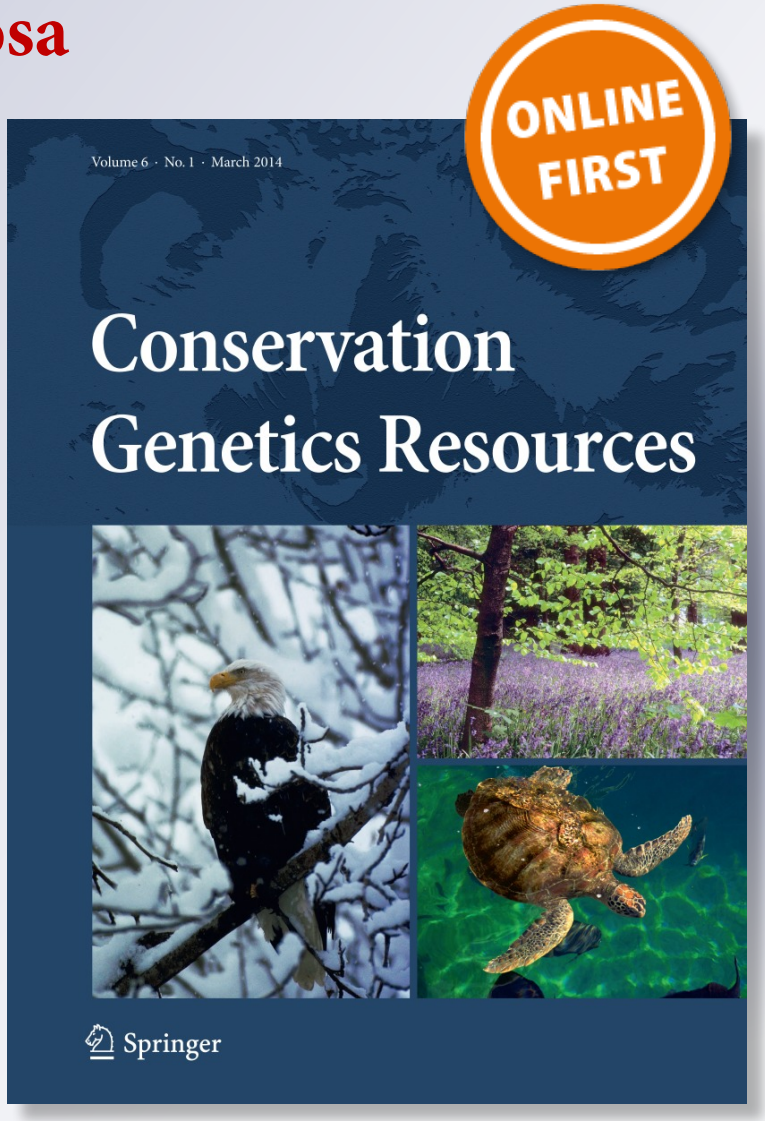

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MICROSATELLITE LETTERS

Microsatellite markers for the endangered shrub *Myrceugenia rufa* (Myrtaceae) and three closely related species

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Abstract *Myrceugenia rufa* is an endangered shrub endemic to the coast range of central Chile, which has suffered strong degradation during recent decades. We developed nine microsatellite markers for this species and tested them on *M. correifolia*, *M. lanceolata*, and *M. exsucca*. Six loci amplified and were polymorphic in all species; the remaining loci were polymorphic in at least two species. The number of alleles per locus ranged from 1 to 10, the expected and observed heterozygosities ranged from 0.07 to 0.90 and 0.12–0.84, respectively. Characterized microsatellites will be useful to estimate the genetic diversity and population structure of *M. rufa* and its sister species.

Keywords Coastal range · Mediterranean forest · Myrceugenia · Rare species

Myrceugenia rufa is an evergreen shrub endemic to Chile. This species is currently restricted to remnants of forest and shrublands in the summits and deep creeks of Costal range of Central Chile. These areas, characterized by high species richness and endemism, have suffered strong degradation during recent decades due to forest plantations, tourism development and urbanization. In addition, *Myrceugenia rufa* is frequently parasitized by insects that destroy fruits and seeds. Given its narrow distribution range (<3,500 km^2), the accelerated degradation of its habitat and the low levels of natural regeneration, *M. rufa* has been listed as Endangered (EN, Hechenleitner et al. 2005) and near threatened (NT, Environmental Minnistery of Chile) and programs of artificial breeding and *ex-situ* conservation have been initiated (Botanical Gardens of Viña del Mar and Chagual).

Myrceugenia rufa is closely related to *M. correifolia*, *M. lanceolata* and *M. exsucca* (Murillo et al. 2012). The former two species have narrow distributions and like *M. rufa* are endemic to the Coast Range of central Chile (Landrum 1981). *M. exsucca* has a broader distribution, reaching the temperate forest of Chile and Argentina. In this study we isolated nine microsatellite markers for *M. rufa* and we tested them in its sister species. Development of microsatellites will be useful to characterize the patterns of genetic diversity of *Myrceugenia* species, and in this way improve in situ and ex situ conservation efforts.

Total genomic DNA was isolated from foliar samples of *M. rufa*. DNA was sent to OMICS solution Sequencing Center (Santiago, Chile) to perform pyrosequencing in the 454 Jr Genome Sequencer. We obtained 26,550 reads with an average read length of 404 bp. We selected 20 microsatellite loci from a total of 745 identified loci. We tested five individuals for PCR amplifications as follows: 17 ng DNA, 1.5 pmol reverse primer, 1.5 pmol M13-tailed forward primer, 1.5 pmol fluorescently labeled M13 universal primer, 5 μL 2 × GoTaq Master Mix, 3 mM $MgCl_2$, 0.5 μL BSA to a final volume of 10 μL. Cycling conditions were 94 °C, 5 min; (94 °C, 1 min; 56–58 °C, 1 min; 72 °C, 1 min) 30 cycles; 72 °C, 10 min. For genotyping, samples were run on the ABI PRISM 310 (Applied

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Table 1 Characteristics of nine microsatellite primers developed in *Myrceugenia rufa*

Locus	Primer sequences (5′–3′)	Repeat	Size	Accession number
M3	TAAGAGATGGCCCAATGTCC TGGAGCTCAGCTGGTTCTTC	$(GA)_{12}$	232–252	KJ570899
M4	AGGCCCTCTTTCTAAGATGATAA ATCCCTGCAGCTTAGAGGGT	$(CT)_{12}$	208–224	KJ570900
M5	TGCAATCATTTGCTTTGCAT TTATTGAATGGCCATCGGAC	$(CT)_{11}$	225–327	KJ570901
M6	GCAAACAATGCCCTAACAGG TACGCCATGCACACTCTTTG	$(GC)_{11}$	250–302	KJ570902
M11	CCACGATCACTCTCCTCTCCC CCTCGGAGTGAATCAAACAAG	$(CTT)_{17}$	210–247	KJ570903
M15	GAAGGGTGACCGAATGTGAG AGACTTTGGCTTGTTGACGG	$(GA)_{15}$	218–317	KJ570904
M17	AACAATAATAACCAAGGAGGTGGA TGCAGGGTGTCATAAAGGCT	$(AG)_{14}$	219–278	KJ570905
M20	TCTCTCTGGAAACGTGCGAT CACATGCCATCACAATC	$(CT)_{11}$	282–317	KJ570906
MR9	TGCCAAAGGCCAGAAAGTTA ATCAAAGCAGCTAACGGCAA	$(GA)_{16}$	205–237	KJ570907

Shown for each primer pair are the forward and reverse sequence, repeat type, size of the original fragment (bp), annealing temperature when run individually (Ta) and the GenBank accession number

Table 2 Number of alleles (*A*), observed (H_O) and expected (H_E) heterozygosity for populations of *M. rufa*, *M. correifolia*, *M. lanceolata*, and *M. exsucca*

		P3	P4	P5	P6	P11	P17	P15	P20	P9
M. rufa										
Los Vilos (n = 15)	A	4	5	6	3	6	9	1	2	1
31°15′S, 72°31′W	H_O	0.60	**0.47**	0.73	0.67	0.73	0.60	0	0.33	0
	H_E	0.64	0.73	0.70	0.65	0.74	0.83	0	0.50	0
Santa Inés (n = 15)	A	5	7	7	2	8	5	2	2	1
32°09′S, 71°29′W	H_O	0.53	0.67	0.73	0.40	0.43	0.33	0.13	0.40	0
	H_E	0.68	0.64	0.71	0.32	0.67	0.40	0.12	0.40	0
M. correifolia										
Talinay (n = 14)	A	2	3	3	6	6	6	X	X	3
30°28′S, 71°37′W	H_O	0.14	**0.07**	0.21	0.57	0.50	**0.29**			0.64
	H_E	0.13	0.31	0.47	0.65	0.63	0.67			0.48
Santa Inés (n = 10)	A	3	4	6	6	5	5	X	X	5
32°09′S, 71°29′W	H_O	0.30	0.40	0.30	0.50	0.90	**0.30**			0.30
	H_E	0.61	0.47	0.66	0.76	0.69	0.71			0.59
M. exsucca										
Los Vilos (n = 14)	A	5	2	11	6	8	5	10	5	5
PC	H_O	0.43	0.21	0.50	0.86	0.50	0.50	0.79	0.71	0.64
32°04′S, 71°29′W	H_E	0.71	0.50	0.81	0.73	0.78	0.65	0.82	0.79	0.60
Pichidangui (n = 16)	A	8	2	6	5	7	7	8	7	2
32°34′S, 71°27′W	H_O	0.69	0.43	**0.13**	0.63	0.50	**0.50**	**0.17**	0.50	0.33
	H_E	0.81	0.48	0.42	0.69	0.80	0.70	0.84	0.64	0.28
M. lanceolata										
Córdova (n = 10)	A	5	7	6	8	8	7	8	5	4
33°26′S, 71°40′W	H_O	0.85	0.44	0.13	0.80	0.50	0.33	**0.30**	0.50	0.50
	H_E	0.74	0.59	0.49	0.73	0.51	0.68	0.83	0.64	0.57

Location (latitude and longitude) and sample size (n) for each population are also shown. Crosses indicate no amplification. Significant deviations from Hardy–Weinberg equilibrium after sequential Bonferroni corrections are shown in bold

Biosystems, Foster City, California, USA) and analyzed using Peak Scanner Software version 1.0 (Applied Biosystems). We used GENALEX 6.5 (Peakall and Smouse 2006) to estimate allele diversity, heterozygosity levels and deviations from Hardy–Weinberg equilibrium. Adjusted p-values were obtained using sequential Bonferroni test.

Nine loci gave reliable amplifications for *M. rufa* (Table 1). Loci were scored on a total of 94 individuals, including *M. rufa* (n = 30), *M. exsucca* (n = 30), *M. correifolia* (n = 24) and *M. lanceolata* (n = 10) from one to two localities per species (Table 2). Six loci amplified and were polymorphic in all studied populations. The remaining loci amplified and were polymorphic in at least two species. The number of alleles per locus per population ranged from one to ten. Expected and observed heterozygosities in polymorphic loci ranged from 0.07 to 0.90 and from 0.12 to 0.84, respectively. Up to three loci per population exhibited significant deviation from Hardy–Weinberg equilibrium following Bonferroni corrections (Table 2).

The polymorphic loci described here will be useful to estimate genetic diversity and population structure of *Myrceugenia rufa* and its sister species. Microsatellites would also be a valuable tool for detecting ongoing hybridization between these species.

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