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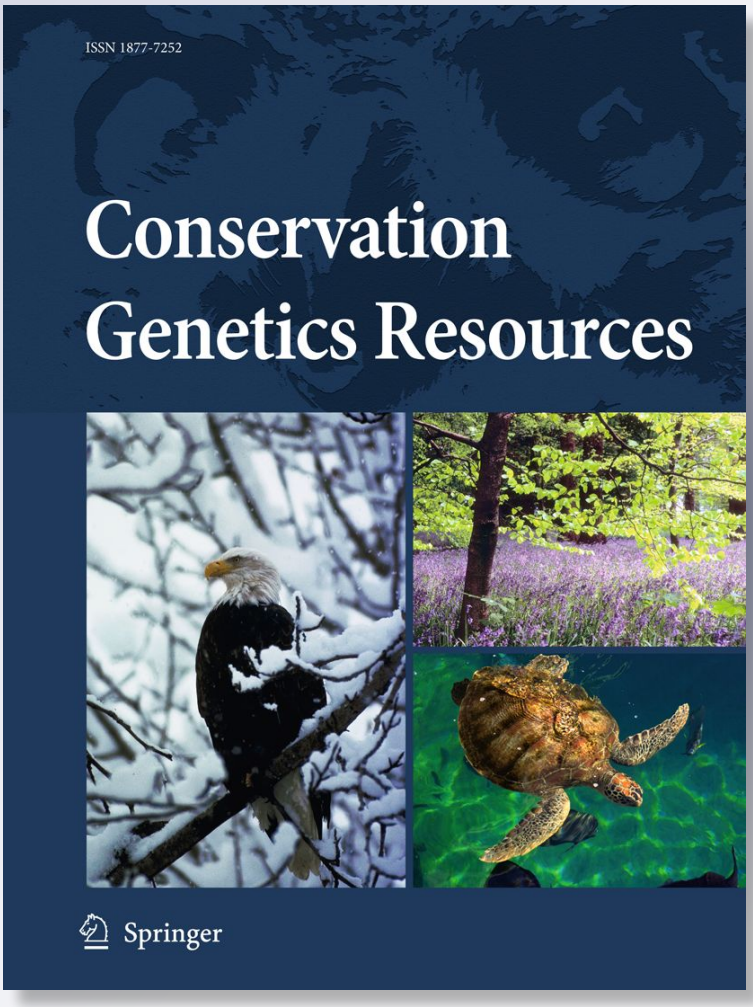

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TECHNICAL NOTE

Ten nuclear microsatellites markers cross-amplifying in *Scaevola montana* and *S. coccinea* (Goodeniaceae), a locally common and a narrow endemic plant species of ultramafic scrublands in New Caledonia

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Abstract We developed ten microsatellites markers to study the population genetic structure of two ultramafic scrublands species in New Caledonia, *Scaevola montana* a common species used in ecological restoration of mining sites, and *S. coccinea* a narrow endemic species of the Tontouta valley. The markers were isolated from *S. montana*. Nine loci are polymorphic in *S. montana*; eight were polymorphic in *S. coccinea*. The narrow endemic species showed lower variation at these loci than *S. montana*. There is a significant heterozygosity deficit in *S. montana* at four loci, and a global significant inbreeding coefficient across all loci ($F_{IS} = 0.159$). In contrast, the tested populations of *S. coccinea* did not show significant deviations from Hardy–Weinberg expectations. These markers are now being used in conservation genetic studies aiming to understand the biology of these species and to provide information to guide restoration programmes.

Keywords Microsatellites · *Scaevola* · Goodeniaceae · Cross-species amplification · Conservation · New Caledonia

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Scaevola montana and *S. coccinea* (Goodeniaceae) grow in ultramafic scrublands in New Caledonia. *S. montana* is locally common and indigenous to New Caledonia and the neighbouring island of Vanuatu. As well as ultramafic soils, it also occurs on substrates such as limestone and metamorphic rocks (Müller 1990). *S. coccinea,* is a narrow endemic species, restricted to ultramafic soils in a single valley system—the Tontouta valley. The extraction of ores from ultramafic substrates is a major industrial activity in New Caledonia and is a source of large-scale environmental destruction. Mining companies undertake restoration programs and microsatellite markers can be useful for estimating levels of genetic diversity and differentiation among potential source populations, and checking for cryptic genetic bottlenecks during translocation programmes. In addition, from a more academic perspective, comparative estimates of population genetic structure in narrow endemic and widespread sister species are of interest for evaluating the genetic consequences of rarity.

Microsatellite sequences were isolated by ecogenics GmbH (Switzerland) using a modified high-throughput genomic sequencing approach as described by Abdelkrim et al. (2009). In brief, size-selected fragments from genomic DNA were enriched for SSR content by using

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Table 1 Primer sequences, size range, and measures of genetic variation for 10 microsatellite markers in *Scaevola montana* and *Scaevola coccinea*

Scaevola montana Locus	Repeat motif	Primer sequence (5′–3′)	NCBI Probe accession no	*TA* (°C)	Size range (bp)	*N*	*A*	H_o	H_e	F_{IS}
Scamon 002472	(GT)17	b F: AAAACATCCAGTTGTGCTCG R: TACCAGGCTTCATTCCTCCC	12324994	56	86–122	52	12	0.808	0.792	−0.020^{ns}
Scamon 003354	(TTG)9	c F: ATCACAAGGAAGGCAGGAGG R: TCATGTGAAAAGCAAAAGAACGAC	12324995	56	245–260	52	6	0.481	0.449	−0.070^{ns}
Scamon 004222	(TTG)13	c F: CGAAAAAGTCTTGCATCGGC R: ACAACATAAGGAACAAGCAAACC	12324996	56	100–133	52	11	0.731	0.756	0.033^{ns}
Scamon 004339	(AC)16	d F: TTGACACCAAAAGAAGAAATTCAC R: GATGCCGATTGTGATGTCCC	12324997	56						
Scamon 004448	(AAC)12	c F: AACGGAGGTAACACCTGTGG R: TGGATTCCTACATAGTTTTGCAGTC	12324998	56	103–154	52	14	0.577	0.781	0.261***
Scamon 004635	(TC)12	a F: TTGCTTCCTGAGTTCCCTCC R: CCTCGCACTTGCCTTTAGTC	12324999	56	120–184	52	15	0.788	0.807	0.023^{ns}
Scamon 007130	(AC)13	d F: CACTCTTATACCACTTACGTTCCAG R: TTGTGGCAGCTAACTCTCCC	12325000	56	131–161	49	11	0.438	0.719	0.371***
Scamon 010065	(CTC)7	b F: TCTCTATCCACCACCGCTTC R: TAATCCGTACCGACGTAGCC	12325001	56	151–172	52	8	0.846	0.781	−0.083^{ns}
Scamon 010114	(CAA)13	b F: ACCACGTAGTAAGAATTACACACC R: TGGATCAAACAAGATTTGCAAGC	12325002	56	182–335	52	21	0.404	0.850	0.525***
Scamon 012182	(TTG)19	d F: TGACCATCCAAAGTCCAAGG R: AGACATGGTTGATTGTGAGTTC	12325003	56	117–159	52	12	0.596	0.823	0.275***
Mean						51.7	12.2	0.630	0.751	0.159*
Scaevola coccinea Locus	Repeat motif	Primer sequence (5′–3′)	NCBI Probe accession no	*TA* (°C)	Size range (bp)	*N*	*A*	H_o	H_e	F_{IS}
Scamon 002472	(GT)17	b F: AAAACATCCAGTTGTGCTCG R: TACCAGGCTTCATTCCTCCC	12324994	56	80–102	52	6	0.538	0.484	−0.113^{ns}
Scamon 003354	(TTG)9	c F: ATCACAAGGAAGGCAGGAGG R: TCATGTGAAAAGCAAAAGAACGAC	12324995	56	239–263	52	2	0.096	0.088	−0.087^{ns}
Scamon 004222	(TTG)13	c F: CGAAAAAGTCTTGCATCGGC R: ACAACATAAGGAACAAGCAAACC	12324996	56	97–115	52	4	0.635	0.587	−0.082^{ns}
Scamon 004339	(AC)16	d F: TTGACACCAAAAGAAGAAATTCAC R: GATGCCGATTGTGATGTCCC	12324997	56	162–182	52	10	0.519	0.550	0.056^{ns}
Scamon 004448	(AAC)12	c F: AACGGAGGTAACACCTGTGG R: TGGATTCCTACATAGTTTTGCAGTC	12324998	56						
Scamon 004635	(TC)12	a F: TTGCTTCCTGAGTTCCCTCC	12324999	56	134–154	52	7	0.731	0.711	−0.027^{ns}

Table 1 continued

Scaevola coccinea Locus	Repeat motif	Primer sequence (5′–3′)	NCBI Probe accession no	*TA* (°C)	Size range (bp)	*N*	*A*	H_o	H_e	F_{IS}
		R: CCTCGCACTTGCCTTTAGTC								
Scamon 007130	(AC)13	d F: CACTCTTATACCACTTACGTTCCAG	12325000	56	133–155	52	8	0.596	0.644	0.075^{ns}
		R: TTGTGGCAGCTAACTCTCCC								
Scamon 010065	(CTC)7	b F: TCTCTATCCACCACCGCTTC	12325001	56						
		R: TAATCCGTACCGACGTAGCC								
Scamon 010114	(CAA)13	b F: ACCACGTAGTAAGAATTACACACC	12325002	56	200–305	52	9	0.423	0.693	0.390^{ns}
		R: TGGATCAAACAAGATTTGCAAGC								
Scamon 012182	(TTG)19	d F: TGACCATCCAAAGTCCAAGG	12325003	56	90–132	52	6	0.712	0.622	−0.145^{ns}
		R: AGACATGGTTGATTGTGAGTTC								
Mean						52	6.5	0.531	0.547	0.029^{ns}

M13 fluorescent labelling: a = red; b = blue; c = yellow; d = green. *TA* primer annealing temperature, *N* number of plants genotyped, *A* number of alleles, H_o observed heterozygosity, H_e expected heterozygosity, F_{IS} inbreeding coefficient (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, *ns* not significant)

Dynabeads M-280 Streptavidin from Invitrogen and biotin-labelled CT and GT repeat oligonucleotides (Microsynth AG, Switzerland). The SSR enriched library was analyzed on a Roche 454 platform using the GS FLX titanium reagents. The total 13,498 reads had an average length of 248 base pairs. Of these, 312 contained a microsatellite insert suitable for primer design with a tetra- or a trinucleotide of at least 6 repeat units or a dinucleotide of at least 10 repeat units. Primers were designed for 36 microsatellite inserts, of which 28 were tested for polymorphism. Polymorphism was determined by using the procedure described by Schuelke (2000). Ten primers which showed clear amplification profiles and reliable amplification, and which were polymorphic in at least one of the two species are described in Table 1. The test set is based on 52 samples coming from two different populations for each species (Col d'Amoss and the Tontonta Valley for *S. montana;* one population near the Tontouta river and the summit of the Kokoreta for *S. coccinea*).

Genomic DNA was extracted from young leaves with Qiagen DNeasy© Plant Mini Kit. Loci were PCR amplified in an Applied Biosystem Verity 96 well thermal cycler. They were visualised with universal M13 fluorescent labelling (Schuelke 2000). Reactions of 10 µl volumes contained 200 µM each dNTP, 0.04 µM of M13 tailed locus specific forward primer, 0.16 µM of locus specific reverse primer, 0.16 µM of universal M13 primer 5′-end labelled with one of four dyes from Applied Biosystems (VIC, FAM, NED or PET), 1× buffer (Qiagen, containing Tris–HCl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$, pH 8.7. The PCR profile was 95 °C for 15 min, followed by 30 cycles of 30 s at 95 °C, 45 s at annealing temperature (Table 1), and 45 s at 72 °C, then eight cycles of 30 s at 95 °C, 45 s at 53 °C, and 45 s at 72 °C, and finally 30 min at 72 °C. PCR products were sized on an AB3130xl Genetic Analyser and genotypes were assigned with GeneMapper (Applied Biosystems Inc). Number of alleles (*A*), observed (H_o) and expected (H_e) heterozygosity, and the inbreeding coefficient (F_{IS}) were assessed using FSTAT v 2.9.3.2 (Goudet 1995). The significance of F_{IS} was assessed via permutation tests.

We detected 6–21 different alleles per locus (mean 12.2) for *S. montana* and between 2 and 10 (mean 6.5) alleles for *S. coccinea*. Higher values of expected heterozygosity were found for *S. montana* (mean $H_e = 0.751$, range 0.449–0.850) whereas they were more variable and on average lower for *S. coccinea* (mean $H_e = 0.547$, range 0.088–0.711). *S. montana* showed a deficit of heterozgosity in four loci and a globally significant inbreeding coefficient, whereas all *S. coccinea* loci were in Hardy–Weinberg equilibrium (HWE). *S. montana* is strongly protandrous and sets limited seed when pollinators are excluded (Wulff unpublished). Thus the significant inbreeding coefficient is

unlikely to be due to intra-flower selfing. Some level of geitonogamy or bi-parental inbreeding may explain these deviations from HWE. An alternative possibility is null alleles. The loci most likely to contain null alleles are *Scamon 007130* and *Scamon 010114* as these loci have more than double the average inbreeding coefficient.

There are currently few genetic studies on the New Caledonian flora (e.g. Bottin et al. 2005; Kettle et al. 2007), despite its importance as a global biodiversity hotspot. These microsatellites markers will be useful to study the genetic structure of *Scaevola* populations in New Caledonia and will give the opportunity to use this information in conservation programmes and studies of evolutionary diversification.

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