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TECHNICAL NOTE

Development of polymorphic microsatellite markers of the endangered and endemic *Vateriopsis seychellarum* (Dipterocarpaceae), a relict canopy tree of the Seychelles

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Abstract The Dipterocarpaceae are a globally significant family of tropical timber trees. They are especially dominant in lowland rainforests of Southeast Asia, but have a pan tropical distribution. *Vateriopsis seychellarum* is the sole representative of this family on the Seychelles. Historically one of the dominant canopy trees on Mahé, extensive overexploitation of this species for its timber has led to its virtual extinction. The last individuals of this species are found in small fragmented populations at lower altitudes (up to 400 m) on the island of Mahé where the total number of known reproductive adults is 112. We developed ten polymorphic microsatellite loci for this species to enable us to quantify the levels of diversity in remnant populations and to study genetic structure and contemporary gene flow. In addition we tested for cross amplification of these alleles in the closely related but geographically disjunct species *Vateria indica*. In *Vateriopsis seychellarum* the number of alleles per locus ranged from 6 to 20 (mean of 11.4 per locus) with an average polymorphic information content of 0.73 across loci. Expected heterozygosity ranged from 0.40 to 0.71 with 3 of the 10 loci showing deviation from Hardy–Weinberg expectations. 8 of the 10 primers showed cross amplification in *Vateria indica*. These markers will help to provide a better understanding of the significance of historic distributions, gene flow and recent anthropogenic habitat degradation for the survival of widespread species in recently fragmented landscapes.

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Vateriopsis seychellarum is an endangered and endemic tree species of the Seychelles, occurring in lowlands up to 400 m on the main island of Mahé. It is the sole representative of the family of Dipterocarpaceae in the Seychelles. Historically this species dominated the forest canopy across much of the Seychelles island of Mahé (Procter 1984), but extensive overexploitation for timber has led to its virtual extinction. Today there are only 112 known adults left, dispersed in nine populations on Mahé, at least two of which are known to be planted. Understanding the distribution and extent of the remaining genetic diversity in these populations, as well as the contemporary potential for gene dispersal by pollen and seed, will help to inform in situ and ex-situ conservation. *Vateriopsis seychellarum* also provides a useful study system for exploring the genetic consequences of habitat fragmentation of highly endangered dominant tropical canopy species.

To this end we developed microsatellite markers for *V. seychellarum*. Enriched libraries were established from size selected genomic DNA ligated into SAULA/SAULB-linker (Armour et al. 1994) using magnetic bead selection with biotin-labelled (CT)13 and (GT)13 oligonucleotide repeats (Gautschi et al. 2000a, b). Of 352 recombinant colonies screened, 255 gave a positive signal after hybridization. Plasmids from 72 positive clones were sequenced and primers were designed for 24 microsatellite inserts, of which 18 were tested for polymorphism. Of these, only the ten most variable loci were optimized and labeled with an M13-tag at its 5′-end described by Schuelke (2000) (Table 1). Polymorphism of the ten

Table 1 Characteristics of ten polymorphic microsatellite loci in *V. seychellarum*

Locus	GenBank accession no.	Primer sequence (5′–3′)	Repeat motif	*Vateriopsis seychellarum*						*Vateria indica*		
				Size range (bp)	T_a (°C)	*A*	H_o	H_e	PIC	Cross-species amplification	Size range (bp)	*A*
01	GU591481	F: TCATTTCAAACCCAGCAATG R: TCATGCTGCTGATGAAGACC	$(CA)_{17}$	214–234	56	6	0.48	0.45	0.46	Yes	217–227	2
10	GU591482	F: TGCGAGAATCAGCCTATGAG R: CATAAAAGCATGGACCTCAGC	$(CT)_{17}$	140–178	56	12	0.46	0.54	0.68	Yes	132–158	6
11	GU591483	F: TCAAGCCATAGGACACTTGC R: GATCGGCCTGCTAAACATTC	$(CT)_{19}$	201–249	56	20	0.68	0.70	0.87	Yes	191–247	13
12	GU591484	F: GGAATCAAGCGCGAATTAAG R: TCATCATCTTTACCCCATTATCAG	$(CT)_{16}$	199–223	56	9	0.25	0.51	0.70	Yes	177–215	9
14	GU591485	F: CTTTTGCCATATGCATGCTC R: ATCGTCACAGCCTCATTACG	$(TC)_3TT(TC)_{16}$	100–130	56	15	0.71	0.74	0.89	Yes	99–109	4
15	GU591486	F: ATTAGGGCTTTGGGTGAGTG R: GCCAGAACCAGTGGATGAG	$(GA)_{22}$	142–174	56	8	0.42	0.39	0.56	Yes	156–225	6
20	GU591487	F: TTTACAGTCTCGAAAATTGTGACTAAG R: AACAACCTGGGTTGGAGATGC	$(GA)_{15}$	114–158	56	11	0.65	0.71	0.82	Yes	102–129	8
21	GU591488	F: TATTCCCTCATCGTGGAACC R: TTCGGGTATAAGAGGGAGGAG	$(CT)_4GGTTG(CT)_{22}$	188–220	56	12	0.58	0.68	0.82	No	–	–
22	GU591489	F: TTTTGATAACGTTCAAGGCTTC R: ATTCAGCCATTGTTGTGCAG	$(CT)_{21}$	168–198	56	10	0.56	0.65	0.73	No	–	–
23	GU591490	F: TATGGCTTCGCTCAATTGTC R: TTCGTCAGTTTTGGAGTTGG	$(AG)_{22}$	204–230	56	11	0.58	0.70	0.81	Yes	198–226	3

F forward primer, *R* reverse primer, T_a annealing temperature, *A* number of alleles, H_o observed heterozygosity, H_e expected heterozygosity, *PIC* Polymorphism information content; 98 individuals were analysed for each locus in *Vateriopsis seychellarum* and 23–35 individuals in *Vateria indica*

PCR-primers generating the expected PCR products was tested with 98 *V. seychellarum* adult tree samples collected from six different sites on Mahé.

Genomic DNA was extracted from silica dried leaves of *V. seychellarum* ($n = 98$) using the QIAGEN DNeasy Plant Maxi Kit, following the manufacturer's protocol. PCR was carried out in 10 µl reactions with 2 µl of 1× PCR buffer (Promega colorless Flexi GoTaq PCR buffer), 15 mM MgCl2, 0.2 µM dNTPs, 0.2 µl of the 0.04 µM M13 forward primer, 0.8 µl of the 0.16 µM reward primer and 0.8 µl of the 0.16 µM M13 primer, 0.025 U *Taq* polymerase (Promega), and 2 µl DNA template (*c.* 10 ng). Cycling conditions were as follows: 1× (95°C for 15 min), 30× (95°C for 30 s, primer-specific temperature (56°C) for 45 s, 72°C for 45 s), 8× (95°C for 30 s, primer-specific temperature (53°C) for 45 s, 72°C for 45 s), 1× (72°C for 30 min) (Table 1) carried out in a Bio-Rad Dyad Cycler. We used an ABI3730 for genotyping and genemapper 3.5 software (Applied Biosystems) for fragment analysis.

Descriptive statistics (number of alleles, observed and expected heterozygosities), deviations from Hardy–Weinberg equilibrium (HWE) were generated using GenAlEx 6.2 (Peakall and Smouse 2006). The polymorphism information content (PIC) was calculated in Cervus 3.0 (Kalinowski et al. 2007). Linkage disequilibrium was tested using GENEPOP (Raymond and Rousset 1995). All ten loci were polymorphic with 6–20 alleles and a total number of 114 alleles detected over all analysed populations for *V. seychellarum*. Observed heterozygosity values ranged from 0.25 to 0.71. There was no evidence for scoring error due to stuttering and no evidence for large allele dropout according to microchecker 2.2.3 (Oosterhout et al. 2004) but evidence for the presence of null alleles in 2 loci (12 and 23). Significant deviations from Hardy–Weinberg equilibrium (HWE, $P < 0.05$) were detected in three loci (12, 20 and 23). No significant linkage disequilibrium was detected suggesting that all 10 loci segregate independently of each other. These results indicate that the 10 primers will provide a valuable tool for evaluating genetic diversity and the reproductive ecology of this rare and emblematic tree species.

In our preliminary test for cross species amplification with *Vateria indica* we applied the same PCR conditions and used 1.3 µl of template DNA. Of the 10 microsatellites tested 8 amplified, we checked for polymorphism in these 8 loci and found 51 alleles in 23–35 individuals sampled from a single region in the Western Ghats, India (Table 1). The results indicate that this set of 10 microsatellite primers will be valuable for quantifying the genetic diversity and gene flow in the remaining populations of *Vateriopsis seychellarum.* Our preliminary assessment of cross amplification in *Vateria indica* also suggests that these markers may prove useful for studies in other closely related taxa.

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