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TECHNICAL NOTE

Development of polymorphic microsatellite markers for the critically endangered and endemic Indian dipterocarp, *Vateria indica* L. (Dipterocarpaceae)

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Abstract *Vateria indica* (Dipterocarpaceae) is an economically and ecologically important canopy tree endemic to the Western Ghats, India. The species has undergone extensive habitat loss and overexploitation and is therefore listed as 'critically endangered' on the 2012 IUCN Red List. We developed ten polymorphic microsatellite loci for *V. indica.* In addition, we confirm cross amplification and variation in two loci isolated from the closely related but geographically disjunct species *Vateriopsis seychellarum*, previously published by Finger et al. Conserv Genet Resour, 2 (S1):309–311, (2010). The twelve microsatellite primers screened on 48 adult samples of *V. indica* had 5–11 alleles per locus (mean of 8.5 per locus) with an average polymorphic information content of 0.64 across loci. Expected heterozygosity ranged from 0.44 to 0.84. These markers will enable us to quantify population genetic diversity in habitat fragments and to study fine scale spatial genetic structure and contemporary gene flow.

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Vateria indica (Dipterocarpaceae) is an economically and ecologically important canopy tree endemic to the Western Ghats, India. The species is listed as 'critically endangered' on the 2012 IUCN Red List because it has undergone extensive habitat loss and overexploitation (Ashton 1998). *Vateria indica* also provides non-timber forest products including gum resin used for incense and in traditional medicine for its anti-microbial properties (Grover and Rao 1981).

The genus *Vateria* is principally bee pollinated (Ashton 1988). Dipterocarp species generally have limited dispersal (Kettle 2012), we thus predict similarly limited dispersal of the wingless fruit of *V. indica*. Understanding the consequences of habitat degradation and fragmentation for genetic diversity and contemporary gene dispersal by pollen and seed in *V. indica,* will help to inform conservation. Furthermore, *V. indica* provides the opportunity to increase our understanding of the reproductive ecology of dipterocarp tree species in highly fragmented habitats (Kettle et al. 2012; Finger et al. 2012).

To this end we developed 10 *de novo* microsatellite markers for *V. indica*. A 454 sequencing library was generated from size selected sheared fragments of genomic DNA using standard Roche reagents. The 454 library was analyzed on a Roche 454 platform using the GS FLX titanium chemistry. The total 41,308 reads had an average length of 336 base pairs. Of these, 452 contained a microsatellite insert with a tetra- or a trinucleotide of at least 6 repeat units or a dinucleotide of at least 10 repeat units. Suitable primer design was possible in 107 reads, of which

Table 1 Characteristics of twelve microsatellite loci in *Vateria indica*

Locus	GenBank accession no.	Multiplex reaction	Primer sequence (5′–3′)	Repeat motif	Size range (bp)	T_a (°C)	*A*	H_o	H_e	*PIC*
Vi93	KC146392	1	F: *FAM*-GGGTTTCAGATGGATGGCAG	$(AT)_{13}$	73–95	56	10	0.771	0.736	0.706
			R: ATGAGACAACGGACCAATGC							
Vi15	KC146393	1	F: *ATTO565*- ATTGGGAGGCCTGACTGATG	$(AT)_{14}$	119–143	56	11	0.771	0.836	0.822
			R: ACTATGCTAACCTCTTGTCTTTCG							
Vi16	KC146394	1	F: *ATTO532*-AGAGTGTGTAGGACAGAAAATAAC	$(AT)_{12}$	187–199	56	6	0.646	0.594	0.563
			R: AGTGAGCTATGCATCCAAGAG							
Vi55	KC146395	1	F: *ATTO550*-ACATCCAAAGTCAAGCCAGC	$(AATA)_{14}$	198–254	56	10	0.688	0.709	0.675
			R: TGAGTGACTAAGCGGTGAGC							
Vi45	KC146396	1	F: *ATTO550*-TGTTTTTGCTCCTTTTCGTGAG	$(TAA)_7$	151–183	56	5	0.417	0.441	0.398
			R: TGACTTTGGGGGATCGATTTTC							
Vi13	KC146397	2	F: *ATTO550*-TAGAACAGGAGACACGTCAG	$(TTA)_9$	124–145	56	6	0.604	0.637	0.599
			R: TGTTAATCCGTCATGCAGTCC							
Vi60	KC146398	2	F: *FAM*-CATGCAAATGCGGTTATGGC	$(ATT)_8$	211–229	56	5	0.646	0.671	0.608
			R: AAACTTGCCACCACTCAACG							
Vi91	KC146399	2	F: *ATTO565*-TCGCAAGACTCAAATAAATCACG	$(ATT)_8$	227–254	56	7	0.660	0.664	0.627
			R CCGGATGGTGTAAAACTGCC							
Vi18	KC146400	3	F: *ATTO565*-AGCTCAATTTGGTTTGCTTAGG	$(TAT)_{15}$	213–249	52	6	0.542	0.595	0.564
			R: ACCGCTGGCTATATGGATGG							
Vi78	KC146401	3	F: *ATTO532*-TGACAGTTTTTGTTTGTGCTTTTG	$(AAT)_8$	232–271	52	8	0.875	0.817	0.793
			R: AGCTCAGTGGATAGAGCGTC							
Vat14[a]	GU591485	2	F: *ATTO532*-CTTTTGCCATATGCATGCTC	$(TC)_3TT(TC)_{16}$	87–97	56	5	0.688	0.672	0.626
			R:ATCGTCACAGCCTCATTACG							
Vat10[a]	GU591482	3	F: *FAM*-TGCGAGAATCAGCCTATGAG	$(CT)_{17}$	114–162	52	8	0.750	0.729	0.697
			R:CATAAAAGCATGGACCTCAGC							

Fluorescent labels are specified in italic font at the 5′ end of the respective forward primer, 48 individuals were analysed for each locus in *Vateria indica*

F forward primer, *R* reverse primer, T_a annealing temperature, *A* number of alleles, H_o observed heterozygosity, H_e expected heterozygosity, *PIC* Polymorphic information content

[a] See Finger et al. (2010) for full details

20 were labeled with an M13-tag at its 5′-end described by Schuelke (2000) and tested for polymorphism on 15 samples. Of these 20 primers, the ten most variable loci were optimized and assembled in three multiplex PCR with fluorescent labels on the 5′-end of the forward primer (Table 1). Polymorphism of these ten primers was tested with 48 *V. indica* adult tree samples collected from a single river catchment in Kodagu district, southern Karnataka, which lies within the Western Ghats biodiversity hotspot.

Genomic DNA was extracted from silica dried leaves of *V. indica* ($n = 48$) using a CTAB extraction method (Sambrook et al. 1989). PCR was carried out in 10.3 μL reactions with 2.06 μL of 5× PCR buffer (Promega colorless Flexi GoTaq PCR buffer), 0.66 μL of 25 mM MgCl2, 1.03 μL of 0.2 mM dNTPs, 0.12 μL of the 5 μM labeled forward primer, 0.25 μL of the 10 μM unlabeled forward primer, 0.62 μL of the 5 μM reverse primer, 0.1 μL of 5 U/μL *Taq* polymerase (Promega), and 1.3 μL DNA template (*c.* 10 ng). The missing volume to reach 10.3 μL was adjusted for each of the 3 multiplex PCR. PCR were carried out in a Bio-Rad Dyad Cycler with the following cycling conditions: an initial denaturation of 95 °C for 5 min followed by 30 cycles of 94 °C for 30 s, primer-specific temperature (56 °C for multiplex reaction 1 and 2 or 52 °C for multiplex reaction 3) for 1 min and 72 °C for 1 min. The reaction was ended with a final extension time of 72 °C for 5 min. We used an ABI3730 for fragment analysis and Genemapper 3.5 software (Applied Biosystems) to score the fragments relative to a LIZ 500 size standard.

Descriptive statistics (number of alleles, observed and expected heterozygosities) were generated using GenAlEx 6.4 (Peakall and Smouse 2006). The polymorphism information content (PIC) and deviations from Hardy–Weinberg equilibrium was calculated in Cervus 3.0 (Marshall et al. 1998). Linkage disequilibrium was tested using GENEPOP (Raymond and Rousset 1995) and the resulting *p*-values were adjusted with a sequential Bonferroni correction described in Rice (1989). All twelve loci were polymorphic with 5–11 alleles and a total number of 87 alleles detected over all samples. Observed heterozygosity values ranged from 0.42 to 0.88 and expected heterozygosity from 0.44 to 0.84. There was no evidence for scoring error due to stuttering or due to large allele dropout and no evidence for the presence of null alleles according to MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004). No significant deviation from Hardy–Weinberg equilibrium was detected in any loci. The test for linkage disequilibrium revealed some association between loci Vi18 and Vi55 and between Vi55 and Vi60 ($p < 0.05$). Two primers isolated from the closely related Seychelles endemic dipterocarp *Vateriopsis seychellarum* (Finger et al. 2010) also provide useful molecular markers for *V. indica* (see Table 1). These 12 primers will provide a valuable tool for studying the genetic diversity and reproductive ecology in this important Indian tree species.

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