Twenty Microsatellite Markers for the Endangered Vatica mangachapoi (Dipterocarpaceae)

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PRIMER NOTE

TWENTY MICROSATELLITE MARKERS FOR THE ENDANGERED *VATICA MANGACHAPOI* (DIPTEROCARPACEAE)[1]

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- *Premise of the study:* Microsatellite markers were developed for *Vatica mangachapoi* (Dipterocarpaceae), an endangered species indigenous to Southeast Asia and southern China.
- *Methods and Results:* Twenty microsatellite markers, including 12 polymorphic markers, were identified from *V. mangachapoi* using high-throughput sequencing. Polymorphism at each marker was evaluated using 87 individuals from three natural populations. The number of alleles per polymorphic locus ranged from six to 15, and the observed and expected heterozygosity varied from 0.000 to 0.926 and from 0.177 to 0.864, respectively. These markers were transferred successfully to the endangered species *V. guangxiensis*.
- *Conclusions:* These markers may be used to investigate the genetic diversity and gene flow of *V. mangachapoi* and *V. guangxiensis*.

Key words: conservation genetics; cross-species transferability; Dipterocarpaceae; microsatellite markers; next-generation sequencing; *Vatica mangachapoi*.

Vatica mangachapoi Blanco (Dipterocarpaceae), a typical component of the tropical rainforest, is mainly distributed in Indonesia, Malaysia, the Philippines, Thailand, Vietnam, and China (Li et al., 2007). Its heartwood is in great demand due to its hard texture, fine structure, and strong resistance to decay (Appanah and Turnbull, 1998). In addition, this species produces secondary metabolites in its leaves and stems that can be used in herbal medicines (Qin et al., 2011). In China, this species occurs on Hainan Island. Its natural areas have declined rapidly due to over-harvesting and the conversion of its habitats into arable lands or fruit orchards. Consequently, its genetic resources are heavily eroded (Huang et al., 2008). The species has thus been listed in the China Species Red List (Wang and Xie, 2004) and in the Red List of Threatened Species (IUCN, 2014). Hence, it is necessary to assess its genetic diversity, genetic structure, and gene flow among populations so that conservation strategies can be developed. To this end, we developed 12 polymorphic microsatellite loci.

METHODS AND RESULTS

One silica gel–dried leaf sample was used in a simple sequence repeat (SSR) scan at the whole genome level. Total genomic DNA was extracted with a modified cetyltrimethylammonium bromide (CTAB) method (Zeng et al., 2002) and was then fragmented into lengths of 300–1500 bp by ultrasonication. DNA fragments shorter than 500 bp were removed using an agarose gel DNA purification kit (Aidlab Biotech Ltd., Beijing, China). The remaining fragments were then sequenced in a one-sixth run on a Roche 454 GS FLX+ platform (454 Life Sciences, a Roche Company, Branford, Connecticut, USA). The sequencing library was prepared following Roche 454 standard protocols. The method of Lu et al. (2015) was used to control the quality of raw sequencing data and to identify microsatellite markers.

A total of 133,569 reads were generated with an average length of 406 bp. Of these reads, 2530 contained microsatellite loci with di-, tri-, tetra-, penta-, or hexanucleotide units of at least five repeats, and 1657 reads could be used to design PCR primers with an expected product size ranging from 100 to 447 bp. The raw data of the sequences have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (accession no. SRP094881).

Fifty-eight microsatellite loci with more than five repeats were selected to design PCR primers using Primer Premier version 5.0 (PREMIER Biosoft International, Palo Alto, California, USA). The amplification of these primers was tested using three individuals each from three natural populations: Shimeiwan, Bawangling, and Baishui Forest Farm on Hainan Island, China. The PCR reaction mixture (10 μL) contained 50 ng of DNA template, 150 μM dNTPs, 2.0 μM $MgCl_2$, 0.5 μM forward and reverse primers, 1× PCR buffer (Tiangen Biotech Ltd., Beijing, China), and 0.04 U/μL of *Taq* DNA polymerase (Tiangen Biotech Ltd.). PCR was carried out on an Applied Biosystems Veriti thermal cycler (Applied Biosystems, Waltham, Massachusetts, USA) using the following program: 94°C for 3 min, 94°C for 30 s, annealing temperature (see Table 1) for 30 s, 72°C for 30 s (35 cycles), and 72°C for 10 min. Among the 58 loci detected, 20 generated specific PCR products.

Polymorphism of the 20 microsatellite loci was further evaluated using 87 individuals from three natural populations of *V. mangachapoi* (Appendix 1). The fluorescence-labeled dUTP method as described by Li and Gan (2011) was applied with the PCR reaction conditions described above and the reaction system modified with 100 μM dNTPs and 10 μM dUTPs. The PCR products were analyzed by an automated sequencer (ABI 3730XL, Applied Biosystems). Genotyping was performed using GeneMapper version 4.0 software (Applied Biosystems). MICRO-CHECKER 2.2.3 (van Oosterhout et al., 2004) was used to detect and correct the presence of null alleles, and POPGENE version 1.31

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TABLE 1. Characterization of 20 microsatellite loci for *Vatica mangachapoi*.

Loci	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	T_a (°C)	*A*	GenBank accession no.
VM03	F: GCACAGGGAGGAAATATCAGGT R: TGACCAGTTTTAATCAGCACGC	$(AGG)_5$	178	56	1	KY056575
VM04	F: ATCAAACTCCATGGAGCCACAA R: GCACCAACTTCCAACTGTGTAC	$(GA)_7$	138	58	1	KY056576
VM05	F: GATAGCGTTCCTGACACAAACG R: TACTGGTAGGCTTTTCGGGTTC	$(AT)_7$	245	55	1	KY056577
VM09	F: GAACCCTTATTGGCCTGCCTAC R: GGGACCAAATGACTTGAGTAATCT	$(AT)_{11}$	166–184	54	9	KY034644
VM12	F: ACCCTAACAATTCTCTTTGTTTCCT R: CCCCAATCTCAGTAAGGACTCA	$(TAA)_{11}$	152–195	59	14	KY034645
VM14	F: CTTGTGTCGAGCATGCATGTAT R: TGCTGGCCTTTTATGTTAGGGT	$(AT)_{11}$	175–191	59	9	KY034646
VM15	F: CTGAAGACGACGAATGCGAATC R: TCTGCTCAATCGTTCCAAAAGC	$(CGG)_5$	150	56	1	KY056578
VM16	F: CAACCCTCTACCCCCAGAATTC R: AACCAAGCGACAATAGCACAAC	$(TA)_8$	197	57	1	KY056579
VM19	F: ATAGCAGGCACTTCGGAAGTAC R: CCTGAGAAACAAAGCAACGCAT	$(TA)_8$	261–277	56	10	KY034647
VM22	F: ACCTTAATGGCTGGTTACCTGG R: AGCCGGTTCACTTTCAATGAGA	$(CTT)_6$	275	59	1	KY056580
VM23	F: TGGTTTGTGAAAAACTTGCATGG R: AAGGTGGCTCGGTCATCTAATC	$(TA)_6$	238	58	1	KY056581
VM26	F: GCACTAGCACTAGCACTAGCTT R: GGCTTTTCCAATTTCCATGGCT	$(CT)_{11}$	218–226	58	7	KY034648
VM29	F: AGTTAAGGGACCAAATTTAGCGT R: GTGTTTGTCAACTGGGCTTCAA	$(TA)_7$	259–269	56	6	KY034649
VM33	F: AAATTGGAGGGGAGGGGAAGA R: CCCTCCCCTCTCCTCATTTAGA	$(TTA)_5$	101	58	1	KY056574
VM37	F: CCCATGTGCTAGGCTAATGCTA R: AAATCAGCATGAAACTTCTCCATT	$(AT)_6$	229–239	56	6	KY034650
VM43	F: CACCACCACAGGCTTGAGTATA R: GAAGGCCAACTAATCAAGCTGC	$(TA)_7$	168–182	59	6	KY034651
VM47	F: TCATTTCTGTCTCACTCGACCC R: TCATCGACGAATCACTGTTCGA	$(TTC)_{10}$	148–168	59	7	KY034652
VM49	F: ACGGATAAGTTAACGGACTAGACA R: AGATTTTCCCCCAGTCATCGAC	$(TA)_{10}$	215–227	54	11	KY034653
VM52	F: GCTGGCACTTAGGATGCCTTAA R: AGCAACCAATTAGCTCAAATCAA	$(ATT)_{11}$	138–150	58	15	KY034654
VM53	F: GGGCAGCCTCGTAAATCAATTAC R: ATTACCTGGCACAACCTTAGCC	$(ATT)_{13}$	225–249	59	10	KY034643

Note: *A* = number of alleles; T_a = annealing temperature.

(Yeh et al., 1999) was used to calculate the number of alleles, expected and observed heterozygosity, and to assess deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD).

Twelve loci were found to be polymorphic and eight were monomorphic. Among the 12 polymorphic loci, the number of alleles per locus ranged from six to 15 (Table 1), with an average of 9.2. The observed and expected heterozygosity

TABLE 2. Genetic diversity data of 12 polymorphic microsatellite loci in three *Vatica mangachapoi* populations.[a]

	Shimeiwan (*N* = 32)				Bawangling (*N* = 28)				Baishui Forest Farm (*N* = 27)			
Locus	*A*	H_o	H_e	HWE[b]	*A*	H_o	H_e	HWE[b]	*A*	H_o	H_e	HWE[b]
VM09	9	0.844	0.790	0.683	6	0.607	0.768	0.301	5	0.815	0.802	0.231
VM12	11	0.844	0.826	0.000**	7	0.714	0.677	0.988	10	0.630	0.800	0.000**
VM14	8	0.750	0.804	0.298	7	0.857	0.823	0.469	8	0.852	0.854	0.490
VM19	9	0.594	0.793	0.004**	8	0.423	0.771	0.000**	9	0.778	0.791	0.000**
VM26	5	0.688	0.657	0.879	6	0.571	0.700	0.766	4	0.667	0.637	0.977
VM29	6	0.594	0.727	0.747	5	0.571	0.686	0.012*	6	0.653	0.740	0.658
VM37	6	0.594	0.591	0.997	3	0.000	0.434	0.000**	5	0.630	0.620	0.968
VM43	5	0.563	0.554	0.265	3	0.107	0.200	0.002**	4	0.185	0.177	0.999
VM47	5	0.656	0.738	0.583	7	0.750	0.744	0.936	6	0.926	0.773	0.768
VM49	7	0.625	0.782	0.052	7	0.679	0.770	0.487	10	0.500	0.864	0.000**
VM52	5	0.438	0.660	0.022*	10	0.607	0.770	0.355	13	0.593	0.860	0.000**
VM53	9	0.875	0.794	0.970	8	0.786	0.853	0.102	8	0.889	0.860	0.520

Note: *A* = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; HWE = Hardy–Weinberg equilibrium probabilities; *N* = number of individuals sampled.

[a]Locality and voucher information are provided in Appendix 1.

[b]Deviations from HWE were statistically significant at $*P < 0.05$ and $**P < 0.01$.

TABLE 3. Cross-species usability of 20 microsatellite loci for *Vatica guangxiensis*.

Loci	*A*	H_o	H_e
VM03	1	0	0
VM04	1	0	0
VM05	1	0	0
VM09	4	0.750	0.821
VM12	2	0	0.423
VM14	5	1.000	0.857
VM15	1	0	0
VM16	1	0	0
VM19	5	0.750	0.857
VM22	1	0	0
VM23	1	0	0
VM26	3	0.500	0.750
VM29	4	0.500	0.786
VM33	1	0	0
VM37	4	0.750	0.643
VM43	3	0.5	0.679
VM47	4	1	0.750
VM49	5	0.750	0.786
VM52	3	0.750	0.714
VM53	5	0.750	0.857

Note: *A* = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity.

of these loci ranged from 0.000 to 0.926 and from 0.177 to 0.864 in three populations, respectively (Table 2). Four loci (VM19, VM37, VM43, and VM52) likely contained null alleles (95% confidence interval). After correction of these null alleles, departure from HWE and LD were tested. Three loci (VM12, VM19, and VM52) showed significant deviation from HWE in Shimeiwan, four loci (VM19, VM29, VM37, and VM43) in Bawangling, and four loci (VM12, VM19, VM49, and VM52) in Baishui Forest Farm (Table 2). No significant LD was detected for all pairwise analyses of the 12 polymorphic loci.

The 20 loci were further tested using five individuals of *V. guangxiensis* S. L. Mo from Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (Appendix 1). This species is also an endangered dipterocarp indigenous to Guangxi and Yunnan, China. Testing indicated that all of these loci could be successfully amplified in this species (Table 3).

CONCLUSIONS

Twenty microsatellite markers, including 12 polymorphic markers, for *V. mangachapoi* are reported here for the first time. All of the polymorphic markers cross-amplified successfully in *V. guangxiensis*. These markers can be applied to investigate the genetic diversity and population genetic structure of both species, which would contribute to the conservation of their genetic resources.

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APPENDIX 1. Voucher specimen information for populations used in this study. Specimens are deposited at the Herbarium of the Research Institute of Tropical Forestry, Chinese Academy of Forestry, Guangzhou, China.

Species	Voucher specimen accession no.[a]	Collection locality	Geographic coordinates	*N*
Vatica mangachapoi Blanco	VM-RITF-GSZ-SMW-04[b]	Shimeiwan, Hainan, China	19.1167°N, 109.1000°E	1
V. mangachapoi	VM-RITF-GSZ-SMW-16	Shimeiwan, Hainan, China	19.1167°N, 109.1000°E	32
V. mangachapoi	VM-RITF-GSZ-BWL-08	Bawangling, Hainan, China	18.7000°N, 109.8333°E	28
V. mangachapoi	VM-RITF-GSZ-BSF-02	Baishui Forest Farm, Hainan, China	18.6500°N, 110.2500°E	27
V. guangxiensis X. L. Mo	VG-RITF-GSZ-ML-01	Mengla County, Yunnan, China	21.6833°N, 109.4167°E	5

Note: *N* = number of individuals sampled.

[a]VM = *Vatica mangachapoi*; VG = *Vatica guangxiensis*; RITF = Research Institute of Tropical Forestry; GSZ = Junjie Guo, Shuaibin Shang, and Jie Zeng, collectors.

[b]This sample was used for designing the primer sequences and was included in the population analysis.