Twenty Microsatellite Markers for the Endangered Vatica mangachapoi (Dipterocarpaceae)

Authors: Guo, Jun-Jie, Shang, Shuai-Bin, Wang, Chun-Sheng, Zhao, Zhi-Gang, and Zeng, Jie

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Twenty microsatellite markers for the endangered *Vatica mangachapoi* (Dipterocarpaceae)

Jun-Jie Guo, Shuai-Bin Shang, Chun-Sheng Wang, Zhi-Gang Zhao, and Jie Zeng

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, 2350 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 MgCl₂, 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 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 was used to calculate and compare the observed and expected heterozygosities.
### 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 | (AGG)_5       | 178                    | 56       | 1 | KY056575              |
|      | R: TGACCACTTTATACCTGACGCC | (GA)_7       | 138                    | 58       | 1 | KY056576              |
| VM04 | F: ATCAACCTCTGAGGCTGAGCAT | (AT)_7       | 245                    | 55       | 1 | KY056577              |
|      | R: TACTGTAGGCTTCTCAGGTTTC |             |                        |          |   |                       |
| VM09 | F: GAAACCTTAATTGCGCTCCTAC | (AT)_11      | 166–184                | 54       | 9 | KY034644              |
|      | R: GGGACAAATGACTGAGTAACTT |             |                        |          |   |                       |
| VM12 | F: ACCTCAACATTTCTGTATTTCTC | (TAA)_11     | 152–195                | 54       | 9 | KY034645              |
| VM14 | F: CTCGCTGAGGATGCTAGTTACC | (GA)_7       | 175–191                | 59       | 9 | KY034646              |
| VM15 | F: CTGAAGGCGAGAATGGGAAAT | (CGG)_5      | 150                    | 56       | 1 | KY056578              |
|      | R: TCTGCTCAATTCCAGCAAGAC  |             |                        |          |   |                       |
| VM16 | F: GAAACCTCTACCCACAAGATTT | (TA)_6       | 197                    | 57       | 1 | KY056579              |
| VM19 | F: ATGACGGCACTGGAGTACAG | (CGG)_5      | 261–277                | 56       | 10| KY034647              |
|      | R: TCTGCTCAATTCCAGCAAGAC  |             |                        |          |   |                       |
| VM22 | F: ACCTTAATTGCCGTTCACCTGG | (CTT)_6      | 275                    | 59       | 1 | KY056680              |
|      | R: AGGCGGCTCACTTTCCATAGA |             |                        |          |   |                       |
| VM23 | F: TGAGTTGTAGAAAACCTTGGTT | (TA)_6       | 238                    | 58       | 1 | KY056581              |
| VM26 | F: GCCTAGGCTGCACTATACCTA | (AT)_7       | 218–226                | 54       | 9 | KY034648              |
|      | R: GGTCTCCACATTTCCAGGCTC |             |                        |          |   |                       |
| VM29 | F: AGTAAAGGGACCACAATTTAGCT | (TA)_11      | 259–269                | 56       | 6 | KY034649              |
|      | R: GTGTTGCTCAGCGGGCTCCTAA |             |                        |          |   |                       |
| VM33 | F: AAATGGAGGGGGGAAAGGAGA | (TTA)_5      | 101                    | 58       | 1 | KY056574              |
|      | R: CCCCTCCCCCTCTCCATTTAGA |             |                        |          |   |                       |
| VM37 | F: CCAAGAGGGCAGCTGTTAGTAC | (AT)_7       | 229–239                | 56       | 6 | KY034650              |
|      | R: AAATCAGCAGGAAATCCAGGCT |             |                        |          |   |                       |
| VM43 | F: CACCACCAAGGCTTGGATAA | (CTT)_6      | 168–182                | 59       | 6 | KY034651              |
|      | R: GAAGGGCAACTTAACTCAGGCT |             |                        |          |   |                       |
| VM47 | F: TCAATTCTGCTACCTGCAGCC | (TTT)_6      | 148–168                | 59       | 7 | KY034652              |
|      | R: TCTGACGGAATACCTGGTTGA |             |                        |          |   |                       |
| VM49 | F: AGCGGATTTAAGAAGCGACAG | (TA)_10      | 215–227                | 54       | 11| KY034653              |
|      | R: AGTACGCTCCCTCAGGTACGA |             |                        |          |   |                       |
| VM52 | F: GCTGACCTTATGCGTTCATAA | (ATT)_11     | 138–150                | 58       | 15| KY034654              |
|      | R: AGACCAACTATTGCTGCTAACA |             |                        |          |   |                       |
| VM53 | F: GGGACACCCTGTAATGTTACTC | (ATT)_11     | 225–249                | 59       | 10| KY034643              |
|      | R: ATTACCTGGCCACACAGTTACG |             |                        |          |   |                       |

**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

| Locus | Shimeiwan (N = 32) | Bawangling (N = 28) | Baishui Forest Farm (N = 27) |
|-------|-------------------|---------------------|-----------------------------|
|       | A | H_o | H_e | HWE | A | H_o | H_e | HWE | A | H_o | H_e | HWE |
| 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 | 7 | 0.571 | 0.700 | 0.766 | 4 | 0.667 | 0.637 | 0.967 |
| 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 | 0.999 | 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_o = observed heterozygosity; H_e = expected 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.

http://www.bioone.org/loi/apps
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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**CONCLUSIONS**

Twenty microsatellite markers, including 12 polymorphic markers, for *V. mangachapoi* are reported here for the first time.