

# Isolation and characterization of microsatellite loci from *Pterocarya stenoptera* (Juglandaceae)

Peng-Fei Wang<sup>1</sup>, Yong Li<sup>1,3</sup>, Zhi-Hao Qian<sup>1</sup>, Jia-Xin Li<sup>1</sup>, and Xue-Jun Ge<sup>2</sup>

Manuscript received 9 August 2018; revision accepted 18 October 2018.

<sup>1</sup> Innovation Platform of Molecular Biology, College of Forestry, Henan Agricultural University, Zhengzhou 450002, People's Republic of China

<sup>2</sup> South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, People's Republic of China

<sup>3</sup> Author for correspondence: liyongrui1@126.com

**Citation:** Wang, P.-F., Y. Li, Z.-H. Qian, J.-X. Li, and X.-J. Ge. 2018. Isolation and characterization of microsatellite loci from *Pterocarya stenoptera* (Juglandaceae). *Applications in Plant Sciences* 6(12): e1205.

doi:10.1002/aps3.1205

**PREMISE OF THE STUDY:** Microsatellite markers of *Pterocarya stenoptera* (Juglandaceae) were developed for future studies on the population genetic diversity and spatial genetic structure of the species.

**METHODS AND RESULTS:** Based on Illumina sequencing of the transcriptome of *P. stenoptera*, a total of 2452 microsatellites were identified from 83,674 assembled unigenes. One hundred microsatellites were randomly selected to design amplification primer pairs. Of these, 15 were successfully amplified and displayed polymorphism. For these markers, the number of alleles per locus and population ranged from one to six. The levels of observed and expected heterozygosity varied from 0.000 to 1.000 and 0.000 to 0.718, respectively. Furthermore, all of the 15 loci were successfully cross-amplified in another congeneric species (*P. hupehensis*) and were demonstrated to be polymorphic.

**CONCLUSIONS:** The microsatellite loci described here can be used for future population genetic and landscape genetic studies on *P. stenoptera*.

**KEY WORDS** Illumina sequencing; Juglandaceae; microsatellite marker; *Pterocarya stenoptera*.

*Pterocarya stenoptera* C. DC. (Juglandaceae) is a deciduous broad-leaved tree that is native to China and the Korean Peninsula. The species is cultivated as an urban landscaping tree because of its large crown and graceful drooping racemes. Previous studies on this plant have focused on its cultivation physiology (Yang et al., 2013; Xu et al., 2015; Yang and Li, 2016). To date, there have been no reports of the genetic diversity and population structure of *P. stenoptera*. Fan et al. (2013) developed 22 microsatellite loci for *P. stenoptera* using primer pairs from *Cyclocarya paliurus* (Batal.) Iljinsk. This number of microsatellite loci is insufficient for landscape genetic studies, which require the molecular markers to be distributed within the genome with high density (Hall and Beissinger, 2014). As an important urban landscaping tree, it is necessary to develop a large number of reliable molecular markers for the assessment of wild germplasm resources and the development of molecular-assisted breeding.

Next-generation sequencing makes it possible to rapidly isolate a large number of microsatellite markers (Kumar et al., 2014). Here, we report the isolation of transcript-based microsatellite markers for *P. stenoptera* using Illumina shotgun sequencing. The transcript-based microsatellite markers are more suited to locating adaptive loci in landscape genetic studies. Although loci identified through neutral markers can be linked to adaptive genes, microsatellites

found within genic regions can provide a clearer link to a causal gene.

## METHODS AND RESULTS

A total of 60 individuals of *P. stenoptera* were sampled from three wild populations in Henan and Shandong provinces (Nanzhao, Henan Province [HNNZ]; Jigong Mountain, Henan Province [HNJG]; Meng Mountain, Shandong Province [SDMM]; Appendix 1). Twenty individuals of *P. hupehensis* Skan were sampled from one natural population in Henan Province (Yaoshan, Henan Province [HNYS]; Appendix 1). Vouchers were deposited at the herbarium of the College of Forestry, Henan Agricultural University, Zhengzhou, China (Appendix 1).

Total RNA was extracted from fresh leaves of one cultivated *P. stenoptera* individual in Henan Agricultural University using a Quick RNA Isolation Kit (BioTeke, Beijing, China) according to the standard protocol of the manufacturer. RNA concentration was estimated using the UV-Vis spectrophotometer (catalog no. ND5000; BioTeke). The construction of a cDNA library and transcriptome sequencing were completed by the Biomarker Biotechnology Corporation (Beijing, China). Sequencing was

TABLE 1. Primer sequences and characterization for 15 polymorphic microsatellite loci isolated from *Pterocarya stenoptera*.

| Locus | Primer sequences (5'–3')                              | Repeat motif                            | T <sub>a</sub> (°C) | Allele size range (bp) | Fluorescent label | GenBank accession no. | BLAST top hit                                                                   |                       |            |
|-------|-------------------------------------------------------|-----------------------------------------|---------------------|------------------------|-------------------|-----------------------|---------------------------------------------------------------------------------|-----------------------|------------|
|       |                                                       |                                         |                     |                        |                   |                       | Putative function                                                               | GenBank accession no. | E-value    |
| Ps2   | F: GTTACTACTAGTCTCCGGC<br>R: TCCGGATCCCTTCTTCTT       | (AC) <sub>6</sub>                       | 48                  | 260–262                | FAM               | MH676105              | Methyltransferase 17-like [ <i>Fragaria vesca</i> subsp. <i>vesca</i> ]         | XP_004307746.1        | 2.605E-98  |
| Ps13  | F: ACGGCGTAGATTTCATCGTC<br>R: TTTTGTGTTTCATTGCTGTG    | (CCT) <sub>6</sub>                      | 52                  | 184–195                | TAMRA             | MH676116              | Hypothetical protein PHAVU_008G280600g [ <i>Phaseolus vulgaris</i> ]            | XP_007142439.1        | 0.000      |
| Ps23  | F: GACGGCACATATTTCAATTC<br>R: GATTGAGTTGCGAGGAAGC     | (GA) <sub>9</sub>                       | 54                  | 236–246                | HEX               | MH676126              | Hypothetical protein PRUPE_ppa007664mg [ <i>Prunus persica</i> ]                | XP_007222111.1        | 0.000      |
| Ps27  | F: AGCTTCTCGAGAGTTGCAAG<br>R: AACCCCAAAGGATATAACGGA   | (TC) <sub>9</sub>                       | 48                  | 217–236                | TAMRA             | MH676130              | U-box domain-containing protein 4 [ <i>Prunus mume</i> ]                        | XP_008229780.1        | 0.000      |
| Ps28  | F: AGCGAGTCTGTAAGACGA<br>R: CCGCCCTTAATTCACAAAA       | (AG) <sub>6</sub>                       | 48                  | 275–281                | FAM               | MH676131              | RING-H2 finger protein ATL47 [ <i>Eucalyptus grandis</i> ]                      | XP_010025372.1        | 3.775E-136 |
| Ps29  | F: GCTCTCTCGGAGCTCTTT<br>R: GAGACTCCACACGGTTGGTT      | (ACC) <sub>5</sub>                      | 58                  | 114–150                | TAMRA             | MH676132              | rho GTPase-activating protein 2 [ <i>Vitis vinifera</i> ]                       | XP_002270566.1        | 0.000      |
| Ps31  | F: ACTTGCTGGAGTAAGCTCGC<br>R: TTCACCGACTTGTAAGGGG     | (AT) <sub>6</sub>                       | 50                  | 227–236                | HEX               | MH676134              | Hypothetical protein L484_002942 [ <i>Morus notabilis</i> ]                     | XP_007206294.1        | 1.147E-60  |
| Ps53  | F: AAAAGCACCTTGCCATTITG<br>R: CCAATCCCAAAAACAAACC     | (GT) <sub>6</sub>                       | 60                  | 114–149                | TAMRA             | MH676156              | Protein strawberry notch [ <i>Vitis vinifera</i> ]                              | XP_003634816.1        | 0.000      |
| Ps59  | F: TGGCTGAGGTGCTCTCTCT<br>R: ATGATGGTGGTAGGGTGAT      | (CAT) <sub>7</sub> CC(TCA) <sub>5</sub> | 62                  | 182–188                | TAMRA             | MH676162              | Prostatic spermine-binding protein-like [ <i>Prunus mume</i> ]                  | XP_008219536.1        | 1.097E-09  |
| Ps65  | F: TGTCTCTGAATCGATACGC<br>R: ACGAGGCCAGATTATTGCAG     | (AT) <sub>6</sub>                       | 56                  | 260–268                | FAM               | MH676168              | No hit                                                                          | —                     | —          |
| Ps69  | F: CTCCTCTCCAAGCCCTAGT<br>R: GGAGACGATTTCAGCATTTGT    | (CTC) <sub>3</sub>                      | 56                  | 236–253                | HEX               | MH676172              | Hypothetical protein Csa_5G601540 [ <i>Cucumis sativus</i> ]                    | KGN51804.1            | 0.000      |
| Ps70  | F: CATGTCCAGCTCCGATACT<br>R: AAACAGCTGTCCCGATTG       | (AAT) <sub>6</sub>                      | 52                  | 209–213                | HEX               | MH676173              | No hit                                                                          | —                     | —          |
| Ps75  | F: ACCGTGAACGAAGCTCAGTC<br>R: CTCAGCTCTCTCCAGTCCCC    | (GCG) <sub>6</sub>                      | 60                  | 243–284                | FAM               | MH676178              | Unnamed protein product [ <i>Vitis vinifera</i> ]                               | CBI32594.3            | 8.241E-45  |
| Ps82  | F: GGACGATGAGGACGAAGAAG<br>R: CTCGAAGCTTTGGGTTTCCAAAG | (GAT) <sub>3</sub>                      | 48                  | 204–231                | HEX               | MH676185              | Pentatricopeptide repeat-containing protein At1g09900 [ <i>Vitis vinifera</i> ] | XP_002272135.2        | 0.000      |
| Ps83  | F: TCACGGTGTTGTAACCCGAC<br>R: GACTAACACAGGCCACGGT     | (AG) <sub>6</sub>                       | 62                  | 127–131                | TAMRA             | MH676186              | Conserved hypothetical protein [ <i>Ricinus communis</i> ]                      | XP_002510114.1        | 1.281E-30  |

Note: A = number of alleles; T<sub>a</sub> = annealing temperature.

**TABLE 2.** Genetic diversity parameters for 15 polymorphic microsatellite loci in three populations of *Pterocarya stenoptera*.<sup>a</sup>

| Locus | HNNZ population (N = 20) |                |                |                 | SDMM population (N = 20) |                |                |                 | HNJG population (N = 20) |                |                |                 |
|-------|--------------------------|----------------|----------------|-----------------|--------------------------|----------------|----------------|-----------------|--------------------------|----------------|----------------|-----------------|
|       | A                        | H <sub>e</sub> | H <sub>o</sub> | F <sub>is</sub> | A                        | H <sub>e</sub> | H <sub>o</sub> | F <sub>is</sub> | A                        | H <sub>e</sub> | H <sub>o</sub> | F <sub>is</sub> |
| Ps2   | 2                        | 0.050          | 0.050          | 0.000           | 2                        | 0.053          | 0.053          | 0.000           | 2                        | 0.056          | 0.056          | 0.000           |
| Ps13  | 2                        | 0.482          | 0.500          | −0.016          | 4                        | 0.405          | 0.474          | −0.174          | 3                        | 0.398          | 0.333          | 0.167           |
| Ps23  | 6                        | 0.441          | 0.421          | 0.046           | 4                        | 0.468          | 0.250*         | 0.472           | 4                        | 0.486          | 0.611          | −0.268          |
| Ps27  | 6                        | 0.653          | 0.700          | −0.075          | 3                        | 0.417          | 0.389          | 0.070           | 2                        | 0.143          | 0.143          | 0.000           |
| Ps28  | 4                        | 0.483          | 0.300          | 0.385           | 3                        | 0.465          | 0.421          | 0.097           | 5                        | 0.718          | 0.882*         | −0.237          |
| Ps29  | 4                        | 0.388          | 0.150*         | 0.620           | 5                        | 0.579          | 0.400*         | 0.315           | 3                        | 0.300          | 0.222          | 0.265           |
| Ps31  | 4                        | 0.645          | 1.000*         | −0.573          | 4                        | 0.562          | 0.737          | −0.323          | 5                        | 0.592          | 0.778          | −0.326          |
| Ps53  | 2                        | 0.235          | 0.158          | 0.333           | 5                        | 0.579          | 0.350*         | 0.402           | 6                        | 0.450          | 0.316*         | 0.303           |
| Ps59  | 3                        | 0.199          | 0.211          | −0.059          | 1                        | 0.000          | 0.000          | —               | 2                        | 0.056          | 0.056          | 0.000           |
| Ps65  | 5                        | 0.658          | 0.800*         | −0.223          | 3                        | 0.642          | 0.750          | −0.173          | 4                        | 0.684          | 0.632          | 0.079           |
| Ps69  | 3                        | 0.304          | 0.300          | 0.013           | 3                        | 0.152          | 0.158          | −0.038          | 3                        | 0.383          | 0.412          | −0.077          |
| Ps70  | 2                        | 0.475          | 0.611          | −0.299          | 2                        | 0.422          | 0.368          | 0.131           | 2                        | 0.513          | 0.800*         | −0.583          |
| Ps75  | 4                        | 0.633          | 0.667          | −0.054          | 3                        | 0.411          | 0.421          | −0.025          | 6                        | 0.504          | 0.600          | −0.197          |
| Ps82  | 1                        | 0.000          | 0.000          | —               | 4                        | 0.191          | 0.100*         | 0.483           | 1                        | 0.000          | 0.000          | —               |
| Ps83  | 2                        | 0.286          | 0.222          | 0.227           | 3                        | 0.465          | 0.474          | −0.019          | 2                        | 0.059          | 0.059          | 0.000           |

Note: A = number of alleles; F<sub>is</sub> = inbreeding coefficient; H<sub>e</sub> = expected heterozygosity; H<sub>o</sub> = observed heterozygosity; N = number of individuals tested.

<sup>a</sup>Voucher and locality information are provided in Appendix 1.

\*Significant deviation from Hardy–Weinberg equilibrium ( $P < 0.05$ ).

performed on an Illumina HiSeq 2500 system (Illumina, San Diego, California, USA). We obtained a total of 20,410,837 paired-end reads of 150 bp (6.10 Gbp; GenBank Sequence Read Archive accession no. SRP154982). The raw reads were subsequently cleaned by removing adapter sequences using Trimmomatic version 0.35 (Bolger et al., 2014) and then assembled using Trinity version 2.6.6 with the default parameters (Grabherr et al., 2011), resulting in a total of 83,674 unigenes. Microsatellites from the unigenes were identified using MISA (Thiel et al., 2003). The filtering conditions of microsatellites are as described in Shi et al. (2018). Parameters were designed for dinucleotides with a minimum of six repeats, and for tri-, tetra-, penta-, and hexanucleotides with a minimum of five repeats. Of these unigenes, 2452 containing microsatellites were selected and deposited in GenBank (MH676104–MH678555). The first 100 microsatellites were selected to design amplification primer pairs using Primer3 (Rozen and Skaletsky, 1999). The primer design parameters were set using the default values except product size = 100–400 bp. These primers were then tested in two steps. First, the 100 primer pairs were tested on four randomly selected *P. stenoptera* individuals. PCR was conducted in a 20-μL PCR reaction mixture consisting of 20 ng of genomic DNA, 10 mM of PCR buffer, 0.2 mM of each dNTP, 0.2 μM of each primer, and 1 unit of *Taq* polymerase (Tiangen, Beijing, China). In a Mastercycler Nexus thermocycler (Eppendorf, Hamburg, Germany), PCR was started with an initial denaturation at 94°C for 5 min; followed by 35 cycles at 94°C for 40 s, locus-specific annealing temperature for 40 s, and 72°C for 40 s; a final extension at 72°C for 5 min; and hold at 4°C. PCR products were visualized on 1.5% agarose gel with a DL2000 DNA ladder (TaKaRa Biotechnology Co., Dalian, Liaoning, China). Of the 100 primer pairs, 43 were unsuccessful or had off-target amplification and 57 were retained for second step validation. The remaining 57 primer pairs were then tested on 12 *P. stenoptera* individuals each from populations HNNZ and SDMM. PCR reaction conditions were the same as above. PCR products were separated by gel electrophoresis on 8% native polyacrylamide gel and visualized by silver staining. The band size was reported using a 50-bp DNA ladder (TaKaRa Biotechnology Co.). Of the 57 primer pairs, 42 were not considered further due

to single or excessive bands, and the remaining 15 primer pairs exhibited polymorphism (Table 1). To further assess the polymorphism of these microsatellites, genotyping was performed on all sampled individuals of *P. stenoptera*. The forward primers were 5' labeled with FAM, HEX, or TAMRA. All reaction conditions were the same as above. PCR products were analyzed on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, California, USA) at BGI (Beijing, China). PCR product size was measured according to GeneScan 500 ROX Size Standard (Applied Biosystems). The peaks of the microsatellite loci were read using GeneMarker 2.2.0 (SoftGenetics, State College, Pennsylvania, USA). Observed heterozygosity (H<sub>o</sub>), expected heterozygosity (H<sub>e</sub>), and Hardy–Weinberg equilibrium (HWE) were obtained using GENETOP 4.2 (Rousset, 2008). Number of alleles (A) and inbreeding coefficient (F<sub>is</sub>) were estimated using FSTAT 2.9.3.2 (Goudet, 1995).

**TABLE 3.** Cross-amplification of 15 polymorphic microsatellites developed for *Pterocarya stenoptera* in *P. hupehensis*.<sup>a</sup>

| Locus | Allele size (bp) | A | H <sub>e</sub> | H <sub>o</sub> | F <sub>is</sub> |
|-------|------------------|---|----------------|----------------|-----------------|
| Ps2   | 260              | 1 | 0.000          | 0.000          | —               |
| Ps13  | 175–187          | 4 | 0.431          | 0.316*         | 0.273           |
| Ps23  | 240–263          | 9 | 0.874          | 0.800          | 0.087           |
| Ps27  | 217–229          | 6 | 0.359          | 0.350          | 0.026           |
| Ps28  | 277–287          | 4 | 0.476          | 0.450          | 0.055           |
| Ps29  | 114–150          | 3 | 0.278          | 0.056*         | 0.738           |
| Ps31  | 213–234          | 5 | 0.628          | 0.800          | −0.283          |
| Ps53  | 114–139          | 7 | 0.740          | 0.556          | 0.254           |
| Ps59  | 176–197          | 5 | 0.496          | 0.474*         | 0.047           |
| Ps65  | 262–274          | 6 | 0.804          | 0.588          | 0.274           |
| Ps69  | 247–250          | 2 | 0.328          | 0.300          | 0.088           |
| Ps70  | 209–216          | 3 | 0.494          | 0.278          | 0.444           |
| Ps75  | 249–270          | 5 | 0.675          | 0.556          | 0.181           |
| Ps82  | 201–214          | 3 | 0.160          | 0.056*         | 0.660           |
| Ps83  | 127–129          | 2 | 0.322          | 0.278          | 0.141           |

Note: A = number of alleles; F<sub>is</sub> = inbreeding coefficient; H<sub>e</sub> = expected heterozygosity;

H<sub>o</sub> = observed heterozygosity; N = number of individuals tested.

<sup>a</sup>Voucher and locality information are provided in Appendix 1.

\*Significant deviation from Hardy–Weinberg equilibrium ( $P < 0.05$ ).

In *P. stenoptera*,  $A$ ,  $H_o$ ,  $H_e$ , and  $F_{is}$  ranged from one to six, 0.000–1.000, 0.000–0.718, and –0.583–0.620, respectively (Table 2). Of the 15 polymorphic microsatellite loci, three, four, and three loci showed significant deviations from HWE in populations HNNZ, SDMM, and HNJG, respectively (Table 2). The cross-amplification of these markers was tested in 20 individuals of *P. hupehensis*. All markers were successfully amplified, and  $A$  per locus ranged from one to nine (Table 3).

CONCLUSIONS

In this study, 2452 microsatellites were discovered in the *P. stenoptera* transcriptome. One hundred primer pairs were randomly selected to verify the efficiency of amplification. Of these tested primer pairs, 15 displayed polymorphism. This is the first set of microsatellite markers developed specifically for *P. stenoptera*; it will be helpful for future population genetic and landscape genetic studies of the species. All markers were successfully amplified in the related species *P. hupehensis*, suggesting that they may also be used to study other related species in *Pterocarya*.

ACKNOWLEDGMENTS

Funding for this project was provided by the National Natural Science Foundation of China (31770225), the National Natural Science Foundation of Henan Province (182300410039), the Opening Project of Guangdong Provincial Key Laboratory of Plant Resources (PlantKF09), the Funding Scheme of Young Backbone Teachers of Higher Education Institutions in Henan Province (2015GGJS-081), and the Henan Agricultural University Science and Technology Innovation Fund (KJCX2016A2).

DATA ACCESSIBILITY

Raw reads were submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (accession no. SRP154982). Unigenes containing microsatellites were deposited in GenBank (MH676104–MH678555). Sequence information for the

developed primers has been deposited to NCBI; GenBank accession numbers are provided in Table 1.

LITERATURE CITED

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30: 2114–2120.

Fan, D. M., L. J. Ye, Y. Luo, W. Hu, S. Tian, and Z. Y. Zhang. 2013. Development of microsatellite loci for *Cyclocarya paliurus* (Juglandaceae), a monotypic species in subtropical China. *Applications in Plant Sciences* 1: 1200524.

Goudet, J. 1995. FSTAT (version 1.2): A computer program to calculate F-statistics. *Journal of Heredity* 86: 485–486.

Grabherr, M. G., B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, et al. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology* 9: 644–652.

Hall, L. A., and S. R. Beissinger. 2014. A practical toolbox for design and analysis of landscape genetics studies. *Landscape Ecology* 29: 1487–1504.

Kumar, S., N. Shah, V. Garg, and S. Bhatia. 2014. Large scale in-silico identification and characterization of simple sequence repeats (SSRs) from de novo assembled transcriptome of *Catharanthus roseus* (L.) G. Don. *Plant Cell Reports* 33: 905–918.

Rousset, F. 2008. GENEPOP'007: A complete reimplementa-tion of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8: 103–106.

Rozen, S., and H. J. Skaletsky. 1999. Primer3 on the WWW for general users and for biologist programmers. In S. Misener and S. A. Krawetz [eds.], *Methods in molecular biology*, vol. 132: Bioinformatics: Methods and protocols, 365–386. Humana Press, Totowa, New Jersey, USA.

Shi, X. G., H. D. Wu, W. X. Li, W. X. Guo, Y. Zheng, S. X. Yu, and Y. L. Huang. 2018. Development of polymorphic EST-SSR markers in *Itea chinensis* (Iteaceae) and cross-amplification in related species. *Applications in Plant Sciences* 6: e1013.

Thiel, T., W. Michalek, R. K. Varshney, and A. Graner. 2003. Exploiting EST databases for the development and characterization of gene-derived SSR markers in barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics* 106: 411–422.

Xu, L. P., Y. L. Pan, and F. Y. Yu. 2015. Effects of water-stress on growth and physiological changes in *Pterocarya stenoptera* seedlings. *Scientia Horticulturae* 190: 11–23.

Yang, Y., and C. Li. 2016. Photosynthesis and growth adaptation of *Pterocarya stenoptera* and *Pinus elliottii* seedlings to submergence and drought. *Photosynthetica* 54: 120–129.

Yang, Y. J., C. X. Li, J. Li, R. Schneider, and W. Lamberts. 2013. Growth dynamics of Chinese wingnut (*Pterocarya stenoptera*) seedlings and its effects on soil chemical properties under simulated water change in the Three Gorges Reservoir Region of Yangtze River. *Environmental Science and Pollution Research* 20: 7112–7123.

APPENDIX 1. Voucher and locality information for *Pterocarya* species used in this study.\*

| Species                             | Population code | Voucher no. | Collection locality            | Geographic coordinates  | N  |
|-------------------------------------|-----------------|-------------|--------------------------------|-------------------------|----|
| <i>Pterocarya stenoptera</i> C. DC. | HNNZ            | LiPS2017001 | Nanzhao, Henan, China          | 33°35'24"N, 112°10'48"E | 20 |
|                                     | HNJG            | LiPS2017002 | Jigong Mountain, Henan, China  | 31°48'36"N, 114°04'48"E | 20 |
|                                     | SDMM            | LiPS2017003 | Meng Mountain, Shandong, China | 35°33'36"N, 117°57'36"E | 20 |
| <i>Pterocarya hupehensis</i> Skan   | HNYS            | LiPH2018001 | Yaoshan, Henan, China          | 33°42'22"N, 112°20'00"E | 20 |

Note: N = number of individuals analyzed.  
\*All voucher specimens are deposited at the herbarium of the College of Forestry, Henan Agricultural University, Zhengzhou, China.