# Microsatellite Loci for an Old Rare Species, Pseudotaxus chienii, and Transferability in Taxus wallichiana var. mairei (Taxaceae)

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PRIMER NOTE

# MICROSATELLITE LOCI FOR AN OLD RARE SPECIES, *PSEUDOTAXUS CHIENII*, AND TRANSFERABILITY IN *TAXUS WALLICHIANA* VAR. *MAIREI* (TAXACEAE)[1]

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- *Premise of the study:* Microsatellite loci were developed for *Pseudotaxus chienii*, an old rare species endemic to China, and which provided a useful tool for investigating the patterns of population genetic structure, phylogeography, evolutionary history, and adaptive potential. Transferability was assayed in the related species, *Taxus wallichiana* var. *mairei*.
- *Methods and Results:* A total of 15 microsatellite loci were targeted in *P. chienii* using the Fast Isolation by AFLP of Sequences COntaining Repeats (FIASCO) protocol. Polymorphism was evaluated in five populations of *P. chienii* and five populations of *T. wallichiana* var. *mairei*. Of these loci, 13 were polymorphic in *P. chienii*, whereas 15 were polymorphic in *T. wallichiana* var. *mairei*.
- *Conclusions:* The 15 microsatellite loci developed lay a solid foundation for further studies on population genetic variability and investigations of local adaptation. Additionally, cross-species amplification in *T. wallichiana* var. *mairei* showed that these loci may also have potential utility in other genera of Taxaceae.

**Key words:** genetic diversity; microsatellites; *Pseudotaxus chienii*; *Taxus wallichiana* var. *mairei*; transferability.

*Pseudotaxus chienii* (W. C. Cheng) W. C. Cheng belongs to *Pseudotaxus* W. C. Cheng (Taxaceae), which is a monotypic genus endemic to China (Fu et al., 1999). The species (white-berry yew) has a restricted distribution in northern Guangdong, northern Guangxi, Hunan, southwestern Jiangxi, and southern Zhejiang provinces (Fu et al., 1999). It should be regarded as an "old rare species," which is well adapted to habitat isolation and ecological heterogeneity in a wide range of climatic and soil conditions (Wang et al., 2006; Su et al., 2009). As an evergreen shrub or small tree that grows up to 4 m tall, *P. chienii* is closely related to the sister genus *Taxus* L. Morphological differences include the white stomatal bands and arils (Fu et al., 1999). In addition, its dioecy with low fertilization rates and fruit production lead to poor natural regeneration (Fu et al., 1999). Environmental factors and human-induced disturbances, such as climate change, habitat destruction, and overexploitation, have been causing population size to continuously decrease in *P. chienii* over the past decades (Fu and Jin, 1992; Yang et al., 2005). As early as 1992, *P. chienii* was categorized as an endangered species in the *Red List of Endangered Plants in China* (Fu and Jin, 1992). Although we have known that *P. chienii* is able to maintain high variation in isolated populations from previous studies using random-amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers (Wang et al., 2006; Su et al., 2009), its evolutionary history, phylogeography, and adaptive potential remain unresolved. Codominant microsatellite markers are urgently needed to further survey the pattern of population genetic structure and local adaptation processes in *P. chienii*. In this study, 15 microsatellite loci of *P. chienii* were developed and applied to assess their transferability in the closely related *T. wallichiana* Zucc. var. *mairei* (Lemée & H. Lév.) L. K. Fu & Nan Li.

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## METHODS AND RESULTS

Microsatellite loci were targeted in *P. chienii* following the Fast Isolation by AFLP of Sequences COntaining Repeats (FIASCO) protocol (Zane et al., 2002). Genomic DNA was prepared from the silica gel–dried leaves of one individual from Bijiashan population according to a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). Approximately 500 ng of genomic DNA was completely digested with the restriction enzyme *Mse*I (New England Biolabs, Ipswich, Massachusetts, USA), and then ligated to an *Mse*I adapter pair (5′-TACTCAGGACTCAT-3′/5′-GACGATGAGTCCTGAG-3′) using $T_4$ DNA ligase (New England Biolabs). The ligation was diluted by 10× and

amplified using the adapter-specific primer *Mse*I-N (5′-GATGAGTCCTGAGTAAN-3′) with the following PCR program: 24 cycles of 94°C for 30 s, 53°C for 60 s, and 72°C for 60 s. A 20-μL reaction volume consisted of 5 μL of diluted product, 1× PCR buffer ($Mg^{2+}$ free), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 μM primer, and 1 U *Taq* DNA polymerase (TaKaRa Biotechnology Co., Dalian, Liaoning, China). The amplified product was denatured at 95°C for 3 min and hybridized with a 5′-biotinylated $(AC)_{10}$ probe at room temperature for 15 min. The probe-bound fragments were captured by streptavidin-coated magnetic beads (Promega Corporation, Madison, Wisconsin, USA) to enrich the fragments containing microsatellite repeats. The enriched fragments were reamplified with the primer *Mse*I-N using the PCR conditions described above. The recovered products were purified with E.Z.N.A. Cycle-Pure Kit (Omega Bio-Tek, Norcross, Georgia, USA), then ligated to a pMD-18T vector (TaKaRa Biotechnology Co.), and transformed into DH5α competent cells. Positive clones were tested by PCR with universal M13 primers. A total of 154 positive clones were randomly selected and sequenced on an ABI PRISM 3730 automated DNA sequencer (Applied Biosystems, Foster City, California, USA). Ninety-nine sequences contained simple sequence repeats. Of these, 60 sequences were discarded due to short flanking regions or unsuitability for primer design. The remaining 39 sequences with sufficient flanking regions were used to design primers using Primer Premier 5.0 software (PREMIER Biosoft International, Palo Alto, California, USA). The primers were commercially synthesized by BGI (Beijing Genomics Institute, Shenzhen, Guangdong, China), and the annealing temperature was optimized by a gradient PCR. The 20-μL PCR reaction volume contained 20 ng of genomic DNA, 1× PCR buffer ($Mg^{2+}$ free), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 μM of each primer, and 1 U *Taq* DNA polymerase (TaKaRa Biotechnology Co.). The final PCR program was carried out as follows: initial denaturation at 94°C for 5 min; 40 cycles of 94°C for 45 s, 47–57°C for 45 s, 72°C for 45 s; and a final extension at 72°C for 10 min (Table 1). Amplified products were separated on 6% denaturing polyacrylamide gels and visualized by silver staining. Sizes of fragments were determined by a 50-bp DNA ladder (TaKaRa Biotechnology Co.). Approximately 38% (15 of 39) successfully amplified PCR products.

The 15 microsatellite loci were measured in 50 individuals of *P. chienii* from five natural populations (10 samples per population), including Dayuanwei from Zhejiang Province, Damingshan from Guangxi Zhuang Autonomous Region, Tianzishan from Hunan Province, and Sanqingshan and Bijiashan from Jiangxi Province (Fig. 1; Appendix 1). Voucher specimens were deposited at the herbarium of Sun Yat-sen University (Appendix 1). Genetic parameters, null alleles, and linkage disequilibrium (LD) were calculated using GenAlEx version 6.41, MICRO-CHECKER version 2.2.3, and GENEPOP version 4.1.3, respectively (Van Oosterhout et al., 2004; Peakall and Smouse, 2006; Rousset, 2008). Of the 15 loci, 13 were polymorphic (all but PTC14 and PTC15; Table 1). The actual number of alleles (*A*) per polymorphic locus ranged from one to seven, the effective number of alleles ($A_e$) ranged from 1.000 to 6.061, observed heterozygosity ($H_o$) per locus varied from 0.000 to 1.000, and expected heterozygosity ($H_e$) varied from 0.000 to 0.835 (Table 2). PTC11 significantly deviated from Hardy–Weinberg equilibrium (HWE) in the Dayuanwei, Sanqingshan, and Bijiashan populations. Null alleles were only detected at one locus (PTC04) in the Dayuanwei, Damingshan, and Bijiashan populations. No loci pairs demonstrated significant LD.

Fifty individuals of *T. wallichiana* var. *mairei* from Longqishan (Fujian), Fenshui (Jiangxi), Lianzhou (Guangdong), Jinyunshan (Chongqing), and Tuankou (Zhejiang) were used to assess cross-species amplification of the 15 microsatellite loci (Fig. 1; Appendix 1). All 15 loci were polymorphic (Table 1). *A* ranged from one to nine and $A_e$ varied between 1.000 and 4.481. $H_o$ and $H_e$ were 0.000–1.000 and 0.000–0.777, respectively (Table 3). No null alleles or significant LD were detected. Moreover, PTC14 was found to significantly deviate from HWE in the Longqishan, Lianzhou, and Tuankou populations, respectively.

## CONCLUSIONS

The 15 microsatellite loci isolated from *P. chienii* can provide a useful tool to detect population genetic structure and candidate loci for local adaptation. Additionally, the cross-species amplifications in *T. wallichiana* var. *mairei* showed that these loci may also be valuable for population genetic studies of other *Taxus* species.

Table 1. Characterization of 15 microsatellite loci developed in *Pseudotaxus chienii*.[a]

| Locus | Primer sequences (5′–3′) | Repeat motif | $T_a$ (°C) | Size (bp) | GenBank accession no. |
|---|---|---|---|---|---|
| PTC01 | F: ACAGTTCCTGACAGTCGTTAGA | $(CA)_7$ | 56 | 139 | JX512258 |
| | R: TACACCATTGAGGGTATTTGA | | | | |
| PTC02 | F: GGGGAAAATGTAGACACCAA | $(AC)_{14}$ | 54 | 270 | JX512259 |
| | R: CAACAATCCTTTAGCCAGAGT | | | | |
| PTC03 | F: TAGATTGTAGCCTTGGTGTAG | $(TG)_{18}$ | 50 | 193 | JX512260 |
| | R: TCATTATGTTTTGATGGGTT | | | | |
| PTC04 | F: ATAGTCCCTTTGGGCACAT | $(CA)_{10}$ | 55 | 176 | JX512261 |
| | R: TCATCCTTGAGGTCCTTTCT | | | | |
| PTC05 | F: GTCAAGAGCACAAAAGTGAACAT | $(AC)_{16}$ | 57 | 104 | JX512262 |
| | R: AGGAGGAGGAAAGTAAAATCG | | | | |
| PTC06 | F: ATAGAACTCATTTTGAAGCCATA | $(AC)_4AT(AC)_{15}ATAG(AT)_5$ | 54 | 251 | JX512263 |
| | R: CAAGGTTTTGTGACCATTTTA | | | | |
| PTC07 | F: TCCTACACATTGTTTCCTGG | $(AC)_7$ | 54 | 165 | JX512264 |
| | R: CACTCTACCTTTTTTAGTTTCTGA | | | | |
| PTC08 | F: TGACTATGTGATTGAAAGAGAA | $(AC)_{15}$ | 54 | 151 | JX512265 |
| | R: GACCCCAACTGTTACGAA | | | | |
| PTC09 | F: CAGAAGCAAAGAAATGTATG | $(CA)_8$ | 47 | 108 | JX512266 |
| | R: TGTGAAAGAATCAATGAGAAA | | | | |
| PTC10 | F: CACGGACTCCCAAACAT | $(GT)_{16}A(TG)_{15}$ | 54 | 119 | JX512267 |
| | R: CGCTTGCGAGATAGATAAT | | | | |
| PTC11 | F: ACATTAGTTCCCATCGCA | $(TG)_7$ | 51 | 110 | JX512268 |
| | R: GTGGTAGTATGAATAAGACAAGG | | | | |
| PTC12 | F: TGCTATCAGTGTTGGAGGG | $(TG)_7$ | 52 | 241 | JX512269 |
| | R: CCATCGCATCATCGCC | | | | |
| PTC13 | F: AAATGCTTAGTATGTCGGC | $(TG)_{15}$ | 50 | 130 | JX512270 |
| | R: ATAAATCTACAAAGAGTAAACCA | | | | |
| PTC14 | F: CCTGGGTGGAAATCATAAAGT | $(GT)_{11}$ | 53 | 147 | JX512271 |
| | R: TAAGAAAAGGGTCCCGAAGT | | | | |
| PTC15 | F: GACATTTCTACTTTGCTGGAT | $(AC)_8$ | 51 | 129 | JX512272 |
| | R: ACTGATGCTTGTTATTGGTTTA | | | | |

*Note*: $T_a$ = annealing temperature.

[a]Voucher: *Q Fan 201107*, *BJS7*, SYSU. See Appendix 1 for location information.

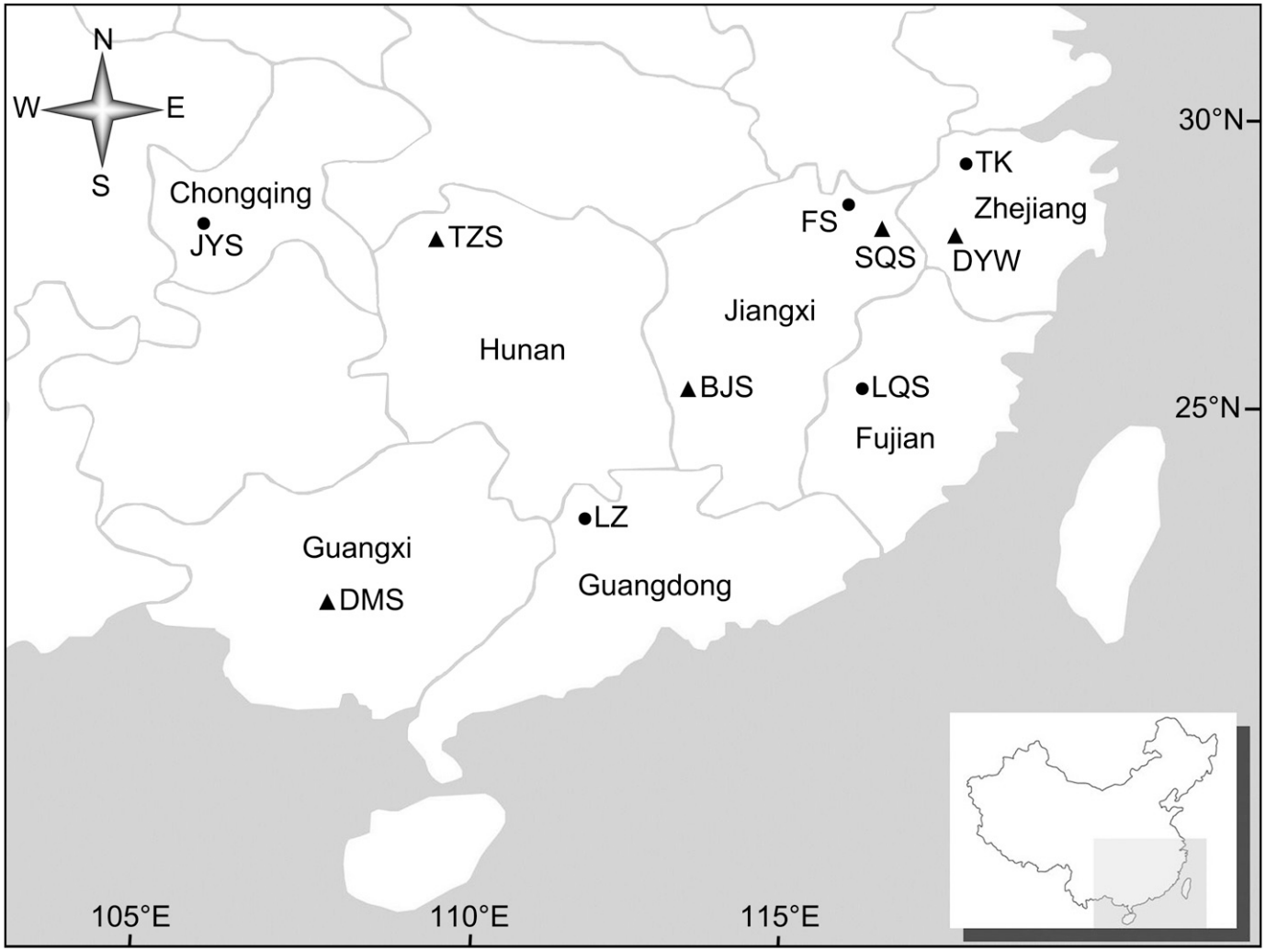


Fig. 1. The population locations of *Pseudotaxus chienii* (solid triangle) and *Taxus wallichiana* var. *mairei* (solid dots). BJS = Bijiashan; DMS = Damingshan; DYW = Dayuanwei; FS = Fenshui; JYS = Jinyunshan; LQS = Longqishan; LZ = Lianzhou; SQS = Sanqingshan; TK = Tuankou; TZS = Tianzishan.

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TABLE 2. Genetic analysis and results of polymorphism in *Pseudotaxus chienii.*

| | Zhejiang Province, Dayuanwei ($N$ = 10) | | | | Guangxi Zhuang Autonomous Region, Damingshan ($N$ = 10) | | | | Hunan Province, Tianzishan ($N$ = 10) | | | | Jiangxi Province | | | | | | | | Total ($N$ = 50) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | Sanqingshan ($N$ = 10) | | | | Bijiashan ($N$ = 10) | | | | | | | |
| Locus[a] | $A$ | $A_e$ | $H_o$ | $H_e$ | $A$ | $A_e$ | $H_o$ | $H_e$ | $A$ | $A_e$ | $H_o$ | $H_e$ | $A$ | $A_e$ | $H_o$ | $H_e$ | $A$ | $A_e$ | $H_o$ | $H_e$ | $A$ | $A_e$ | $H_o$ | $H_e$ |
| PTC01 | 2 | 1.471 | 0.400 | 0.320 | 2 | 1.923 | 0.800 | 0.480 | 2 | 1.471 | 0.400 | 0.320 | 2 | 1.980 | 0.900 | 0.495 | 2 | 1.923 | 0.800 | 0.480 | 2 | 1.873 | 0.660 | 0.466 |
| PTC02 | 3 | 2.740 | 0.900 | 0.635 | 3 | 2.740 | 0.900 | 0.635 | 3 | 2.532 | 1.000 | 0.605 | 3 | 2.597 | 0.900 | 0.615 | 3 | 2.410 | 0.600 | 0.585 | 3 | 2.675 | 0.860 | 0.626 |
| PTC03 | 3 | 2.899 | 0.900 | 0.655 | 4 | 2.564 | 0.700 | 0.610 | 3 | 2.667 | 1.000 | 0.625 | 3 | 2.597 | 1.000 | 0.615 | 3 | 2.381 | 1.000 | 0.580 | 4 | 2.919 | 0.920 | 0.657 |
| PTC04 | 3 | 2.632 | 0.200 | 0.620 | 5 | 4.167 | 0.200 | 0.760 | 3 | 2.299 | 0.700 | 0.565 | 5 | 4.167 | 0.500 | 0.760 | 3 | 2.632 | 0.200 | 0.620 | 5 | 3.915 | 0.360 | 0.745 |
| PTC05 | 4 | 2.740 | 0.900 | 0.635 | 3 | 2.899 | 0.500 | 0.655 | 2 | 1.923 | 0.600 | 0.480 | 4 | 3.226 | 0.700 | 0.690 | 2 | 2.000 | 1.000 | 0.500 | 4 | 2.664 | 0.740 | 0.625 |
| PTC06 | 2 | 1.105 | 0.100 | 0.095 | 5 | 1.942 | 0.600 | 0.485 | 4 | 2.469 | 0.900 | 0.595 | 2 | 1.220 | 0.200 | 0.180 | 2 | 1.105 | 0.100 | 0.095 | 6 | 1.498 | 0.380 | 0.332 |
| PTC07 | 7 | 6.061 | 0.900 | 0.835 | 5 | 3.774 | 0.900 | 0.735 | 3 | 2.020 | 0.700 | 0.505 | 4 | 3.175 | 0.900 | 0.685 | 2 | 1.835 | 0.700 | 0.455 | 7 | 4.429 | 0.820 | 0.774 |
| PTC08 | 3 | 2.174 | 0.800 | 0.540 | 3 | 2.062 | 0.800 | 0.515 | 3 | 1.852 | 0.600 | 0.460 | 3 | 2.020 | 0.700 | 0.505 | 3 | 2.632 | 1.000 | 0.620 | 3 | 2.211 | 0.780 | 0.548 |
| PTC09 | 2 | 1.342 | 0.300 | 0.255 | 2 | 1.835 | 0.700 | 0.455 | 1 | 1.000 | 0.000 | 0.000 | 1 | 1.000 | 0.000 | 0.000 | 3 | 1.361 | 0.200 | 0.265 | 3 | 1.827 | 0.240 | 0.453 |
| PTC10 | 2 | 1.923 | 0.800 | 0.480 | 6 | 2.198 | 0.600 | 0.545 | 2 | 1.105 | 0.100 | 0.095 | 5 | 2.174 | 0.700 | 0.540 | 2 | 1.342 | 0.300 | 0.255 | 6 | 1.719 | 0.500 | 0.418 |
| PTC11 | 2 | 2.000 | 1.000 | 0.500* | 2 | 1.600 | 0.500 | 0.375 | 2 | 1.835 | 0.700 | 0.455 | 5 | 2.985 | 1.000 | 0.665* | 2 | 2.000 | 1.000 | 0.500* | 6 | 4.600 | 0.840 | 0.783 |
| PTC12 | 3 | 2.198 | 1.000 | 0.545 | 2 | 1.724 | 0.600 | 0.420 | 3 | 2.740 | 1.000 | 0.635 | 3 | 2.062 | 0.800 | 0.515 | 3 | 2.532 | 1.000 | 0.605 | 4 | 3.501 | 0.880 | 0.714 |
| PTC13 | 3 | 1.942 | 0.700 | 0.485 | 3 | 2.198 | 0.800 | 0.545 | 3 | 2.062 | 0.800 | 0.515 | 3 | 1.504 | 0.400 | 0.335 | 2 | 1.980 | 0.900 | 0.495 | 3 | 2.036 | 0.720 | 0.509 |
| Mean | 3.000 | 2.402 | 0.685 | 0.508 | 3.462 | 2.433 | 0.662 | 0.555 | 2.615 | 1.998 | 0.654 | 0.450 | 3.308 | 2.362 | 0.669 | 0.508 | 2.462 | 2.010 | 0.677 | 0.466 | 4.308 | 2.759 | 0.669 | 0.588 |

*Note*: $A$ = actual number of alleles; $A_e$ = effective number of alleles; $H_e$ = expected heterozygosity; $H_o$ = observed heterozygosity; $N$ = sample size.

[a] PTC14 and PTC15 are not included because they are monomorphic.

* Deviation from Hardy–Weinberg equilibrium ($P < 0.05$).

TABLE 3. Genetic analysis and results of transferability in *Taxus wallichiana* var. *mairei.*

| | Fujian Province, Longqishan ($N$ = 10) | | | | Jiangxi Province, Fenshui ($N$ = 11) | | | | Guangdong Province, Lianzhou ($N$ = 11) | | | | Chongqing Municipality, Jinyunshan ($N$ = 8) | | | | Zhejiang Province, Tuankou ($N$ = 10) | | | | Total ($N$ = 50) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | $A$ | $A_e$ | $H_o$ | $H_e$ | $A$ | $A_e$ | $H_o$ | $H_e$ | $A$ | $A_e$ | $H_o$ | $H_e$ | $A$ | $A_e$ | $H_o$ | $H_e$ | $A$ | $A_e$ | $H_o$ | $H_e$ | $A$ | $A_e$ | $H_o$ | $H_e$ |
| PTC01 | 5 | 2.985 | 0.800 | 0.665 | 6 | 2.881 | 0.909 | 0.653 | 2 | 2.000 | 1.000 | 0.500 | 4 | 2.246 | 0.750 | 0.555 | 4 | 2.198 | 0.800 | 0.545 | 9 | 2.618 | 0.860 | 0.618 |
| PTC02 | 3 | 2.247 | 0.200 | 0.555 | 3 | 2.305 | 0.455 | 0.566 | 2 | 1.984 | 0.182 | 0.496 | 4 | 2.510 | 0.875 | 0.602 | 4 | 2.778 | 0.600 | 0.640 | 4 | 3.544 | 0.440 | 0.718 |
| PTC03 | 2 | 1.835 | 0.700 | 0.455 | 3 | 2.123 | 0.364 | 0.529 | 2 | 1.541 | 0.455 | 0.351 | 3 | 2.169 | 0.625 | 0.539 | 2 | 1.980 | 0.500 | 0.495 | 3 | 2.408 | 0.520 | 0.585 |
| PTC04 | 2 | 1.471 | 0.400 | 0.320 | 4 | 1.793 | 0.364 | 0.442 | 5 | 4.033 | 0.818 | 0.752 | 2 | 1.600 | 0.500 | 0.375 | 2 | 1.835 | 0.100 | 0.455 | 5 | 2.860 | 0.440 | 0.650 |
| PTC05 | 3 | 2.299 | 0.500 | 0.565 | 4 | 3.270 | 0.909 | 0.694 | 3 | 2.469 | 0.909 | 0.595 | 4 | 3.657 | 0.875 | 0.727 | 3 | 1.681 | 0.500 | 0.405 | 4 | 3.501 | 0.740 | 0.714 |
| PTC06 | 2 | 1.835 | 0.700 | 0.455 | 2 | 1.308 | 0.273 | 0.236 | 1 | 1.000 | 0.000 | 0.000 | 2 | 1.969 | 0.875 | 0.492 | 2 | 1.220 | 0.200 | 0.180 | 3 | 1.465 | 0.380 | 0.317 |
| PTC07 | 3 | 2.532 | 1.000 | 0.605 | 3 | 1.754 | 0.364 | 0.430 | 3 | 1.766 | 0.545 | 0.434 | 3 | 2.462 | 0.250 | 0.594 | 3 | 2.062 | 0.600 | 0.515 | 3 | 2.130 | 0.560 | 0.531 |
| PTC08 | 3 | 2.469 | 0.700 | 0.595 | 5 | 4.481 | 0.909 | 0.777 | 5 | 3.507 | 0.727 | 0.715 | 4 | 2.723 | 0.750 | 0.633 | 4 | 2.857 | 0.700 | 0.650 | 5 | 4.122 | 0.760 | 0.757 |
| PTC09 | 2 | 1.471 | 0.400 | 0.320 | 4 | 2.659 | 0.909 | 0.624 | 4 | 2.180 | 0.727 | 0.541 | 2 | 1.438 | 0.375 | 0.305 | 3 | 1.802 | 0.600 | 0.445 | 4 | 1.952 | 0.620 | 0.488 |
| PTC10 | 2 | 1.471 | 0.400 | 0.320 | 4 | 2.988 | 0.636 | 0.665 | 4 | 2.547 | 0.636 | 0.607 | 3 | 1.855 | 0.625 | 0.461 | 3 | 2.469 | 0.700 | 0.595 | 4 | 2.473 | 0.600 | 0.596 |
| PTC11 | 2 | 1.835 | 0.700 | 0.455 | 3 | 1.716 | 0.545 | 0.417 | 4 | 2.373 | 0.636 | 0.579 | 4 | 1.939 | 0.250 | 0.484 | 3 | 2.532 | 1.000 | 0.605 | 5 | 3.408 | 0.640 | 0.707 |
| PTC12 | 2 | 2.000 | 0.600 | 0.500 | 4 | 2.916 | 0.727 | 0.657 | 4 | 2.782 | 1.000 | 0.640 | 3 | 2.133 | 0.750 | 0.531 | 2 | 1.835 | 0.700 | 0.455 | 6 | 2.664 | 0.760 | 0.625 |
| PTC13 | 3 | 2.899 | 0.300 | 0.655 | 4 | 2.000 | 0.455 | 0.500 | 3 | 2.469 | 0.545 | 0.595 | 3 | 1.662 | 0.500 | 0.398 | 3 | 2.247 | 0.600 | 0.555 | 4 | 3.388 | 0.480 | 0.705 |
| PTC14 | 2 | 2.000 | 1.000 | 0.500* | 2 | 1.936 | 0.636 | 0.483 | 2 | 2.000 | 1.000 | 0.500* | 2 | 1.969 | 0.875 | 0.492 | 2 | 2.000 | 1.000 | 0.500* | 2 | 1.993 | 0.900 | 0.498 |
| PTC15 | 4 | 2.740 | 0.500 | 0.635 | 4 | 3.143 | 0.091 | 0.682 | 2 | 1.308 | 0.091 | 0.236 | 2 | 1.969 | 0.625 | 0.492 | 4 | 3.175 | 0.700 | 0.685 | 4 | 3.236 | 0.380 | 0.691 |
| Mean | 2.667 | 2.139 | 0.593 | 0.507 | 3.667 | 2.485 | 0.570 | 0.557 | 3.067 | 2.264 | 0.618 | 0.503 | 3.000 | 2.154 | 0.633 | 0.512 | 2.933 | 2.178 | 0.620 | 0.515 | 4.333 | 2.784 | 0.605 | 0.613 |

*Note*: $A$ = actual number of alleles; $A_e$ = effective number of alleles; $H_e$ = expected heterozygosity; $H_o$ = observed heterozygosity; $N$ = sample size.

* Deviation from Hardy–Weinberg equilibrium ($P < 0.05$).

Appendix 1. Information on GPS coordinates of each population for *Pseudotaxus chienii* and *Taxus wallichiana* var. *mairei*. Representative voucher specimens were deposited at the herbarium of Sun Yat-sen University (SYSU).

| Species | Population | GPS coordinates | Voucher specimens |
|---|---|---|---|
| *Pseudotaxus chienii* | Dayuanwei, Zhejiang Province | 28°43′N, 118°57′E | *Y Jiang 200308*, *DYW4* |
| | Damingshan, Guangxi Zhuang Autonomous Region | 22°42′N, 107°46′E | *Y Jiang 200308*, *DMS2* |
| | Tianzishan, Hunan Province | 29°22′N, 110°30′E | *Y Jiang 200308*, *TZS4* |
| | Sanqingshan, Jiangxi Province | 28°54′N, 118°04′E | *WB Liao 200808*, *SQS3* |
| | Bijiashan, Jiangxi Province | 26°30′N, 114°09′E | *Q Fan 201107*, *BJS7* |
| *Taxus wallichiana* var. *mairei* | Longqishan, Fujian Province | 26°31′N, 117°16′E | *ZY Li 200608*, *LQS174* |
| | Fenshui, Jiangxi Province | 28°56′N, 118°02′E | *WB Liao 200808*, *FS1* |
| | Lianzhou, Guangdong Province | 24°59′N, 112°14′E | *WB Liao 201108*, *LZ1* |
| | Jinyunshan, Chongqing Municipality | 29°50′N, 106°22′E | *WB Liao 200808*, *JYS1* |
| | Tuankou, Zhejiang Province | 30°00′N, 119°03′E | *ZY Li 200608, TK198* |