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Authors: Wang, Si-Si, Zhang, Yang, Liu, De-Chen, Sun, Xiao-Wei,

Wang, Rong, et al.

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PRIMER NOTE

ISOLATION AND CHARACTERIZATION OF 30 MICROSATELLITE LOCI FOR *CUNNINGHAMIA LANCEOLATA* (TAXODIACEAE)¹

Si-Si Wang², Yang Zhang², De-Chen Liu², Xiao-Wei Sun², Rong Wang², and Yuan-Yuan Li^{2,3}

²School of Ecological and Environmental Sciences, Tiantong National Station of Forest Ecosystem, Shanghai Key Laboratory for Urban Ecology and Restoration, East China Normal University, Shanghai 200241, People's Republic of China

- *Premise of the study:* To quantify the population-level genetic characteristics of *Cunninghamia lanceolata* (Taxodiaceae), an important timber conifer, we developed 30 pairs of microsatellite primers based on the nuclear genome.
- Methods and Results: Using the streptavidin-biotin capture system, we developed 14 polymorphic and 16 monomorphic micro-satellites. Polymorphisms were detected in 14 loci using 94 individual trees that were collected from three C. lanceolata populations in Hubei and Zhejiang provinces and in Chongqing Municipality, China. There were three to 30 alleles per locus, and the observed and expected heterozygosities ranged from 0.0313–0.8333 and from 0.0313–0.9246, respectively. Cross-species amplification showed that two to seven polymorphic loci were functional in three of the five related species that were collected.
- Conclusions: Our newly developed microsatellite primers provide neutral molecular markers that are beneficial to future studies of population genetics and germplasm conservation of *C. lanceolata*.

Key words: cross-amplification; Cunninghamia lanceolata; genetic diversity; microsatellite; Taxodiaceae.

Cunninghamia lanceolata (Lamb.) Hook. (Taxodiaceae), known as Chinese fir, is an evergreen, outcrossing, and long-lived conifer that is widely distributed in southern China and northern Vietnam. Because of its relatively low nutrient demands, fast rate of growth, and strong resistance to corrosion and insect attacks, C. lanceolata is an important timber source that has been cultivated for more than 2000 yr (Yeh et al., 1994). Its present plantations cover about 4 million hectares accounting for 20–25% of the total commercial production of timber in China (Bao and Jiang, 1998; Huang et al., 2005). Understanding its genetic background is therefore critical to selecting germplasm resources and managing forests.

Some codominant molecular markers have been reported for *C. lanceolata*, e.g., 10 pairs of polymorphic microsatellite primers based on the nuclear genome (Li et al., 2015), 28 polymorphic expressed sequence tag–simple sequence repeat (EST-SSR) markers (Wen et al., 2013), and 97 polymorphic SSR loci based on transcript data (Xu et al., 2016). Microsatellite loci located in noncoding regions are neutral and usually show higher mutation rates than those located in encoding regions of the genome (Charlesworth et al., 1994). Neutral markers can be used to study population genetic diversity that is not related to adaptive

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³Author for correspondence: yyli@des.ecnu.edu.cn

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traits, and therefore can better reveal spatial genetic structure, gene flow, and historical events (e.g., bottlenecks and founder effects) that contribute to conservation of germplasms. However, detecting gene flow patterns, especially in fine-scale analyses such as parentage analyses, requires genetic resolution high enough to distinguish every individual and is thus dependent on a large number of neutral markers with high polymorphism. It is therefore necessary to develop more informative neutral molecular markers for *C. lanceolata*. Here, 14 polymorphic and 16 monomorphic microsatellite loci were isolated and characterized in the nuclear genome of *C. lanceolata* to facilitate future studies on population genetics and germplasm conservation.

METHODS AND RESULTS

Total genomic DNA was extracted from dried leaves of one C. lanceolata individual (located in Tiantong in Zhejiang Province, China [Appendix 1]) using the Plant Genomic DNA Extraction Kit (Tiangen, Beijing, China). Approximately 250 ng of DNA were digested with the restriction enzyme MseI (New England Biolabs, Beverly, Massachusetts, USA), and fragments of 200-800 bp were fractionated. The fragments were linked with an MseI-adapter pair (F: 5'-TACTCAGGACTCAT-3'; R: 5'-GACGATGAGTCCTGAG-3'). The diluted products were amplified by an MseI-N primer (5'-GATGGTCCTGAGTAAN-3') under the following conditions: an initial step at 95°C for 3 min, followed by 20 cycles of 30 s at 94°C, 1 min annealing at 53°C, and 1 min at 72°C. The products were hybridized with 5'-biotinylated probes (AG)₁₅ in a 250-µL reaction system at 48°C for 2 h. The hybridization products were adhered by streptavidin-coated magnetic beads (Promega Corporation, Madison, Wisconsin, USA). The washed and eluted DNA fragments were further amplified with the MseI-N primer using the conditions given above for 30 cycles. The products were purified using a multifunctional DNA Extraction Kit (BioTeke, Beijing, China) and were ligated to a pMD19-T vector (TaKaRa Biotechnology Co., Dalian, China). Then, the products were transformed into Escherichia coli

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Table 1. Characterization of 14 polymorphic and 16 monomorphic microsatellite loci developed in Cunninghamia lanceolata.^a

CL22 F. ** *COGNOTATION CAGGARAT* (GA); 170	Locus		Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	A	$T_{\rm a}$ (°C)	Fluorescent dyeb	GenBank accession no.
R. CAGGARATICAGACARACAGC 176-190 5 61 6-FAM KY769205 134 F. TOTCAMAGACTOCTAGGARD (CT) 176-190 5 61 6-FAM KY769205 136-100-100-100-100-100-100-100-100-100-10	CL22	F:	TGGTAGTACTCGCAGGAAAT	(GA) ₅	170	1	64	HEX	KY769227
R: OCAGGARAGAGGCACACATAG C109		R:	CAGAGAATGGACACAAACAG	, ,,,					
CL90 F. CARAGRAGOTCCACACACC (AG) ₈ 214-222 5 65 TAMRA KY769206 R. ACTOCAMOGRAGATICOCTG (GA) ₇ 108-124 6 64 HEX KY769207 KY769226 KY769226 KY769226 KY769226 KY769226 KY769226 KY769229	CL34*			$(CT)_5$	176–190	5	61	6-FAM	KY769205
R. ACTISCANAGGGGATACGGCTG	GT OO:			(1.6)	244 222	_		T-1.15D.4	***********
CLION	CL90*			$(AG)_8$	214–222	5	65	TAMRA	KY/69206
R: COCTATOCTACTCAGTCACC 164	CI 100*			$(G\Lambda)$	100 124	6	64	LIEV	VV760207
CLIO	CLIUG			$(OA)_7$	100-124	U	04	IILA	K1709207
R: GAAGHTTCTCTTTGGGC (L255 P: GAACHGGGATTTTCCAGCA (AG) ₁ 118 1 63 ROX KY769229 R: TTCACCTTAAGGGTTTTC (L256 P: GGTTGCTCAGGGTTTGGA (AG) ₁ 107 1 64 ROX KY769230 R: TGTCCCTTACCTCTA (L278 P: GAACHGGGATGGAGAG (TC) ₁ 114 1 65 ROX KY769231 R: GACAGGGGATCGCAAGAGGA (TC) ₁ 114 1 65 ROX KY769231 R: GACAGGGGATCGCAAGAGGA (AG) ₁ 169 1 65 6-FAM KY769220 CL287 P: GACGCAATACACACAGATA (AG) ₂ 169 1 65 6-FAM KY769220 CL288 P: GACGCAATACACACACATAGAG (AG) ₂ 176 1 62 6-FAM KY769221 CL291 P: AATACATTGCTCCAGATA CL291 P: AATGGAATTGTTGCAGC (AG) ₂ 153 1 61 ROX KY769232 R: AATGCAATTGTCGGGGT (AG) ₂ 153 1 61 ROX KY769232 CL295 P: AATGCAATTGCAGCACTCGGAG (CT) ₈ 157-163 4 65 ROX KY769208 R: CAGAGTCACACACACACACTCGGAG (CT) ₈ 157-163 4 65 ROX KY769208 R: CAGAGTCACACACACACACACTCGGAG (CT) ₈ 157-163 4 65 ROX KY769208 R: CAGAGTCACACACACACACTCGGAG (CT) ₈ 157-163 4 65 ROX KY769208 R: CAGAGTCACACACACACACTCGGAG (AG) ₁ 197-311 30 61 HEX KY769209 CL343 P: CACACAGAGATCACACACGGGAG (AG) ₁ 197-311 30 61 HEX KY769209 R: GTAAAGGGGAAAGGGAGT (AG) ₁ 113 1 65 ROX KY769209 CL346 P: ACACAGAGAATCAGACC (AG) ₁ 147-171 10 63 6-FAM KY769210 CL346 P: CACAGACACACACACACACACACACACACACACACACAC	CL164			(CT) ₁₂	124	1	63	6-FAM	KY769226
R: TTCACCTGTAAGGGTTTTC R: TGTCCTCTACGTTAGA R: TGTCCTCTACGTCTCA R: GGTTGCTCAGGGGAGAG R: GGTGCTCAGGGGAGAG R: GGAGCTAGACCGTGGGAGAG R: GGAGCTAGACCGTGGAGAGG CL287 F: GGAGCTAGACCGTCAGAGG CL287 F: GGAGCTAGACCGTCAGAGG CL288 F: GCAGCATAGCACCACAGAGA R: ACAAGTGTCAAAGACTCCTCAGAGAG CL288 F: GCAGCATAGCACACACATAGAG CL288 F: GCAGCATAGCACACACATAGAG CL291 F: AATGCAGTTTGCAGAGAG CL291 F: AATGCAGATTTGCAGC CL292 R: AATGCAGTTTGCAGCT CL295 F: ATGCGGAATTGCAGCC CL293 R: GCAGCATACACACATAGAG CL295 F: ATGCGAGATTGCAGCTGAGAG CL295 F: AATGCAGTTTGCAGGT CL295 F: TGCAGGTGTGAGTTAGCTT CL296 F: GCAGATTGGAGGTGATTAGCTT CL343 F: TGCAGGAGTGAGGTGTTAGCTT CL346 F: ACACACACAATAGAACC CL347 F: TGCAGGTGTGAGTTAGCTT CL348 F: GGAGATTGAGAGGTGAGTTAGCTT CL349 F: GGAGATTGAAAGAGCT CL340 F: ACACACACAATAGAACC CL340 F: ACACACACAATAGAACC CL340 F: ACACACACAATAGAACC CL340 F: CACACACACAATAGAACC CL340 F: CACACACACAATAGCACC CL340 F: CACACACACAATAGCACC CL340 F: CACACACACAATACGACT CL340 F: CACACACACAATACGACT CL340 F: CACACACACAATACGACT CL340 F: CACACACACAATACGAC CL340 F: CACACACACACACGACACACACACACACACACACACACA				()12					
R: TTCACCTGTAAGGGTTTCC 12.26 F: GGTTGCTCAGGGTTCCA R: TGTCCCTCTACCTCCA R: GGTTGCTCAGGTTCCAC R: GGTGCTCAGGTTCCAC CL278 F: GGGTGCTCAGGTGGGAGAG R: GGGGGATCCACACCAGAA R: GGGGGATCCACACCAGAA R: GGGGATCCACACTCCACACAGAA R: GGGGATCACCACCAGAAC CL287 F: GGACGATAGCACCACAGAAC CL287 F: GGACGATAGCACCACAGAAC CL288 F: GGACGATAGCACCACACATAGAC CL288 F: GGACGATAGCACCACACATAGAC CL288 F: GGACGATAGCACACACATAGAC CL288 F: GGACGATAGCACCACACATAGAC CL291 F: AATGGGATATTCCAGC R: TACCGAATTGCTCCAGATA CL291 F: AATGGGATATTCCACC CL295 F: ATGGGAATTATCCACC CL295 F: ATGGGAATTATCCACC R: AATGGAATTATCCACC R: GGACATCACACATAGACACCTGAGA R: GGACAATCAGACTGAGAC CL295 F: TACCGAACTGAACTGAGAC CL295 F: TACCGAACTGAAATGGACCC CC343 F: TCCACACTGAAATGGACC CC343 F: TCCACACTGAAATGGACC CC343 F: TCCACACTGAAATGGACC CC343 F: TCCACACTGAAATGGACC CC346 F: GCACACCACAATAGAACC CC346 F: CACCACCACAATAGAACC CC347 F: TCCACACTGAAATGGACC CC347 F: TCCACACCACAATAGAACC CC347 F: TCCACACCACAATAGCACC CC347 F: TCCACACCACAATAGCACC CC347 F: TCCACACACCAATAGCACC CC347 F: TCCACACACCAATAGCACC CC347 F: TCCACACCACAATAGCAC CC347 F: TCACATAGAACCATTGGACC CC347 F: TCACATAGAACCATTGGC CC347 F: TCACATAGAACCATTGGC CC347 F: TCACATAGACCACTACC CC35 F: AACCACACAATACCACC CC35 F: AACCACACAATACCACCACAC CC347 F: TCACATAGACCACACCACCACCACCACCACCACCACCACCACCACC	CL255			$(AG)_{21}$	118	1	63	ROX	KY769229
R: TOTOCCTCTACCTGCACA R: GAGACTAGGCTGGGAGAG (TC)s 114 1 65 ROX KY769231 R: GAGACGGGATCGGAAGAG (TC)s 114 1 65 6-6-FAM KY769220 R: GAGACGGGATCCCATAGCACCAGAT (AG)s 169 1 65 6-FAM KY769220 R: ACAGTGCAAAGACTCCTAAGGAG CL288 F: GCACCATAGCACACATAGAGAG CL288 F: GCACCATAGCACACACATAGAGAG CL288 F: GCACCATAGCACACACATAGAGAG CL291 F: AATGGAGATATTGCAGC (AG)s 153 1 61 ROX KY769221 R: TACCGAATATTGCAGC (AG)s 153 1 61 ROX KY769232 R: AATGGAGATATTGCAGC (CT)s 157-163 4 65 ROX KY769232 R: AATGCACACACACTAGCACCTGGAG (CT)s 157-163 4 65 ROX KY769208 R: GCACATACTAGCACCTGGAG (CT)s 197-511 30 61 HEX KY769209 R: GTAAAAGCACACTTGCACCTGGAG (AG)s 197-511 30 61 HEX KY769209 R: TOCAACTGTACACACACATTAGCCT (GA)s 17-711 10 63 6-FAM KY769232 CL396 F: ACACACACACATTAGCACC (AG)s 147-171 10 63 6-FAM KY769210 CL396 F: GCACATTCTAGACGCGACCT (CT)s 111 1 65 ROX KY76924 R: GCACACACACACACACTTGCAGCAC (AG)s 147-171 10 63 6-FAM KY769210 CL396 F: GCACATTCTACACCACACCCTCCC (AG)s 147-171 10 63 6-FAM KY769210 CL596 F: CCATGTGCCACCTCC (CT)s 111 1 65 ROX KY76924 R: GGACATTCTACACACAGA TCGCACAGC (CT)s 111 1 65 ROX KY769211 CL540 F: GGACATTCTACACCAGCAGC (CT)s 111 1 65 ROX KY769211 CL540 F: GGACACTCCACCTC (CT)s 111 1 65 ROX KY769211 CL540 F: TACACACACACACTGCACAGG (CT)s 111 1 65 ROX KY769211 CL540 F: TACACACACACTGCACAGG (CT)s 204-238 15 65 HEX KY769211 CL540 F: TACACACACACTGCACACG (CT)s 204-238 15 65 HEX KY769212 CL540 F: TACACACACACTGCACACTGCACC (CT)s 204-238 15 65 HEX KY769212 CL540 F: TACACACCACTGCACTCTCC (CT)s 204-238 15 65 ROX KY769212 CL540 F: TACACACCACTGCACTCTCC (CT)s 204-238 15 65 HEX KY769214 CL540 F: TACACACCACTGCACACG (CT)s 204-238 15 65 HEX KY769214 CL540 F: TACACACCACTGCACACG (CT)s 204-238 15 65 HEX KY769214 CL540 F: TACACACCACTACACCACCACACG (TC)s 214-227 5 63 TAMRA KY769215 CL540 F: TACACTCCACCACACACG (TC)s 214-227 5 63 TAMRA KY769215 R: CACCACACACACACACACC (CT)s 214-227 5 63 TAMRA KY769216 R: TACACCACACACACACACACC (CT)s 224 16 65 HEX KY769226 R: TACACCACACACACACACC (CT)s 224 16 65 HEX KY769217 R: GCACACACACACACACC (CT)s 224 16 65 HEX KY769217 R: GCACACACACACACAC		R:	TTCACCTGTAAGGGTTTTC						
CL278 F. GAGACTAGACCTGGAAGAG CL287 F. GCAGCATACACACAGACA CAG) CL287 F. GCAGCATACACACACAGACA CAG) CL288 F. GCAGCATACACACACACACACACACACACACACACACACA	CL256			$(AG)_{15}$	107	1	64	ROX	KY769230
R: GAGAGGGATCGAAAGAG R: ACAAGTGCAACAGCAGAAT (AG) ₉ 169 1 65 6-FAM KY769220 R: ACAAGTGCCAAAGACCCTAAGAG C1288 F: GCAGCAATACACACACATAG (AG) ₉ 176 1 62 6-FAM KY769221 R: TACCGAATTGCCCCAAGAG R: TACCGAATTGCCCCCAAGAG R: TACCGAATTGCCCCCAAGAG R: TACCGAATTGCCCCCAGAT R: TACCGAATTGCCCCCAGAT R: TACCGAATTGCCCCCAGAT R: TACCGAATTGCCCCCAGAT R: AATACATCAACACAATTACACCCCGAGG R: AATACATCAACACAATTACCACCTGAGG C1295* F: ATCATCAACACAATTACCACCTGAGG C1295* F: ATCATCAACACAATTACCACCTGAGG R: AATACATCAACACAATTACCACCTGAGG C1343* F: TGCAACTGCAACGTGAGTTAGCTT C1343* F: TGCAACTGCAACGTGAGTCAGCC C1346 F: ACACACAGAATTACACCCGAGGT C1346 F: ACACACAGAATTACACCCGAGGT C1346 F: ACACACAGAATTACACCCCGAGGT C1346 F: ACACACAGAATTACACCCCGAGGC C1346 F: ACACACAGAATTACACCCCCAGGC C1346 F: GAGATGTAGAATGGAGCC C1346 F: CAGATGCATGCCCCCCCC C1346 F: CAGATGCAACACAACACCCCCAGGACC C1346 F: CAGATGCAACACCAACCCCCCCCCCCCCCCCCCCCCCCC	CT 270			(TC)	114			DOM	1717760001
CL287 F. GCAGCAATACACACAGAAT (AG) ₈ 169 1 65 6-FAM KY769220 R. CAGAGTOTCAAGAGCTCCTAAGAGAGC (AG) ₈ 176 1 62 6-FAM KY769221 R. TACCGAATTGTCTCCAGATA (AG) ₈ 153 1 61 ROX KY769232 R. AATACATTGTCCGGGTTA (CT) ₈ 157-163 4 65 ROX KY769232 R. AATACAATTGTCCGGGTTAGCT (TA) ₁₁ 157-163 4 65 ROX KY769208 R. GCAGATCATCAGACTTAGCTC (AG) ₁₁ 197-311 30 61 HEX KY769208 KY76920	CL2/8			$(1C)_5$	114	1	65	ROX	KY/69231
R: ACAGCTTCCAAGGACTCCTAAGAAG AGB	CI 287			(ΔG) .	160	1	65	6-FAM	KV760220
CL288 F. GCAGCAATACACAACTARAS CAG) 176 1 62 6-FAM KY76922 R. TACCAATTCCTCCAGACTA	CLZ07			(AO) ₉	10)	1	03	0-1 Alvi	K1707220
R: TACCGAATTOTCTCCAGATA CAG CA	CL288			(AG) _o	176	1	62	6-FAM	KY769221
R: AATACATGTGCCGGGTF (CL295* F: ATCATCAACACAATTGACCCTCGAG (CT) ₈ 157-163 4 65 ROX KY769208 R: GCAGATCATCCGAACTGAGTTAGCTT (CL343* F: TGCAAGTGTGAATAGAACC (GA) ₂₁ 197-311 30 61 HEX KY769209 R: GTAAAAGGGGAAACGGAGT (CL346 F: ACACCACAATGTAGGCAG (AG) ₁₇ 113 1 65 ROX KY769233 CL386 F: ACACCACAATGTAGGCAG (AG) ₁₅ 147-171 10 63 6-FAM KY769210 R: CTTTTCTGTGTTGTGAGGAGGAGGC (CL366 F: CCATGGCCTCACTCTC (CT) ₅ 111 1 1 65 ROX KY769214 R: GGTAAGAGGTCAACCTCTC (CT) ₅ 111 1 1 65 ROX KY769244 R: GGTAGAGGTCAACCTCTC (CT) ₅ 111 1 1 65 ROX KY769214 R: GGTAGAGGATACTACGAGAGA (TC) ₁₀ A(CA) ₁₀ 118-160 19 63 ROX KY769214 R: AGAAACCAGAATTGAGGGAGG (CT) ₁₂ 204-238 15 65 HEX KY769212 CL546* F: TGACATGAACCTTGGACTTAGC (CT) ₁₂ 204-238 15 65 HEX KY769212 R: TACAAACTGTGAGCTTAGATG (TC) ₀ 208-224 6 6 63 6-FAM KY769213 R: GTTTTCTGGTATCCAACTAGG CL616 F: TGGTGAGGAGGATTAGACCTTGTGACT CL637 F: ACCCAAACGAGGATTCACCCCACCACCACCACCACCACCACCACCACCACCA				. ,,					
CL295 F. ATCATCAACACAATTAGCACCTGGGG CT) ₈ 157-163 4 65 ROX KY769208 R. GCGGATCATCATGGTT CTCATCGGAGGTGGAGTTGGATT CTCATCGGAGGTGAGTTGGAT CTCATCGGAGGTGAGTGAGTGAGT CTCATCGGAGGTGAGATGGAGC CGA) ₂₁ 197-311 30 61 HEX KY769209 R. GCGAGATCAGGGAGT CTCATCGGAGGGAGGGGGT CTCATCGGAGGGGAGGGGGGGGGGGGGGGGGGGGGGGGG	CL291	F:	AATGGAGATATTGCAGC	$(AG)_{24}$	153	1	61	ROX	KY769232
R. GCAGATCATCGAACGTGAGTTAGCTT									
CL343* F: TGCAAGGGAAAGGAAC	CL295*			$(CT)_8$	157–163	4	65	ROX	KY769208
R: GTAAAAGGGAAAGGAACT	CI 242*			(CA)	107 211	20	61	HEV	VV760200
CL346 F: ACCACAGAGATGTAGGCAG CAG) ₁₇ 113 1 65 ROX KY769233 R: ATTGTCAGGTTTTGAGT CL389* F: GGAGATTGTAAAATGGACTCTAGCC CAG) ₁₅ 147-171 10 63 6-FAM KY769210 R: CTTTTCTTGTCTTCTGGAGAGGC CC136 TI11 T 1 1 1 1 1 1 1 1	CL345"			$(GA)_{21}$	197–311	30	01	ПЕЛ	K I 709209
R: ATTGTCCAGGGTTTGGAGT	CL346			(AG),,	113	1	65	ROX	KY769233
CL389* F: GGGATTGTAAAATGGACTCTAGCC	020.0			(110)[/	110	•	00	11011	111,0,200
R: CLTTTTCTGTTCTTCGGGGGGC	CL389*			$(AG)_{15}$	147-171	10	63	6-FAM	KY769210
R: GGTTAGGGGTTCAGGTT		R:	CTTTTTCTTGTTCTTTCGGAGGAGC						
CL540* F: GGGTAGTGATCATGGAAGA (TC) ₁₀ A(CA) ₁₀ 118-160 19 63 ROX KY769211 CL564* F: TAGAAGCAGATATCGGTTAGC (CT) ₁₂ 204-238 15 65 HEX KY769212 CL586* F: TACAAAACTGTGGGCTTGATGATG (TC) ₉ 208-224 6 63 6-FAM KY769213 CL631 F: TGGTAGGAAGATTCAGCCGACAG (TG) ₅ 273 1 65 ROX KY769222 CL653 F: AATGGAGTATTGCAAC (AG) ₂₀ 114 1 59 ROX KY769223 CL654* F: TCTCTCCTCCTTTGCTTAGC (TC) ₈ 126-144 9 63 HEX KY769214 CL723* F: ATCTCTGGTTAGAGAGTATCG (TC) ₆ 211-227 5 63 TAMRA KY769215 CL723* F: ATCTCTGCTTTTTGGATAGG (TC) ₆ 211-227 5 63 TAMRA KY769215 CL753 F: TAGCTGTAGACCCAAGAGAGGC (GA) ₉ 232 </td <td>CL396</td> <td></td> <td></td> <td>$(CT)_5$</td> <td>111</td> <td>1</td> <td>65</td> <td>ROX</td> <td>KY769234</td>	CL396			$(CT)_5$	111	1	65	ROX	KY769234
R: AGAAAGCAGATATCGGTTG	CT 7.40*			(TG) 1 (G1)	110 160	10	(2)	DOM	17177700011
CL564* F: TGAACTTGACCTTGTGACTTAGC R: TACAAAACTGTGGCCTTCATGATGATG (CT) ₁₂ 204-238 15 65 HEX KY769212 CL586* F: CAGCAAACACACGCTATGGT R: GTTTTGTGGTATCCAACTAGG (TC) ₉ 208-224 6 63 6-FAM KY769213 CL631 F: TGGTGAGGAAGGATTCAGCCGACAG R: TCAGTTCCGGTTAGGCTCAGTACAC (TG) ₅ 273 1 65 ROX KY769222 CL653 F: AATGAGGTATTGCAAC (AG) ₂₀ 114 1 59 ROX KY769223 CL654* F: TCTCTCCTCCTTTTGCTTACG R: CCATGCGTGAAGGAGTATCG (TC) ₈ 126-144 9 63 HEX KY769214 CL723* F: ATCTCTGCTCTTTTGCTTACG R: GGAATTATTGTGGGGTTAGG (TC) ₆ 211-227 5 63 TAMRA KY769215 CL753 F: TAGAATCACAGAGAAAGG R: ACTCAAAAACATGACTCGGTAGC (GA) ₉ 232 1 65 HEX KY769236 CL761 F: CCTCTTATGACACATTGGT R: TTTCAGATGACTCTCGGA (CT) ₆ 138 1 59 HEX KY769224 CL776* F: ACTGCAAAGAGAGTACGCTGAAGG R: GCAA	CL540*			$(1C)_{10}A(CA)_{10}$	118–160	19	63	ROX	KY /69211
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	CI 564*			(CT)	204 238	15	65	HEV	KV760212
CL586* F: CAGCAAAGAAACGGTTATGGT R: GTTTTGTGGTATCCAACTAGG (TC)9 208-224 6 63 6-FAM KY769213 CL631 F: TGGTGAGGAAGGATTCAGCCGACAG R: TCAGTTCCGGTTAGGCTCAGTACAC (TC)3 273 1 65 ROX KY769222 CL653 F: AATGGAGGTATTCCAC R: (AG)20 114 1 59 ROX KY769223 R: ACCTGTAAGGGTTTCC (TC)8 126-144 9 63 HEX KY769214 CL654* F: TCTCTGCTCTTTGCTACG (TC)8 126-144 9 63 HEX KY769214 R: CCATGCGTTGAGAGAAGTATCG (TC)6 211-227 5 63 TAMRA KY769215 R: GAATTATGTTGGGGTTAGG (GA)9 232 1 65 HEX KY769236 CL753 F: TAGAATCACACTAGGT (CT)6 138 1 59 HEX KY769224 R: TTTCAGATGACCTCTGGTA (CT)6 138 1 59	CL304			(C1) ₁₂	204-236	13	03	IILA	K1709212
R: GTTTTGTGGTATCCAACTAGG	CL586*			(TC) _o	208-224	6	63	6-FAM	KY769213
R: TCAGTTCCGGTTAGGCTCAGTACAC				\ -/9					
CL653 F: AATGGAGGTATTGCAAC (AG) ₂₀ 114 1 59 ROX KY769223 R: ACCTGTAAGGGTTTCC (TC) ₈ 126–144 9 63 HEX KY769214 R: CCATGGGTTGAAGAAGTATCG (TC) ₆ 211–227 5 63 TAMRA KY769215 R: GGAATTATTGTTGGGGTTAGG (GA) ₉ 232 1 65 HEX KY769236 CL753 F: TAGAACAACATGACTCGGTAGC (CT) ₆ 138 1 59 HEX KY769236 CL761 F: CCTCTTATGACACATTGGT (CT) ₆ 138 1 59 HEX KY769224 R: TTTCAGATGACACTCTGGA (TC) ₈ 221–225 3 65 TAMRA KY769216 CL776* F: ACTGCAAAGAGGTCCACAATACA (TC) ₈ 221–225 3 65 TAMRA KY769216 CL776* F: ACTGCAAAAGAGGTCCACAATACA (TC) ₈ 183 1 65 HEX KY769237 CL852* F: CTAGGTTCCAAAAAAGAGCA (CT) ₉ 136–148 7 63 <td< td=""><td>CL631</td><td>F:</td><td>TGGTGAGGAAGGATTCAGCCGACAG</td><td>$(TG)_5$</td><td>273</td><td>1</td><td>65</td><td>ROX</td><td>KY769222</td></td<>	CL631	F:	TGGTGAGGAAGGATTCAGCCGACAG	$(TG)_5$	273	1	65	ROX	KY769222
R: ACCTGTAAGGGTTTTCC CL654* F: TCTCTCCTCCCTTTGCTTACG (TC) ₈ 126–144 9 63 HEX KY769214 R: CCATGCGTTGAAGAAGTATCG CL723* F: ATCTCTGTCTTTGCACTCTC (TC) ₆ 211–227 5 63 TAMRA KY769215 R: GGAATTATTGTTGGGGTTAGG CL753 F: TAGAATCAACGCACAAGAAAGGC (GA) ₉ 232 1 65 HEX KY769236 R: ACTCAAAAACATGACTCGGTAGC CL761 F: CCTCTTATGACACTTGGT (CT) ₆ 138 1 59 HEX KY769224 R: TTTTCAGATGACTCTGGA CL776* F: ACTGCAAAAGGAGAAGGC (TC) ₈ 221–225 3 65 TAMRA KY769216 R: GACGCAAAAGAGGTCCACAATACA CL783 F: CTAGATACGAGTGTCGAAGA (TC) ₆ 183 1 65 HEX KY769237 R: GCAATACACATACACACAGA CL852* F: CTAGATTCCAAAAAAGAGC (CT) ₉ 136–148 7 63 ROX KY769217 R: GAGATATGAGTAGAATGAG CL871 F: TGGTCCGCGGTTACAAGAAGGG CL871 F: TGGTCCGCGGTTACAAGAATCAC CL878* F: CAGGGTAGCCTTTGAAACA (AG) ₈ 147–163 7 64 ROX KY769218									
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	CL653			$(AG)_{20}$	114	1	59	ROX	KY769223
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	CI (5.1*			(TC)	126 144	0	(2	HEV	VV760214
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	CL054**			$(1C)_8$	120-144	9	0.3	HEX	K 1 /09214
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	CL723*			(TC)	211–227	5	63	TAMRA	KY769215
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	CETES			(10)6	211 227	5	0.5	TI HVIICE	111707213
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	CL753			$(GA)_{9}$	232	1	65	HEX	KY769236
$ \begin{array}{c} R: \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ $									
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	CL761	F:		$(CT)_6$	138	1	59	HEX	KY769224
R: GACGCAAAAGAGGTCCACAATACA CL783 F: CTAGATACGAGTGTCGAAGA (TC) ₆ 183 1 65 HEX KY769237 R: GCAATACACACAGA CL852* F: CTAGTGTCCAAAAAAGAGCA (CT) ₉ 136–148 7 63 ROX KY769217 R: GAGATATGAGATACACAGG CL871 F: TGGTCCGCGTTACAAGTATACATG (GT) ₅ 228 1 65 HEX KY769225 R: ACTCTGCCCTTTTCACTATTCTGC CL878* F: CAGGGTAGCCTTTGAAACA (AG) ₈ 147–163 7 64 ROX KY769218	CT 77.64			(TC)	221 227	2		TILD A	TTTT(0016
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	CL//6*			$(1C)_8$	221–225	3	65	TAMRA	KY /69216
R: GCAATACACATACACAGA CL852* F: CTAGTGTCCAAAAAAGAGCA (CT)9 136–148 7 63 ROX KY769217 R: GAGATATGAGTAGAATGAGG CL871 F: TGGTCCGCGTTACAAGTATACATG (GT)5 228 1 65 HEX KY769225 R: ACTCTGCCCTTTTCACTATTCTGC CL878* F: CAGGGTAGCCTTTGAAACA (AG)8 147–163 7 64 ROX KY769218	CI 783			(TC)	183	1	65	HEX	KV760237
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	CL103			(10)6	103	1	03	IILA	K1707237
R: GAGATATGAGTAGAATGAGG CL871 F: TGGTCCGCGTTACAAGTATACATG (GT) $_5$ 228 1 65 HEX KY769225 R: ACTCTGCCCTTTTCACTATTCTGC CL878* F: CAGGGTAGCCTTTGAAACA (AG) $_8$ 147–163 7 64 ROX KY769218	CL852*			(CT) _o	136-148	7	63	ROX	KY769217
CL871 F: TGGTCCGCGTTACAAGTATACATG (GT) $_5$ 228 1 65 HEX KY769225 R: ACTCTGCCCTTTTCACTATTCTGC CL878* F: CAGGGTAGCCTTTGAAACA (AG) $_8$ 147–163 7 64 ROX KY769218	-			` /9	-		-	-	
CL878* F: CAGGGTAGCCTTTGAAACA $(AG)_8$ 147–163 7 64 ROX KY769218	CL871			$(GT)_5$	228	1	65	HEX	KY769225
		R:	ACTCTGCCCTTTTCACTATTCTGC						
R: GGCTCCATATAACAACATC	CL878*			$(AG)_8$	147–163	7	64	ROX	KY769218
		R:	GGCTCCATATAACAACATC						

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

^a All values are based on samples representing three populations located in Lichuan in Hubei Province, Lin'an in Zhejiang Province, and Wanxian in Chongqing Municipality, China.

^bFluorescent dyes (i.e., HEX, ROX, 6-FAM, and TAMRA) used for fragment analysis.

^{*}Polymorphic microsatellite loci.

Table 2. Genetic properties of 14 newly developed polymorphic microsatellites of Cunninghamia lanceolata.^a

Locus	Lichuan population				Lin'an population				Wanxian population			
	n	A	$H_{\rm o}$	H_{e}	n	A	$H_{\rm o}$	H_{e}	n	A	$H_{\rm o}$	H_{e}
CL34	30	4	0.2000*	0.5847	25	3	0.2000*	0.5200	29	4	0.2069*	0.5850
CL90	32	5	0.6563	0.6880	32	4	0.3750	0.5709	30	4	0.5667	0.6356
CL108	32	5	0.1875	0.2336	32	4	0.3125	0.3021	30	3	0.2333	0.3203
CL295	32	4	0.5938	0.6047	32	4	0.6563	0.5843	30	4	0.5000	0.5701
CL343	32	16	0.1875*	0.8750	32	17	0.1563*	0.9246	29	14	0.2759	0.8209
CL389	30	7	0.2667	0.3819	32	5	0.1250	0.1220	30	5	0.2000	0.1904
CL540	29	15	0.3793*	0.9165	29	14	0.5172*	0.9201	28	12	0.5000	0.8935
CL564	32	12	0.8125	0.8705	32	11	0.6250	0.6999	30	10	0.8333	0.8621
CL586	31	3	0.1613	0.2089	32	6	0.2813	0.3105	30	4	0.2667	0.2706
CL654	32	7	0.5625	0.6002	32	6	0.6250	0.6969	30	6	0.6333	0.6418
CL723	32	5	0.0938*	0.4454	32	3	0.1563*	0.4043	30	4	0.1000*	0.5028
CL776	32	3	0.3438	0.3031	32	2	0.0313	0.0313	30	2	0.3667	0.3045
CL852	32	4	0.2500	0.2555	32	6	0.4688	0.3973	30	4	0.6000	0.4729
CL878	32	6	0.1875	0.2073	32	5	0.2813	0.4772	30	3	0.2000	0.1859

Note: A = number of alleles; $H_e = \text{expected heterozygosity}$; $H_o = \text{observed heterozygosity}$; n = number of individuals genotyped.

strain JM109 (TaKaRa Biotechnology Co.) through transient thermal stimulation following the TaKaRa *E. coli* JM109 competent cell protocol.

A total of 1400 colonies were selected and tested by PCR with (AG)₁₀ and M13+/M13- as primers, producing 469 positive sequences. The positive PCR products were sequenced on an ABI 3730 DNA Sequence Analyzer (Applied Biosystems, Foster City, California, USA). We designed 263 primer pairs using the software Primer Premier 5.0 (PREMIER Biosoft International, Palo Alto, California, USA). The criteria for primer design were: (1) primer length between 18-28 bp and amplicon length between 100-300 bp; (2) melting temperature $(T_{\rm m})$ in the range of 45–65°C; (3) GC content of the sequence between 40–60%, with no more than three Gs or Cs in the last five bases at the 3' end of the primer without mismatch or secondary structures. Polymorphisms were detected using 24 individuals selected randomly from three populations from Lichuan (Hubei Province, China), Lin'an (Zhejiang Province, China), and Wanxian (Chongqing Municipality, China), according to the method proposed by Schuelke (2000). The nested PCR used three primers: a sequence-specific forward primer with an M13(-21) tail (5'-TGTAAAACGACGGCCAGT-3') at its 5'-end, a universal M13(-21) primer labeled with a fluorescent dye, and a sequence-specific reverse primer. In the first 30 cycles, the forward primer was incorporated into the PCR products. Then these products were marked with the fluorescently labeled M13(-21) primer, which was incorporated during the following eight cycles at 53°C. Each reaction was run in a 20-µL system containing the following: 40 ng of DNA, 1× PCR buffer, 2.0 mM Mg²⁺, 0.2 mM of dNTPs, 0.1 μ M M13(-21) primer labeled with HEX, ROX, or 6-FAM (Sangon Biotech, Shanghai, China), 0.025 µM forward primer with an M13(-21) tail, 0.1 µM reverse primer, and 2 units Taq DNA polymerase (Sangon Biotech). Thermocycling conditions were as follows: 5 min of denaturation at 94°C; 30 cycles of 30 s at 94°C, 45 s at 59–65°C (Table 1), and 45 s at 72°C; followed by eight cycles of 30 s at 94°C, 45 s at 53°C, and 45 s at 72°C; and a 10-min extension at 72°C. We genotyped the amplification products on an ABI 3730 automated sequencer using GeneScan 500 LIZ Size Standard (Applied Biosystems), and alleles were identified using GeneMapper 4.0 software (Applied Biosystems). Among the 263 primer pairs tested, 186 were not amplified in any samples, 32 did not produce clear and single-target bands, 12 could not be successfully amplified in the individuals used for testing, and three were

duplicates of previous markers in Li et al. (2015). Finally, 14 polymorphic and 16 monomorphic microsatellite loci were obtained (Table 1).

All polymorphic loci were further characterized using 94 *C. lanceolata* individuals from the same three populations mentioned above. One of four fluorescent dyes (HEX, ROX, 6-FAM, TAMRA) labeled the forward primers. PCRs were set up in 10-µL reaction volumes containing 40 ng of template DNA, 1× PCR buffer, 2.5 mM Mg²⁺, 0.2 mM of each dNTP, 0.1 µM forward and reverse primer, and 1 unit of *Taq* DNA polymerase. We used the following conditions: 5 min of denaturation at 94°C; 30 cycles of 30 s at 94°C, 45 s at 61–65°C (Table 1), and 1 min at 72°C; and a final extension at 72°C for 10 min. The amplification products were scanned on an ABI 3730 sequencer using GeneScan 500 LIZ Size Standard (Applied Biosystems), and alleles were called and binned using GeneMapper 4.0 software (Applied Biosystems).

The number of alleles varied from three to 30 with an average of 9.4 using FSTAT 2.9.3 software (Goudet, 1995) (Table 1). The observed and expected heterozygosities ranged from 0.0313–0.8333 and 0.0313–0.9246, respectively, analyzed by the software TFPGA version 1.3 (Miller, 1997) (Table 2). After the sequential Bonferroni adjustment (Rice, 1989), only two loci (CL34 and CL723) displayed significant deviations from Hardy–Weinberg equilibrium (P < 0.05) in all three populations, and two loci (CL343 and CL540) deviated significantly from Hardy–Weinberg equilibrium in the Lichuan and Lin'an populations. No loci exhibited significant linkage disequilibrium after sequential Bonferroni adjustment by GENEPOP version 4.0 (Rousset, 2008). Null alleles were likely to be present in the loci CF34, CF343, CF389, CF540, and CF723 using MICRO-CHECKER version 2.2.3 (van Oosterhout et al., 2004).

We also tested the performance of 14 polymorphic primer pairs in five related species belonging to the same family (Taxodiaceae), using one to 10 individuals from each species (Table 3; Appendix 1). In *Metasequoia glyptostroboides* Hu & W. C. Cheng, successful amplifications occurred in seven loci (CL90, CL295, CL343, CL723, CL776, CL852, and CL878), of which three (CL90, CL723, and CL776) could also be amplified in *Cryptomeria fortunei* Hooibr. ex Otto & A. Dietr. and two (CL852 and CL878) in *Taxodium ascendens* Brongn. No polymorphic loci amplified successfully in *Cryptomeria japonica* (Thunb. ex L. f.) D. Don or in *Sequoia sempervirens* (D. Don) Endl. (Table 3). Unfortunately,

Table 3. Allele size ranges tested in five additional taxa for cross-amplification trials of SSR loci isolated from Cunninghamia lanceolata.

Locus	$Cryptomeria\ japonica \ (n=1)$	$Cryptomeria\ fortunei$ $(n = 5)$	$Metasequoia\ glyptostroboides \ (n = 10)$	Sequoia sempervirens $(n=2)$	Taxodium ascendens $(n = 2)$
CL90	_	268	218–220	_	_
CL295	_	_	159–165	_	_
CL343	_	_	193	_	_
CL723	_	261	237–247	_	_
CL776	_	225	217–225	_	_
CL852	_	_	142	_	142
CL878	_	_	145–155	_	145

Note: — = primers could not be amplified.

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^aLocality and voucher information for the populations are available in Appendix 1.

^{*}Indicates significant deviation from Hardy–Weinberg equilibrium (P < 0.05).

we were unable to sample the only congeneric species of *C. lanceolata* (*C. konishii* Hayata), and thus the cross-amplification capability of these primers is likely underestimated.

CONCLUSIONS

Of the 30 markers reported here for *C. lanceolata*, 14 microsatellite loci showed a high level of polymorphism. These loci will be used to study population genetic diversity, gene flow, and mating systems. Combined with the previously isolated loci, these markers will facilitate the further investigation of parentage analyses and kinships between the planted and natural populations of *C. lanceolata*, all of which are relevant to germplasm conservation and forest management of this timber species.

LITERATURE CITED

- Bao, F. C., and Z. H. Jiang. 1998. Wood properties of main tree species from plantation in China. China Forestry Publishing House, Beijing,
- Charlesworth, B., P. Sniegowski, and W. Stephan. 1994. The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature* 371: 215–220.
- GOUDET, J. 1995. FSTAT (version 1.2): A computer program to calculate *F*-statistics. *Journal of Heredity* 86: 485–486.
- HUANG, Z., Z. XU, S. BOYD, AND D. WILLIAMS. 2005. Chemical composition of decomposing stumps in successive rotation of Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.) plantations. *Chinese Science Bulletin* 50: 2581–2586.

- LI, Y. X., Z. S. WANG, J. K. SUI, Y. F. ZENG, A. G. DUAN, AND J. G. ZHANG. 2015. Isolation and characterization of microsatellite loci for *Cunninghamia lanceolata* (Lamb.) Hook. *Genetics and Molecular Research* 14: 453–456.
- MILLER, M. P. 1997. Tools for population genetic analyses (TFPGA) 1.3: A Windows program for the analysis of allozyme and molecular population genetic data. Website http://www.marksgeneticsoftware.net/ tfpga.htm [accessed 28 August 2017].
- RICE, W. R. 1989. Analyzing tables of statistical tests. Evolution 43: 223–225.
- ROUSSET, F. 2008. GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8: 103–106.
- Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* 18: 233–234.
- VAN OOSTERHOUT, C., W. F. HUTCHINSON, D. P. M. WILLS, AND P. SHIPLEY. 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4: 535–538.
- WEN, Y., S. UENO, W. HAN, AND Y. TSUMURA. 2013. Development and characterization of 28 polymorphic EST-SSR markers for *Cunninghamia lanceolata* (Taxodiaceae) based on transcriptome sequences. *Silvae Genetica* 62: 137–141.
- Xu, Y., R. Zheng, Z. Wang, Y. Wang, Z. Hong, L. Yang, Y. Lu, et al. 2016. Identification and characterization of genic microsatellites in *Cunninghamia lanceolata* (Lamb.) Hook. (Taxodiaceae). Archives of Biological Sciences 68: 417–425.
- YEH, F. C., J. SHI, R. YANG, J. HONG, AND Z. YE. 1994. Genetic diversity and multilocus associations in *Cunninghamia lanceolata* (Lamb.) Hook. from The People's Republic of China. *Theoretical and Applied Genetics* 88: 465–471.

APPENDIX 1. Locality information of *Cunninghamia lanceolata* and its related species used in this study. Voucher specimens were deposited in East China Normal University (HSNU), Shanghai, China.

Species	Population	Collection locality	Geographic coordinates	N
Cunninghamia lanceolata (Lamb.) Hook.	Tiantong	Tiantong, Zhejiang, China	29°48′19″N, 121°47′43″E	1
	Lichuan	Lichuan, Hubei, China	30°10′37″N, 108°37′03″E	32
	Lin'an	Lin'an, Zhejiang, China	30°19′14″N, 119°26′04″E	32
	Wanxian	Wanxian, Chongqing, China	30°39′43″N, 108°45′05″E	30
Cryptomeria japonica (Thunb. ex L. f.) D. Don	Shanghai	Shanghai Botanic Garden, Shanghai, China	31°08′48″N, 121°26′50″E	1
Cryptomeria fortunei Hooibr. ex Otto & A. Dietr.	Shanghai	Shanghai Botanic Garden, Shanghai, China	31°08′48″N, 121°26′50″E	5
Sequoia sempervirens (D. Don) Endl.	Nanjing	Nanjing Botanic Garden, Nanjing, China	32°04′15″N, 118°48′25″E	1
	Hangzhou	Hangzhou Botanic Garden, Hangzhou, China	30°15′19″N, 120°07′22″E	1
Metasequoia glyptostroboides Hu & W. C. Cheng	Lichuan	Lichuan, Hubei, China	30°10′22″N, 108°39′32″E	10
Taxodium ascendens Brongn.	Nanjing	Nanjing Botanic Garden, Nanjing, China	32°04′15″N, 118°48′25″E	2

Note: N = number of individuals.

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