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PRIMER NOTE

DEVELOPMENT AND CHARACTERIZATION OF MICROSATELLITE LOCI FOR *LINDERA GLAUCA* (LAURACEAE)[1]

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- *Premise of the study:* Microsatellite primers were developed to investigate population genetic structure in *Lindera glauca* (Lauraceae).
- *Methods and Results:* Twenty-five microsatellite primers were developed and optimized for *L. glauca* using Illumina's Solexa sequencing technology. These novel primers were found to be polymorphic in nine wild *L. glauca* populations with 81 total alleles confirmed and genotyped via capillary gel electrophoresis. The total number of alleles, observed heterozygosity, and expected heterozygosity for each population ranged from one to four, from 0.00 to 0.90, and from 0.00 to 0.79, respectively. In addition, the 25 primers were tested in 10 additional individuals of the related species *L. communis*, and all but four markers showed good amplification results.
- *Conclusions:* This set of microsatellite primers is the first specifically developed for *L. glauca* and will facilitate studies of genetic diversity and evolution among populations of this species.

Key words: genetic diversity; Lauraceae; *Lindera glauca*; microsatellite; polymorphism.

Lindera glauca Blume is a deciduous shrub or small tree that belongs to the family Lauraceae. It is extensively distributed in mountainous regions at low altitudes in central and southern China and is also found in Japan, Korea, and Taiwan. It is of potentially great economic value and ecological importance owing to its various valuable properties, including its natural abundance, the medicinal value of its leaves and roots, its high-quality wood, and the wide applications of its volatile oil in the biochemical and medicinal industries (Liu et al., 1992; Seki et al., 1994; Wang et al., 1994, 2011; Sun et al., 2011; Huh et al., 2014). However, few studies have investigated its population genetic diversity and genetic relationships among germplasms and breeding populations. Male individuals of *L. glauca* trees are very rare in China, and only female individuals are found in Japan (Dupont, 2002), although male individuals have been known from continental Asia in the past several decades (Wang, 1972; Li, 1982). Consequently, understanding the genetic diversity of this species is relevant to the utilization and conservation of its germplasm resources, to population genetic studies, and to the evolution of apomixis in this dioecious species.

Microsatellites, or simple sequence repeats (SSRs), have been widely used as genetic markers owing to their multiallelic nature, codominant inheritance, and thorough genome coverage (Powell et al., 1996). They are a powerful tool and an effective way to analyze population genetic structure, marker-assisted breeding, gene flow, levels of inbreeding, and germplasm identification (Varshney et al., 2005). However, no studies have previously published SSR markers for this species. Therefore, we used a next-generation transcriptome sequencing approach (Illumina's Solexa sequencing technology) to develop microsatellites specifically for *L. glauca.*

METHODS AND RESULTS

***Plant materials and DNA extraction*—**Leaves and fruits of wild *L. glauca* were collected from nine locations in China in 2014 and 2015 (Appendix 1). Genomic DNA was extracted from the leaves of one individual from each of nine total populations using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987).

***Development of SSRs and primer design*—**In this study, we used transcriptome data from Niu et al. (2015) to develop microsatellite markers. We used the 163,427 unigenes from the transcriptome data for SSR exploitation using QDD version 3.1 software (Meglécz et al., 2014) with at least five, five, four, four, three, and two SSR motif repeat units for di-, tri-, tetra-, penta-, hexa-, and heptanucleotide and higher-order nucleotides, respectively. A total of 8969 putative SSRs (excluding mononucleotide repeats) were detected, with the majority of repeats being dinucleotide (66.83%), followed by trinucleotide (33.77%), tetranucleotide (1.87%), pentanucleotide (0.50%), and hexanucleotide (1.04%). With this detailed information, the program PRIMER 5 (PRIMER-E, Auckland, New Zealand) was then used to design 27,350 primer pairs with primer lengths of 18–25 bp, amplification product sizes of 100–400 bp, GC contents from 40% to 60%, and annealing temperatures ranging from 55°C to 65°C.

***PCR amplification and fragment analysis*—**An initial polymorphism screening of 120 primer pairs, including 50 primer pairs for dinucleotide motifs, 40 for trinucleotide motifs, 15 for tetranucleotide motifs, 10 for pentanucleotide motifs, and five for hexanucleotide motifs, was performed using

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TABLE 1. Characteristics of 25 microsatellite loci developed for *Lindera glauca*.

Locus	Primer sequences (5′–3′)	Fluorescent dye	Repeat motif	Allele size (bp)	T_a (°C)	GenBank accession no.
XBLG-013	F: CGAGGGAGAGATCGACGC	FAM	$(AG)_5$	190	58	KX545436
	R: ATGGCACCACGAAGTGTGTT					
XBLG-033	F: CGGGATGACAATTTGCATGT	HEX	$(AG)_5$	259	58	KX545437
	R: TGGAGCAGATTATGGTTTCCA					
XBLG-036	F: CATCACCTCCCTCAAATCCC	FAM	$(AG)_7$	263	58	KX545438
	R: GTTTCCGAAATTCTCGAGGC					
XBLG-049	F: TTTCACAACCAGGGTGGCTA	TAM	$(AC)_6$	191	58	KX545439
	R: CACTGGGACTAAGACACGGC					
XBLG-051	F: CAAACAGAACCAAGACATCCAA	HEX	$(ATAC)_6$	148	55	KX545440
	R: ATGGAGGAGCATGATTCGAG					
XBLG-053	F: TCCTCTTATTCTCTTTCCTTTCTGA	TAM	$(AT)_7$	268	55	KX545441
	R: TCAGACCAACAGGAACATGC					
XBLG-055	F: CCTCTTCAAACCAAACCTCC	FAM	$(AAG)_5$	236	55	KX545442
	R: CTGCAACTCCATGTGAGGG					
XBLG-056	F: CAACTGTACGTGCTGTGGGT	ROX	$(AG)_8$	283	55	KX545443
	R: AGCCCACACCAGATCTTCAC					
XBLG-058	F: AGTCCAGGCTAACCAGACTCC	HEX	$(AAC)_6$	277	55	KX545444
	R: CCCAGTTTGCCAGGTAAGAA					
XBLG-060	F: ATTCCACCCATTCCCTTCTT	FAM	$(AAG)_6$	197	55	KX545445
	R: GATTCTAAGAAGAAGAAGAAAGTACCC					
XBLG-062	F: AACATCATTCCCTCCATCCA	ROX	$(AATCC)_5$	192	55	KX545446
	R: CCAGCCAGTTAGGGTTTCAC					
XBLG-063	F: CATGGCAACGCAAATCCTAT	TAM	$(ATC)_6$	196	55	KX545447
	R: CTAGATCCTTTGGCCATGTTT					
XBLG-066	F: GTCGACGAGGACGAGGAC	HEX	$(CCG)_5$	187	55	KX545448
	R: TCGAATGAGGAAAGTTTGGC					
XBLG-073	F: ACCACAAAGATAAGCTACAATGC	FAM	$(ACGC)_5$	219	55	KX545449
	R: GGGCCTTAATGTCTATGGCA					
XBLG-076	F: GGATGCTCTAAGGTGCTTGC	ROX	$(AG)_7$	182	55	KX545450
	R: GGAATCGCCATTCTCCCT					
XBLG-082	F: TGTGGAAACAGAACCCATGA	TAM	$(AGC)_5$	168	55	KX545451
	R: ACAAAGCAGAGCTGCTGACA					
XBLG-083	F: CTCTCTCATCGATCCACCG	HEX	$(AAG)_5$	186	55	KX545452
	R: AAACCCAACACTGTACAACCTAAA					
XBLG-084	F: AAGTGAGGCGATACGATTGG	FAM	$(AGG)_5$	144	55	KX545453
	R: ACATGACCATAAACATGGGTGA					
XBLG-086	F: TTGGGACTAGGCTTTGATCG	TAM	$(ACC)_6$	190	55	KX545454
	R: CCCATCATCAATGTGGTTATAGA					
XBLG-089	F: TGTCTTGTGATCGAAATCAGG	FAM	$(AG)_7$	177	55	KX545455
	R: ACTTCAGAGGCATTCCAGCA					
XBLG-092	F: CTCAAGCCGATTGATGATCC	TAM	$(AG)_8$	144	55	KX545456
	R: TCATAACATGTCACATTCAAAGGA					
XBLG-097	F: TTTGGGAAAGTCCCATGAAA	TAM	$(ATC)_6$	193	55	KX545457
	R: GGGTACAAGTGGATACAATGAGG					
XBLG-099	F: TGCAAGGGTACATGCCATAG	ROX	$(AC)_7$	165	55	KX545458
	R: CCAAACATTTGCCCACTTCT					
XBLG-111	F: GAGAGGTACAACCACCCACG	HEX	$(ACT)_6$	192	58	KX545459
	R: GCCCGAAGTTAAGTAAATGGAT					
XBLG-119	F: GCATGGTGTGTTTGGTCAAG	ROX	$(AAG)_5$	350	58	KX545460
	R: TCTCAACAGACCCTCGTCG					

Note: T_a = annealing temperature.

polyacrylamide gel electrophoresis. We hand-selected 120 loci based on desired criteria (representative loci with different repeat unit lengths), of which 25 (20.83%) were successfully amplified and found to be polymorphic in the nine wild *L. glauca* populations (Appendix 1, Table 1), while 71 (59.17%) primer pairs produced no product, 21 (17.50%) amplified monomorphic markers or identical heterozygotic genotypes, and three (2.50%) produced larger or smaller products than the expected size. Forward primers of the 25 primer pairs were further labeled with fluorescently labeled nucleotides (M13: 5′-TGTAAAACGACGGCCAGT-3′). PCR reactions were performed in a total reaction volume of 15 µL, which contained 7.5 µL of 2× *Taq* PCR MasterMix (Aidlab, Beijing, China), 1.0 µL of 30 ng/µL DNA, 5.5 µL of ddH_2O, 0.5 µL of 10 µM reverse primer, 0.2 µL of 10 µM forward primer, and 0.3 µL of 10 µM fluorescent dyes (M13F-FAM, M13F-HEX, M13F-TAM, and M13F-ROX). Thermocycling program conditions included a 5-min melting step of 94°C; then 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 35 s; and a final extension step of 72°C for 10 min. Ten microliters each of all M13F-labeled PCR products were sent to the Ruibo Biotechnology Center DNA Sequencing Facility (Beijing, China) for fragment analysis using an ABI 3730XL DNA Analyzer with a GeneScan 500 LIZ Size Standard (Applied Biosystems, Changping, Beijing, China). Allele genotyping was performed using GeneMarker version 2.2.0 software (SoftGenetics, State College, Pennsylvania, USA).

***Detection of SSR polymorphism and data analysis*—**The 25 novel polymorphic SSRs yielded 81 total alleles confirmed and genotyped via capillary gel electrophoresis. Using GENEPOP 3.2 software (Rousset, 2008) for each population, the resulting genotypic data from the capillary gel electrophoresis were analyzed to obtain standard descriptive statistics and to test for deviations from Hardy–Weinberg equilibrium (HWE) assumptions (Table 2). The total number of alleles ranged from one to four with a mean of 3.240. The observed and expected heterozygosity ranged from 0.00 to 0.90 and from 0.00 to 0.79 with averages of 0.201 and 0.479, respectively. HWE and linkage disequilibrium using Bonferroni correction were tested for every locus. Less than half of the loci (five,

TABLE 2. Descriptive statistics of the 25 newly developed polymorphic microsatellites of *Lindera glauca*.[a]

	ATM ($n = 10$)				JGS ($n = 10$)				LDZ ($n = 10$)				SJG ($n = 10$)				NTB ($n = 10$)				YTH ($n = 10$)				DBS ($n = 10$)				HMF ($n = 10$)				TMS ($n = 10$)			
Locus	*A*	H_o	H_e	HWE[b]	*A*	H_o	H_e	HWE[b]	*A*	H_o	H_e	HWE[b]	*A*	H_o	H_e	HWE[b]	*A*	H_o	H_e	HWE[b]	*A*	H_o	H_e	HWE[b]	*A*	H_o	H_e	HWE[b]	*A*	H_o	H_e	HWE[b]	*A*	H_o	H_e	HWE[b]
XBLG-013	3	0.30	0.54	**	1	0.00	0.00	M	2	0.00	0.19	***	4	0.00	0.69	***	4	0.00	0.69	***	4	0.20	0.67	**	2	0.00	0.51	***	2	0.00	0.34	***	4	0.00	0.61	***
XBLG-033	1	0.00	0.00	M	3	0.00	0.65	***	2	0.40	0.51	n.s.	3	0.00	0.59	***	1	0.00	0.00	M	1	0.00	0.00	M	2	0.10	0.39	**	2	0.00	0.51	***	2	0.20	0.19	n.s.
XBLG-036	2	0.30	0.52	n.s.	2	0.20	0.19	n.s.	2	0.00	0.19	***	3	0.00	0.59	***	2	0.10	0.27	*	2	0.00	0.19	***	2	0.00	0.19	***	2	0.10	0.10	n.s.	3	0.10	0.68	***
XBLG-049	3	0.20	0.56	n.s.	3	0.40	0.69	n.s.	3	0.10	0.65	***	3	0.10	0.53	***	2	0.20	0.19	n.s.	2	0.10	0.27	*	3	0.50	0.68	n.s.	3	0.10	0.64	**	2	0.00	0.51	***
XBLG-051	3	0.20	0.61	***	2	0.70	0.48	n.s.	3	0.90	0.65	**	2	0.30	0.27	n.s.	3	0.30	0.43	n.s.	3	0.30	0.54	n.s.	3	0.00	0.36	***	2	0.20	0.34	n.s.	3	0.10	0.68	n.s.
XBLG-053	3	0.40	0.47	n.s.	2	0.40	0.34	n.s.	3	0.60	0.69	n.s.	2	0.00	0.51	***	2	0.00	0.44	***	3	0.10	0.62	***	2	0.20	0.19	n.s.	3	0.10	0.59	***	3	0.50	0.43	n.s.
XBLG-055	3	0.30	0.69	*	3	0.60	0.57	***	2	0.00	0.19	***	2	0.00	0.51	***	2	0.00	0.19	**	3	0.00	0.48	***	3	0.30	0.54	**	3	0.00	0.65	***	3	0.80	0.69	*
XBLG-056	1	0.00	0.00	M	1	0.00	0.00	M	2	0.00	0.19	***	3	0.20	0.35	n.s.	2	0.00	0.19	***	2	0.30	0.48	n.s.	2	0.00	0.19	***	2	0.00	0.34	***	2	0.00	0.34	***
XBLG-058	3	0.30	0.53	***	4	0.60	0.50	n.s.	2	0.70	0.48	n.s.	4	0.60	0.66	n.s.	4	0.30	0.72	***	4	0.30	0.62	***	4	0.40	0.69	**	3	0.20	0.61	**	4	0.20	0.65	**
XBLG-060	3	0.30	0.43	***	1	0.00	0.00	M	4	0.20	0.36	**	4	0.70	0.79	n.s.	3	0.20	0.57	***	4	0.20	0.36	**	2	0.20	0.34	n.s.	2	0.10	0.10	n.s.	2	0.10	0.27	*
XBLG-062	1	0.00	0.00	M	1	0.00	0.00	M	1	0.00	0.00	M	3	0.30	0.28	n.s.	2	0.10	0.10	n.s.	4	0.30	0.44	*.	1	0.00	0.00	M	1	0.00	0.00	M	3	0.20	0.19	n.s.
XBLG-063	2	0.20	0.44	n.s.	3	0.10	0.43	***	2	0.00	0.19	***	2	0.20	0.21	*	3	0.30	0.59	***	3	0.30	0.56	n.s.	3	0.20	0.35	n.s.	2	0.00	0.51	***	2	0.10	0.52	**
XBLG-066	1	0.00	0.00	M	1	0.00	0.00	M	2	0.00	0.19	***	2	0.10	0.10	n.s.	1	0.00	0.00	M	2	0.10	0.10	n.s.	2	0.90	0.52	*	2	0.50	0.39	n.s.	2	0.50	0.39	n.s.
XBLG-073	1	0.00	0.00	M	2	0.20	0.19	n.s.	2	0.00	0.19	***	1	0.00	0.00	M	1	0.00	0.00	M	2	0.10	0.27	n.s.	1	0.00	0.00	M	2	0.30	0.27	n.s.	2	0.10	0.10	n.s.
XBLG-076	1	0.00	0.00	M	1	0.00	0.00	M	2	0.60	0.44	n.s.	2	0.50	0.39	n.s.	2	0.30	0.27	n.s.	2	0.40	0.34	n.s.	3	0.40	0.35	n.s.	2	0.50	0.39	n.s.	3	0.20	0.54	n.s.
XBLG-082	3	0.20	0.19	n.s.	3	0.20	0.19	n.s.	3	0.30	0.42	n.s.	3	0.50	0.54	n.s.	3	0.30	0.62	*	3	0.30	0.59	***	2	0.00	0.34	***	3	0.30	0.62	**	3	0.20	0.66	**
XBLG-083	3	0.30	0.54	n.s.	2	0.30	0.27	n.s.	3	0.00	0.36	***	3	0.30	0.58	*	3	0.10	0.42	**	3	0.10	0.56	*	2	0.00	0.19	***	2	0.10	0.10	n.s.	2	0.00	0.19	***
XBLG-084	2	0.10	0.52	*	2	0.00	0.44	***	2	0.00	0.53	***	3	0.00	0.61	***	2	0.40	0.51	n.s.	2	0.30	0.48	n.s.	2	0.00	0.34	***	3	0.10	0.53	***	3	0.20	0.54	n.s.
XBLG-086	1	0.00	0.00	M	1	0.00	0.00	M	2	0.20	0.34	n.s.	2	0.10	0.27	*	2	0.20	0.34	n.s.	2	0.00	0.51	***	3	0.20	0.54	*	3	0.20	0.65	**	2	0.30	0.27	n.s.
XBLG-089	2	0.30	0.27	n.s.	1	0.00	0.00	M	1	0.00	0.00	M	1	0.00	0.00	M	2	0.40	0.51	n.s.	1	0.00	0.00	M	1	0.00	0.00	M	2	0.60	0.44	n.s.	2	0.20	0.53	*
XBLG-092	2	0.40	0.51	n.s.	3	0.80	0.57	n.s.	2	0.00	0.19	***	4	0.40	0.76	*	2	0.10	0.39	**	3	0.50	0.67	*	2	0.20	0.34	n.s.	3	0.10	0.51	**	4	0.50	0.66	*
XBLG-097	3	0.60	0.68	*	3	0.90	0.59	n.s.	3	0.10	0.28	***	3	0.40	0.48	***	3	0.20	0.57	n.s.	4	0.30	0.60	n.s.	3	0.20	0.19	n.s.	3	0.20	0.19	n.s.	4	0.70	0.66	n.s.
XBLG-099	3	0.20	0.57	***	3	0.20	0.56	n.s.	2	0.10	0.10	n.s.	2	0.00	0.51	***	3	0.00	0.65	***	3	0.10	0.69	***	2	0.10	0.39	**	3	0.50	0.47	n.s.	4	0.30	0.75	***
XBLG-111	3	0.20	0.36	***	3	0.10	0.43	***	3	0.60	0.65	**	3	0.20	0.62	**	2	0.00	0.19	***	2	0.20	0.53	**	2	0.00	0.44	***	3	0.20	0.28	*	2	0.30	0.52	n.s.
XBLG-119	2	0.30	0.48	n.s.	2	0.10	0.39	**	2	0.10	0.48	**	2	0.30	0.39	n.s.	2	0.10	0.52	**	2	0.20	0.53	*	2	0.10	0.52	**	2	0.10	0.52	**	2	0.00	0.19	***
Mean	2.20	0.20	0.36		2.00	0.23	0.30		2.14	0.20	0.34		2.64	0.21	0.46		2.32	0.14	0.37		2.64	0.22	0.44		2.24	0.16	0.34		2.40	0.18	0.40		2.72	0.27	0.47	

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

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

[b] Asterisks indicate significant deviation from Hardy–Weinberg equilibrium (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$); M = monomorphic; n.s. = not significant.

TABLE 3. Cross-amplification results for the 25 polymorphic cDNA-SSR loci developed for *Lindera glauca* in 10 individuals of *L. communis*.[a]

Locus	LC001	LC002	LC004	LC005	LC009	LC010	LC011	LC019	LC021	LC022
XBLG-013	0	0	1	0	0	0	1	0	1	0
XBLG-033	0	1	1	0	1	0	0	0	0	0
XBLG-036	1	0	0	1	0	1	1	0	0	0
XBLG-049	1	1	1	1	0	1	1	1	0	1
XBLG-051	1	1	1	1	1	1	1	1	1	1
XBLG-053	1	1	1	1	1	1	1	1	1	1
XBLG-055	1	1	1	1	1	1	1	0	1	1
XBLG-056	1	1	1	1	0	1	1	1	1	1
XBLG-058	0	0	1	1	1	1	1	1	1	1
XBLG-060	1	1	1	1	0	1	1	1	1	1
XBLG-062	1	1	1	1	1	1	1	1	1	1
XBLG-063	1	1	1	1	1	1	1	1	1	1
XBLG-066	1	1	1	1	1	1	0	1	1	0
XBLG-073	1	1	1	0	1	1	1	1	0	1
XBLG-076	1	1	1	1	1	1	0	1	1	1
XBLG-082	1	0	1	1	1	1	1	1	1	1
XBLG-083	1	1	1	0	0	1	0	1	1	1
XBLG-084	1	0	1	1	1	0	1	1	1	0
XBLG-086	1	1	1	1	0	1	1	0	1	1
XBLG-089	1	1	1	1	0	1	1	1	1	1
XBLG-092	0	1	1	1	1	0	1	1	1	1
XBLG-097	1	0	1	0	1	1	1	1	1	1
XBLG-099	0	1	1	1	1	1	1	1	1	1
XBLG-111	1	0	1	0	1	1	1	0	1	0
XBLG-119	0	0	1	0	0	0	1	0	0	0

Note: 1 = successful amplification; 0 = failed amplification.

[a]LC = population names of *Lindera communis*. Samples were collected in Longjiang County, Yunnan Province, China (geographic coordinates: 24°46′33″N, 98°39′25″E; elevation: 1219 m) and identification codes are kept at the Laboratory of Systematic Evolution and Biogeography of Woody Plants, School of Nature Conservation, Beijing Forestry University, Beijing, China.

five, 12, nine, eight, seven, eight, seven, and seven loci in populations ATM, JGS, LDZ, SJG, NTB, YTH, DBS, HMF, and TMS, respectively) showed significant departure from HWE ($P < 0.001$). Significant linkage disequilibrium was not detected between any pair of loci ($P < 0.001$).

***Cross-species amplifications*—**The 25 primers were tested in 10 individuals of *L. communis* Hemsl. under the same PCR conditions as above. All 25 were found to amplify in at least 21 of the species (Table 3).

CONCLUSIONS

In the current study, we developed 25 novel cDNA-SSR markers that were highly polymorphic in *L. glauca* and used these markers to successfully investigate genetic distances within nine wild populations of *L. glauca*. The collection of SSRs presented herein provide a means to assess genetic diversity and to further investigate large-scale and fine-scale population genetic structure in *L. glauca*. These markers may also be useful for germplasm identification and breeding programs in both this species and other species in the genus *Lindera* Thunb.

LITERATURE CITED

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

DUPONT, Y. L. 2002. Evolution of apomixis as a strategy of colonization in the dioecious species *Lindera glauca* (Lauraceae). *Population Ecology* 44: 293–297.

HUH, G. W., J. H. PARK, J. H. KANG, T. S. JEONG, H. C. KANG, AND N. I. BAEK. 2014. Flavonoids from *Lindera glauca* Blume as low-density lipoprotein oxidation inhibitors. *Natural Product Research* 28: 831–834.

LI, X. W. 1982. Flora Reipublicae Popularis Sinicae, Vol. 31: Lauraceae and Hernandiaceae, 393–394. Science Press, Beijing, China.

LIU, L. D., J. GU, AND J. CHEN. 1992. Studies on the chemical constituents of the leaf of *Lindera glauca* (Sieb et Zucc) Bl and their uses. *Jiangxi Science* 1: 007.

MEGLÉCZ, E., N. PECH, A. GILLES, V. DUBUT, P. HINGAMP, A. TRILLES, R. GRENIER, AND J.-F. MARTIN. 2014. QDD version 3.1: A user-friendly computer program for microsatellite selection and primer design revisited: Experimental validation of variables determining genotyping success rate. *Molecular Ecology Resources* 14: 1302–1313.

NIU, J., X. Y. HOU, C. L. FANG, J. Y. AN, D. L. HA, L. QIU, Y. X. JU, ET AL. 2015. Transcriptome analysis of distinct *Lindera glauca* tissues revealed the differences in the unigenes related to terpenoid biosynthesis. *Gene* 559: 22–30.

POWELL, W., G. C. MACHRAY, AND J. PROVAN. 1996. Polymorphism revealed by simple sequence repeats. *Trends in Plant Science* 1: 215–222.

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

SEKI, K., T. SASAKI, K. HAGA, AND R. KANEKO. 1994. Two methoxybutanolides from *Lindera glauca. Phytochemistry* 36: 949–951.

SUN, H. L., J. X. WANG, X. Z. GU, AND W. Y. KANG. 2011. Analysis of volatile compounds from leaves and fruits of *Lindera glauca. Chinese Journal of Experimental and Traditional Medical Formulae* 7: 033.

VARSHNEY, R. K., A. GRANER, AND M. E. SORRELLS. 2005. Genic microsatellite markers in plants: Features and applications. *Trends in Biotechnology* 23: 48–55.

WANG, R., S. TANG, H. ZHAI, AND H. DUAN. 2011. Studies on anti-tumor metastatic constituents from *Lindera glauca. China Journal of Chinese Materia Medica* 36: 1032–1036.

WANG, W. C. 1972. Higher plants iconography of China. *In* W. C. Wang [ed.], Vol. 1, 853. Science Press, Beijing, China.

WANG, Y., X. GAO, X. YU, S. CHENG, AND L. KONG. 1994. Study on the resource and its utilizations of *Lindera glauca* in China. *Henan Science* 12: 331–334.

Appendix 1. Location and sampling information for *Lindera glauca* individuals used in this study.[a]

Population	Sample accession no.	Geographic coordinates		Elevation (m)	Province in China	County	*n*
		Latitude	Longitude				
ATM	A14-10	31°13′30″N	115°51′35″E	646–834	Anhui	Jinzhai	10
JGS	J13-09	31°52′15″N	114°05′13″E	203–317	Henan	Xinyang	10
LDZ	L14-04	31°56′47″N	114°15′26″E	154–261	Henan	Dongzhai	10
SJG	S14-10	31°44′58″N	115°32′29″E	243–476	Henan	Shangcheng	10
NTB	N14-04	32°19′45″N	113°25′24″E	241–256	Henan	Tongbai	10
YTH	Y14-04	31°03′24″N	115°51′54″E	647–734	Hubei	Yingshan	10
DBS	D14-09	31°00′32″N	115°50′12″E	834–1003	Hubei	Yingshan	10
HMF	H14-09	28°26′51″N	113°00′22″E	224–257	Hunan	Wangcheng	10
TMS	T14-09	30°19′28″N	119°26′56″E	359–432	Zhejiang	Linan	10

Note: *n* = number of individuals sampled.

[a] Sample accession numbers refer to voucher specimens deposited in the Herbarium of the Beijing Forestry University (BJFC), Beijing, China; geographic coordinates and elevation were obtained with a portable GPS receiver.