

Characterization of 31 Microsatellite Markers for *Sinocalycanthus chinensis* (Calycanthaceae), an Endemic Endangered Species

Author(s): Xiao-Yan Wang, Ze-Xin Jin, Jian-Hui Li, and Yuan-Yuan Li

Source: Applications in Plant Sciences, 5(9)

Published By: Botanical Society of America

<https://doi.org/10.3732/apps.1700009>

URL: <http://www.bioone.org/doi/full/10.3732/apps.1700009>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

The monotypic genus *Sinocalycanthus chinensis* W. C. Cheng & S. Y. Chang within the family Calycanthaceae is an endemic, endangered plant species in China. *Sinocalycanthus chinensis* is a diploid ($2n = 22$; Jin et al., 2010), deciduous shrub characterized by large, individual flowers with a diameter of 4.5–7 cm (Cheng and Chang, 1964). Its high ornamental and medicinal value results in overharvesting and a highly restricted geographic distribution (Li 8.5J-0.04ap4/phyloonoal Soc China).

TABLE 1. Characteristics of 31 microsatellite loci developed from *Sinocalycanthus chinensis*.

Locus	Primer sequences (5'–3')	Repeat motif	Allele size range (bp)	T_a (°C)	GenBank accession no.
SC020*	F: GAATAAGGGGAGTGGACG R: GAGAAAGGAAGGAAATAAAA	(TC) ₈	142	57	KY560159
SC056	F: ATAGAAAGCCTTGGTTG R: AGGGAAAACCTCAAAAAGA	(GA) ₉	220–226	54	KY560160
SC061	F: CACTAATGCTACCAACAG R: GAAAAACATACCAACCAAAA	(CT) ₁₆	205–223	54	KY560161
SC078	F: GAACCTACAGAACTTGAC R: GTGTTGTAGATTGGGTGGT	(GT) ₁₀ (GA) ₁₃	174–186	56	KY560162
SC093	F: TTCGGAGAACGAGAT R: TTTAGTCATGCCAATG	(CT) ₂₄	94–112	48	KY560163
SC096*	F: AAACCTCTATTTGGCTCC R: TTTCAAACACCCCTTCACA	(AG) ₁₅	104	47	KY560164
SC098	F: CTGGTAGGTTTTGCTGCTTTT R: CGGATCTCCTTTCTTTCTTCA	(AG) ₁₄	150–184	55	KY560165
SC107-2	F: ACCATCAAATGAAAC R: GAGTCCTGAGAATAAGA	(GA) ₁₀	90–106	57	KY560166
SC124	F: TACGGCGGTAATACAGGG R: CTGAAACGCCATCCGACTC	(AG) ₈ ...(GA) ₉	220–246	60	KY560167
SC136*	F: GACAGGTTTTGGAGATG R: GGAGTGATTCTCTTTGG	(AG) ₇	124	50	KY560168
SC151	F: CCACAAAAGTCAATGAG R: TCTGGATGGGTTGGACTA	(GA) ₂₅	150–180	48	KY560169
SC197*	F: AAAACCAAACCAAGGAAGA R: GCCAACGTCAACATAAGTAGC	(CT) ₁₆	183	52	KY560170
SC220	F: ATGACAAATGCCAGGAGAT R: TCACGCTCCTCTGTTTCT	(GA) ₁₅	203–213	49	KY560171
SC245*	F: GGGTTACTGGTTGGTT R: GGGTCGGACAGTGAGTA	(CT) ₁₅	188	50	KY560172
SC257*	F: GAGATAAGGAGATGGAT R: AAGTTGGACAGTGATGG	(AG) ₁₂	199	45	KY560173
SC264	F: TGGGTTATTTGGTTTCA R: GTCGCAGTCACCTTCTC	(GA) ₉	154–166	54	KY560174
SC280	F: GATTACCTTCTTAGCAC R: CAGGTCCAGACTGATGAC	(CT) ₈ (CA) ₁₂	308–322	52	KY560175
SC296	F: AAAAGAAGGACCATCAGTAT R: GTTGTATTGCATTCAAAGTT	(TC) ₁₅	94–98	52	KY560176
SC301	F: TGTTTACATCATGCCAGT R: GCTCTACTCCCTGATTTT	(CT) ₉	124–128	50	KY560177
SC318*	F: TGAGACTCGAAATCACCAC R: GGAGACAGAAGCAGCAGAAT	(TC) ₇	199	50	KY560178
SC367	F: GAACAATGAAACCGAAGG R: TAGTTCAAATAAGAAGCAGAG	(CT) ₇	170–184	54	KY560179
SC375	F: AAGTGTAATATGCCGTGGA R: GCTGCCTCGAACAAGTCT	(GA) ₇	113–123	50	KY560180
SC388*	F: CCATGATCCCAAGGTAAG R: AAGACAGAATGCCCAAT	(CT) ₁₁	255	56	KY560181
SC424	F: AGAAAGTAGGGAGGGAAGC	(GA) ₇	2220 5.249 78 347.110 0 0 2		

TABLE 2. Genetic diversity of 21 polymorphic microsatellite markers in three *Sinocalycanthus chinensis* populations.^a

Locus	Damingshan (N = 30)				Daleishan (N = 30)				Longxushan (N = 30)				Total (N = 90)			
	n	A	H _o	H _e	n	A	H _o	H _e	n	A	H _o	H _e	n	A	H _o	H _e
SC056	30	2	0.000*	0.444	30	3	0.033*	0.609	30	3	0.000*	0.371	90	4	0.033	0.475
SC061	30	6	0.733	0.776	30	6	0.667	0.727	30	3	0.133*	0.598	90	10	0.511	0.700
SC078	30	4	0.300	0.579	30	7	0.567	0.736	30	5	0.533	0.626	90	7	0.467	0.647
SC093	30	6	0.567	0.767	29	7	0.517*	0.804	30	6	0.733	0.760	89	7	0.606	0.777
SC098	30	2	0.033	0.033	30	6	0.633	0.719	30	6	0.367*	0.617	90	9	0.344	0.456
SC107-2	30	6	0.500	0.661	30	6	0.467*	0.756	27	2	0.296	0.444	87	7	0.421	0.620
SC124	30	10	0.933*	0.811	30	8	0.500*	0.746	30	8	0.800*	0.835	90	14	0.933	0.797
SC151	30	6	1.000	0.752	30	6	0.567	0.779	30	5	0.600	0.562	90	8	0.722	0.698
SC220	30	2	0.267	0.231	30	7	0.567*	0.766	30	2	0.633	0.433	90	7	0.489	0.477
SC264	30	2	0.067*	0.180	30	2	0.133*	0.444	30	1	0.000	0.000	90	2	0.067	0.208
SC280	30	1	0.000	0.000	30	2	0.033	0.033	30	2	0.067	0.064	90	2	0.033	0.032
SC296	30	2	0.000*	0.124	30	3	0.267	0.527	30	2	0.000*	0.491	90	3	0.267	0.381
SC301	30	2	0.033	0.033	30	3	0.667	0.491	30	3	0.267	0.238	90	3	0.322	0.254
SC367	29	3	0.000*	0.585	30	5	0.100*	0.502	29	3	0.069*	0.447	88	5	0.069	0.511
SC375	30	2	1.000*	0.500	30	2	1.000*	0.500	30	2	1.000*	0.500	90	2	1.000	0.500
SC424	30	2	0.000*	0.124	29	9	0.241*	0.795	30	5	0.033*	0.578	89	9	0.137	0.499
SC492	30	8	0.267*	0.642	30	11	0.600*	0.854	30	10	0.833*	0.839	90	20	0.550	0.778
SC512-2	29	2	0.000*	0.408	29	4	0.000*	0.302	27	2	0.556	0.497	85	4	0.556	0.402
SC537	30	4	0.000*	0.611	30	5	0.133*	0.563	30	4	0.033*	0.517	90	5	0.083	0.564
SC556-2	30	4	0.267	0.317	30	4	0.567	0.668	30	4	0.433*	0.686	90	5	0.422	0.557
SC673-2	30	2	0.567	0.455	30	2	0.300	0.339	28	2	0.393	0.316	88	2	0.420	0.370

Note: A = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; N = number of individuals sampled; n = number of individuals successfully amplified.

^aLocality and voucher information are provided in Appendix 1.

*Significant deviation from Hardy–Weinberg equilibrium expectations after Bonferroni correction (P < 0.001).

The number of alleles, observed heterozygosity, expected heterozygosity, and linkage disequilibrium were estimated software FSTAT 2.9.3.2 (Goudet, 2001), and Hardy–Weinberg equilibrium was assessed using GenAlEx 6.3 (Peakall and Smouse, 2006). Of the 31 loci, 21 loci were polymorphic in at least two of the three tested populations, and the remaining 10 loci were monomorphic (Table 1). The number of alleles per locus ranged from one to 20, e.eG an average of 4.677. In the 21 polymorphic markers, the average observed and expected heterozygosity in all three populations were 0.403 ± 0.061 (mean ± SEM [standard error of the mean]) (0.033–1.000 per locus) and 0.510 ± 0.043 (0.032–0.797 per locus), respectively (Table 2). Seven loci (SC056, SC124, SC367, SC375, SC424, SC492, SC537) significantly deviated from Hardy–Weinberg equilibrium in all three tested populations after Bonferroni correction (P < 0.001) (Table 2). Of these 31 loci, 29 were successfully amplified in *C. floridus* and also revealed high levels of polymorphism (Table 3).

CONCLUSIONS

In this study, 31 microsatellite markers were developed from the Chinese endemic endangered plant species *S. chinensis*. Twenty-one loci were polymorphic in three tested populations. The high transferability of these markers will provide a more effective method to research the population genetics and phylogeography of *S. chinensis* and the closely related species *C. floridus*.

LITERATURE CITED

CHENG, W. . , AND S. . CHANG. 1964. New genus in the family Calycanthaceae—genus *Sinocalycanthus*. *Acta Phytotaxonomica Sinica* 9: 135–138.

GOUDET, J. 2001. FSTAT (version 2.9.3): A program to estimate and test gene diversities and fixation indices. Institute of Ecology, Lausanne, Switzerland. Website <http://www.unil.ch/popgen/software/fstat.htm> [accessed 20 December 2016].

JIN, Z. X., AND J. M. LI. 2007. ISSR analysis on genetic diversity of endangered relic shrub *Sinocalycanthus chinensis*. *Journal of Applied Ecology* 18: 247–253.

JIN, Z. X., J. M. LI, S. S. KE, C. M. BIAN, AND W. B. ZHANG. 2010. Conservation biology of *Sinocalycanthus chinensis*. Science Press, Beijing, China.

LI, J. M., AND Z. X. JIN. 2006. High genetic differentiation revealed by RAPD analysis of narrowly endemic *Sinocalycanthus chinensis*, Cheng et S.Y. Chang, an endangered species of China. *Biochemical Systematics and Ecology* 34: 725–735.

LI, J. M., Z. X. JIN, AND T. TAN. 2012. Genetic diversity and differentiation of *Sinocalycanthus chinensis* populations revealed by chloroplast microsatellite (cpSSRs) markers. *Biochemical Systematics and Ecology* 41: 48–54.

PEAKALL, R., AND P. E. SMOUSE. 2006. GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

YE, Q., Y. X. QIU, Y. Q. QUO, J. X. CHEN, S. Z. YANG, M. S. ZHAO, AND C. X. FU. 2006. Species-specific SCAR markers for authentication of *Sinocalycanthus chinensis*. *Journal of Zhejiang University. Science. Series B* 7: 868–872.

ZANE, L., L. BARGELLONI, AND T. PATARNELLO. 2002. Strategies for microsatellite isolation: A review. *Molecular Ecology* 11: 1–16.

ZHAO, H., L. ZHOU, H. LIU, AND Z. BAO. 2014. Genetic effects of different mating modes in *Sinocalycanthus chinensis* (Cheng et S.Y. Chang) Cheng et S.Y. Chang, an endangered species endemic to Zhejiang Province, China. *Biochemical Systematics and Ecology* 54: 8–14.

APPENDIX 1. Locality information for the *Sinocalycanthus chinensis* and *Calycanthus floridus* samples used in this study.^a

Species	Population ID	Collection locality	Geographic coordinates	Collector	Collection no.	N
<i>Sinocalycanthus chinensis</i> W. C. Cheng & S. Y. Chang	DMS	Damingshan, Zhejiang, China	30.039817°N, 118.972933°E	Xiao-Yan Wang	DLS1-30	30
<i>Sinocalycanthus chinensis</i>	DLS	Daleishan, Zhejiang, China	28.988717°N, 120.811367°E	Xiao-Yan Wang	DMS1-30	30
<i>Sinocalycanthus chinensis</i>	LXS	Longxian, Anhui, China	30.039817°N, 118.972933°E	Xiao-Yan Wang	DMS1-30	30