

Supplementary

Table S1. The location and voucher information of *M. sprengeri* samples used in this study.

P ¹	Location	Latitude	Longitude	Alt. (m)	N ²	Collection number
1	Longnan, Gansu	33.66298	106.37503	2220	1	K_ZSZ 845
2	Tongren, Guizhou	27.54302	108.42152	1911	3	K_ZSZ 800,808,851
3	Qiandong, Guizhou	26.22922	108.11905	1913	5	K_ZSZ 753,803,804,805, 847
4	Tongren, Guizhou	27.19493	108.03560	1719	1	K_ZSZ 783
5	Tongren, Guizhou	27.26102	108.05311	1056	3	K_ZSZ 806,849,850
6	Zunyi, Guizhou	28.14137	107.09382	1585	5	K_ZSZ 784,785,786,812, 855
7	Tongren, Guizhou	27.54680	108.38585	1558	3	K_ZSZ 801,802,809
8	Shennongjia, Hubei	31.45694	110.31537	1578	1	K_ZSZ 872
9	Wufeng, Hubei	30.27883	110.67747	1592	3	K_ZSZ 701,702,709
10	Wufeng, Hubei	30.27391	110.64335	1371	2	K_ZSZ 703,721
11	Wufeng, Hubei	30.28387	110.46231	1328	2	K_ZSZ 712,713
12	Wufeng, Hubei	30.18847	110.56368	1204	3	K_ZSZ 707,711,722
13	Wufeng, Hubei	30.18385	110.85734	1200	4	K_ZSZ 704,705,706,708
14	Wufeng, Hubei	30.17791	110.86651	1004	3	K_ZSZ 710,714,719
15	Zhangjiajie, Hunan	29.46388	110.04043	1291	5	K_ZSZ 744,745,746,747, 748
16	Zhangjiajie, Hunan	29.04751	110.47443	1419	5	K_ZSZ 742,743,788,789, 790
17	Zhangjiajie, Hunan	29.04956	110.47765	842	1	K_ZSZ 569
18	Shangluo, Shaanxi	33.81434	108.99731	1093	1	K_ZSZ 876
19	Baoji, Shaanxi	33.72666	106.76983	1365	4	K_ZSZ 717,874,877,879
20	Hanzhong, Shaanxi	33.58956	107.76553	1150	3	K_ZSZ 522,530,844
21	Baoji, Shaanxi	34.08907	107.70639	1136	1	K_ZSZ 881
22	Baoji, Shaanxi	34.08771	107.75167	1227	3	K_ZSZ 715,718,875
23	Baoji, Shaanxi	34.04856	107.61392	1268	1	K_ZSZ 716

24	Ankang, Shaan-xi	33.44365	108.48758	1456	2	K_ZSZ 523,537
25	Ankang, Shaan-xi	33.44455	108.45597	2051	1	K_ZSZ 720
26	Ankang, Shaan-xi	32.36209	109.44273	502	1	K_ZSZ 880
27	Ankang, Shaan-xi	32.08625	109.53163	921	1	K_ZSZ 868
28	Chengdu, Si-chuan	30.90973	103.55639	1160	1	K_ZSZ 527
29	Fengjie, Chong-qing	30.39481	109.25114	1303	5	K_ZSZ 871,892,893,894,895
30	Guangyuan, Sichuan	32.41695	106.55074	1694	1	K_ZSZ 773
31	Jiangyou, Si-chuan	31.52290	104.39013	1580	3	K_ZSZ 526,529,772
32	Mianyang, Si-chuan	32.65456	104.11153	1613	1	K_ZSZ 525
33	Mianyang, Si-chuan	31.89665	104.68127	1208	1	K_ZSZ 524
34	Bazhong, Si-chuan	30.52463	103.20626	1639	1	K_ZSZ 528
35	Linan, Zhejiang	30.34932	119.42419	1481	1	K_ZSZ 775
36	Nanchuan, Chongqing	29.04287	107.17184	1637	5	K_ZSZ 760,761,762,763,782

¹ Populations number; ² Number of samples.

Table S2. Primers used for DNA barcoding studies.

Region	Primer	Sequence	Annealing temperature	expected length	Reference
<i>matK</i>	3F	5'-CGTACAGTAC TTTT- GTGTTTAC- GAG-3'	50 °C	837 bp	Chao et al., 2014 [1]
	1R	5'-ACCCAGTCCA TCTG- GAAATCTT- GGTTC-3'			
<i>trnH-psbA</i>	psbA-F	5'-GTTATGCATG AAC- GTAATGCTC-3'	58 °C	465 bp	Sang et al., 1997 [2]
	trnH-R	5'-CGCGCATGGT GGAT- TCACAATCC-3'			
<i>rbcL</i>	F	5'-ATGTCACCAC AAACAGA- GACTAAAGC-3'	55 °C	553 bp	Kress & Erickson, 2007 [4]
	R	5'-GTAAAATCAA GTCCACCGCG-3'			

Table S3. Variable sites of the aligned sequences of the three chloroplast DNA (cpDNA) fragments (*matK*, *trnH-psbA* and *rbcL*) from which six cpDNA haplotypes of *Magnolia sprengeri* were identified.

cpDNA haplo- types	<i>matK</i> (1-765) ¹				<i>trnH-psbA</i> (766-1126)				<i>rbcL</i> (1127-1650)	
	0	0	0	0	0	0	1	1	1	1
	1	2	6	7	8	9	0	0	2	5
	1	6	1	5	5	8	3	4	9	6
	4	7	7	4	1	1	8	2	6	4
HP1	A	G	C	T	A	G	G	C	A	T
HP2	T
HP3	C
HP4	.	.	G
HP5	G	A	.	G	.	.	A	T	G	C
HP6	.	A	.	G	.	.	A	T	G	C

¹ The total alignment length was 1,650 bp. The fragments are united with *matK* (765bp), *trnH-psbA* (361bp) and *rbcL* (524bp). Nucleotide position is at the bottom of this column; ² All haplotype sequences are edited with reference to HP1.

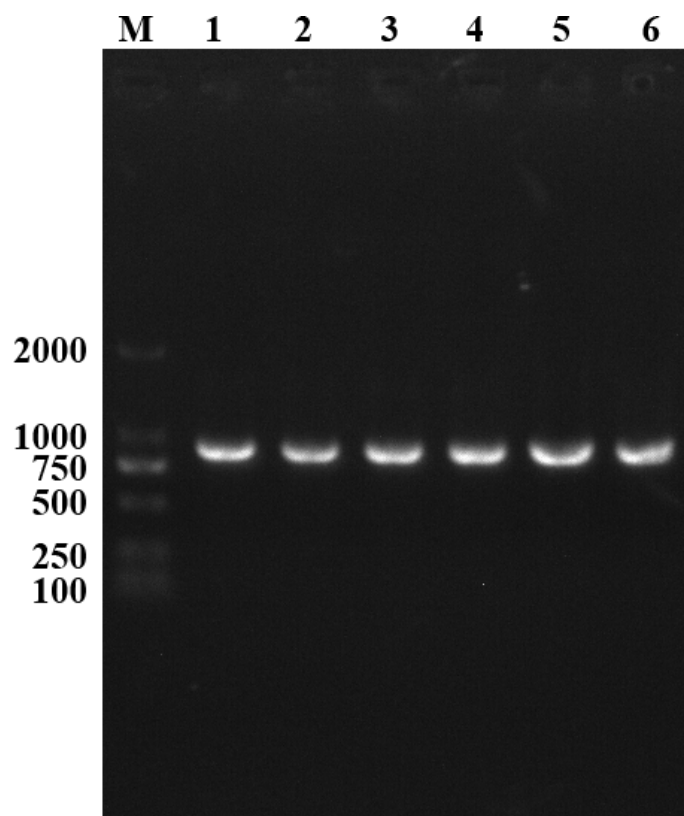


Figure S1. Electrophoretic map of *matK*'s primers. M, 2000bp DNA Marker; 1-6, six materials of *M. sprengeri*. The results showed that the primers could amplify the target bands in *M. sprengeri*, the size of which was about 800bp.

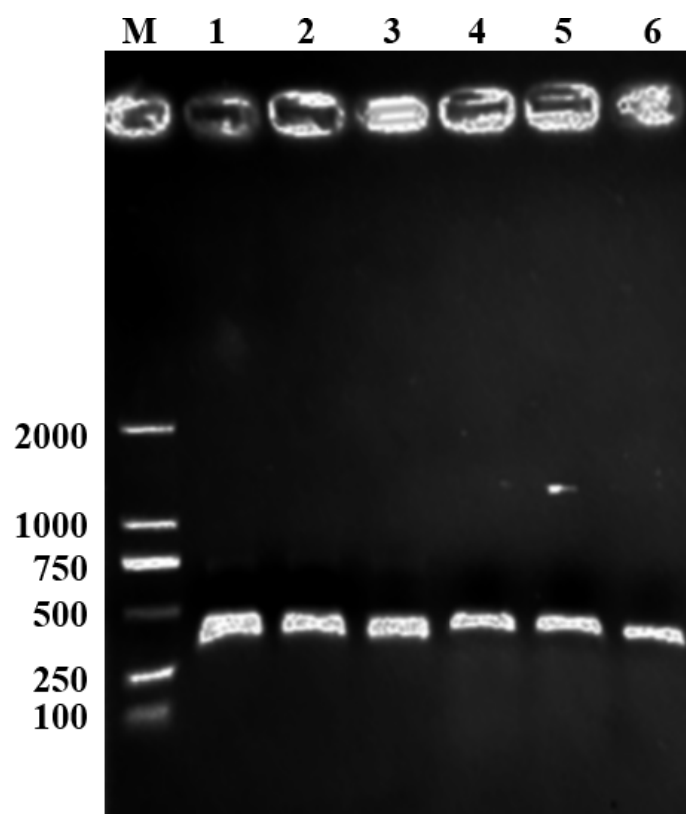


Figure S2. Electrophoretic map of *trnH-psbA*'s primers. M, 2000bp DNA Marker; 1-6, six materials of *M. sprengeri*. The results showed that the primers could amplify the target bands in *M. sprengeri*, the size of which was about 450bp.

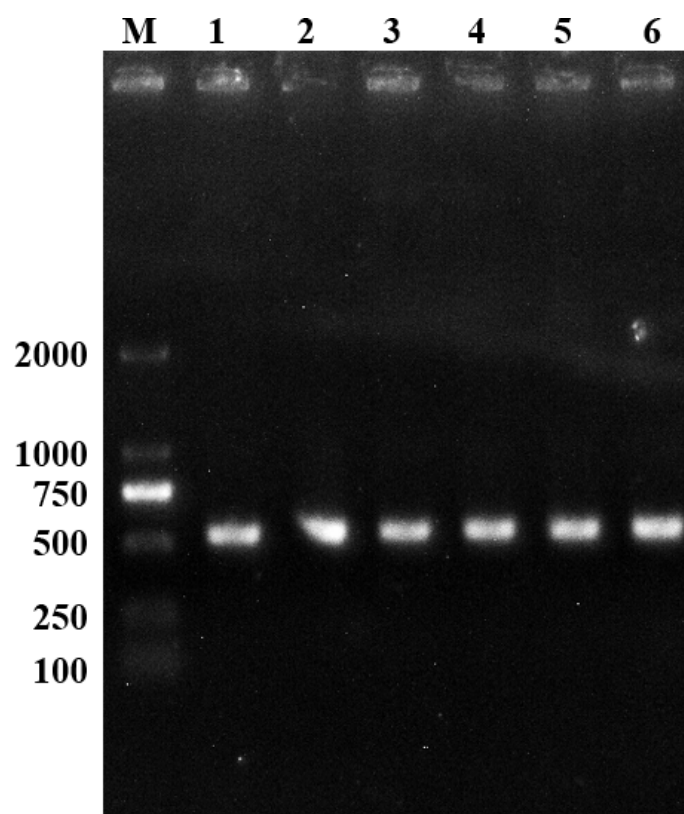


Figure S3. Electrophoretic map of *rbcL*'s primers. M, 2000bp DNA Marker; 1-6, six materials of *M. sprengeri*. The results showed that the primers could amplify the target bands in *M. sprengeri*, the size of which was about 550bp.

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	1450	1460	1470	1480	1490	1500
SP15-1	A	T	T	T	G	G
SP15-5	A	T	T	T	G	G
SP1-1	A	T	T	T	G	G
SP2-1	A	T	T	T	G	G
SP2-2	A	T	T	T	G	G
SP2-3	A	T	T	T	G	G
SP3-1	A	T	T	T	G	G
SP3-2	A	T	T	T	G	G
SP3-3	A	T	T	T	G	G
SP3-4	A	T	T	T	G	G
SP3-5	A	T	T	T	G	G
SP4-1	A	T	T	T	G	G
SP5-1	A	T	T	T	G	G
SP5-2	A	T	T	T	G	G
SP5-3	A	T	T	T	G	G
SP6-1	A	T	T	T	G	G
SP6-2	A	T	T	T	G	G
SP6-3	A	T	T	T	G	G
SP6-4	A	T	T	T	G	G
SP6-5	A	T	T	T	G	G
SP7-2	A	T	T	T	G	G
SP7-3	A	T	T	T	G	G
SP10-1	A	T	T	T	G	G
SP10-2	A	T	T	T	G	G
SP11-1	A	T	T	T	G	G
SP11-2	A	T	T	T	G	G
SP12-1	A	T	T	T	G	G
SP12-2	A	T	T	T	G	G
SP12-3	A	T	T	T	G	G
SP19-1	A	T	T	T	G	G
SP13-1	A	T	T	T	G	G
SP13-2	A	T	T	T	G	G
SP13-3	A	T	T	T	G	G
SP13-4	A	T	T	T	G	G
SP14-1	A	T	T	T	G	G
SP14-2	A	T	T	T	G	G
SP22-2	A	T	T	T	G	G
SP14-3	A	T	T	T	G	G
SP15-2	A	T	T	T	G	G
SP15-3	A	T	T	T	G	G
SP16-1	A	T	T	T	G	G
SP16-2	A	T	T	T	G	G
SP16-3	A	T	T	T	G	G
SP16-4	A	T	T	T	G	G
SP16-5	A	T	T	T	G	G
SP18-1	A	T	T	T	G	G
SP19-2	A	T	T	T	G	G
SP19-3	A	T	T	T	G	G
SP19-4	A	T	T	T	G	G
SP20-1	A	T	T	T	G	G
SP24-1	A	T	T	T	G	G
SP26-1	A	T	T	T	G	G
SP36-4	A	T	T	T	G	G
SP36-5	A	T	T	T	G	G
SP34-1	A	T	T	T	G	G
SP36-1	A	T	T	T	G	G
SP36-2	A	T	T	T	G	G
SP36-3	A	T	T	T	G	G
SP32-1	A	T	T	T	G	G
SP33-1	A	T	T	T	G	G
SP31-2	A	T	T	T	G	G
SP31-3	A	T	T	T	G	G
SP30-1	A	T	T	T	G	G
SP31-1	A	T	T	T	G	G
SP29-4	A	T	T	T	G	G
SP29-5	A	T	T	T	G	G
SP29-1	A	T	T	T	G	G
SP29-2	A	T	T	T	G	G
SP27-1	A	T	T	T	G	G
SP28-1	A	T	T	T	G	G
SP24-2	A	T	T	T	G	G
SP25-1	A	T	T	T	G	G
SP21-1	A	T	T	T	G	G
SP22-1	A	T	T	T	G	G
SP20-2	A	T	T	T	G	G
SP20-3	A	T	T	T	G	G
SP29-3	A	T	T	T	G	G
SP23-1	A	T	T	T	G	G
SP22-3	A	T	T	T	G	G
SP9-1	A	T	T	T	G	G
SP9-2	A	T	T	T	G	G
SP9-3	A	T	T	T	G	G
SP8-1	A	T	T	T	G	G
SP17-1	A	T	T	T	G	G
SP35-1	A	T	T	T	G	G
SP7-1	A	T	T	T	G	G

[illegible]

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Figure S4. All alignments sequences of *M. sprengeri* used in this study. SP represents *M. sprengeri*. The total alignment length was 1,650 bp, and 10 nucleotide substitutions revealed six haplotypes (Table S3, HP1-6).

References

1. Jiang, C.; Cao, L.; Yuan, Y.; Chen, M.; Jin, Y.; Huang, L. Barcoding melting curve analysis for rapid, sensitive, and discriminating authentication of saffron (*Crocus sativus* L.) from its adulterants. *Biomed Res. Int.* **2014**, *2014*, 809037.
2. Swift, J.F.; Lance, R.F.; Guan, X.; Britzke, E.R.; Lindsay, D.L.; Edwards, C.E. Multifaceted DNA metabarcoding: Validation of a noninvasive, next-generation approach to studying bat populations. *Evol. Appl.* **2018**, *11*, 1120–1138.
3. Tate, J.A.; Simpson, B.B. Breeding system evolution in *Tarasa* (Malvaceae) and selection for reduced pollen grain size in the polyploid species. *Am. J. Bot.* **2004**, *91*, 207–213.
4. Kress, W.J.; Erickson, D.L. A two-locus global DNA barcode for land plants: the coding *rbcL* gene complements the non-coding *trnH-psbA* spacer region. *PLoS One.* **2007**, *2*, e508.