

Two PEL of cpDNA of *Magnolia Accisa*, a New Species (Magnoliaceae) from China

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Abstract: *Magnolia* sect. *Rytidospermum* Spach is an important taxon and has an important position in the evolutionary and taxonomic system of Magnoliaceae. A new species of the section was discovered in the investigation of the plant resources in China, named *Magnolia accisa* D. L. Fu, H. Fu et R. M. Duan, sp. nov. for its leaves with accisus apices and viridescent lower surfaces, which had been confused with *M. officinalis* var. *biloba* Rehd. & Wils. for many years. In order to determine its evolutionary taxonomic status, two similar species, *M. officinalis* Rehd. & Wils. with the variety *M. officinalis* var. *biloba* Rehd. & Wils. and *M. rostrata* W. W. Smith were also collected, and two partial sequences of chloroplast genome, *matK* and *trnL-ndhJ*, were amplified and sequenced respectively. The partial sequences were analyzed and compared with 39 samples of the chloroplast complete genomes of Magnoliaceae in the NCBI (National Center for Biotechnology Information, USA) database, and the results indicated that the new species *Magnolia accisa* has two PEL (particularly evolutionary loci): *matK* (...ACAAGGTGGGATTTC...) and *trnL-ndhJ* (...GTCTAGTCTATAGGAGT...), for that the oppositely evolutionary loci of *matK* (...ACAAGGTGAGATTTC...) and *trnL-ndhJ* (...GTCTAGTCTAGAGGAGT...) are possessed by all other contrasted samples including the experimental materials and the data of NCBI of Magnoliaceae. So, the new species is described and illustrated according International Code of Botanical Nomenclature, which will speed up the investigation of plant resources of *Magnolia* sect. *Rytidospermum* Spach in China.

Keywords: PEL (Particularly Evolutionary Loci), *Magnolia Accisa*, *matK*, *trnL-ndhJ*, New Species, Magnoliaceae

1. Introduction

Magnolia sect. *Rytidospermum* Spach [1-3] is an important taxon and has an important position in the evolutionary and taxonomic system of Magnoliaceae, which was even regarded as a new genus, *Houpoea* N. H. Xia & C. Y. Wu [4, 5]. The species of the section had been cultivated more than 2,000 years in China, because its barks has been used as a Chinese medicine, commonly known as Houpo, a traditional Chinese herbal medicines and traditional Chinese exported medicines. The trees grow rapidly, adapt strongly,

distribute and are cultivated widely, the flowers are big and fragrant, and the leaves gather at the end of perennial often non-divaricating branches as “umbrella tree” called in North America, so they have unique ornamental features and occupy an important position in the cultivation of Forestry. The species of *Magnolia* sect. *Rytidospermum* Spach native to China are mainly three species described: *M. officinalis* Rehd. & Wils.[6], *M. rostrata* W. W. Smith [1-4, 7], and *M. chathayana* D. L. Fu et T. B. Zhao [8]. In the investigation of

the plant resources of *Magnolia* sect. *Rytidospermum* in Henan, Hunan, Hubei, Sichuan, Yunnan, Xizang and other provinces in recent years, a new species, named *Magnolia accisa* D. L. Fu, H. Fu et R. M. Duan, sp. nov., had been found by the authors. The new species has the particularity of the leaves with accisus apices and viridescent lower surfaces, but it had been confused with *M. officinalis* var. *biloba* Rehd. & Wils. [6, 7, 9] for many years by botanists [3, 10-12]. In order to determine its evolutionary taxonomic status, along with two similar species, *M. officinalis* Rehd. & Wils. with its variety *M. officinalis* var. *biloba* Rehd. & Wils., and *M. rostrata* W. W. Smith were collected, and two partial sequences of chloroplast genome, *matK* and *trnL-ndhJ*, were amplified and sequenced respectively. The partial sequences were analyzed and compared with 39 samples of the chloroplast complete genomes of Magnoliaceae in NCBI (National Center for Biotechnology Information, USA) database, and the results are as follows.

2. Materials & Methods

2.1. Plant Materials

The leaves of 3 species and 1 variety of *Magnolia* sect. *Rytidospermum* Spach were collected from China: the new species of *M. accisa* D. L. Fu, H. Fu et R. M. Duan and the similar species *M. officinalis* Rehd. et Wils. and its variety *M. officinalis* var. *biloba* Rehd. et Wils. from Beichuan County of Sichuan province, the other similar species *Magnolia rostrata* W. W. Smith from Gongshan and Tengchong County of Yunnan province (see Table 1).

2.2. PCR Primer Design

Two pairs of primers, *matK*-Y01-F and *matK*-Y01-R, and *trnL-ndhJ*-Y01-F and *trnL-ndhJ*-Y01-R were designed using Primer Premier 6, the sequences of primers and the length of amplification and sequencing of cpDNA, see Table 2.

Table 1. Experimental materials of *Magnolia* sect. *Rytidospermum*.

Species name	Experimental material	Collected place
<i>Magnolia accisa</i>	leaves	Beichuan, Sichuan, China
<i>M. officinalis</i> var. <i>biloba</i>	leaves	Beichuan, Sichuan, China
<i>M. officinalis</i>	leaves	Beichuan, Sichuan, China
<i>M. rostrata</i>	leaves	Gongshan, Yunnan, China
<i>M. rostrata</i>	leaves	Tengchong, Yunnan, China

Table 2. Two designed primers for amplification and sequencing of partial cpDNA of Magnoliaceae.

Primer name	Primer sequences	Length of amplification and sequencing /bp
<i>matK</i> _Y01	F: 5'-GAGCCAAAGTTCTAGCACACG-3' R: 5'-CACTGCTGGATACAAGATGCC-3'	832
<i>trnL-ndhJ</i> _Y01	F: 5'-TTAGAAATCGTGAGGGTTCAAGTC-3' R: 5'-TTCGGATGAGTAGAGCCAATAGG-3'	980

Table 3. Contrasted chloroplast genomes of Magnoliaceae in NCBI.

Species	DNA number in NCBI	Species	DNA number in NCBI
<i>Liriodendron chinense</i>	NC030504.1	<i>Magnolia pyramidata</i>	NC023236.1
<i>Liriodendron tulipifera</i>	DQ899947.1	<i>Magnolia sinica</i>	NC023241.1
<i>Magnolia aromatica</i>	NC037000.1	<i>Magnolia tripetala</i>	NC024027.1
<i>Magnolia conifera</i>	NC037001.1	<i>Magnolia yunnanensis</i>	NC024545.1
<i>Magnolia dandyi</i>	NC037004.1	<i>Michelia cathartii</i>	NC023234.1
<i>Magnolia dealbata</i>	NC023235.1	<i>Michelia laevifolia</i>	NC035956.1
<i>Magnolia duclouxii</i>	NC037002.1	<i>Michelia odora</i>	NC023239.1
<i>Magnolia fordiana</i> var. <i>calcareo</i>	MF990562.1	<i>Michelia</i> sp.	KY921716.1
<i>Magnolia glaucifolia</i>	NC037003.1	<i>Yulania acuminata</i>	JX280391.1
<i>Magnolia grandiflora</i>	JN867584.1	<i>Yulania biondii</i>	KY085894.1
<i>Magnolia grandiflora</i>	JN867587.1	<i>Yulania denudata</i>	JN227740.1
<i>Magnolia grandiflora</i>	NC020318.1	<i>Yulania denudata</i>	JN867577.1
<i>Magnolia insignis</i>	MF990566.1	<i>Yulania denudata</i>	JX280394.1
<i>Magnolia kwangsiensis</i>	HM775382.1	<i>Yulania diva?</i>	NC023242.1
<i>Magnolia officinalis</i>	JN867579.1	<i>Yulania kobus</i>	NC023237.1
<i>Magnolia officinalis</i>	JN867581.1	<i>Yulania liliiflora</i>	NC037005.1
<i>Magnolia officinalis</i>	JN867582.1	<i>Yulania liliiflora</i>	NC023238.1
<i>Magnolia officinalis</i>	KY085916.1	<i>Yulania liliiflora</i>	JX280397.1
<i>Magnolia officinalis</i>	NC020316.1	<i>Yulania salicifolia</i>	NC023240.1
<i>Magnolia officinalis</i> var. <i>biloba</i>	JN867580.1		

2.3. PCR Amplification

Total genomic DNA was isolated from silica-dried leaves of 5 materials belong to 3 species and 1 variety of *Magnolia*

sect. *Rytidospermum* using a modified CTAB method [13]. The primers of PCR amplification are *matK*_Y01 and *trnL-ndhJ*_Y01 (see Table 2) respectively. All PCR reactions were completed in T-gradient (Biometra). PCR amplifications were performed in 15 µL volume: 7.5 µL 2x

Es Taq MasterMix, 0.2 µL forward primer and 0.2 µL reverse primer, 6.1 µL ddH₂O, and 1 µL genomic DNA, with the following cycles: 5 min initial denaturation at 94°C, 10 cycles of 30 s at 94°C, 45 s at 61°C and 2 min at 72°C, 27 cycles of 30 s at 94°C, 45 s at 56°C and 2 min at 72°C, and final extension 5 min at 72°C. The PCR products were extracted and purified using the Gel Extraction Kit (OMEGA).

2.4. DNA Sequencing

PCR amplifications were performed in 15 µL volume: 7.5 µL 2x Es Taq MasterMix, 0.2 µL forward primer and 0.2 µL reverse primer, and 1 µL purified DNA, 6.1 µL ddH₂O, with the following cycles: 3 min initial denaturation at 95°C, 26 cycles of 10 s at 95°C, 10 s at 50°C and 4 min at 60°C, using ABI 3730 XL.

2.5. DNA Sequence Analysis

The absolutely coincident partial sequences using the forward primer and reverse primer were correct ones of the samples, which could be analyzed. The particularly evolutionary loci could be easily found out and verified using Microsoft Word or other software. Table 3 listed the contrasted chloroplast complete genomes of Magnoliaceae in the NCBI database.

3. Two PEL (Particularly Evolutionary Loci) of cpDNA of Magnolia Accisa

3.1. PEL of Partial Sequence of *matK* of the New Species

There are 706 bp absolutely coincident sequences of *Magnolia accisa*, using the forward primer and reverse primer of *matK*-Y01 respectively, which can be regarded as the correct partial cpDNA sequence of the new species. The sequence is as follow:

Magnolia accisa, partial sequence of *matK* of cpDNA

ACTTTATTCGATACAAAGTCTGTTTTTTTGAGGAT
CCACTGTGATAATGAGAAAGATTTCTGTATATCCGC
CCAAATCGATTGATAATATCAGAATCTGACGAATCG
GCCCGGACCGACTTACTAATGGGATGCCCTGATACG
TTACAAAATTTGCTTTAGCCACTGATCCAATCAGA
GGAATAATTGGGACTAGGGTCTCGAATTTATTAATA
GAAGTATCTATTAGAAATGAATTCTCTAGCATTGTA
ATCCTTACCACCGAAGTGTTTAGTCGTACACTTGAA
AGATAGCCCAGAAAATATAAGGAATGATTGTATAA
TTGGTTTATATGGATCCTGTCCGGTAGAGACCACAA
GTAAAAATGACATTGCCAAAAATGGACAAGGTGGG
ATTTCATTTCTTCATCAGAAGATGAGTCCCCTTTGA
AGCCAGAATGGATTTTCTTGATATCTGACATAATG
CATGAAAGGGTCCTTGAACAACCATAGGGTCTTCTG
AAAATCATTACGAAGCACTACTACAAGATGTTCTAT
TTTTCCATAGAAATGTGTTTCGCTCAAGAAAAGTTCC
AGAGGATGTTGATAGTAAATGAGAAGATTGTTTACG
GAGAAACACTAATACGGATTACATTTCATATACATG
AGAATTATATAGTAACAAGAAGATCTTTGATTCTC

TTTTGAAAAAAGAGAAATGGATT

Compared to the similar species, *M. officinalis* var. *biloba*, *M. officinalis* and *M. rostrata*, and the other DNA data of Magnoliaceae in NCBI, it can be concluded that the evolutionary locus (...ACAAGGTGGGATTTC...) is particular because the oppositely evolutionary locus of transition (...ACAAGGTGAGATTTC...) is possessed by the similar species and variety in the research, and all other contrasted chloroplast complete genomes data of Magnoliaceae in NCBI.

3.2. PEL of Partial Sequence of *trnL-ndhJ* of the New Species

There are 765 bp absolutely coincident sequences of *Magnolia accisa*, using the forward primer and reverse primer of *trnL-ndhJ*_Y01 respectively, which can be regarded as the correct partial cpDNA sequence of the new species. The sequence is as follow:

Magnolia accisa, partial sequence of *trnL-ndhJ* of cpDNA

AAATGTTTCTCTCTTATCACAAGTCTTGATAGATA
TATGATATACGTACAAATGCCCATATTATGGGCAAG
GAATCTCCATTATTGAATCATTACAGTGCATATCA
TTACTCTTACACTTACAAAGTCTTCTTTTTGAAGATC
CAAGAAATTCCAGGACCTAGGTAAGATTTTGAAGATG
CTTTTGTAGTCCCTTTAATTGACATAGACCCAAGTCC
TCTAGTAGGATGACGCATCGGAATGGTCGGGATA
GCTCAGCTGGTAGAGCAGAGGACTGAAAATCCTCG
TGTCACCAGTTCAAATCTGGTTCCTGACACGTGGTT
AATGTATCAAACGGATACTCATACAAATGAATCGAT
ATGGATAGGGATCCATATTTATTAATAGTCTAATAG
TCTAGACCATGATACATAAGTCTAGACCATGATACA
TACTTATCCATCTAGGTATATAAATGGATACCTCCC
TTTTTGATAGTGGGTAAAGAATATATGGGTAAGGTA
AAGAAAAGAATTAGATTCTATCCCTCTTCTTTTTTG
TTGTTTATACTGTACCTCCTCTCGCTCAAAAAGAAT
GTTAATACTTCATACATATCCGAAGTTAGTTGGCTT
GGCTGAAAAACCCAAAAGTCTAGTCTATAGGAGTG
GAAGGATAGGAATAGACAGGATTCATTTCAGATAC
AATACAAAGAAAGAAAATCCGATCCCTTTTCATTTT
CATTTCTGAATTCATATTTTCTTTCCTATTCTATTTC
TTCACCTT

Compared to the similar species, *M. officinalis* var. *biloba*, *M. officinalis* and *M. rostrata*, and the other DNA data of Magnoliaceae in NCBI, it can be concluded that the evolutionary locus (...GTCTAGTCTATAGGAGT...) is particular because the oppositely evolutionary locus of transversion (...GTCTAGTCTAGAGGAGT...) is possessed by all similar species and variety, and all contrasted data of Magnoliaceae in NCBI.

So it can be concluded that there are at least two particularly evolutionary loci of cpDNA of *Magnolia accisa*, which are *matK* (...ACAAGGTGGGATTTC...) and *trnL-ndhJ* (...GTCTAGTCTATAGGAGT...). The new species is supported by cpDNA evolutionomy based on the evolutionary continuity principle [14] and the evolutionary particularity principle. According International Code of Botanical

Nomenclature [15], the new species is described and illustrated.

4. *Magnolia Accisa* D. L. Fu, H. Fu et R. M. Duan, sp. Nov., Figures 1, 2

Magnolia biloba (Rehd. et Wils.) Cheng, auct. non Rehd. et Wils. (1913): in *Iconographia Cormophytorum Sinicorum* 1: 787. figure.1574. 1972; *Magnolia officinalis* subsp. *biloba* (Rehd. et Wils.) Law, auct. non Rehd. et Wils. (1913): in *Fl. Reipubl. Popul. Sin.*, 30 (1): figure 29 (2). 1996.

Magnolia officinalis Rehd. & Wils. var. *punicea* T. B. Zhao et Z. X. Chen, in L. G. Song et al., *J. Henan For. Sci. & Techn.*, 18 (1): 6-7. 1998. Type: non designated.

Magnolia officinalis Rehd. & Wils. ssp. *biloba* (Rehd. & Wils.) Law var. *rubicunda* Yi, *J. Sichuan For. Sci. & Techn.*, 35 (5): 1-2. 2014. Type: T. P. Yi 14003 SAUD (non vidi).



Figure 1. *Magnolia accisa* D. L. Fu, H. Fu et R. M. Duan, sp. nov. 1. branchlet with leaves and flower, 2. fruit aggregate, 3. beak of follicle (from *Iconog. Cormophyt. Sin.*, 1972, misidentified as *M. biloba* [10]).

Arbor decidua 10 m alta. Ramuli crassi 1.2~1.8 cm diam., cinerei, partibus minute flavibis, glabri, stipuli-cicatricibus annulare manifestis. Gemmae juveniles conicae glabrae. Folia veris saepe 10~12 in veris ramulo conferta, crasse chartacea anguste subovato-elliptica 32.5~45.5 cm longa 14.0~20.5 cm lata, apice accisa 2.5~4.0 cm alto, basi cuneata margine integra, supra virida nitida glabra costis minute recavis glabris, subtus pallide viridula albi-tomentosa, costis conspicue elevatis cinereo-albi-tomentosis, nervis lateralibus 22~32-jugis elevatis post trito-laevibus brunneolis; petioli 3.0~6.0 cm longi flavo-virentes primo villosi post glabri vel interdum villosi; stipulae glabrae vel rare albi-pubescentes, cicatricibus stipularum longitudinem 1/2~2/3 petiolorum partes aequantibus. Folia helicalterna in ramulis aestivis usque autumnalis. Alabastra terminalia longi-ovoidea in ramulis veris 5.0~7.5 cm longa 2.5~3.5 cm diam., apice conica basin cylindrica; bractea spathacea una coriacea nitidi glabra; pedicelli grossi 2.5~4.0 cm longi et 1.0~1.5 cm diam. nitidi

viridi glabri. Flores bisexuales post folia aperti, fragrantis, purpureo-rubri, rubelli vel albi; tepala 12~13 in quoque flore, extrema 3 in florescentia reflexa, tenuiter coriacea spathuli-elliptica vel oblongi-elliptica 7.5~9.0 cm longa 4.5~5.0 cm lata apice obtusa basi truncata, interna 9~10 coriacea elliptici-spathulata vel anguste elliptici-spathulata 6.5~9.5 cm longa 1.5~4.5 cm lata apice obtusa basi cuneata ad anguste cuneata. Stamina ca. ± 260 , 1.9~2.3 cm longa, filamentis purpureo-rubris 4~6 mm longis antheris albis 1.4~1.8 cm longis introrsi-longitudinalibus dehiscentibus connectivis apice triangulate mucronatis; Gynoecium longi-ovoidium 3.5~4.5 cm longum et 1.1~2.5 cm diam.; simplici-pistillis disjunctis ca. ± 180 , glabris, stylis et stigmatibus 4~5 mm longis apice revolutis minute flavo-viridulis; 2-ovulis in quoque simplici-pistillo. Fructus oblongo-ovoideus, 12.5~16.5 cm longus et 5.0~7.0 cm diam. apice rotundatus basi rotundatus vel truncatus. Folliculi lignei monosperma vel duosperma supra breviter rostratis rostro ca. 6 mm longo.

Sichuan: Beichuan county, Guixi town, Linfeng village, Yaowang Valley, D. L. Fu 2009051901 (Holotypus, hic designatus, CAF) collected in a secondary forest, alt. 1300 m. on May. 19, 2009.

This species has been confused with *Magnolia officinalis* Rehd. & Wils. or *M. officinalis* var. *biloba* Rehd. & Wils. [3, 10-12]. But with the complete materials it becomes obvious that they are taxonomically different. *Magnolia officinalis* Rehd. & Wils. and *M. officinalis* var. *biloba* Rehd. & Wils. has the leaves often with rotund, short acuminate, obtuse, truncate, emarginate or even 3 cm bilobate apices and glaucescent lower surfaces often sparse villous (see Figure 2. left); flowers white and tepals carnosae; fruit aggregates often with truncate apices and rotundate bases often with abruptly contract and follicles often with 3 mm long beak. But the new species has the leaves with 2.5~4 cm accisus apices and viridescent lower surfaces often tomentose (see Figure 2. right); flowers purplish red, pink or white and tepals coriaceous; fruit aggregates often with rotundate apices and rotundate or truncate bases not with abruptly contract and follicles often with 6 mm long beak.



Figure 2. Lower surfaces of leaves of *M. officinalis* var. *biloba* and *Magnolia accisa*. (Left) glaucescent of *Magnolia officinalis* var. *biloba* Rehd. et Wils.; (Right) viridescent of *Magnolia accisa* D. L. Fu, H. Fu et R. M. Duan.

Magnolia accisa D. L. Fu, H. Fu et R. M. Duan is distributed in Sichuan, Hubei, Henan, Hunan, Jiangxi, Zhejiang, Fujian, Guangdong, Guangxi and Guizhou, and also cultivated as a main species for Houpo, a Chinese traditional medicine. It is also a good ornamental species for its congested leaves and beautiful, fragrant and red or white flowers.

5. Conclusion

The new species *Magnolia accisa* has at least two PEL of cpDNA, which are *matK* (...ACAAGGTGGGATTTC...) and *trnL-ndhJ* (...GTCTAGTCTATAGGAGT...). The leaves with accisus apices and viridescent lower surfaces are also particular in *Magnolia* sect. *Rytidospermum*. So the new species is supported by genomic and morphological evolutionomy based on the evolutionary continuity principle and the evolutionary particularity principle. The description of the new species will speed up the investigation of plant resources of *Magnolia* sect. *Rytidospermum* Spach in China.

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