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Two Particularly Evolutionary Loci of *mat*K of cpDNA of Genera of Magnoliaceae

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Abstract: In order to quickly identify Magnoliaceae plants and scientifically correct the misidentification of the samples of chloroplast complete genomes in the NCBI (National Center for Biotechnology Information, USA) database, total 260 samples of Magnoliaceae, 196 of Yulania Spach, 40 of Magnolia L., 19 of Michelia L., and 5 of Liriodendron L. were collected and the partial sequences of *mat*K gene were amplified and sequenced respectively. The results indicated that there are two particular loci in the partial sequences, *mat*K (...AAGNAATGATTGTATAA...CCAAAAATMGACAAGGTG...) (N = A, G; M = A, G, T) of Magnoliaceae, which can be used to identify the family because they are not possessed by other families. They are also PEL (particularly evolutionary loci) of genera of Magnoliaceae, which can be used to quickly identify the genera of the family, for all samples of the genus of Yulania Spach with the loci of *mat*K (...AAGGAATGATTGTATAA...CCAAAAATAGACAAGGTG...), all samples of Magnolia L. with *mat*K (...AAGGAATGATTGTATAA...CCAAAAATGGACAAGGTG...), all samples of Michelia L. with *mat*K (...AAGAAATGATTGTATAA...CCAAAAATGGACAAGGTG...), and all samples of Liriodendron L. with *mat*K (...AAGGAATGATTGTATAA....CCAAAAATTGACAAGGTG...). So, In four genera of Magnoliaceae, Yulania Spach, Michelia L. and Liriodendron L. have the respective PEL, and Magnolia L. can also be easily identified for having the oppositely evolutionary loci of other 3 genera. Based on the two PEL, the misidentified samples of chloroplast complete genome in NCBI were listed, which included 11 samples of Yulania Spach and 3 samples of Michelia L.. Being simple and reliable, PEL is a scientific method to identify the evolutionary taxa, which can effectively overcome the limitations of being partial and subjective in Taxonomy and Phylogeny.

Keywords: PEL (Particularly Evolutionary Loci), *mat*K, Genera, Magnoliaceae, Evolutionomy

1. Introduction

Magnoliaceae Juss. [1-14] have many primitive characters, such as the branchlets with annular stipular scars, the flowers with spathaceous perules and bracts, and androecium and gynoecium spirally arranged on the elongate receptacle, which are the most primitive taxa of Fructophyta [15] and take an extremely important role in the study of evolutionomy of fruit plants. Some of them are rare taxa, with narrow geographical distribution, and extremely high scientific research value, such as Yulania puberula D. L. Fu. Many tree species such as Michelia L. and Magnolia L. are important tree species of the evergreen broad-leaved forests from the central subtropical to the southern subtropical zone. They are also important broad-leaved timber species, which are of good quality and are used for fine furniture. Some trees with large, beautiful and aromatic flowers, are important species for garden viewing, such as Yulania denudata (Desr.)

D. L. Fu, Y. liliiflora (Desr.) D. L. Fu, Y. campbellii (Hook. f. et Thom.) D. L. Fu and Magnolia grandiflora L.. Some species are important aromatic and medicinal species, such as Magnolia officinalis Rehd. & Wils and Yulania biondii (Pamp.) D. L. Fu, have significant economic benefits. Some tree species, such as Yulania biondii (Pamp.) D. L. Fu have strong vitality, strong adaptability and developed root system, which are important tree species for barren hill greening and preservation of water and soil.

Scientific identification of taxa of Magnoliaceae is the basis for the research and utilization of the plant resources. Due to the complexity of morphological evolution, the traditional morphological taxonomy often leads to the misidentification of the taxa of Magnoliaceae even by the taxonomic experts of the family. The genomes of plants have a large number of evolutionary codes, which are scientific means for identifying the evolutionary taxa of plants. However, in the process of chloroplast genomic research of Yulania Spach, it was found that the chloroplast genomes of Magnoliaceae in the NCBI (National Center for Biotechnology Information, USA) database have also the phenomenon of misidentification of evolutionary taxa as the traditional taxonomy, which maybe is inseparable with the possible existence of the partiality and subjectivity of traditional taxonomy and phylogenic theory. How to scientifically avoid this unscientific phenomenon has always been the problem that the authors have begun to solve. It has been found that, based on the evolutionary continuity principle [15] and evolutionary particularity principle, the PEL (particularly evolutionary loci) of evolutionary taxa can be used to quickly identify the plants of Magnoliaceae and scientifically to distinguish different evolutionary taxa of Magnoliaceae.

2. Materials & Methods

2.1. Plant Materials

The leaves of 4 genera of Magnoliaceae, total 260 samples, 196 of Yulania Spach, 40 of Magnolia L., 19 of Michelia L., and 5 of Liriodendron L., were collected from Henan, Shanxi, Sichuan, Yunnan, Guizhou, Hubei, Anhui, Jiangsu, Zhejiang, Guangdong province, and Xizang Autonomous Region of China. The samples include some representative species of the family, such as Liriodendron chinense L., L. tulipifera L.; Magnolia decidua (Q. Y. Zheng) V. S. Kumar, M. delavayi Franchet, M. fordiana (Oliver) Hu, M. globosa Hook. f. & Thomson, M. grandiflora L., M. henryi Dunn, M. hodgsoni (Hook. f. & Thom.) H. Keng, M. insignis Wallich, M. kwangsiensis Figlar & Nooteboom, M. officinalis Rehd.& Wils., M. omeiensis (W. C. Cheng) Dandy, M. rostrata W. W. Smith, M. sieboldii K. Koch; Michelia alba DC., M. baillonii Finet & Gagnep, M. balansae Dandy, M. chapensis Dandy, M. figo (Lour.) Spreng, M. odora (Chun) Noot. & B. L. Chen; Yulania acuminata (L.) D. L. Fu, Y. biondii (Pamp.) D. L. Fu, Y. campbellii (Hook. f. & Thomson) D. L. Fu, Y. cylindrica (Wils.) D. L. Fu, Y. dawsoniana (Rehd.& Wils.) D. L. Fu, Y. kobus (DC.) Spach, Y. liliiflora (Desr.) D. L. Fu, Y. pendula D. L. Fu et al., Y. puberula D. L. Fu, Y. salicifolia (Sieb.& Zucc.) D. L. Fu, Y. sargentiana (Rehd. & Wils.) D. L. Fu, Y. shizhenii D. L. Fu et F. W. Li, Y. sinostellata (P. L. Chiu & Z. H. Chen) D. L. Fu, Y. sprengeri (Pamp.) D. L. Fu, Y. stellata (Sieb. & Zucc.) D. L. Fu, Y. urceolata D. L. Fu, B. H. Xiong et X. Chen, Y. viridula D. L. Fu, T. B. Zhao et G. H. Tian, Y. zenii (Cheng) D. L. Fu, etc. (see Table 1).

Table 1. *Experimental materials of Magnoliaceae in the study.*

Genus	Samples	Species	Collected place
Liriodendron	5	Liriodendron chinense, L. tulipifera	Henan, Shanxi
Magnolia	40	Magnolia accisa, M. albosericea, M. chingii, M. coco, M. decidua, M. delavayi, M. fordiana, M. globosa, M. grandiflora, M. henryi, M. hodgsoni, M. insignis, M. kwangsiensis, M. lucida, M. megaphylla, M. moto, M. nitida, M. officinalis, M. omeiensis, M. otungensis, M. pachyphylla, M. rostrata, M. sieboldii, M. tripetala, M. yunnanensis, M. yuyuanensis, etc.	Henan, Shanxi, Sichuan, Yunnan, Hubei, Jiangsu, Guangdong, Xizang
Michelia	19	Michelia alba, M. baillonii, M. balansae, M. chapensis, M. crassipes, M. elegans, M. figo, M. gioi, M. macclurei, M. martinii, M. maudiae, M. mediocris, M. odora, M. platypetala, M. wilsonii, etc.	Henan, Sichuan, Yunnan, Guangdong, Jiangsu
Yulania	196	Yulania acuminata, *Y. amoena**, *Y. anhueiensis*, Y. axilliflora, Y. baotaina, Y. biondii, Y. campbellii, *Y. cuneatifolia*, Y. cylindrica, Y. dawsoniana, Y. denudata, *Y. dimorpha*, Y. diva, *Y. elliptigemmata, Y. elliptilimba*, *Y. funiushanensis, Y. honanensis, Y. huainingensis,* Y. jigongshanensis, Y. kobus, Y. liliiflora, Y. pendula, Y. pilocarpa, Y. puberula, Y. pyriformis, Y. salicifolia, Y. sargentiana*, Y. shirenshanensis,* Y. shizhenii, Y. sinostellata, Y. sprengeri, Y. stellata, Y. urceolata, Y. viridula, *Y. wufengensis, Y. wugangensis, Y. xingyangensis,* Y. zenii, *Y. zhaoyangyulan*, etc.	Henan, Shanxi, Sichuan, Yunnan, Guizhou, Hubei, Anhui, Jiangsu, Zhejiang, Guangdong, Xizang

* The italic names of Yulania Spach are initially determined to be synonyms.

2.2. PCR Primer Design

A pairs of primers, *mat*K-Y01-F and *mat*K-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 2. *The designed primers for amplification and sequencing of partial cpDNA of Magnoliaceae.*

Primer name	Primer sequences	Length of amplification and sequencing /bp
*mat*K_Y01	F: 5'-GAGCCAAAGTTCTAGCACACG-3' R: 5'-CACTGCTGGATACAAGATGCC-3'	832

2.3. PCR Amplification

Total genomic DNA was isolated from silica-dried leaves of 260 samples of 4 genera, 196 of Yulania Spach, 40 of Magnolia L., 19 of Michelia L. and 5 of Liriodendron L., using a modified CTAB method [16]. The primers of PCR amplification are *mat*K_Y01 (see Table 2). PCR amplifications were performed in 15 μL volume containing 1μL genomic DNA, 7.5 μL 2x Es Taq MasterMix, 0.2 μL forward primer and 0.2 μL reverse primer, 6.1 μL ddH_2O, and 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 5 min final extension at 72°C. PCR reactions were carried out in T-gradient (Biometra). The amplified products were extracted and purified with the Gel Extraction Kit (OMEGA).

2.4. DNA Sequencing

Purified DNAs were sequenced using ABI 3730 XL. PCR amplifications were performed in15μL volume containing 1μL purified DNA, 7.5 μL 2x Es Taq MasterMix, 0.2 μL forward primer and 0.2 μL reverse primer (Table 2), 6.1 μL ddH_2O, and with the following cycles: 3 min initial denaturation at 95°C; and 26 cycles of 10 s at 95°C, 10 s at 50°C and 4 min at 60°C.

2.5. DNA sequence Analysis

The partial sequences of absolutely coincident sequencing using the forward primer and reverse primer were analysis. The particularly evolutionary loci could be easily found out and verified using some software or even just Microsoft Word. The contrasted chloroplast complete genomes of Magnoliaceae in NCBI see Table 3.

3. The PEL of Partial Sequences of *mat*K of Genera of Magnoliaceae

There are 620 bp absolutely coincident sequences of all samples of Magnoliaceae, using the forward primer and reverse primer of *mat*K-Y01 respectively, which can be simply compared for all samples of the family. The sequences of four selected representative species of genera of Magnoliaceae are as follow:

Yulania dawsoniana, partial sequence of *mat*K of cpDNA.

TTTTGAGGATCCACTGTGATAATGAGAAAGATTTC
TGTATATCCGCCCAAATCGATTGATAATATCAGAATCT
GACGAATCGGCCCGGACCGACTTACTAATGGGATGC
CCTGATACGTTACAAAATTTTGCTTTAGCCACTGATC
CAATCAGAGGAATAATTGGGACTAGGGTATCGAATTT
ATTAATAGAAGTATCTATTAGAAATGAATTCTCTAGCA
TTTGAATCCTTACCACCGAAGTGTTTAGTCGTACACT
TGAAAGATAGCCCAGAAAATATAAGGAATGATTGTAT
AATTGGTTTATATGGATCCTGTCCGGTAGAGACCACA
AGTAAAAATGACATTGCCAAAAATAGACAAGGTGAG
ATTTCCATTTCTTCATCAGAAGATGAGTCCCCTTTGA
AGCCAGAATGGATTTTCCTTGATATCTGACATAATGC
ATGAAAGGGTCCTTGAACAACCATAGGGTCTTCTGA
AAATCATTACGAAGCACTACTACAAGATGTTCTATTT
TTCCATAGAAATGTGTTCGCTCAAGAAAAGTTCCAG
AGGATGTTGATCGTAAATGAGAAGATTGTTTACGGA
GAAACACTAATACGGATTCACATTCATATACAT

Magnolia decidua, partial sequence of *mat*K of cpDNA

TTTTGAGGATCCACTGTGATAATGAGAAAGATTTC
TGTATATCCGCCCAAATCGATTGATAATATCAGAATCT
GACGAATCGGCCCGGACCGACTTACTAATGGGATGC
CCTGATACGTTACAAAATTTCGCTTTAGCCACTGATC
CAATCAGAGGAATAATTGGGACTAGGGTCTCGAATTT
ATTAATAGAAGTATCTATTAGAAATGAATTCTCTAGCA
TTTGAATCCTTACCACCGAAGTGTTTAGTCGTACACT
TGAAAGATAGCCCAGAAAATATAAGGAATGATTGTAT
AATTGGTTTATATGGATCCTGTCCGGTAGAGACCACA
AGTAAAAATGACATTGCCAAAAATGGACAAGGTGAG
ATTTCCATTTCTTCATCAGAAGATGAGTCCCCTTTGA
AGCCAGAATGGATTTTCCTTGATATCTGACATAATGC
ATGAAAGGGTCCTTGAACAACCATAGGGTCTTCTGA
AAATCATTACGAAGCACTACTACAAGATGTTCTATTT
TTCCATAGAAATGTGTTCGCTCAAGAAAAGTTCCAG
AGGATGTTGATCGTAAATGAGAAGATTGTTTACGGA
GAAACACTAATACGGCTTCACATTCATATACAT

Michelia maudiae, partial sequence of *mat*K of cpDNA

TTTTGAGGATCCACTGTGATAATGAGAAAGATTTC
TGTATATCCGCCCAAATCGATTGATAATATCAGAATCT
GACGAATCGGCCCGGACCGACTTACTAATGGGATGC
CCTGATACGTTACAAAATTTCGCTTTAGCCACTGATC
CAATCAGAGGAATAATTGGGACTAGGGTCTCGAATT
TCTTAATAGAAGTATCTATTAGAAATGAATTCTCTAGC
ATTTGAATCCTTACCACCGAAGTGTTTAGTCGTACAC
TTGAAAGATAGCCCAGAAAAGATAAGAAATGATTGT
ATAATTGGTTTATATGGATCCTGTCCGGTAGAGACCA
CAAGTAAAAATTACATTGCCAAAAATGGACAAGGTG
AGATTTCCATTTCTTCATCAGAAGATGAGTCCCCTTT
GAAGCCAGAATGGATTTTCCTTGATATCTGACATAAT
GCATGAAAGGGTCCTTGAACAACCATAGGGTCTTCT
GAAAATCATTACGAAGCACTACTACAAGATGTTCTAT
TTTTCCATAGAAATGTGTTCGCTCAAGAAAAGTTCCA
GAGGATGTTGATCGTAAATGAGAAGATTGTTTACGG
AGAAACACTAATACGGATTCACATTCATATACAT

Liriodendron chinense, partial sequence of *mat*K of cpDNA

TTTTGAGGATCCACTGTGATAATGAGAAAGATTTC
TGTATATCTGCCCAAATCGATTTATAATATCAGAATCT
GACGAATCGGCCCGGACCGACTTACTAATGGGATGC
CCTGATACGTTACAAAATTTCGCTTTAACCACTGATC
CAATCAGAGAAATAATTGGGACTAGGGTCTCGAATTT
ATTAATAGAAGTATCTATTAGAAATGAATTCTCTAGCA
TTTGAATCCTTACCACCGAAGTGTTTAGTCGTACACT
TGAAAGATAGCCCAGAAAATAGAAGGAATGATTGTA
TAATTGGTTTATATGGATCCTGTCCGGTCGAGACCAC
AAGTAAAAATGACATTGCCAAAAATTGACAAGGTGA
GATTTCCATTTCTTCATCAGAAGATGAGTCCCCTTTG
AAGCTAGAATGTATTTTCCTTGATATCTGACATAATGC
ATGAAAGGGTCCTTGAACAACCATAGGGTTTTCTGA

AAATCATTACGAAGCACTACTACAAGATGTTCTATTT TTCCATAGAAATGTGTTCGCTCAAGAAAAGTTCCAG AGGATGTTGATCGTAAATGAGAAGATTGTTTACGGA GAAACACTAATACGGATTCACATTCATATACAT

Compared to the samples of the same genus and other genera, it can be found that all samples of the genus of Yulania Spach with the loci of *mat*K (...AAGGAATGATTGTATAA...CCAAAAATAGACAAGG TG...) and the PEL (particularly evolutionary locus) of the genus is *mat*K (...CCAAAAATAGACAAGGTG...), all samples of the genus of Michelia L. with the loci of *mat*K (...AAGAAATGATTGTATAA...CCAAAAATGGACAAGG TG...) and the PEL of the genus is *mat*K (...AAGAAATGATTGTATAA...), all samples of the genus of Liriodendron L. with the loci of *mat*K (...AAGGAATGATTGTATAA....CCAAAAATTGACAAGG TG...) and the PEL of the genus is *mat*K (...CCAAAAATTGACAAGGTG...), all samples of the genus of Magnolia L. with the loci of *mat*K (...AAGGAATGATTGTATAA...CCAAAAATGGACAAGG TG...), which can also be easily identified for having the oppositely evolutionary loci of the PEL of other 3 genera.

Compared to the chloroplast complete genomes of close families in the NCBI database such as Winteraceae R. Br. ex Lindl., Calycanthaceae Lindl., Chloranthaceae R. Br. ex Sims, Myristicaceae R. Br., Lauraceae Juss., Hernandiaceae Blume, Annonaceae Adans., etc., it can be concluded that the two loci of the partial sequences of Magnoliaceae Juss., *mat*K (...AAGNAATGATTGTATAA...CCAAAAATMGACAAGG TG...) (N = A, G; M = A, G, T) are also the particular loci of the family of Magnoliaceae, because the other families do not possess the loci. So two loci both can be used to quickly distinguish the plants of Magnoliaceae.

Table 3. *Some misidentifications (in italic) of chloroplast complete genomes of the genera of Magnoliaceae in NCBI.*

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

4. Misidentification of Chloroplast Complete Genomes of Magnoliaceae

Mainly based on the two PEL of partial sequences of *mat*K, 39 chloroplast complete genomes of different samples of Magnoliaceae in the NCBI database were compared. The misidentified samples of chloroplast complete genomes were listed in italic and the correct names were given (see Table 3), which included 11 samples of Yulania Spach and 3 samples of Michelia L. Those are NC023234.1_Michelia cathcartii, NC035956.1_Michelia laevifolia, KY921716.1_Michelia sp., JX280391.1_Yulania acuminata, KY085894.1_Yulania biondii, JN227740.1_Yulania denudata, JN867577.1_Yulania denudata, JX280394.1_Yulania denudata, NC023242.1_Yulania diva? NC023237.1_Yulania kobus, NC037005.1_Yulania liliiflora, NC023238.1_Yulania liliiflora, JX280397.1_Yulania liliiflora, NC023240.1_Yulania salicifolia.

5. Conclusion

Based on the evolutionary continuity principle and evolutionary particularity principle, with large numbers of repeated samples of Yulania Spach and Liriodendron L. and some representative samples of Magnolia L. and Michelia L., two particularly evolutionary loci of *mat*K of cpDNA of genera of Magnoliaceae are found, and total 14 misidentified samples of chloroplast complete genomes of Magnoliaceae in NCBI were scientifically corrected. Being simple and reliable, PEL is a scientific method to identify the evolutionary taxa, which can effectively overcome the limitations of being partial and subjective in Taxonomy and Phylogeny.

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