Two Particularly Evolutionary Loci of *matK* 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 *matK* gene were amplified and sequenced respectively. The results indicated that there are two particular loci in the partial sequences, *matK* (...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 *matK* (...AAGGAATGATTGTATAA...CCAAAAATAGACAAGGTG...), all samples of Magnolia L. with *matK* (...AAGGAATGATTGTATAA...CCAAAAATGGACAAGGTG...), all samples of Michelia L. with *matK* (...AAGAAATGATTGTATAA...CCAAAAATGGACAAGGTG...), and all samples of Liriodendron L. with *matK* (...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), *matK*, 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 phylogenetic 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	<i>Liriodendron chinense</i> , <i>L. tulipifera</i>	Henan, Shanxi
Magnolia	40	<i>Magnolia accisa</i> , <i>M. albosericea</i> , <i>M. chingii</i> , <i>M. coco</i> , <i>M. decidua</i> , <i>M. delavayi</i> , <i>M. fordiana</i> , <i>M. globosa</i> , <i>M. grandiflora</i> , <i>M. henryi</i> , <i>M. hodgsoni</i> , <i>M. insignis</i> , <i>M. kwangsiensis</i> , <i>M. lucida</i> , <i>M. megaphylla</i> , <i>M. moto</i> , <i>M. nitida</i> , <i>M. officinalis</i> , <i>M. omeiensis</i> , <i>M. otungensis</i> , <i>M. pachyphylla</i> , <i>M. rostrata</i> , <i>M. sieboldii</i> , <i>M. tripetala</i> , <i>M. yunnanensis</i> , <i>M. yuyuanensis</i> , etc.	Henan, Shanxi, Sichuan, Yunnan, Hubei, Jiangsu, Guangdong, Xizang
Michelia	19	<i>Michelia alba</i> , <i>M. baillonii</i> , <i>M. balansae</i> , <i>M. chapensis</i> , <i>M. crassipes</i> , <i>M. elegans</i> , <i>M. figo</i> , <i>M. gioi</i> , <i>M. macclurei</i> , <i>M. martinii</i> , <i>M. maudiae</i> , <i>M. mediocris</i> , <i>M. odora</i> , <i>M. platypetala</i> , <i>M. wilsonii</i> , etc.	Henan, Sichuan, Yunnan, Guangdong, Jiangsu
Yulania	196	<i>Yulania acuminata</i> , <i>Y. amoena</i> *, <i>Y. anhweiensis</i> , <i>Y. axilliflora</i> , <i>Y. baotaina</i> , <i>Y. biondii</i> , <i>Y. campbellii</i> , <i>Y. cuneatifolia</i> , <i>Y. cylindrica</i> , <i>Y. dawsoniana</i> , <i>Y. denudata</i> , <i>Y. dimorpha</i> , <i>Y. diva</i> , <i>Y. elliptigemmata</i> , <i>Y. elliptilimba</i> , <i>Y. funiushanensis</i> , <i>Y. honanensis</i> , <i>Y. huainingensis</i> , <i>Y. jigongshanensis</i> , <i>Y. kobus</i> , <i>Y. liliiflora</i> , <i>Y. pendula</i> , <i>Y. pilocarpa</i> , <i>Y. puberula</i> , <i>Y. pyriformis</i> , <i>Y. salicifolia</i> , <i>Y. sargentiana</i> , <i>Y. shirenschanensis</i> , <i>Y. shizhenii</i> , <i>Y. sinostellata</i> , <i>Y. sprengeri</i> , <i>Y. stellata</i> , <i>Y. urceolata</i> , <i>Y. viridula</i> , <i>Y. wufengensis</i> , <i>Y. wugangensis</i> , <i>Y. xingyangensis</i> , <i>Y. zenii</i> , <i>Y. zhaoyangyulan</i> , 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, *matK*-Y01-F and *matK*-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
<i>matK</i> _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 *matK*_Y01 (see Table 2). PCR amplifications were performed in 15 µL volume containing 1 µL genomic DNA, 7.5 µL 2x Es Taq MasterMix, 0.2 µL forward primer and 0.2 µL reverse primer, 6.1 µL ddH₂O, 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 in 15 µL volume containing 1 µL 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 ddH₂O, 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 *matK* of Genera of Magnoliaceae

There are 620 bp absolutely coincident sequences of all samples of Magnoliaceae, using the forward primer and reverse primer of *matK*-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 *matK* of cpDNA.

TTTTGAGGATCCACTGTGATAATGAGAAAGATTTC
TGTATATCCGCCCAAATCGATTGATAATATCAGAATCT
GACGAATCGGCCCGGACCGACTTACTAATGGGATGC
CCTGATACGTTACAAAATTTGCTTTAGCCACTGATC
CAATCAGAGGAATAATTGGGACTAGGGTATCGAATTT
ATTAATAGAAGTATCTATTAGAAATGAATTCTCTAGCA
TTTGAATCCTTACCACCGAAGTGTTTAGTCGTACACT
TGAAAGATAGCCCAGAAAATATAAGGAATGATTGTAT
AATTGGTTTATATGGATCCTGTCCGGTAGAGACCACA
AGTAAAAATGACATTGCCAAAAATAGACAAGGTGAG
ATTTCCATTTCTTCATCAGAAGATGAGTCCCCTTTGA

AGCCAGAATGGATTTTCCTTGATATCTGACATAATGC
ATGAAAGGGTCCTTGAACAACCATAGGGTCTTCTGA
AAATCATTACGAAGCACTACTACAAGATGTTCTATTT
TTCCATAGAAATGTGTTTCGCTCAAGAAAAGTTCCAG
AGGATGTTGATCGTAAATGAGAAGATTGTTTACGGA
GAAACACTAATACGGATTACATTCATATACAT

Magnolia decudua, partial sequence of *matK* of cpDNA

TTTTGAGGATCCACTGTGATAATGAGAAAGATTTC
TGTATATCCGCCCAAATCGATTGATAATATCAGAATCT
GACGAATCGGCCCGGACCGACTTACTAATGGGATGC
CCTGATACGTTACAAAATTTGCTTTAGCCACTGATC
CAATCAGAGGAATAATTGGGACTAGGGTCTCGAATTT
ATTAATAGAAGTATCTATTAGAAATGAATTCTCTAGCA
TTTGAATCCTTACCACCGAAGTGTTTAGTCGTACACT
TGAAAGATAGCCCAGAAAATATAAGGAATGATTGTAT
AATTGGTTTATATGGATCCTGTCCGGTAGAGACCACA
AGTAAAAATGACATTGCCAAAAATGGACAAGGTGAG
ATTTCCATTTCTTCATCAGAAGATGAGTCCCCTTTGA
AGCCAGAATGGATTTTCCTTGATATCTGACATAATGC
ATGAAAGGGTCCTTGAACAACCATAGGGTCTTCTGA
AAATCATTACGAAGCACTACTACAAGATGTTCTATTT
TTCCATAGAAATGTGTTTCGCTCAAGAAAAGTTCCAG
AGGATGTTGATCGTAAATGAGAAGATTGTTTACGGA
GAAACACTAATACGGCTTCATTCATATACAT

Michelia maudiae, partial sequence of *matK* of cpDNA

TTTTGAGGATCCACTGTGATAATGAGAAAGATTTC
TGTATATCCGCCCAAATCGATTGATAATATCAGAATCT
GACGAATCGGCCCGGACCGACTTACTAATGGGATGC
CCTGATACGTTACAAAATTTGCTTTAGCCACTGATC
CAATCAGAGGAATAATTGGGACTAGGGTCTCGAATTT
TCTTAATAGAAGTATCTATTAGAAATGAATTCTCTAGC
ATTTGAATCCTTACCACCGAAGTGTTTAGTCGTACAC
TTGAAAGATAGCCCAGAAAAGATAAGGAATGATTGT
ATAATTGGTTTATATGGATCCTGTCCGGTAGAGACCA
CAAGTAAAAATTACATTGCCAAAAATGGACAAGGTG
AGATTTCCATTTCTTCATCAGAAGATGAGTCCCCTTT
GAAGCCAGAATGGATTTTCCTTGATATCTGACATAAT
GCATGAAAGGGTCCTTGAACAACCATAGGGTCTTCT
GAAAATCATTACGAAGCACTACTACAAGATGTTCTAT
TTTTCCATAGAAATGTGTTTCGCTCAAGAAAAGTTCCA
GAGGATGTTGATCGTAAATGAGAAGATTGTTTACGG
AGAAACACTAATACGGATTACATTCATATACAT

Liriodendron chinense, partial sequence of *matK* of cpDNA

TTTTGAGGATCCACTGTGATAATGAGAAAGATTTC
TGTATATCTGCCCAAATCGATTTATAATATCAGAATCT
GACGAATCGGCCCGGACCGACTTACTAATGGGATGC
CCTGATACGTTACAAAATTTGCTTTAACCCTGATC
CAATCAGAGAAATAATTGGGACTAGGGTCTCGAATTT
ATTAATAGAAGTATCTATTAGAAATGAATTCTCTAGCA
TTTGAATCCTTACCACCGAAGTGTTTAGTCGTACACT
TGAAAGATAGCCCAGAAAATAGAAGGAATGATTGTA
TAATTGGTTTATATGGATCCTGTCCGGTCGAGACCAC
AAGTAAAAATGACATTGCCAAAAATGGACAAGGTGA
GATTTCCATTTCTTCATCAGAAGATGAGTCCCCTTTG
AAGCTAGAATGTATTTTCCTTGATATCTGACATAATGC
ATGAAAGGGTCCTTGAACAACCATAGGGTTTTCTGA

AAATCATTACGAAGCACTACTACAAGATGTTCTATTT
TTCCATAGAAATGTGTTTCGCTCAAGAAAAGTTCCAG
AGGATGTTGATCGTAAATGAGAAGATTGTTTACGGA
GAAACACTAATACGGATTACATTCATATACAT

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 *matK* (...AAGGAATGATTGTATAA...CCAAAAATGACAAGG TG...) and the PEL (particularly evolutionary locus) of the genus is *matK* (...CCAAAAATGACAAGGTG...), all samples of the genus of *Michelia* L. with the loci of *matK* (...AAGGAATGATTGTATAA...CCAAAAATGACAAGG TG...) and the PEL of the genus is *matK* (...AAGGAATGATTGTATAA...), all samples of the genus of *Liriodendron* L. with the loci of *matK* (...AAGGAATGATTGTATAA...CCAAAAATGACAAGG TG...) and the PEL of the genus is *matK*

(...CCAAAAATGACAAGGTG...), all samples of the genus of *Magnolia* L. with the loci of *matK* (...AAGGAATGATTGTATAA...CCAAAAATGACAAGG 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., *matK* (...AAGGAATGATTGTATAA...CCAAAAATGACAAGG 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
<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 cathcartii</i>	<i>NC023234.1</i>
<i>Magnolia dealbata</i>	NC023235.1	<i>Michelia laevifolia</i>	<i>NC035956.1</i>
<i>Magnolia duclouxii</i>	NC037002.1	<i>Michelia odora</i>	NC023239.1
<i>Magnolia fordiana</i> var. <i>calcareae</i>	MF990562.1	<i>Michelia</i> sp.	<i>KY921716.1</i>
<i>Magnolia glaucifolia</i>	NC037003.1	<i>Yulania acuminata</i>	<i>JX280391.1</i>
<i>Magnolia grandiflora</i>	JN867584.1	<i>Yulania biondii</i>	<i>KY085894.1</i>
<i>Magnolia grandiflora</i>	JN867587.1	<i>Yulania denudata</i>	<i>JN227740.1</i>
<i>Magnolia grandiflora</i>	NC020318.1	<i>Yulania denudata</i>	<i>JN867577.1</i>
<i>Magnolia insignis</i>	MF990566.1	<i>Yulania denudata</i>	<i>JX280394.1</i>
<i>Magnolia kwangsiensis</i>	HM775382.1	<i>Yulania diva?</i>	<i>NC023242.1</i>
<i>Magnolia officinalis</i>	JN867579.1	<i>Yulania kobus</i>	<i>NC023237.1</i>
<i>Magnolia officinalis</i>	JN867581.1	<i>Yulania liliiflora</i>	<i>NC037005.1</i>
<i>Magnolia officinalis</i>	JN867582.1	<i>Yulania liliiflora</i>	<i>NC023238.1</i>
<i>Magnolia officinalis</i>	KY085916.1	<i>Yulania liliiflora</i>	<i>JX280397.1</i>
<i>Magnolia officinalis</i>	NC020316.1	<i>Yulania salicifolia</i>	<i>NC023240.1</i>
<i>Magnolia officinalis</i> var. <i>biloba</i>	JN867580.1		

4. Misidentification of Chloroplast Complete Genomes of Magnoliaceae

Mainly based on the two PEL of partial sequences of *matK*, 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 *matK* 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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