

Comparative chloroplast genomes of six Magnoliaceae species provides new insight into intergeneric relationship and phylogeny

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Abstract

The Magnoliaceae plants are industrial tree species with high medicinal value. We published the 6 complete chloroplast genomes of the family Magnoliaceae by using Illumina sequencing. These 6 chloroplast genomes showed a typical quadripartite structure of angiosperm and were 159,901 – 160,008 bp in size, respectively. A total of 324 microsatellite loci and 6 variable intergenic regions ($P_i > 0.01$) were identified in 6 genomes. Compare with other 5 genomes, the contraction and expansion of the IR regions were significantly different in *Manglietia grandis*. For having more thorough understanding of the intergeneric relationship of family Magnoliaceae, we also included 31 published chloroplast genomes of close relative species for phylogenetic analyses. The new insights into intergeneric relationship of family Magnoliaceae was provided based on the combination of our result and the previous morphological, phytochemical and anatomical information. We suggest that the genus *Yulania* should be separated from the genus *Michelia*, and restore systematic position of the genus *Yulania*; the genera *Paramichelia* and *Tsoongiodendron* should be merged into the genera *Michelia*; the genera *Pachylarnax* and *Parakmeria* should be combined into one genus. These findings will provide a theoretical basis for adjustment phylogenetic position of family Magnoliaceae at the molecular level.

Key Message

The new insights into intergeneric relationship of family Magnoliaceae was provided by the combination of our results on chloroplast genome and the previous morphological, phytochemical and anatomical information.

1. Introduction

Chloroplasts were Critical plant organelles that play a prominent part in photosynthesis (Saldaña et al. 2022). Chloroplast genomes (cp genome) were highly conserved because of genetic replication mechanisms of uni-parent inheritance and a relatively high level of genetic variation resulting from the low selective pressure that make it useful for revealing phylogenetic relationships (Shen et al. 2022). With the development of Illumina and assembly technologies, the cp genomes of an increasing number of species had been published (Antunes et al. 2020; Feng et al. 2020; Zhou et al. 2020). These cp genomes provided valuable information about species identification, trait improvement, genealogical geography and conservation of endangered species (Duan et al. 2020; Xie et al. 2019; Zhao et al. 2020).

The Magnoliaceae was one of the most endangered families of angiosperms, and it was listed under Class II National Protection in China (Ngoc et al. 2021). It was considered a key material indispensable for exploring the origin of angiosperms and also an important component of tropical to temperate evergreen broadleaf in deciduous broadleaf forests ecologically important (Timmis et al. 2004). The family Magnoliaceae plants are industrial tree species with high medicinal value (Huang et al., 2021). The leaves, flowers and bark of it were rich in monoterpenes and sesquiterpenes which have good anti-tumor-promoting, anti-carcinogenesis activities and treat inflammation, ulceration diseases (Sun et al. 2019).

The current methods for distinguishing the phylogenetic position of the family Magnoliaceae were mainly according to anatomical and morphological aspects (Zhu et al. 2021). The systematic classification of family Magnoliaceae has been controversial for a long time (Sima et al. 2012). A total of 12 genera were classified in the family by narrow concept of Magnoliaceae for the first time by Dandy in 1964 (Hutchinson 1964), and afterward, it was split into 16 and 18 genera according to the characters of stomatal pores at the leaf epidermis and polygamous flower respectively (Baranova et al. 2000; Liu et al. 2000). Few years later, some taxonomists considered that the family

Magnoliaceae should be divided into the 2 genera (*Magnolia* and *Michelia*) based on the main morphological traits, while the rest of the 16 genera should be combined within both (Chen et al. 2008). In summary, the controversial differences in the classification of the family Magnoliaceae mainly focus on the merging or separation of intergeneric relationships (Sima et al. 2012). In our study, we reconstructed the phylogenetic relationship among the genera *Yulania*, *Michelia*, *Paramichelia*, *Tsoongiodendron*, *Pachylarnax* and *Parakmeria* by using 37 species of the family Magnoliaceae to sequence alignment and phylogenetic analysis of cp genomes. These results provided a molecular level basis for the systematic taxonomic position of the family Magnoliaceae species.

2. Materials And Methods

2.1 Plant Materials and DNA Sequencing

The young green and disease-free leaves of 6 species for the family Magnoliaceae were collected from natural distribution areas (Table 1), and identified by *Assoc. Prof.* Jianhua Qi (College of Forestry, Southwest Forestry University). DNA extraction and sequencing was performed according to the previous study by Wang et al (2022).

2.2 Chloroplast genome assembly and annotation

The cp genome sequences of *Manglietia dandyi* (MF990567) were used as a reference sequence to assemble the 6 cp genomes of the family Magnoliaceae using MEGA5.1 (Kumar et al., 2016). The 6 cp genomes annotation was performed via the Genious 8.1.3 with Sequences of other closely related species. The genomes annotation was the same as Zheng et al (2020). The sequences of 6 cp genomes were deposited in GenBank NCBI (MW415418, MW415419, MW415420, MW415421, MW415416, MW415417). The cp genome map was draw by software OGDRAW37 (Tillich et al. 2017).

2.3 Sequence divergence, genome comparison, and single sequence repeats analysis

The 6 cp genome sequences of the family Magnoliaceae were performed using VISualization Tool in Shuffle-LAGAN mode for Alignments (Mu et al. 2020). We used the software DnaSPv. 5.0 in set the parameter to window length size 600 bp and the distance between each locus to 200 bp to measure nucleotide diversity (π) (Librado et al. 2009). The 6 cp genome sequences were uploaded to the online IRscope software for visualization of their IR/SC boundaries using the .gb format (Huang et al. 2021). The simple sequence repeat (SSR) markers were searched by surveying six genomic sequences of the family Magnoliaceae using MISA v1.0 (Thiel et al. 2003).

2.4 Phylogenetic Analysis

Sequence alignment were performed using the newly assembled 6 cp genomes, and 31 published close relative cp genomes which downloaded from NCBI of the family Magnoliaceae (Table S1). The similar procedure of Phylogenetic analyses was performed according to the previous study by Tamura et al (2013).

3. Results

3.1 Characteristics of the six cp genomes

The 6 cp genomes of the family Magnoliaceae are similar to other angiosperms (Table 2 and Fig. 1). The complete cp genome of its form between 159,901 – 160,008 bp in length, exhibiting a classic four-partition structure with an SSC region (18,800 – 18,803 bp), LSC region (87,753 – 88,534 bp) and two IR regions (26,207-26602bp). Six cp

genomes contained 131 genes (86 protein-coding genes, 37 tRNA genes, 8 rRNA genes), which include 44 photosynthesis genes, 58 translation related genes, and 11 other genes (Table S2).

3.2 Comparative genomic, IR expansion and contraction, and SSR analysis

For investigating the levels of sequence polymorphism, the 6 cp genomes among the family Magnoliaceae species were compared (Fig. 2). The result showed that the structures, orders and contents of these 6 cp genomes were all conserved. The Pi values of these 6 genomes ranged from 0 to 0.0153. Although aligned sequences showed relative low divergences, but some hotspot regions with high variation were also identified. The variable regions with Pi exceeding 0.01 in the six cp genomes were *ndhF-trnL-UAG*, *ndhD-ndhE*, *rpl32-trnL-UAG*, *petG-psaJ*, *psaC-ndhA*, *trnF-GAA-ndhK* (Fig. 3).

The 6 CP genomes were compared for IR/LSC and IR/SSC Boundary structure (Fig. 4). The results exhibited that the IR boundaries of the cp genomes of the 6 Magnoliaceae species were comparatively conserved. Only the *rpl2* gene of *Magnolia grandis* expanded to the LSC region with an expansion length of 308 bp, and the *rpl2* genes of the remaining 5 species were in the IRb region. Among then, the distribution of genes on the IRb/SSC and SSC/IRa boundaries of its was similar for the *ndhF* and *ycf1* genes, and the length of the *ycf1* gene on the SSC/IRa boundary ranged from 5558–5594 bp, all of which were pseudogenes. The characteristics of SSRs in 6 cp genomes were analyzed, a total of 324 repeats were certified in 6 genomes, and most SSRs included of the A/T rather than the G/C motif (Fig. 5b and Table S3). Mono-nucleotide repeats were the highest abundance of SSR in all species, penta-nucleotide repeats were the least abundant. The analysis of long repeats in 6 species revealed more forward and palindromic repeats than reverse and complementary repeats (Fig. 5c and 5d).

3.3 Phylogenetic Analysis

Phylogenetic analysis of 6 species was performed by using the ML method (Fig. 6), the results showed that the most of nodes had 100% bootstrap values. The phylogenetic tree showed that the 37 species of the family Magnoliaceae can be broadly divided into 5 clusters. Among then, the genera *Yulania*, *Paramichelia*, *Michelia*, *Tsoongiodendron*, *Alcimandra*, *Pachylarnax*, *Parakmeria*, and *Woonyoungia* clustered into one group; The *Manglietia*, *Talauma*, *Liriodendron* genera each clustered one group; the genera *Illicium*, *Kadsura*, and *Schisandra* clustered into one group. In our phylogenetic tree, the sister relationship of *Pachylarnax* and *Parakmeria*, and *Michelia* was closely related to the *Paramichelia* and *Tsoongiodendron* was powerfully supported. In addition, we also compared the views of Flora Reipublicae Popularis Sinicae (FRPS) and Flora of China (FOC) of plant classification in family Magnoliaceae, which founded the taxonomic status of the genera *Paramichelia*, *Tsoongiodendron*, *Pachylarnax*, and *Parakmeria* has changed.

4. Discussion

We had first time published the 6 cp genomes of the family Magnoliaceae species, including 4 *Manglietia* species and 2 *Yulania* species. These cp genomes were consistent with the characteristics of most angiosperm species (Han et al. 2022), and didn't differ significantly from each other in structure and length (159,901 – 160,008 bp). In addition, we found that the mean contents of AT and GC of these 6 cp genomes were 61.7% and 39.3%. In the genome, the higher the AT content, the lower the DNA density, and the sequences were more prone to denaturation and mutation (Li et al. 2022). Therefore, we speculated that the 6 cp sequences of the family Magnoliaceae species

were somewhat mutagenic and their chloroplast gene sequences might be more prone to variation than those of other species.

The results of IR boundary analysis showed that the contraction of the IR region (26,207 bp) was the most pronounced in *Manglietia grandis*, as the expansion of the *rpl2* gene in its IR to LSC region by 308 bp, while the *rpl2* genes of the other 5 species were located intact in the IR region. It indicated that the boundary change of LSC /IR is the dominant factor affecting the expansion and contraction of the cp genome IR region of *Manglietia grandis*. This result is similar to the expansion of the family Rosaceae in IR region (Du et al. 2020), but different from the contraction of family Zingiberaceae and Arecaceae (Gao et al. 2019; Santana et al. 2018). It indicated that different species have evolved under the influence of different factors resulting in different degrees of expansion and contraction of IR/SC boundaries, thus showing the diversity of genome length and boundaries (Dong et al. 2012).

The variation of SSRs in cp genomes has a greater taxonomic distance than nuclear and mitochondrial genomes, it was widely used in studies of genetic diversity and germplasm resources of plant populations (Alzahrani et al. 2021). We identified 324 SSRs in cp genomes of six Magnoliaceae, most of which were detected mononucleotide repeats were composed of A/T. These SSRs can be used to develop microsatellite markers for genetic diversity and evolution analysis (Saina et al. 2021). We also screened a total of 7 highly variable regions by nucleotide diversity analysis. Among them, 4 of them are located in the LSC region and 3 in the SSC region. It indicates that the LSC and SSC regions of these 6 Magnoliaceae have high nucleotide variability, these highly variable regions can be used as potential polymorphic molecular markers for evolutionary studies (Menezes et al. 2018).

These 6 cp genome sequences were phylogenetically analyzed with their 31 relatives, the results showed that the most of the nodes had high bootstrap values in our phylogenetic tree. Meanwhile, the result of phylogenetic analysis for monophyletic genus was consistent with previous studies, it indicated that the phylogenetic tree in this study is reliable (Zhu et al. 2021). The plants of the family Magnoliaceae can be divided into two classification situations of broad and narrow concepts due to the morphological differences and by Engels and Hutchinson system (Sima et al. 2012). The broader concept of Magnoliaceae included the genera *Illicium*, *Schisandra* and *Kadsura* etc (Liu 1984). The narrow concept of Magnoliaceae only included the *SubFam* Magnolioideae and the genus *Liriodendron* (Hutchinson 1921). The phylogenetic tree of this study showed that *Liriodendron* is most closely linked to the *SubFam* Magnolioideae, and the genus *Illicium* is sister group to the genera *Schisandra* and *Kadsura*. This result was consistent with morphological classification of Magnoliidae in broader concept (Zhu et al. 2021).

The aim of this study is to clear the intergeneric relationships within the family Magnolioideae, as the systematic classification of Magnoliaceae has been controversial for a long time (Sima et al. 2012). It has previously been demonstrated that the genus *Yulania* is included in the genus *Michelia* based on the characteristics of pre-branching (Figlar 2000). However, the content of volatile oils obtained from flower and the pit vessel characteristics of wood of these two genera was found significantly different in subsequent studies (Canright 1955; Fu et al. 2005). Especially the reproductive isolation was discovered between the two genera that the genus *Yulania* was thus separated from the genus *Michelia* (Gong et al. 2001). This result was consistent with phylogenetic analysis in our study. Furthermore, it was consistent with previous conclusions inferred from *Matk* and *ndhF* sequences (Kim 2001). The similar results were reported in the family Bupleurum that the new insights into the phylogenetic status was provided by combining the information of cp genomes and morphological characteristics of fruits and leaves (Alzahrani et al. 2021; Xie et al. 2021). We thus advised that the genus *Yulania* should be separated from the genus *Michelia*, and restore systematic position of the genus *Yulania*.

In the present study, the genera *Paramichelia*, *Tsoongiodendron* and *Michelia* are clustered in one clade, it was identical to previous result of phylogenetic analysis based on molecular markers (Wang et al. 2020). It suggested that the closely relationship of these 3 genera was strongly supported. It has been argued that the genera *Paramichelia* and *Tsoongiodendron* should be separated from the genus *Michelia* according to the different character of the ripe fruit carpels (Liu et al. 2000). This tiny difference is considered by traditional taxonomists to be the result of parallel evolution (Xu and Wu 1995). In other words, these three genera come from the same ancestor and therefore show the same trend in evolution (Jin et al. 1999). Based all evidence, this opinion of the genera *Paramichelia* and *Tsoongiodendron* should be merged into the genus *Michelia* was shared by us. Similarly, the recent revision by Flora of China to adjust the genus *Paramichelia* and *Tsoongiodendron* genus-level status in the systematic position (Wu 2022).

The genus *Pachylarnax* was established based on the characteristic of polygamous flower (Hu and Zheng 1951), and it is considered to be more closely related to the genus *Manglietia* (Wu 1991). This argument was not consistent with the result of phylogenetic analysis in our study, we suggested that compare with genus *Manglietia* the genus *Parakmeria* is more closely related to the genus *Pachylarnax*. Meanwhile, this view also is consistent with results of the phylogenetic analysis by using B-class MADS-box gene (Xiong et al. 2018). Additionally, the genus *Pachylarnax* and *Parakmeria* both have the recognized high taxonomic value characteristic of curling young leaves in the family Magnoliaceae (Sima et al. 2001). We thus recommend that the genera *Pachylarnax* and *Parakmeria* should be combined into one genus. Furthermore, based on all results of phylogenetic relationships we compared the two classification views and founded the FRPS can locate the species attribution more precisely than FOC in the Magnoliaceae.

5. Conclusions

This study reported the complete cp genome sequence of 6 Magnoliaceae: *M.crassipes*, *M.grandis*, *M.hookeri*, *M.ventii* and *Y.praecocissima*, *Y.soulangeana*. New insights into intergeneric relationships in the Magnoliidae family are provided by combining previous studies, we recommend that the genus *Yulania* should be separated from the genus *Michelia*, and restore systematic position of the genus *Yulania*; the genera *Paramichelia* and *Tsoongiodendron* should be merged into the genera *Michelia*; the genera *Pachylarnax* and *Parakmeria* should be combined into one genus. These results provided a theoretical foundation for the phylogenetic position of family Magnoliaceae at the molecular level.

Declarations

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CRedit Authorship contribution statement: All authors contributed to the study conception and design. Lin Yang: Conceptualization, Methodology, Formal analysis, Writing-original draft. Jinhong Tian: Conceptualization, Methodology, Formal analysis, Writing-original draft. Liu Xu: Validation, Investigation, Software. Xueli Zhao: Methodology, Investigation. Yuyang Song: Writing-editing, Formal analysis. Dawei Wang: Writing-review & editing, Formal analysis, Funding acquisition. All authors read and approved the manuscript.

Data Availability: The datasets generated during and/or analysed during the current study are available in the NCBI repository, [<https://www.ncbi.nlm.nih.gov/>]. The datasets generated during and/or analysed during the current study

are available from the corresponding author on reasonable request. All data generated or analysed during this study are included in this published article [and its supplementary information files].

Compliance with ethical standards

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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Tables

Table 1: The sampling area and six species information of the family Magnoliaceae

Genus	Species	Protection grade	sampling area	Longitude/ Latitude
<i>Manglietia</i>	<i>Manglietia crassipes</i>	☐	GuanXi, China	109°50'E/ 23°40'N
	<i>Manglietia grandis</i>	☐	YunNan, China	104°33'E/ 22°48'N
	<i>Manglietia hookeri</i>	☐	YunNan, China	99°55'E/ 21°10'N
	<i>Manglietia ventii</i>	☐	YunNan, China	102°10'E/ 24°23'N
<i>Yulania</i>	<i>Yulania praecocissima</i>	-	YunNan, China	102°10'E/ 24°23'N
	<i>Yulania soulangeana</i>	-	YunNan, China	102°10'E/ 24°23'N

Notes: ☐: Critically Endangered, ☐: Endangered, ☐: Vulnerable

Table 2 Summary of chloroplast genome characteristics in six Magnoliaceae chloroplast genomes

Species	<i>Manglietia crassipes</i>	<i>Manglietia grandis</i>	<i>Manglietia hookeri</i>	<i>Manglietia ventii</i>	<i>Yulania praecocissima</i>	<i>Yulania soulangeana</i>
Total length (bp)	159901	160008	159905	159950	159778	159778
LSC length (bp)	87959	88534	87973	88008	87840	87753
SSC length (bp)	18800	18803	18776	18800	18734	18734
IR length (bp)	26571	26207	26578	26571	26602	26602
Overall GC content (%)	39.3	39.3	39.3	39.3	39.3	39.3
Total gene number	131	131	131	131	131	131
GenBank accession	MW415418	MW415419	MW415420	MW415421	MW415416	MW415417

Figures

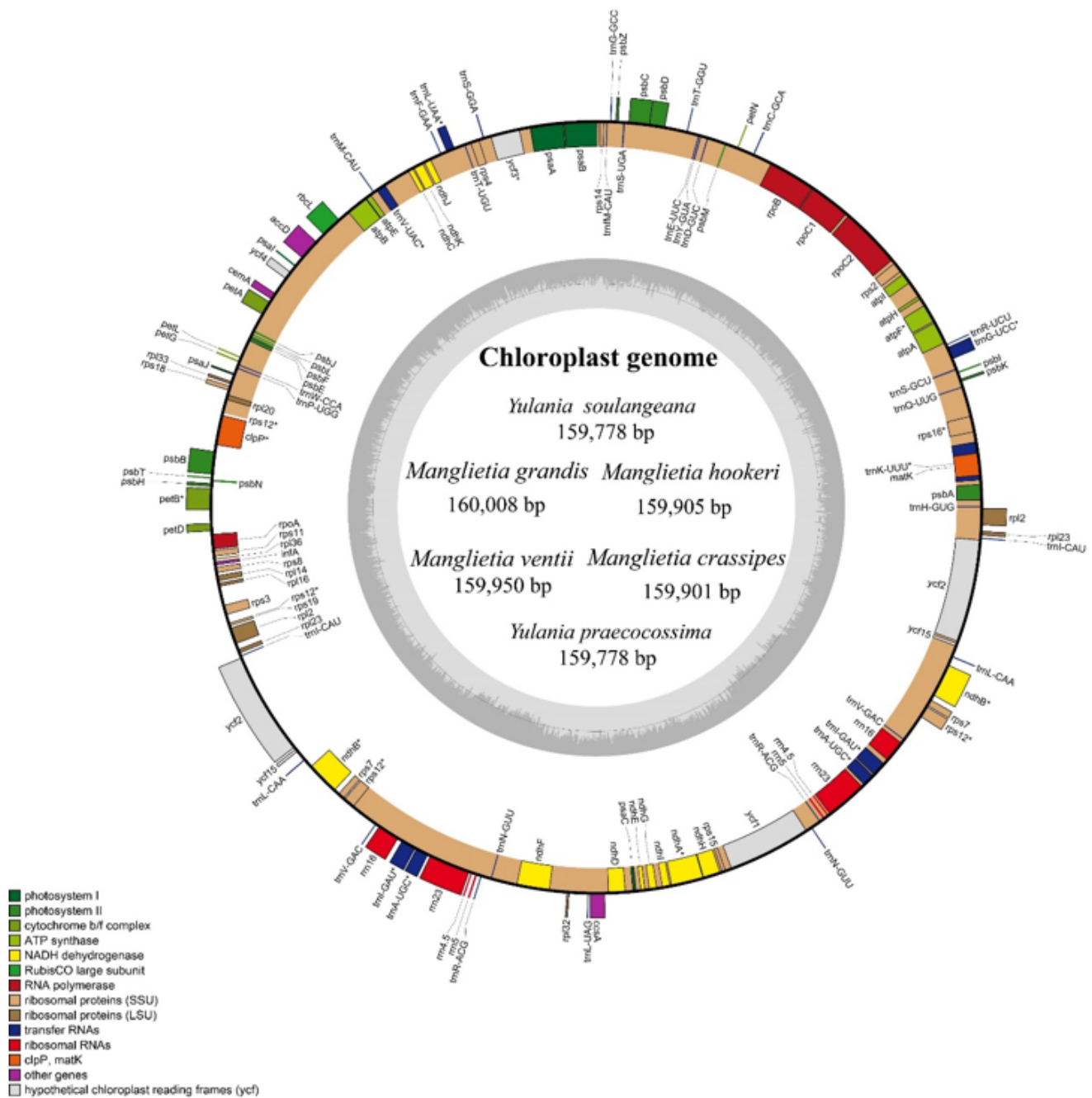


Figure 1

The six chloroplast genomes structural map of the family Magnoliaceae species

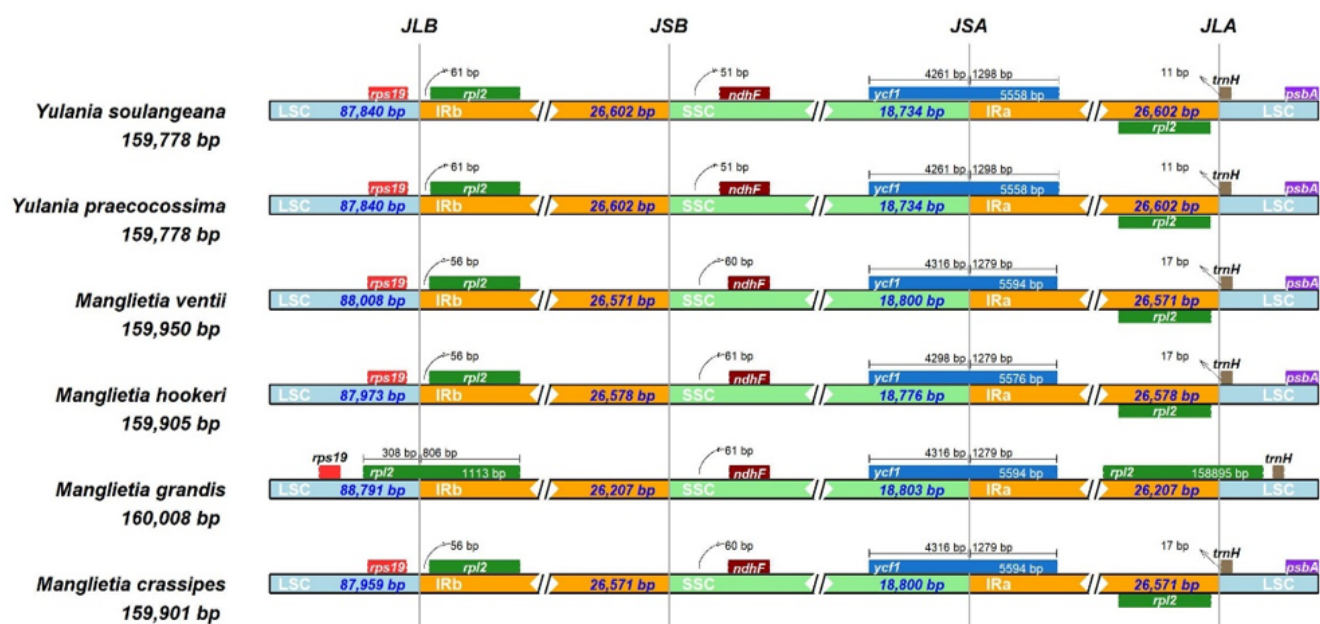


Figure 4

Comparison of the border regions among the six chloroplast genomes of the family Magnoliaceae.

Note: Different genes are denoted by colored boxes. The gaps between the genes and the boundaries are indicated by the base lengths (bp).

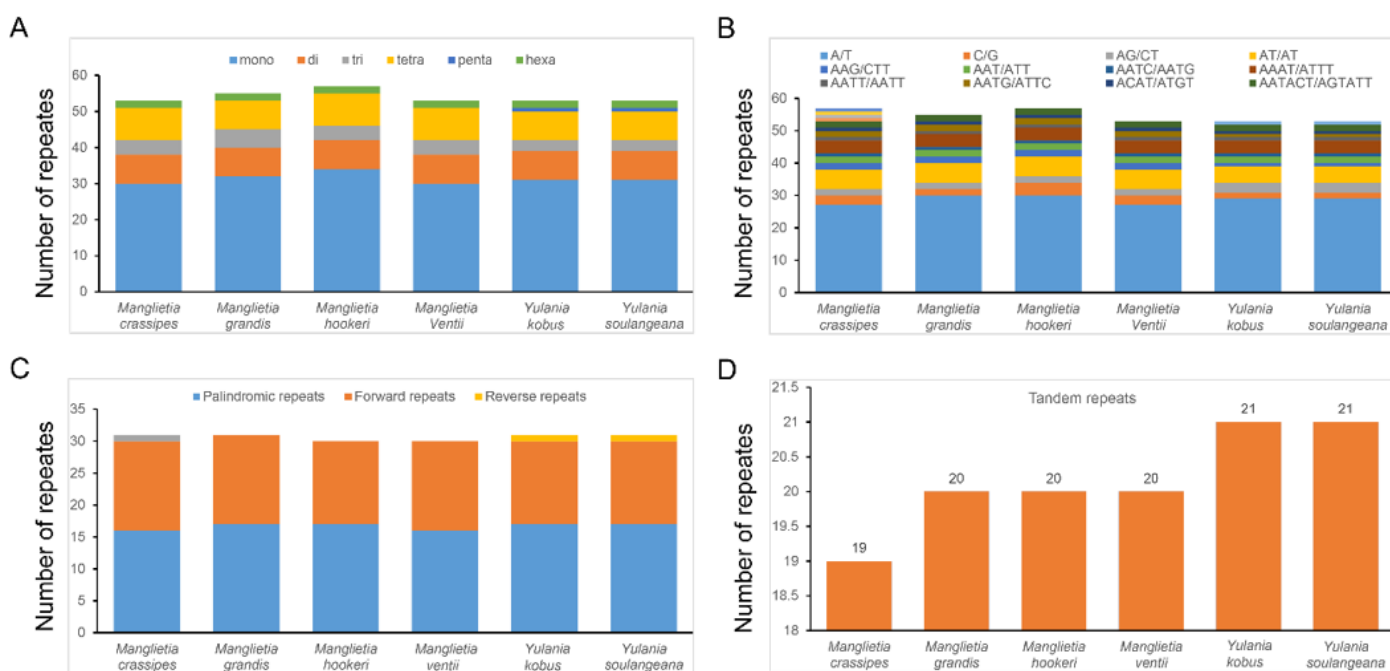


Figure 5

Comparison of repeats in six species of the family Magnoliaceae.

Note: A. The type frequency of different SSR types. B. The type frequency of SSR motifs in different repeat class types. C. The type frequency of different repeat types. D. The type frequency of dispersed repeat sequences.

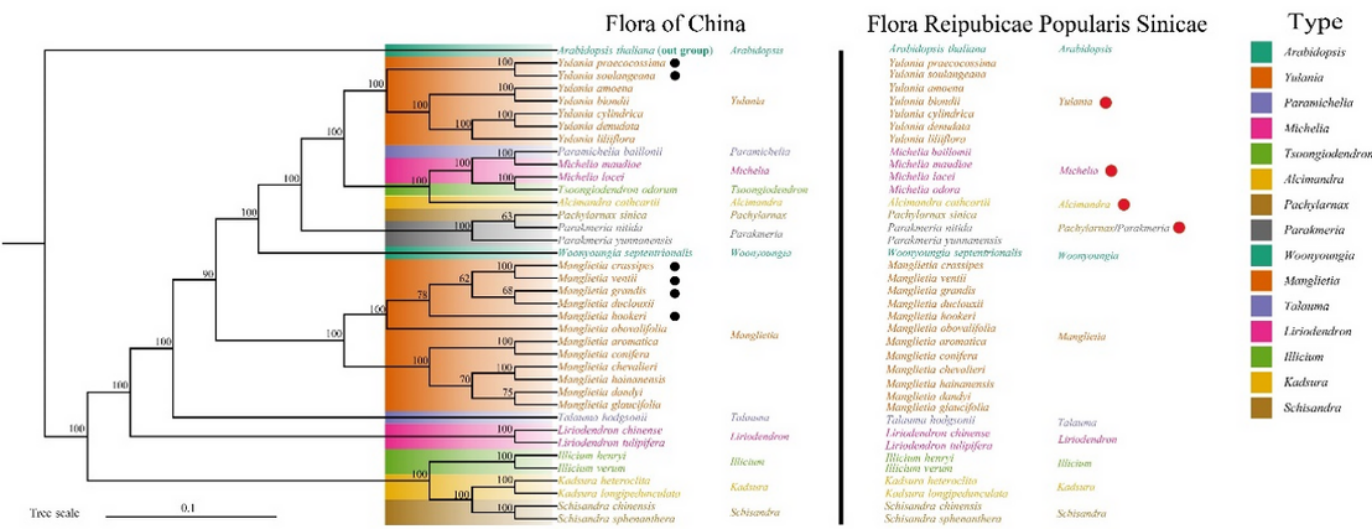


Figure 6

Maximum likelihood (ML) phylogenetic tree based on 37 complete chloroplast genomes of the family Magnoliaceae.

Note: black circles indicate the six species of Magnoliaceae in this study. The red circles indicate the genera whose phylogenetic position was discussed in this study

Supplementary Files

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- [SupplementaryTable.docx](#)