

# Analysis of *Michelia* Magnoliaceae chloroplast genomes: Provide New Insights into Intergeneric and Intrageneric Relationships

**Junxia Shu**

Southwest Forestry University

**Ruiling Wang**

Southwest Forestry University

**Lin Yang**

Southwest Forestry University

**Xi Xia**

Southwest Forestry University

**Dawei Wang** (✉ [wangdawei@swfu.edu.cn](mailto:wangdawei@swfu.edu.cn))

Southwest Forestry University

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## Research Article

**Keywords:** Magnoliaceae, Phylogeny, Chloroplast genome, Classification, Intergeneric relationships

**Posted Date:** January 6th, 2023

**DOI:** <https://doi.org/10.21203/rs.3.rs-2381194/v1>

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# Abstract

**Background:** Among Magnoliaceae, the genus *Michelia* is the second largest and most evolved, which is essential for studying angiosperm origins, development, and evolution. Given the taxonomic confusion among *Magnolia*, *Manglietia*, and *Michelia*, this study provides recommendations for some of the taxonomically confused intergeneric and intrageneric relationships based on chloroplast genome analysis of six *Michelia* plants.

**Results:** The six chloroplast genomes were set up to range in size from 159,703 bp to 160,026 bp. It had abundant simple sequence repeat sites (136-142), and six highly variable regions (*rpl32-trnL*, *ndhC-trnV*, *petA-psbL*, *ccsA-ndhD*, *rps15-ycf2*, *trnN-ndhF*) were detected ( $p > 0.005$ ). Compared with five other genomes, a shift of the *rpl2* gene from the IR region to the LSC region was found only in *Michelia doltsopa*. In this study, 26 species of cp genome were used, and two trees establish methods were used for phylogenetic analysis of Magnoliaceae. The results showed that the two phylogenetic trees had the same topological structure. The six genera *Michelia* were first grouped into a cluster, which was further separated into two sister clades. There was a clustering of *Magnolia sieboldii* and *Manglietia insignis* within the genus *Michelia*, while the genus *Yulania* tended to be more close relatedness to those species.

**Conclusion:** Combining the results of previous morphological, sporological, and molecular marker studies, we offer a new taxonomic view to the classification of Magnoliaceae: there should be a merger between *Manglietia insignis*, *Paramichelia baillonii*, and *Tsoongiodendron odor* in the genus *Michelia*; *Parakmeria yunnanensis* and *Pachylarnax sinica* should be combined into the genus *Pachylarnax*. This study provides six genomic resources of *Michelia* that will be beneficial for the evolutionary study and the phylogenetic construction of Magnoliaceae.

## Background

The Magnoliaceae family is recognized as a representative species for exploring the origin and evolution of angiosperms [1, 2]. It is an important component of temperate forests and an important ornamental, medicinal and aromatic raw material species [3-5]. The Magnoliaceae family's genus *Michelia* is the second largest and most evolved [6, 7]. The species of this genus are beautifully shaped and pleasantly fragrant and are widely used in landscaping [1, 4]. In medicine and chemicals, among the volatile chemical constituents contained in the leaves and flowers of the genus *Michelia*, some phenolic substances have been shown to have inhibitory effects on certain pathogens, while other alcohols are used as fragrance agents in perfumes and cosmetics [8-11].

Chloroplasts are a plastid with energy conversion commonly found in plants and some algae [12]. The cp genome is one of the components of the cytoplasmic genome of higher plants [13]. Compared with the nuclear genome, it has the advantages of moderate sequence length, direct gene homology, and a moderate rate of evolution, so it can be used in studies revealing the origin of species, and evolution [14, 15]. With the progress of sequencing technologies, a large number of cp genomes have been sequenced and assembled using high-throughput sequencing, and the phylogeny, species identification of various species have been evaluated based on these cp genomes [16-19].

Taxonomists classify Magnoliaceae into 2-17 genera and 240-300 species by different classification methods, with approximately 80 species in the genus *Michelia* [3, 7, 20]. There has been much disagreement in the classification of the family, and these disagreements exist mainly in the intergeneric relationships of the genera *Magnolia*, *Manglietia*, and *Michelia* and the interspecific relationships within the genus [3, 20-23]. Given the taxonomic confusion within the Magnoliaceae, six cp genomes of the *Michelia* plant were sequenced, assembled, annotated, and 20 Magnoliaceae species registered in GenBank were added for phylogenetic analysis, which will provide taxonomic information for some controversial species.

## Results

## Characteristics of the Cp Genome

The six cp genomes are typical of a cyclic tetrameric structure containing three regions (LSC, SSC, and IR) and the full lengths ranged from 159,703bp to 160,026 bp (Fig. 1) The value of the total GC content of these cp genomes varied from 39.2%-39.3%, and the total SSR loci were 136-142 (Table 1, Table S1). The cp genome of six *Michelia* shares a similar genomic structure, in which add up to 130 genes was annotated, with 110 unique genes (76 protein-coding genes, 30 transfer RNA genes, and 4 ribosomal RNA genes). Ten of the protein-coding genes and seven of the tRNA genes contain introns; five of the tRNA genes and six of the protein-coding genes have two copies, the *trnM-CAU* gene has three copies, and all of the rRNA genes have two copies.

**Table 1.** The cp genomic characteristics of the genus *Michelia*.

### Codon Usage and Amino Acid Abundance

Codon usage bias in these six species was analyzed using relative synonymous codon usage (RSCU). The highest values of RSCU for these codons were AGA (1.83-1.85 with arginine), followed by GCU (1.82-1.84 with alanine); abundant amino acids 10.12% -10.22% (2173-2655) encode leucine, 1.13% -1.16% (244-303) encode cysteine the most and least (Table S2). In the 33 preferred codons (RSCU > 1), 16 ended with U, 13 ended with A, 3 ended with G, and 1 ended with C (Fig. 2).

### IR Expansion and Contraction

The length of the IR region ranges from 25,037 bp-26,575 bp, and there is no obvious expansion or contraction except for *M. doltsopa* (Fig. 3). Significant variation was observed in *M. doltsopa* the *rpl2* gene was completely transferred to the LSC, while it was located in the IR region in the other five species. In addition, the *ndhF* gene of *M. doltsopa* showed significant contractile in the SSC region compared to the other five species. The *trnH*, *ndhF*, *ycf1*, *rpl2*, and *rps19* are situated at the junction of the LSC/IR and SSC/IR boundaries. The SSC-IRA boundary is embedded in *ycf1* with a length of 5,560bp to 5,576bp.

### Repeat Sequences and SSRs Identification

Six *Michelia* cp genomes were found to have 136 to 142 SSRs, the type of penta (AAAAT/ATTTT) has the lowest number with only 1 or none, and the type of mono (A/T, C/G) has the most number with 113-118. Most SSRs (83.10%-84.56%) were mononucleotide A/T repeats and dinucleotide AT/AT (Fig. 4A and 4B; Table S3 and S4). Furthermore, we identified 29 to 33 repeat sequences in two categories, and the repeat sequences ranged from 22 to 28 (Fig. 4C and 4D, and Table S5 and S6).

### Sequence Divergence Analysis

The result of Sequence polymorphic levels analysis showed that the six sequences were relatively conserved, the genetic order was the same, and the non-coding region variation was more abundant (Fig.5). The Pi values of these six genomes ranged from 0 to 0.01022, the maximum Pi value in the non-coding area was 0.01022 and 0.003 in the coding area. Among the hotspot regions, *petA-psbL* has a Pi value that overtops 0.01. Other hotspot regions include *ndhC-trnV*, *trnN-ndhF*, *rpl32-trnL*, *ccsA-ndhD*, and *rps15-ycf2* (Pi > 0.005) (Fig.6, Table S7).

### Phylogenetic Analysis

In the BI tree, the support rate of 22 out of 24 nodes is 100% (Fig. 7), while in the ML tree, the support rate of 20 out of 24 nodes is more than 90% (Figure S1). This indicates high reliability for both ML and BI trees. In the clade of genus *Michelia* (clade A), *M. macclurei*, *M. cavaleriei*, and *M. hypolampra* are clustered in branch A1, *M. fulva*, *M. lacei*, *M. doltsopa* are clustered in branch A2. *Magnolia siedoldii*, *Manglietia insignis*, *Paramichelia baillonii*, and *Tsoongiodendron odorum* are also clustered in branch A, *Yulania* and *Michelia* are closely related.

# Discussion

## Characteristics of the cp genome

The six cp genomes of the genus *Michelia* ranged in size from 159,703 to 160,026 bp, with an average GC content of 39.26% and codon usage mostly ending in A/U, similar to the other cp genomes of Magnoliaceae, indicating that higher plants have a stable genome structure [5, 24-26]. In this study, the transfer of the gene *rpl2* to the LSC region resulted in a shorter IR region and a longer LSC region in *M. doltsopa*, and this gene transfer is also present in a variety of angiosperms [25, 27-29]. This suggested that the conservation of cp genome sequences in higher plants coexists with IR boundary diversity, which in turn ensures genome diversity [14, 30-32].

SSR markers have a high mutation rate, are easy to genotype, and are commonly used as molecular markers for many plants [33, 34]. Among the six species, the percentage of A/T was the highest of all repeat types, which is similar to higher plants [35, 36]. It suggested that this is because it is easier to transform A to T than G to C [37]. In total, six hotspots with high variability were identified in the regions of SSC and LSC regions, which are often used to study taxonomic or evolutionary drift in plant populations [25, 38, 39]. The high-variance regions identified in this study can be used as references for molecular markers, species identification, and population genetics studies of Magnoliaceae [24, 40].

## Phylogenetic relationship

There is considerable controversy about the classification of Magnoliaceae, in which there is an obvious intergeneric crossover between the genera *Michelia*, *Manglietia*, and *Magnolia*, leading to confusion in intergeneric classification [20, 21, 23, 41]. In the traditional morphological classification, *Manglietia insignis* partitions from the genus *Michelia* by the degree of curvature of the dorsal wall of the epidermis of the leaf and the number of ovules in the carpels [20, 42, 43]. However, in studies at the molecular level, it was found to be more closely related to *Michelia laevifolia* and *Tsoongiodendron odorum* [31, 44, 45]. Genotypes are more stable than phenotypes, therefore in the case of divergent phenotypic and molecular-based taxonomic results, the molecular level is more accurate [16, 44, 46, 47]. In the present study, the relatedness was close that *M. insignis* to the genus *Michelia*, so this study concluded that *M. insignis* can be considered for inclusion in the genus *Michelia*.

Previous studies distinguished the two genera based on the dioeciousness of the male and bisexual flowers (*Parakmeria yunnanensis*) and the distinctive character of the several whorls of carpels arranged on the receptacle (*Pachylarnax sinica*) [3, 20, 41, 48]. However, it was argued that unisexual male flowers are the result of highly immature staminodes, so flower sex is not a sufficient basis for dividing the genus, and it was, therefore, suggested that the genus *Parakmeria* should be included in the genus *Magnolia* [7, 47, 49]. In the present study, *P. yunnanensis* and *P. sinica* are more closely related. Combined with the morphological characteristics of both genera in which the seed's inner epidermal sympodial zone is tubular [50], the young leaves are erect in the bud [51], and the results of comparative cp genome analysis [25, 31]. Therefore, our study agreed that the genus *Parakmeria* should be merged into the genus *Pachylarnax* [52].

In 1984, taxonomists classified the two genera based on the carpel of mature fruit connate (*Paramichelia baillonii*) and carpel of mature fruit free (*Tsoongiodendron odorum*) [20, 41]. Since 1993, it was suggested that fruit carpels connate might be the result of the parallel evolution of carpels from free to connate, so this characteristic feature could not be used as a basis for dividing the genera [7, 21]. Meanwhile, these two genera are similar to the genus *Michelia* in the pollen surface characteristics and size characteristics [53], young leaf coiling characteristics [51], and the height, width, and density of wood rays of wood anatomy [54]. Based on the results of the study of chemical composition, including some alkaloids, terpenoids indicated that they could be included in the genus *Michelia* [8, 9, 55]. The results of the plastid genome sequence indicate that it may form a group and a clade with other taxa of the genus *Michelia* [56-58]. In a word, we suggest that *P. baillonii* and *T. odorum* should be merged into the genus *Michelia*.

## Conclusions

In this study, the cp genomes of six new and 20 reported species of Magnoliaceae were used to explore the phylogenetic relationships of Magnoliaceae. The results of this study combined with previous studies provide a new viewpoint on the genus relationship among Magnoliaceae. There should be a merger between *Manglietia insignis*, *Paramichelia baillonii*, and *Tsoongiodendron odorum* in the genus *Michelia*; *Parakmeria yunnanensis* and *Pachylarnax sinica* could include the genus *Pachylarnax*. The result will provide insight into the taxonomy and evolution of the Magnoliaceae in the future.

## Materials and Methods

### Plant Material

We collected six species of *Michelia* (*Michelia cavaleriei*, *Michelia doltsopa*, *Michelia fulva*, *Michelia hypolampra*, *Michelia lacei*, *Michelia macclurei*) from the Kunming Institute of Botany of the Chinese Academy of Sciences (102°44', 25°8'). Plant samples were identified by Associate Professor Jianhua Qi (Southwest Forestry University) and deposited in the herbarium of Southwest Forestry University (collection numbers: WangDW-2019-034 to WangDW-2019-039).

### DNA Extraction, Sequencing, Assembly, and Annotation

DNA extraction and sequencing were performed concerning the previous study [5] and the cp genome of *Michelia alba* (NCBI accession MF990568.1) was used as a reference for these clean data [59]. Geneious 8.1.3 was used to assemble six cp genomes, and DOGMA was used to annotate the genomes [60].

### Phylogenetic Analysis

Two methods (ML and BI) were used to construct phylogenetic trees concerning Shen [5]. The ML and BI phylogenetic trees were framed using Mega 6.0 and MrBayes v3.2.6, respectively. 20 other species from 10 genera of Magnoliaceae were added, with the *Liriodendron chinense* and *Liriodendron tulipifera* as an outgroup (Table S8).

### Other Analysis

With reference to previous studies, we analyzed the cp genome of six *Michelia* species [5, 52], including genome structure, sequence divergence, genome comparison, SSRs, and codon usage analysis. The value of RSCU and codon usage were determined with the software of Condon W1.4.2 [61]. Six sequenced chloroplasts were aligned and visualized using mVISTA in Shuffle-LAGAN mode, with *Michelia alba* annotations as reference [62]. In order to analyze the nucleotide diversity and find simple sequence repeat markers, the DnaSP 5.10 and the MISA v program were used respectively [63, 64].

## Abbreviations

CP: Chloroplast; IR: Inverted repeat; LSC: Large single-copy; SSC: Small single-copy; SSRs: Simple sequence repeats; GC: Guanine-cytosine; BI: Bayesian inference; ML: Maximum Likelihood.

## Declarations

### Acknowledgments

We thank Associate Professor Qi Jianhua of Southwest Forestry University for the identification of the experimental materials and Mr. Yang Lin for making suggestions on revising the article.

### Funding

This research was supported by the Fund of Ten-Thousand Talents Program of Yunnan

Province, grant number YNWR-QNBJ-2020-230.

### Availability of data and materials

All annotated chloroplast genomes have been deposited in GenBank ([https:// www. ncbi. nlm. nih. gov/ genba nk/](https://www.ncbi.nlm.nih.gov/genbank/)). Other data generated or analyzed in our study are included in the supplementary files.

### Ethics approval and consent to participate

We complied with relevant institutional, national, and international regulations. The leaf material in this study was collected with permission from the staff of the Kunming Botanical Garden and identified by Associate Professor Qi Jianhua.

### Competing interests

Not applicable.

### Consent for publication

Not applicable.

### Authors' contributions

D.W. conceived and designed the study. J.S. wrote the manuscript. J.S., X.X., and L.Y. performed the analyses. R.W. collected the data. All authors reviewed and revised the manuscript. The author(s) read and approved the final manuscript.

### Author details

<sup>1</sup> Key Laboratory for Forest Resources Conservation and Utilization in the Southwest Mountains of China Ministry of Education, Southwest Forestry University, Kunming 650224, China.

<sup>2</sup> Key Laboratory for Forest Genetics and Tree Improvement and Propagation in Universities of Yunnan Province, Southwest Forestry University, Kunming 650224, China.

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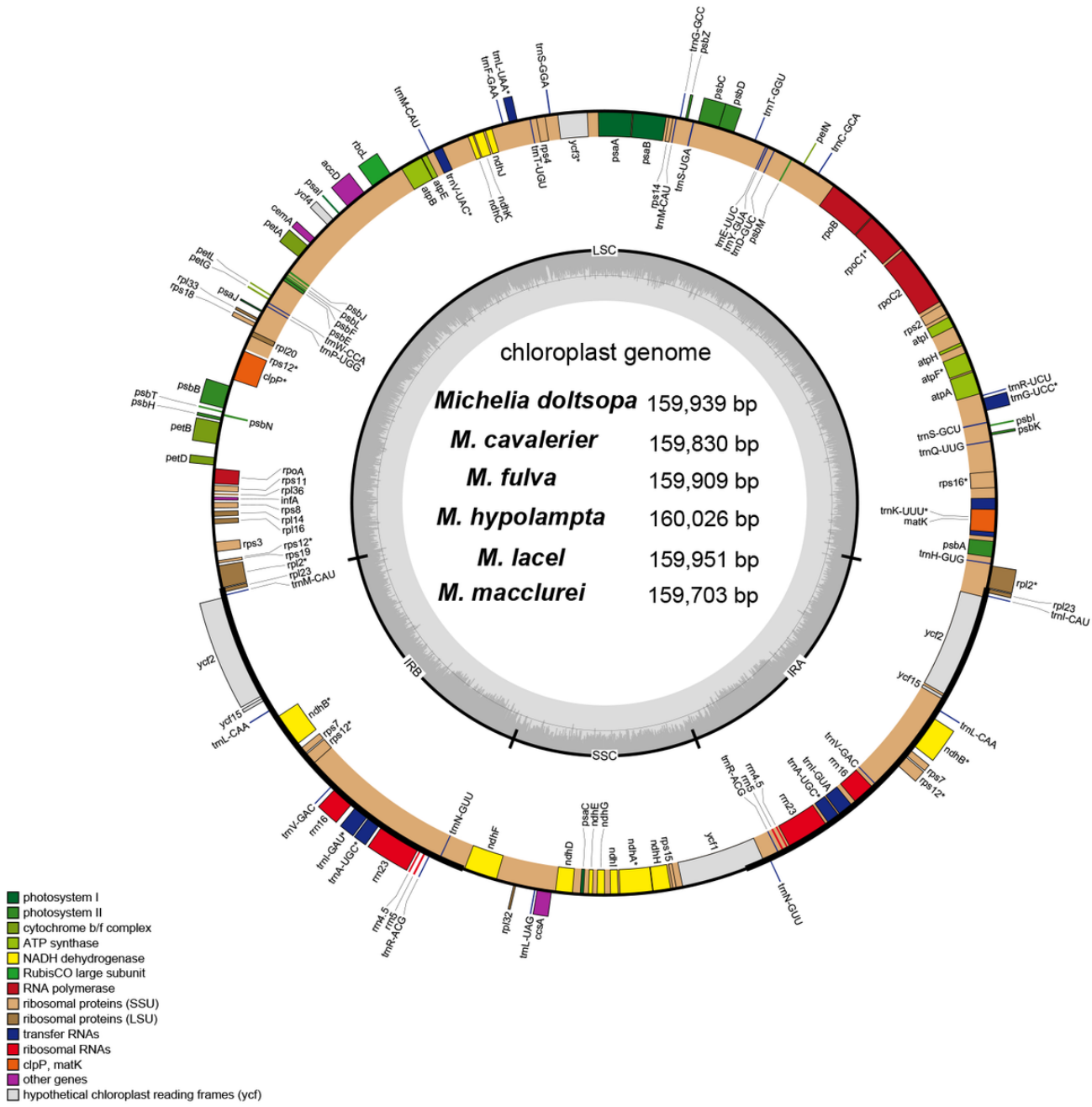
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## Tables

**Table 1** Summary of the cp genomic characteristics of the genus *Michelia*.

Species	<i>M. cavaleriei</i>	<i>M. doltsopa</i>	<i>M. fulva</i>	<i>M. hypolampra</i>	<i>M. lacei</i>	<i>M. macclurei</i>
length (bp)	159,830	159,939	159,909	160,026	159,951	159,703
LSC length (bp)	87,893	91,074	87,980	88,080	88,022	87,730
SSC length (bp)	18,817	18,791	18,795	18,796	18,793	18,822
IR length (bp)	26,560	25,037	26,567	26,575	26,568	26,575
GC content (%)	39.3%	39.3%	39.3%	39.2%	39.2%	39.3%
Gene number	130	130	130	130	130	130
SSR number	136	142	140	141	140	136
GenBank accession	MW480859	MW470943	MW491463	MW470944	MW470942	MW470941

## Figures



**Figure 1**

Gene map of the cp genome of six genera *Michelia*

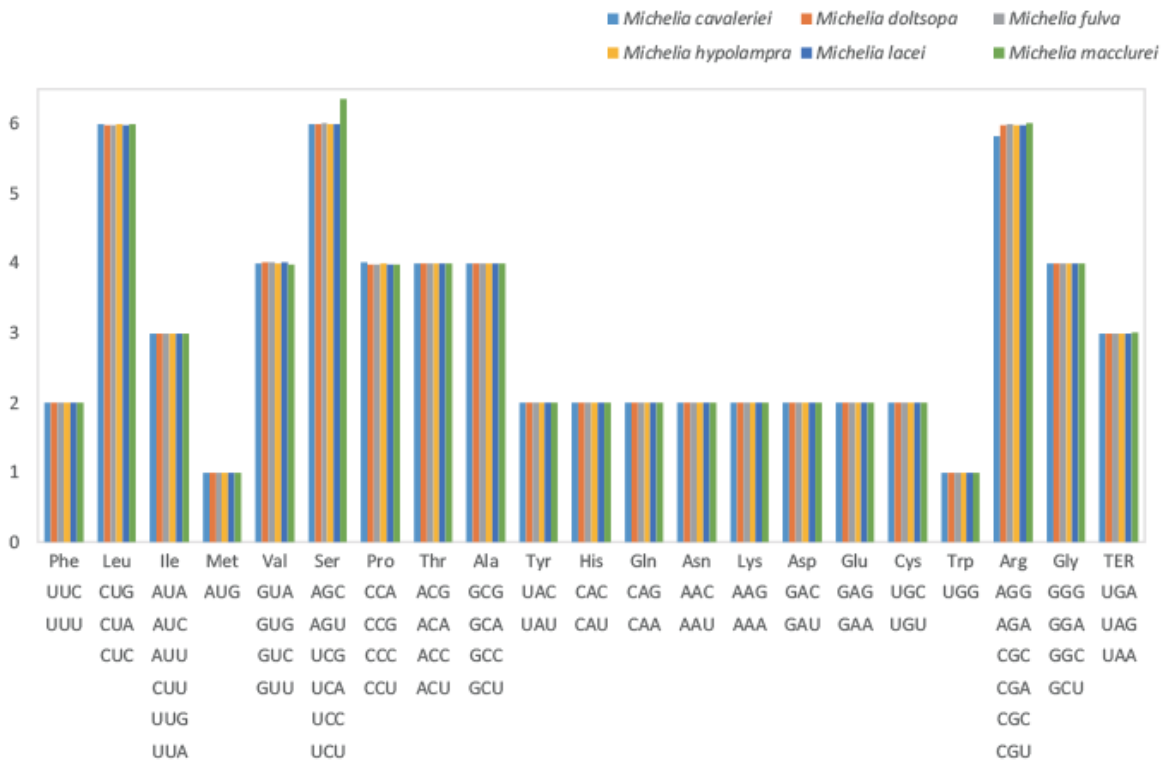


Figure 2

A number of codons in all protein-coding genes in the cp genomes of the six *Michelia*

### Inverted Repeats

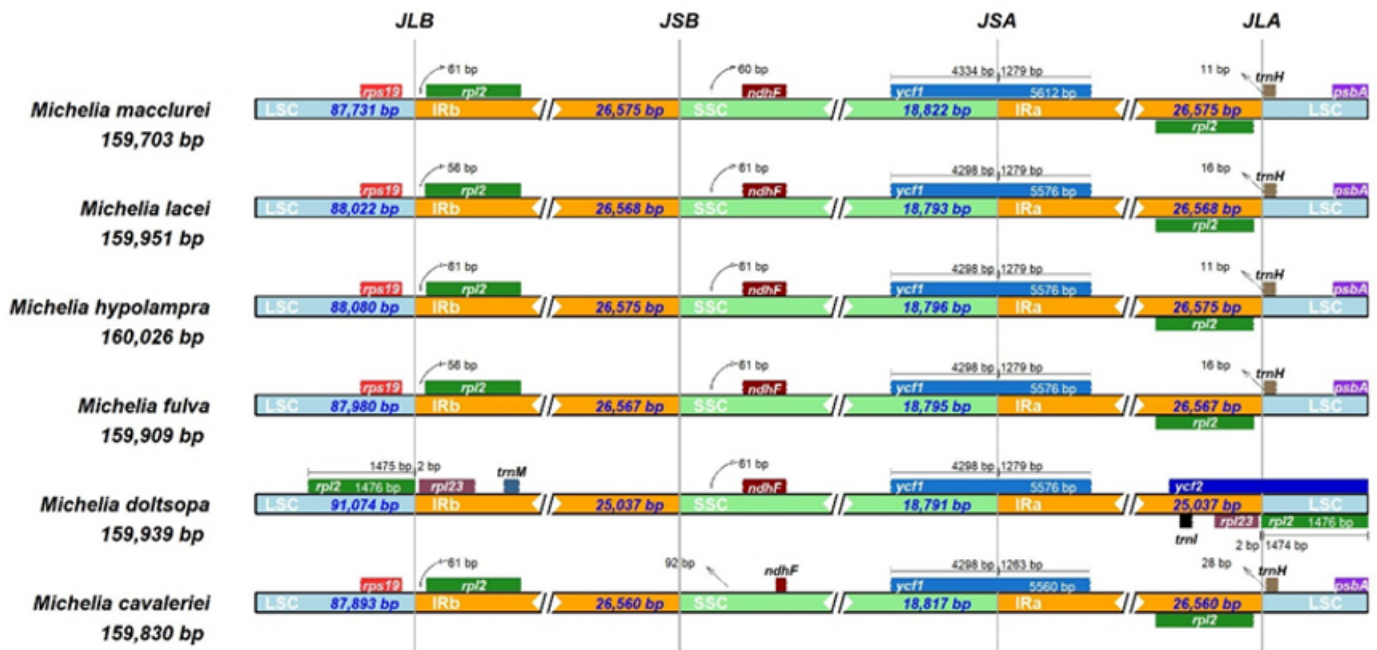
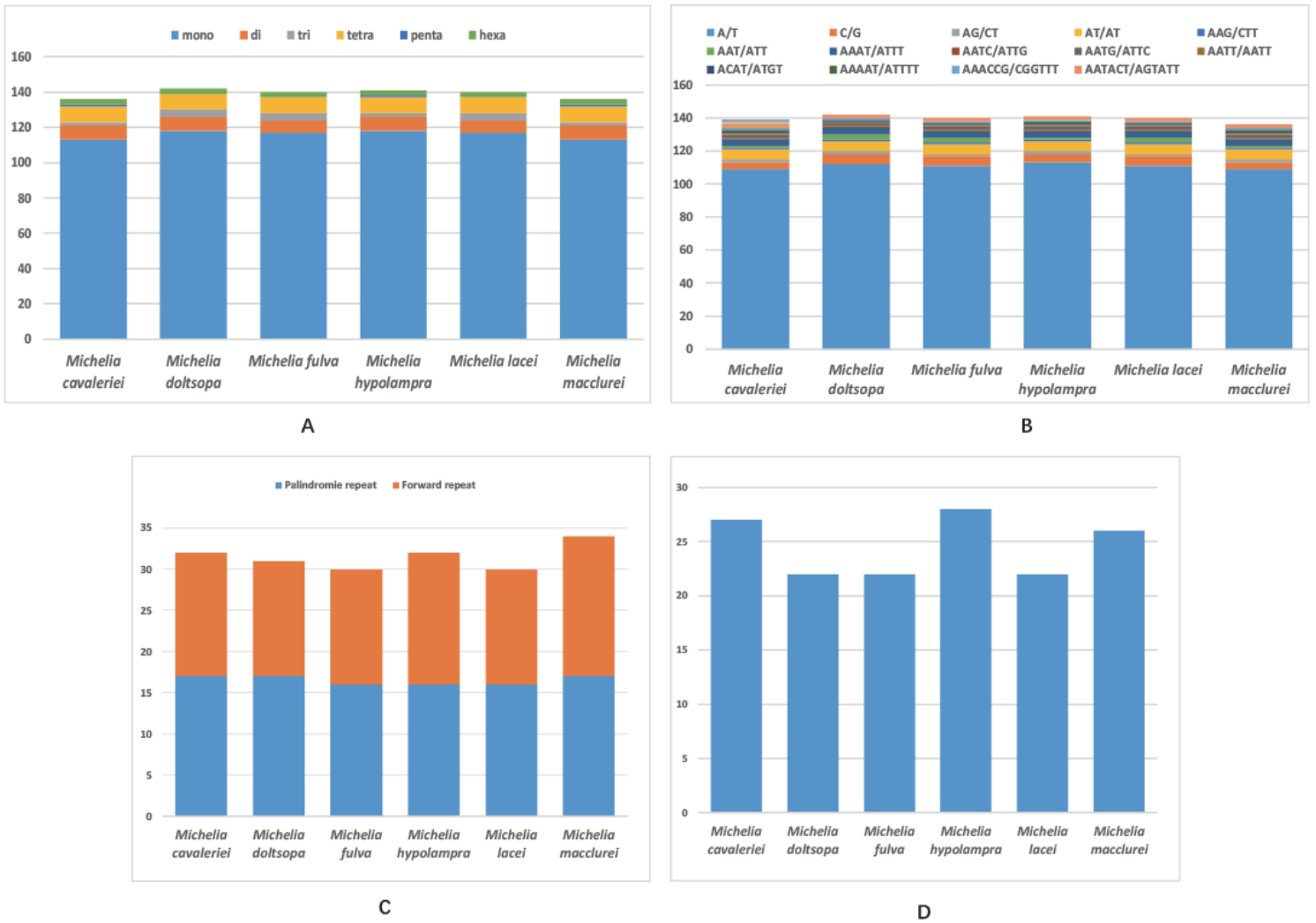


Figure 3

Comparison of the SSC, LSC, and IR border positions in the cp genomes of the six *Michelia* species

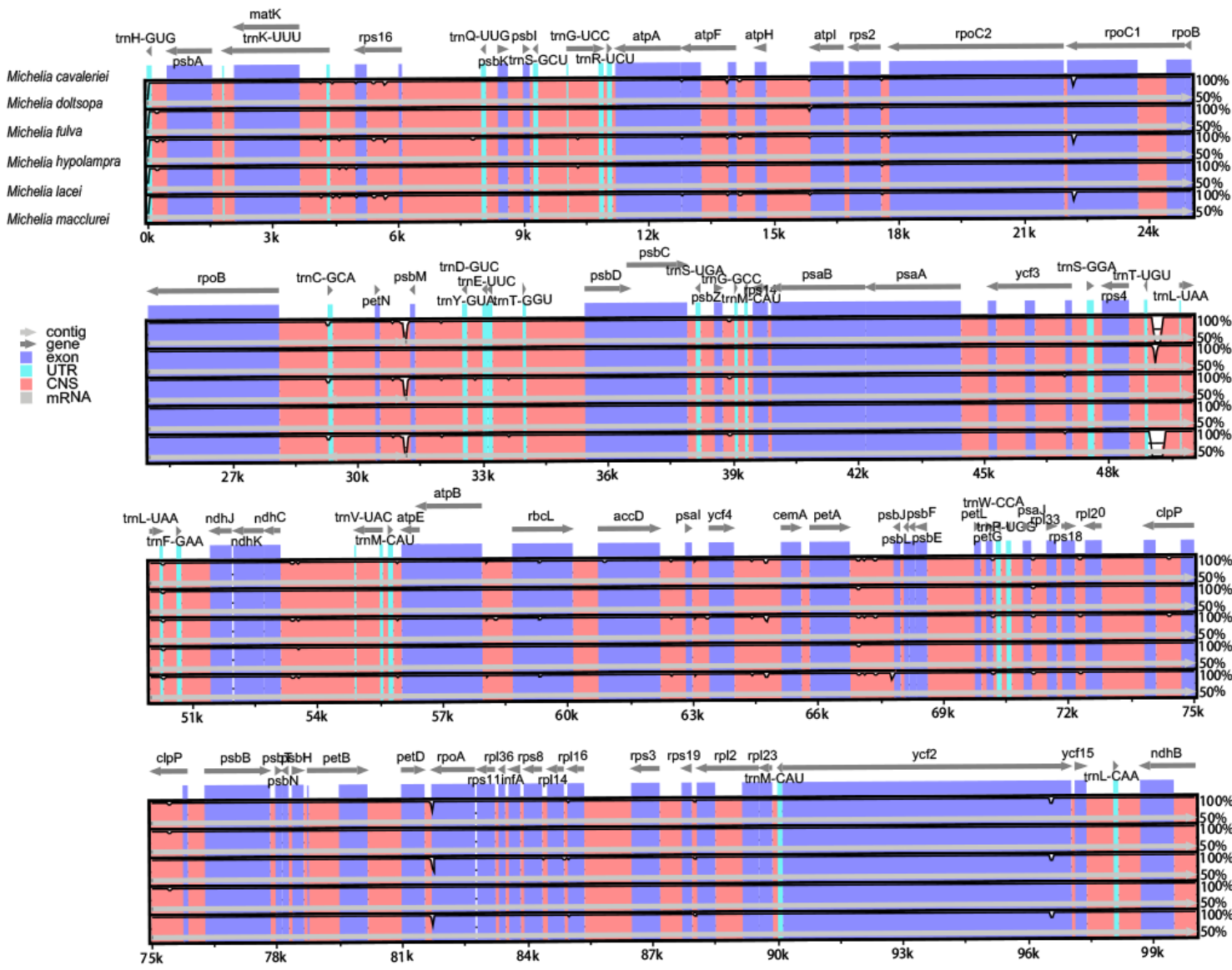


**Figure 4**

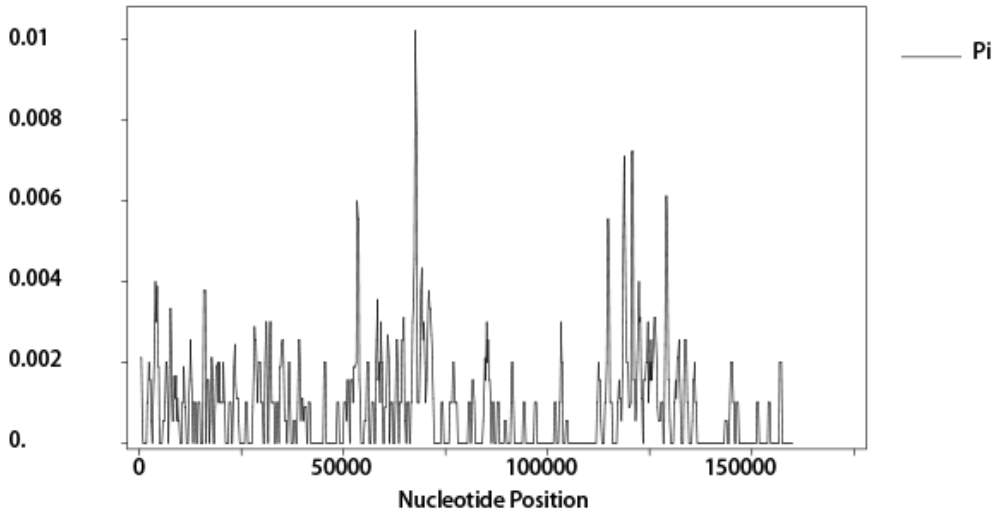
The number and type of SSRs and repeat sequences in the cp genome of six *Michelia* species

A. Number of six SSR types; B. Number of SSR motifs in different repeat class types;

C. Number of repeat types; D. Number of dispersed repeat sequences.



**Figure 5**  
Alignment of cp genomes sequences from the six *Michelia*



**Figure 6**

Nucleotide variability values for the cp genomes of the six *Michelia*

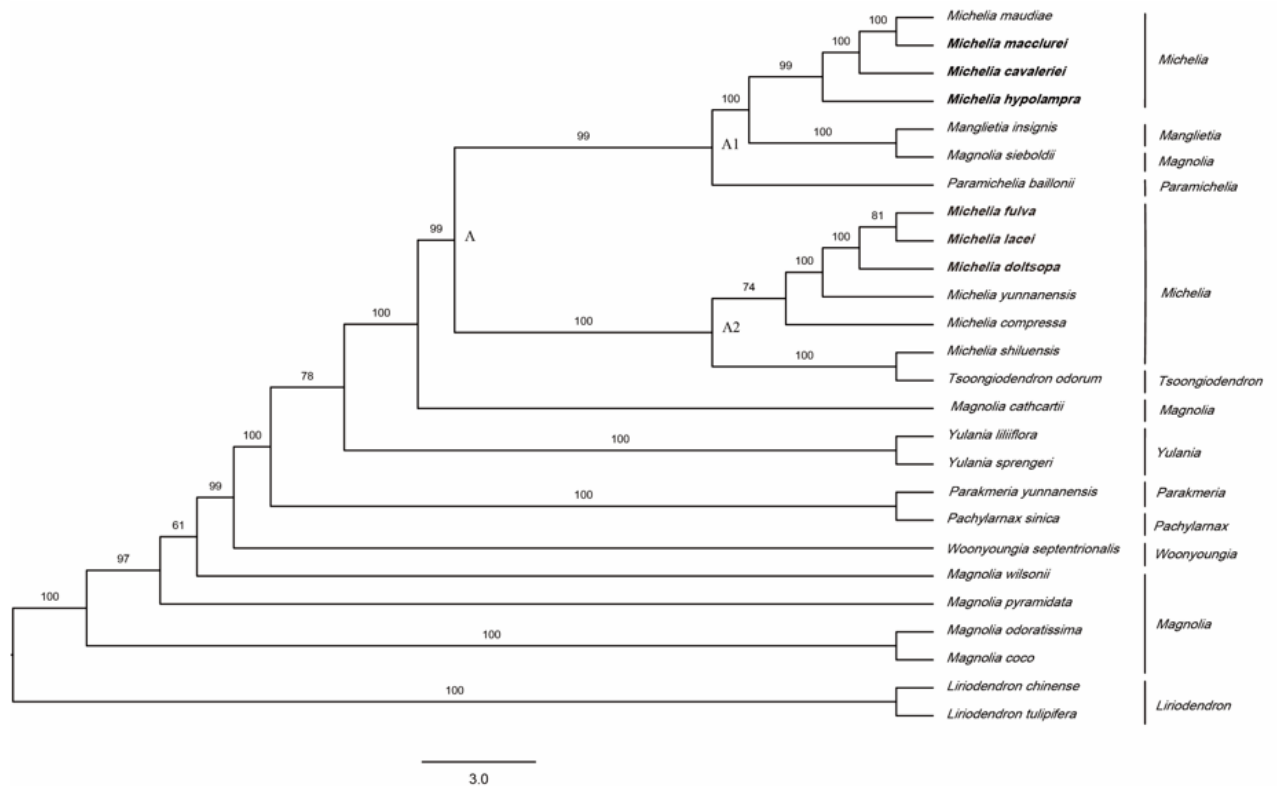


Figure 7

Phylogenetic relationships by Bayesian inference of 26 Magnoliaceae species

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [FigureS1.PhylogeneticrelationshipsbyMaximumLikelihoodof26Magnoliaceaespecies..pdf](#)
- [TableS1.ListofgenesencodedbysixspeciesofMicheliachloroplastgenome..docx](#)
- [TableS2.CodonfrequenciesandrelativesynonymouscodonusageRSCUvaluesofthecpgenomesofsixMicheliaspecies..xlsx](#)
- [TableS3.SeveralsixSSRtypes..xlsx](#)
- [TableS4.SeveralSSRmotifsindifferentrepeatclasstypes..xlsx](#)
- [TableS5.Severalrepeattypes..xlsx](#)
- [TableS6.Severaldispersedrepeatsequences..xlsx](#)
- [TableS7.NucleotidediversityofthesixMicheliachloroplastgenomes.xlsx](#)
- [TableS8.Accessionnumbersofthechloroplastgenomesusedinthephylogeneticanalysis..xlsx](#)