

# Chloroplast genome and historical biogeography of the three Magnolias

**min zhang**

Chengdu University of Traditional Chinese Medicine

**shuai guo**

Chengdu University of Traditional Chinese Medicine

**YIN Yanpeng**

Chengdu University of Traditional Chinese Medicine

**Luojing ZHOU**

Chengdu University of Traditional Chinese Medicine

**REN Bo**

Chengdu University of Traditional Chinese Medicine

**WANG Li**

Sichuan Academy of Forestry Sciences

**Xiaodong SHI**

Chengdu University

**HOU Feixia**

Chengdu University of Traditional Chinese Medicine

**PENG Cheng**

Chengdu University of Traditional Chinese Medicine

**YIN Xianmei** (✉ [xianmeiyincm@163.com](mailto:xianmeiyincm@163.com))

Chengdu University of Traditional Chinese Medicine

**GAO Jihai**

Chengdu University of Traditional Chinese Medicine

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## Research Article

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# Abstract

*Magnolia officinalis*, *M. officinalis* subsp.*biloba* and *M. hypoleuca* are all typical medicinal plants, belonging to genus *Magnolia* and Family Magnoliaceae. Their molecular information, particularly genetic difference, were known less. In this study, the platform Illumina HiSeq was used to sequence and assemble a novel cp (chloroplast) genome of *M. hypoleuca* followed by cp purification. Combined with two published cp rawdata, gene cycles and function annotations were comparably performed for the three plant species. The results indicated that 19 791 019 clean reads was assembled for *M. hypoleuca* cp, Q30 being 91.33%, and genome 160 051 bp. Its GC content is 39.2%, including 37 tRNAs and 8 rRNAs. The *M. hypoleuca* had smaller chloroplast genome and more introns (or exons) than *M. officinalis* and *M. officinalis* subsp.*biloba*. And there were respectively 11 and 8 more functional genes in *M. hypoleuca* cp than that in the other two. Based on cp complete genomes sequences, we constructed the phylogenetic relationship and estimated the divergence time of the three species by ML (Maximum likelihood) method, with other 10 published Magnoliaceae species. The results showed that *M. officinalis* subsp.*biloba* and *M. officinalis* might diverge from *M. hypoleuca* around 18.98 Ma, then they diverged from each other around 15.00 Ma. Additionally, the middle Miocene warming period might play an important role in the demographic and evolutionary histories of the three Magnolias, which provided a novel insight of the origin and dispersal routes of *M. hypoleuca*.

# Introduction

*M. officinalis*, *M. hypoleuca* and *M. officinalis* subsp.*biloba* are the deciduous tree belonging to the genus *Magnolia* [1]. Their bark have been used for thousands of years in Chinese and Japanese traditional medicines and are still widely employed as herbal preparations for their sedative, antioxidant, anti-inflammatory, antibiotic [2], dyspepsia [3] and antispastic effects [4]. Besides, three Magnolias have many other uses besides medicine. For example, *M.hypoleuca*, famous in Korea, Japan and China [5], is widely utilized as a natural packaging material for traditional foods in Japan [6]. Meanwhile, a comparison of three Magnolias found that *M. hypoleuca* owned unique biological characteristics, such as stronger cold resistance ability [7–8], higher  $\beta$ -cineol content [9] and faster growth and maturity rate.. Former researchers [10] found that *M. hypoleuca* was the northernmost of all Magnolias plants and its cold tolerance was better than other Magnoliaceae plants, which is native to the south of the Kuril islands [11]. Till now, *M. hypoleuca* was widely distributed in Janpan, China and Korea. Instead, *M. officinalis* subsp.*biloba* and *M. officinalis* are native to China and were widely distributed in China. The origin of Janpanese islands was the eastern margin of the Eurasian Continent rifted approximately 700–750 Ma [12]. After the formation of Japan, those continental islands have experienced various paleogeographic and paleoclimatologic changes resulting in high endemism and biodiversity of terrestrial species [13–15], such as *Liriodendron* [16], *Magnolia section Rytidospermum* [17], Some of them had been reported with the molecular phylogenetic techniques to study evolutionary patterns or estimate divergence times of disjuncts. It has been proposed that = biogeographic studies of widely distributed plants can be performed to provide insight into the broader patterns of the evolutionary history and geographic

diversification [18]. However, there is no analysis of the evolutionary history and biogeography of the three Magnolias.

Cp-genome (chloroplast genome) possess a highly conserved tetrad structure, containing two inverted repeat (IR) regions (IRa and IRb), a small single-copy (SSC) region and a large single-copy (LSC) region [19–20]. In addition to photosynthesis, cp genome-encoded proteins are involved in other metabolic processes, such as responses to heat, drought, salt, and light [21]. Besides, cp-genome were widely used in species evolution [22], phylogenetic [23–25] and biogeographic studies [26–28]. Meanwhile, organismic and environmental processes played a major role in organismal evolution [29–30]. Currently, with the rapid development of high throughput sequencing technologies, made it possible for researchers to obtain cp genomic sequences to study species evolution, phylogenetic and biogeographic [31]. Therefore, whole cp genome of *M. hypoleuca* was urgently needed, which might significantly provide an insight in plant phylogenetic relationships and contributed to the, domestication, and utilization of Magnoliaceae plants.

In this study, we sequenced cp genome of *M. hypoleuca* and performed the comparative genomes analysis to obtain comprehensive understanding the structure of cp genomes within three Magnolias. Meanwhile, we studied divergence time of three Magnolias, which indicated that it was consistent with Darwinian evolutionary theory. Our study will provide genetic resources for future research in the genus, and decipher the genetic relationship of three Magnolias to provide some reference value for molecular breeding of superior variety, and also provide useful information for identifying three Magnolias species, and providing insight into their evolutionary history.

## Results

### Genome Sequencing, assembly and annotation

A length of 19 816 708 raw reads of *M.hypoleuca* was obtained by Illumina HiSeq PE14 sequencing platform. Through removing the connector and low-quality reads, a length of 19 791 019 clean reads of *M.hypoleuca* were obtained and the Q30 was 91.33%. After sequence assembly and annotation, the results showed that the total length of the cp genome of *M.hypoleuca*, *M. officinalis* subsp.*biloba* and *M. officinalis* were 160 051 bp, 160 099 bp and 160 183 bp, respectively. (Fig 1 and Table 1). Among them, the genome of *M.hypoleuca* had a quadripartite structure with an SSC of 18 771 bp and an LSC of 88 146 bp, which were separated by two IR regions (IRa and IRb, each 26 562 bp). The GC content of the overall cp genome was 39.2%, comprising 8 rRNA and 37 tRNA, respectively (Table 1). Through the comparison of the cp genomes of three Magnolias, we concluded that the cp genomes structure had a typical quadripartite structure, with a circular molecule of 160 051 bp to 160 183 bp in length, and the content of GC, rRNA and tRNA were same in the cp genes of three Magnolias. On the contrary, the total number of genes and coding region were distincted in three Magnolias, with its total number of *M. hypoleuca* were 13 and 14 times higher than *M. officinalis* and *M. officinalis* subsp.*biloba*, respectively (Table 1).

### Comparative analysis of cp genomes

Chloroplast is important organ for plant photosynthesis, which is very conservative in structure. Comparative analysis of cp genomes is an essential step in genomics [32-33]. A comparison of the structural differences among cp genomes of three Magnolias indicated that the cp genome of *M. hypoleuca* was the smallest (Fig 1; *M. hypoleuca*, 160,051 bp; *M. officinalis* subsp.*biloba*, 160,099 bp; *Magnolia officinalis*, 160,183 bp). Instead, Comparison of the functional genes quantity showed that the cp genome of *M. hypoleuca* was the most which indicated *M. hypoleuca* had more functional effects. The result showed that *M. hypoleuca* had 11 more functional genes than *M. officinalis*, including *psbC*, *ycf14*, *ycf1*, *rps14*, *rps12*, *rps12*, *rpl22*, *rpl23*, *petD*, *petL*, *ropC2*, respectively, and had 8 more functional genes than *M. officinalis* subsp.*biloba*, including *psbC*, *ycf14*, *ycf1*, *rpl22*, *rps12*, *rpoC2*, *petD*, *petL*, respectively (Table 3). Studies on ribosomal protein *rpl22* and *rpl23* revealed they were essential of metabolism of organisms, whatever the plant was the stage of developmental or light phase. Moreover, the above all different genes were intensively located in Photosynthetic System II Subunit, large ribosomal subunit and small ribosomal subunit, which mainly control photosynthesis and polypeptide formation in plants [34- 35].

Except for the length of total cp complete genome sequences and amount of functional genes, the frequently divergent regions between three Magnolias were mainly in the introns (or exons) content in genes (Table 2). Introns play important roles in regulating gene expression [ 36]. The genes containing introns (or exons) of cp genomes of *M. hypoleuca* had 2 more than that in *M. officinalis* and *M. officinalis* subsp.*biloba*. They were respectively *petD* and *rpl16* gene (Table 2). It indicated that *petD* and *rpl16* genes of *M. hypoleuca* were more than that in *M. officinalis* and *M. officinalis* subsp.*biloba*. In this study, the length of exon of *ycf3* and *rps12* gene of *M. hypoleuca* were 226 bp longer and 114 bp smaller than the other two species. In all species, the two *rps12* gene were trans-spliced. There were two *rps12* gene in cp genomes of three Magnolias. Those were, the length of intron and exon of another *rps12* gene of *M. hypoleuca* were 535 bp longer and 227 bp smaller than the other two species. Moreover, *rps12* gene encoded the ribosome S12 protein [37], which is usually highly conserved, and its structural change was thought to be the result of evolution [38]. It revealed differences among the three Magnolias.

### **Divergence time estimation based on cp complete genomes**

We used 14 cp complete genomes of Magnoliaceae and Ginkgoaceae for phylogenetic reconstruction and estimation of the divergence times of three Magnolias. Partial phylogenetic tree of 14 species was constructed by ML method (Fig. 2). Results showed the ML phylogenetic tree based on the cpDNA of partial Magnoliaceae was divided into two main clades (Fig. 2). The first clade was Magnoliaceae and the second clade was outgroup, *Ginkgo biloba*. Among them, the first group could be further divided into two secondary groups, including 11 species of *Magnolia* genus as the first groups and *Linriodendron* genus as the second groups. The phylogenetic relationship of 14 species was mostly consistent with the study of *Lirianthe hodgsonii* [39-40]. Moreover, through comparison of the phylogenic relationship of three Magnolias, *M. officinalis* was most closely related to *M. officinalis* subsp.*biloba*, followed by *M. hypoleuca*. The relationship between other Magnoliaceae plants and the three Magnolias was as follows: *M. sinostellata*, *M. yunnanensis*, *M. liliflora*, *M. biondii*, *M. grandiflora*, *M. denudata*, *M. zenii*, *L.*

*tulipifera* and *L. chinense*. Besides, the result showed that the degree of genetic divergence between *M. officinalis* and *M. officinalis* subsp.*biloba* were later than *M. hypoleuca* in three Magnolias. The divergence time of *M. officinalis* was estimated to be 15.00 MYA and it was same as *M. officinalis* subsp.*biloba*, later than divergence time of *M. hypoleuca* which was estimated to be 18.98 MYA. Meanwhile, *Liriodendron* was estimated to have diverged in the mid Miocene based on allozyme and restriction fragment length polymorphism (RFLP) analyses of cpDNA and paleobotanical evidence [41]. It was consistent with the conclusion in this study (Fig 2). The divergence time of 13 Magnoliaceae we selected was subdivided at the period of 14.20-21.78 MYA. It indicated that the evolutionary and biogeography of partial modern Magnoliaceae species mainly occurred in mid Miocene.

## Discussion

The cp genomes of the three Magnolias species were same in the content of GC, rRNA and tRNA, including 37.2% GC, 37 tRNA and 8rRNA. The analysis of codon usage in cp genomes of *Actinidia chinensis* showed that codon content was mainly affected by multiple factors such as base composition, natural selection and gene mutation [42]. And there was a negative correlation between the variation degree of cp genome sequence and the GC content of the sequence [43]. Thus, the rRNA, tRNA and IR regions were relatively conservative in structure due to the GC content of all regions of Magnoliaceae was high in this regions. The stable cp complete genomes structure of three Magnolias indicated that its variation degree was very small. Furthermore, it showed that the relationship of three Magnolias was relatively close and slow in evolution. It was consistent with the discovery that Magnoliaceae evolved respectively slowly [44].

Instead, there were different numbers of functional genes of cp genomes of three Magnolias. Especially functional genes numbers of *M. hypoleuca* were more 11 than *M. officinalis*, and more 8 than *M. officinalis* subsp.*biloba*. Among them, *psbC* and *psbD* genes were key genes of photosynthetic system ☒. Some researchers found the synthesis of *psbC* gene product might occur in the transcript containing *psbD* sequence. That indicated that *psbD* and *psbC* genes jointly coordinated the effect on light [45]. Some scholars [46] found that *psbD* gene in the *Phyllostachys japonicus* leaves played the protective role of photosynthetic system and reduce strong sunlight damage, when the plants were in a stage of growth and color differences. In this study, *psbC* gene number of *M. hypoleuca* was more than that of *M. officinalis* and *M. officinalis* subsp.*biloba*. Meanwhile, the different number of *psbC* gene of three Magnolias might be one of the factors affecting photosynthesis and growth cycle.

In this study, the total number of tRNA in the cp genomes of three Magnolias was same, but the corresponding amino acid types were diversified. *M. hypoleuca* had 3 more tRNA in alanine transport than the other two species and it had unique types of tRNA in histidine transport. Some studies showed that the types and quantities of tRNA will change in different periods at the same organism and will be adjusted accordingly with environmental changes [47]. For example, the natural structure formation and expression of tRNA was controled and affected by temperature and ionic strength [48]. Alanine was one of the main products of anaerobic metabolism in plants, which could accumulate rapidly under stress

conditions [49], and its free form could resist environmental stimulation. The content of free histidine in cp was positively correlated with the SOD (superoxide dismutase) content and it could resist cold and oxidation. In short, the unique types and amounts of tRNA in the cp genome of three Magnolias correspond to their various biological characteristics.

Comparing three Magnolias species cp genes introns (or exons), the results showed that *rps12* gene introns of *M. officinalis* and *M. officinalis* subsp.*biloba* was missing, and its exons content was 0.50 times of *M. hypoleuca*. Furthermore, the exons and introns of *petD* and *rpl16* genes were found only in *M. hypoleuca*. The plastid ribosomal protein S12 encoded by the *rps12* gene was a highly conserved protein, which was located in the functional center of the 30S subunit of the ribosome. Some scholars found that *rps12* was a trans-splicing gene in ferns [50], and the evolution rate of species was affected due to its change in intron deletion and exon position. It indicated that intron loss accelerated the evolution rate. In this study, the different length of *rps12* introns of three Magnolias was consistent with the previous study, revealing the evolutionary relationship between three Magnolias. Furthermore, some scholars [37] found that the diversified expression of *rps12* gene caused the different phenotypes in plants, such as sharp and narrow leaves, serrated or defective leaves.. What's more, according to the *petD* gene of *Ginkgo biloba* [51], it found that the expression level of *petD* was highest in leaves, next to the stem, which was related to the formation of cytochrome b/f complex. The main function of cytochrome b/f complex was to participate in the electron transport process of photosynthesis and enhanced the photosynthetic ability of leaves. In this study, we found that the *petD* gene of *M. officinalis* and *M. officinalis* subsp.*biloba* was probably pseudogenes that they did not gene expression. That revealed that the photosynthetic capacity and growth ability of *M. hypoleuca* were stronger than the other two species. Besides, the phylogenetic tree of 14 species showed that the Magnoliaceae was divided into two branches, *Liriodendron* and *Magnolia*. Among the three Magnolias, the Phylogenic relationship of *M. officinalis* and *M. officinalis* subsp.*biloba* were closer, next to *M. hypoleuca*. It indicated the divergence of three Magnolias was due to geographic movements. Thus, the main disjunction we dated in this study was within three Magnolias based on estimation of the divergence times and phylogenetic reconstruction.

We used the cp complete genomes of 13 Magnoliaeace species and 1 outgroup for phylogenetic reconstruction and estimation of the divergence times. Through a comparison of the divergence time of three Magnolias, the divergence time between *M. officinalis* and *M. officinalis* subsp.*biloba* was estimated to be 15.00 MYA, and divergence time between *M. hypoleuca* and other two species was estimated to be 18.98 MYA. It indicated that divergence time of *M. hypoleuca* was earlier than the other two. Moreover, the leaf shape of Magnolias was divided into three types: the first type of leaf shape was sharply pointed or blunt, the second type had slightly absent or obtuse leaves at the apex and the apex of the leaves of the third type was concaved into 2 shallow lobes, but there was no obvious concave at the apex of the seedlings, which were more obtuse or slightly emarginate [52]. According to the theory of species evolution, the first type is relatively primitive, and the third type has a higher degree of evolution. Among them, the leaf shape of *M. hypoleuca*, *M. officinalis* and *M. officinalis* subsp.*biloba* belonged to the first type, second type and third type, respectively. It indicated that *M. hypoleuca* was relatively more primitive than *M. officinalis* and *M. officinalis* subsp.*biloba* by the diversity of leaf shape of three

Magnolias. Moreover, the Miocene was a period with globally warmer climates than those in the preceding Oligocene, or the subsequent Pliocene [53]. Among them, the middle Miocene warming period was from 13 to 18 MYA [54–55]. Some researchers found that the Miocene floras had many elements in common with the modern mesophytic floras of eastern Asia and eastern North America, supporting the proposal that the divergence of the modern north temperate elements occurred during that period. There are some else to support this proposal. such as, Manta, devil rays, *Pterocarya* [55] and Magnoliaceae in the Northern Hemisphere and so on. This study indicated that time-division of three Magnolias might occur in the period of middle Miocene warming period.

## Conclusions

In this study, we reported and analyzed the cp complete genomes of *M. hypoleuca*, which is the components of deciduous broad-leaved forest in the cold temperate zone. And we compared cp complete genomes of three Magnolias, the results revealed that the genome size of *M. hypoleuca* was smaller than *M. officinalis* subsp.*biloba* and *M. officinalis*. Moreover, we found the distinct difference of three Magnolias from the content and length of introns and exons in genes, and the types and quantity of functional genes. We detected the GC content of three Magnolias cp genome was 39.2%, comprising 8 rRNA and 37 tRNA. In addition, the result showed that *M. hypoleuca* had 11 and 8 more functional genes than *M. officinalis* and *M. officinalis* subsp.*biloba*, respectively. And through constructing the relationship between phylogenetic and divergence time of the three Magnolias, it indicated that the divergence time of three Magnolias might take place during the middle Miocene (13–18 Ma).

## Materials And Methods

### Plant material

The fresh and insect-free leaves samples of *M. hypoleuca* were collected in the State Bank of Chinese Drug Germplasm Resources, Chengdu University of Traditional Chinese Medicine (Chengdu, China). According to check out previous studies, no cp complete genome of *M. hypoleuca* has been reported, even the comparison of cp complete genomes of three Magnolias. Therefore, we decided to analyse and compared the difference of cp complete genomes of three Magnolias. In this study, all methods were performed in accordance with the relevant guidelines and regulations of China.

### Sample material, DNA extraction, and sequencing

We extracted cp complete genome from 20–30 mg of fresh leaf material (dried mass after lyophilization) by using modified CTAB [56] method. Fresh material was lyophilized with liquid nitrogen. The specific extraction steps were as follows: The fresh leaves were smashed in liquid-nitrogen, and suspended in liquid A (50 mmol·L<sup>-1</sup> Tris, 25 mmol·L<sup>-1</sup> EDTA, 1.25 mol·L<sup>-1</sup> NaCl, 0.25 mmol·L<sup>-1</sup> Vc, 1.5% PVP, pH 3.6). Then filtering and retaining the supernatant; The Buffer B (50 mmol·L<sup>-1</sup> Tris, 25 mmol·L<sup>-1</sup> EDTA, 1.25 mol·L<sup>-1</sup> NaCl, 0.25 mmol·L<sup>-1</sup> Vc, 1 mmol·L<sup>-1</sup> DTT, 0.1% Bovine Serum Protein BSA, pH 8.0) was added in the supernatant and samples were placed at room temperature, extracted for 10 min at 2000 g. Moreover, the

cpDNA of *M. hypoleuca* was stored at 4°C to guarantee the quality. In addition, DNA of *M. hypoleuca* was qualified through Qubit 2.0 (BMKcloud <http://www.biocloud.net/>), then fragmented, purified, constructed sequencing library, and sequenced by a high-throughput sequencing platform (Illumina HiSeq PE14).

## Genome assembly and annotation

Raw sequences were submitted to China National Center for Bioinformation (BioProject number PRJCA004348). Pair-end Illumina raw reads were cleaned from adaptors and barcodes, and then quality filtered by Trimmomatic [57]. After quality filtering, we retrieved the cpDNA sequence of *M. officinalis* (NC\_020316) and *M. officinalis* subsp. *biloba* (JN867581) as the references. The plastome was assembled using online platform of Galaxy (<https://usegalaxy.org>) [58]. Then, the cp complete genomes of *M. hypoleuca*, *M. officinalis* and *M. officinalis* subsp. *biloba* were annotated using the online program CpGAVAS2 [59]. And we drew a circular representation of both sequences by using the online tool Genome VX [60].

## Evolutionary and Phylogenetic analysis

To estimate phylogenetic relationships within three Magnolias, a total of 14 cp complete genomes were downloaded from the NCBI database. The Genbank accession numbers for each plant species were follows: *M. grandiflora* (JN867584), *M. sinostellata* (NC039941), *M. biondii* (KY085894), *M. sprengeri* (JX280401), *M. denudata* (JN227740), *M. yunnanensis* (KF753638), *M. zenii* (MH607378), *M. liliflora* (JX280397), *Liriodendron chinense* (NC030504), *Liriodendron Tulipifera* (NC008326) and *Ginkgo biloba* (NC016986). Among them, the last species, *Ginkgo biloba*, was used as outgroup.

The 14 cp complete genomes sequences were aligned using megaX. The program megaX was used to infer ClustalW, with Pairwise alignment of the sequence, according to the pairwise alignment to calculate the pairwise distance matrix. Moreover, model detection tool, "Model", was used for optional model detection. We used the optional model to construct the evolutionary tree using the ML method, with the bootstrap number set as 1000. Among them, only the bootstrap number of Phylogenetic tree was bigger or equal to 50%, it just valued to be reliable. The clades division in figures was mainly based on the clustering results of phylogenetic trees and the morphological classification of Magnoliaceae. Then the tree with ".nwk" format was downloaded for next analysis.

## Divergence time estimates

We used the cp complete genomes data set for dating the divergence times. ML approaches based on a global clock model was usually used in the time estimates. A likelihood ratio test ruled out a global molecular clock ( $P < 0.05$ ) for our data [61]. Time estimates were done based on a global molecular clock and fossil data. That was, we used the final aligned sequences with 14 species, which were converted to MEGA format by using MEGAX software [62], and the phylogenetic tree of 14 species with .nwk format. An ML tree with lengths inferred from GARLI was used in the PL estimate with steps. All analyses were performed using the GTR model of nucleotide substitution with a gamma distribution with four rate categories. The tree calculation models was implemented in each analysis, with rate variation across

branches assumed to be uncorrelated and lognormally distributed. Among them, three clades with an expanded outgroup species was used in the dating analyses, meaning the that individual DNA alignments were pruned to eliminate multiple accessions of the same species [63]. The fossil times of *L. chinense* vs *L. Tulipifera* was 14.2 MYA, *M. zenii* vs *L. chinense* was 55 MYA and *M. sprengeri* vs *M. zenii* was 28.3 MYA. The outgroup fossil times *Ginkgo biloba* vs Magnoliaceae was 313 MYA. Therefore, the phylogeny was calibrated using four Magnoliaceae fossils that were applied to the stem nodes of *L. chinense*, *L. Tulipifera*, *M. zenii* and *M. sprengeri*. Additional fossil of Ginkgoaceae species was used to calibrate the stem node of Magnoliaceae and selected as an outgroup. All analyses were outputted in nwk format. The final estimates were obtained using the model that yielded the highest posterior probability. Posterior distributions of parameters were approximated using two independent analyses of 20 000 000 generations with 10% burn-in. Samples from the two chains which yielded similar results were combined.

## Declarations

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### Author contributions

Zhang Min conceived the experiment, Guo Shuai Processed the data, Yin Yanpeng, Zhou Luoqing and Ren Bo conducted the experiment, Wang Li, Shi Xiaodong and Hou Feixia analysed the results, Peng Cheng, Yin Xianmei and Gao Jihai reviewed the manuscript.

### Additional Information

The corresponding author is responsible for submitting a competing interests statement on behalf of all authors of the paper.

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## Tables

Table 1 Basic information of three Magnolias cp genes

Name	Length(bp)	GC(%)	Total number of genes	Coding region	rRNA	tRNA
<i>Magnolia officinalis</i>	160 183	39.2	126	82	8	37
<i>Magnolia officinalis</i> subsp. <i>biloba</i>	160 099	39.2	128	84	8	37
<i>Magnolia hypoleuca</i>	160 051	39.2	137	97	8	37

Table 2 Comparison of introns and exons of three Magnolias cp genome

	<i>Magnolia officinalis</i>					<i>Magnolia officinalis</i> subsp. <i>biloba</i>					<i>Magnolia hypoleuca</i>				
gene	EpI	InI	EpII	InII	EpIII	EPI	InI	EpII	InII	EpIII	EpI	InI	EpII	InII	EpIII
<i>trnK-UUU</i>	37	2498	35			37	2492	35			36	2491	34		
<i>rps16</i>	42	824	246			42	824	246			39	823	220		
<i>atpF</i>	145	709	410			145	707	410			144	708	410		
<i>rpoC1</i>	432	740	1614			432	734	1614			431	733	1614		
<i>ycf3</i>	124	733	232	729	141	124	734	232	727	141	226	732	232	727	143
<i>trnL-UAA</i>	35	491	50			35	491	50			34	490	49		
<i>trnV-UAC</i>	39	584	37			39	585	37			38	583	36		
<i>rps12</i>	227		227		29	227		227		29	113	535	231		25
<i>clpP</i>	71	786	291	629	244	71	781	291	628	244	70	776	290	627	245
<i>petB</i>	6	784	642			7	784	641			5	783	641		
<i>petD</i>											7	700	472		
<i>rpl16</i>											8	968	398		
<i>ndhB</i>	775	700	758			775	700	758			776	700	755		
<i>trnI-GAU</i>	42	937	35			42	936	35			41	936	34		
<i>trnA-UGC</i>	38	800	35			38	800	35			37	798	34		
<i>ndhA</i>	553	1082	539			553	1102	539			552	1103	540		
<i>trnA-UGC</i>	38	800	35			38	800	35			37	798	34		
<i>trnI-GAU</i>	42	937	35			42	936	35			41	935	34		
<i>rps12</i>	227		29		29	227		29				532	231		25
<i>ndhB</i>	775	700	758			775	700	758			776	699	755		
<i>rpl2</i>	385	661	431			385	649	443			396	660	430		
<i>trnG-UCC/</i>	24	770	48			24	767	48			23	768	47		

<i>trnG-</i>																				
<i>GCC</i>																				

Note: *rps*, Ribosomal subunit; *rpo*, RNA polymerase; *ycf*, Open reading frame; *clp*, *Protease gene*; *pet*, *Cytochrome*; *ndh*, Dehydrogenase complex; *rpl*, Ribosome large subunit; *ep*, Expressed region; *in*, Intron; *psa*, Photosynthesis.

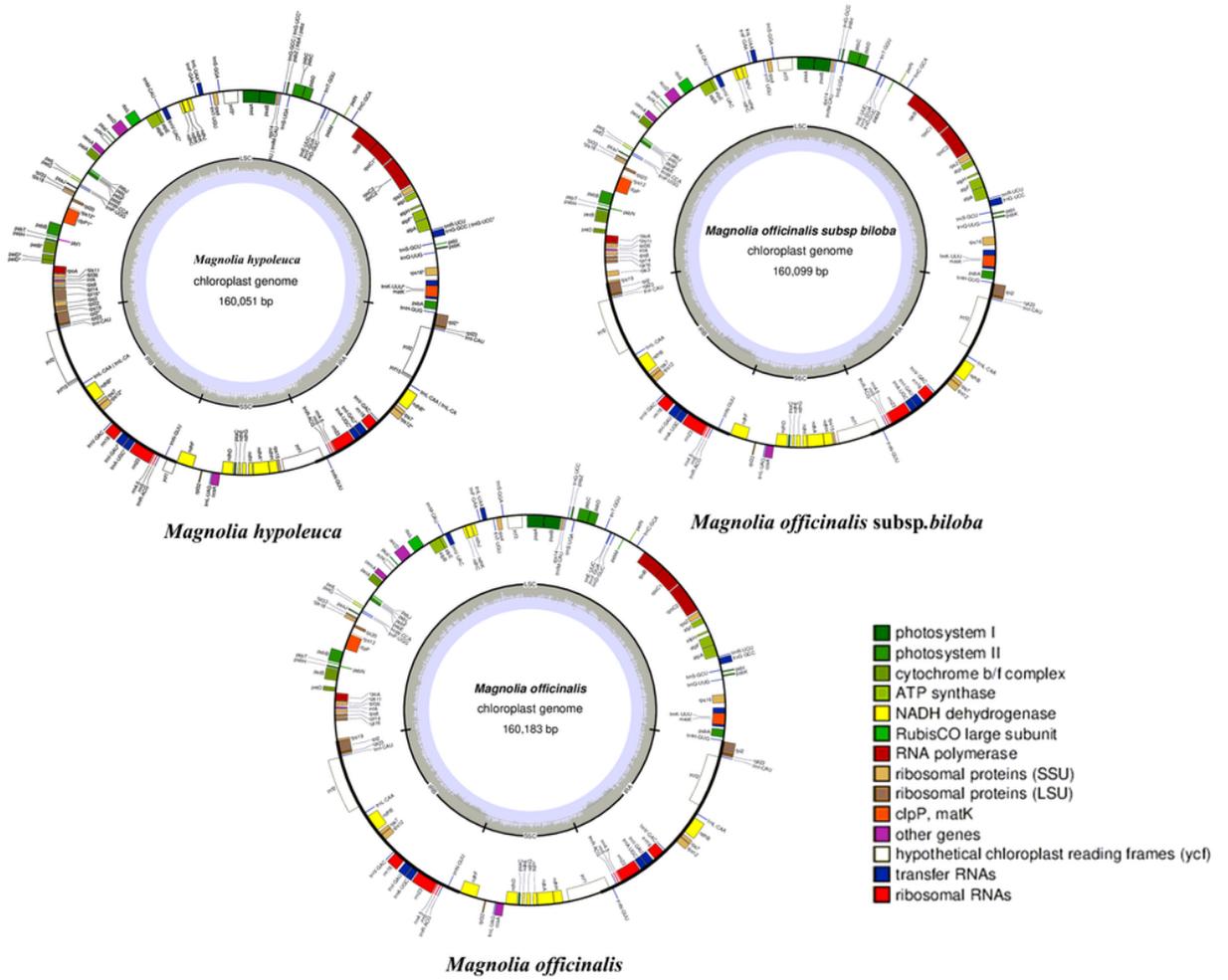
Table 3 Comparison of cp functional genes of three Magnolias plants

Gene function	Gene type	<i>Magnolia hypoleuca</i>	<i>Magnolia officinalis</i>	<i>Magnolia officinalis</i> subsp. <i>biloba</i>
Photosynthesis	ATP synthase subunit	atpA, atpA, atpB, atpE, atpF, atpF, atpH, atpH, atpI, atpI	atpA, atpB, atpE, atpF, atpH, atpI	atpA, atpB, atpE, atpF, atpH, atpI
	Photosynthetic System I Subunit	psaA, psaB, psaC, psaI, psaJ	psaA, psaB, psaC, psaI, psaJ	psaA, psaB, psaC, psaI, psaJ
	Photosynthetic System II Subunit	psbA, psbA, psbB, psbC, psbC, psbD, psbD, psbE, psbF, psbI, psbJ, psbK, psbL, psbM, psbM, psbN, psbT, psbZ, ycf3	psbA, psbB, psbC, psbD, psbE, psbF, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ, ycf3	psbA, psbB, psbC, psbD, psbE, psbF, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ, ycf3
Self copy	NADH dehydrogenase subunit	ndhA, ndhB, ndhB, ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK	ndhA, ndhB, ndhB, ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK	ndhA, ndhB, ndhB, ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK
	Cytochrome b/f complex	petA, petB, petD, petG, petL, petN, petN	petA, petB, petD, petG, petL, petN	petA, petB, petD, petG, petL, petN
	Ribulose diphosphate carboxylase subunit	rbcL	rbcL	rbcL
	Large ribosomal subunit	rpl14, rpl16, rpl2, rpl20, rpl23, rpl32, rpl33, rpl36	rpl14, rpl16, rpl2, rpl2, rpl20, rpl23, rpl23, rpl32, rpl33, rpl36	rpl14, rpl16, rpl2, rpl2, rpl20, rpl23, rpl23, rpl32, rpl33, rpl36
	Small ribosomal subunit	rps11, rps12, rps12, rps14, rps16, rps18,	rps11, rps12, rps12, rps14, rps16,	rps11, rps12, rps12, rps14, rps14 rps16,

		rps19, rps2, rps2, rps4, rps7, rps7, rps8	rps18, rps19, rps2, rps4, rps7, rps7, rps8	rps18, rps19, rps2, rps3, rps4, rps7, rps7, rps8
Other gene	DNA-dependent RNA polymerase subunit	rpoA, rpoB, rpoB, rpoC1, rpoC1, rpoC2, rpoC2	rpoA, rpoB, rpoC1, rpoC2	rpoA, rpoB, rpoC1, rpoC2
	Acetyl-CoA carboxylase subunit	accD	accD	accD
	C-type cytochrome synthase	ccsA	ccsA	ccsA
	Membrane Protein	cemA	cemA	cemA
	Protease	clpP	clpP	clpP
	Translation initiation factor	infA	infA	infA
	Mature enzyme	matK	matK	matK
Unknown functional gene	Conservative open reading frame	ycf1, ycf4	ycf1, ycf2, ycf2, ycf4	ycf1, ycf2, ycf2, ycf4

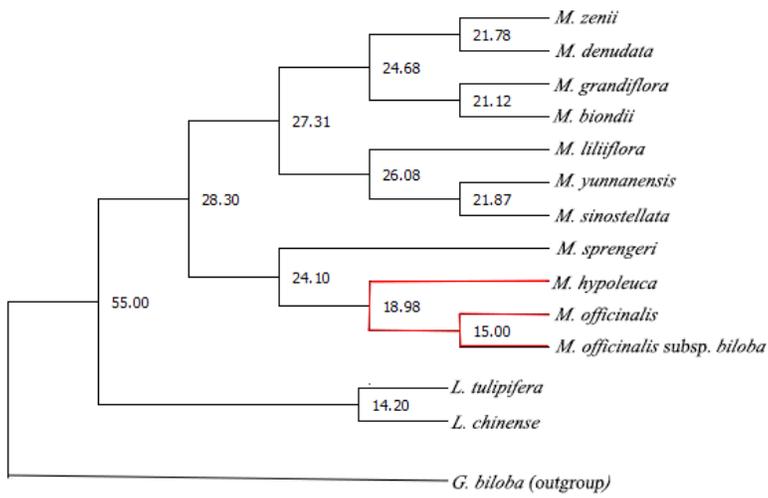
Note: acc, Acetyl-coa carboxylase; rbc, Ribulose biphosphate carboxylase; ccs, *TypeC cytochrome synthesis gene*; cem, *Membrane protein gene*; mat, *Mature enzyme gene*; psb, *Photosystem*.

## Figures



**Figure 1**

Three Magnolias cp gene cycles A *M. hypoleuca* cp genome. B *M. officinalis* subsp. *biloba* cp genome. C *M. officinalis* cp genome. Genes shown outside the circle are transcribed clockwise and those inside counterclockwise. Genes belonging to different functional groups are color-coded



**Figure 2**

Fossil-calibrated phylogenetic tree of three *Magnolias* plants. Species tree constructed using four cpDNA of 14 species in Magnoliaceae and one outgroup, *Ginkgo biloba*. The tree is a clade credibility tree; numbers along the branches of the tree are the average estimated divergence times.