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김 상 태 교수지도  
석사학위 청구논문

Phylogeny of subgenus *Yulania*  
(Magnoliaceae) and related taxa  
based on chloroplast genomes

2012

성신여자대학교 대학원  
생물학과  
송은지

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김 상 태 교수지도

이 논문을 석사학위 논문으로 제출함

2012 년 5 월

성신여자대학교 대학원

생물학과

송은지

# 인 준 서

송은지의 석사학위 논문으로 인준함.

심사위원 \_\_\_\_\_인

심사위원 \_\_\_\_\_인

심사위원 \_\_\_\_\_인

성신여자대학교 대학원

## 논문개요

목련과 식물은 고전적 형태 연구들에 의해 가장 원시적인 피자식물로 제시되어 많은 식물 학자들의 관심의 대상이 되어 왔다. 현대의 분자계통학적 연구는 가장 원시적인 식물로서 *Amborella*를 제시하였고, 목련과 식물들은 그 다음의 원시적 식물 그룹으로 제시되고 있지만, 여전히 원시피자식물군의 식물로서 목련과 식물들에 대한 피자식물의 초기 진화를 이해하기 위한 계통적, 유전적, 진화적 연구들이 진행되고 있다. 지금까지 수행된 목련과 식물에 대한 분자적인 계통학적 연구들은 과내의 주요 분계조를 잘 구분하고 있지만 이들간의 계통유연관계는 여전히 불명확하다. 그러므로, 유전체 수준의 데이터에 의한 계통 분석이 요구된다. 이 연구는 지금까지 진행되어온 목련과 식물에 대한 일련의 분자적 계통 연구의 연장으로서 1) Solexa/Illumina 기술을 이용한 *Yulania* 아속의 하나인 *Magnolia kobus*의 엽록체 전체 유전체 결정과 2) PCR을 이용한 *Yulania* 아속과 이와 연관된 분류군 10개에 대한 엽록체 유전체를 결정하여 분석하였다. 목련과 식물의 엽록체 유전체의 길이는 158,177bp ~ 160,093bp의 범위를 가진다. IR의 길이는 25,651bp (*M. liliiflora*) ~ 26,602bp (*M. acuminata*)이고, 이것은 87,484bp (*M. kobus*) ~ 88,156bp (*M. sinica*)의 범위를 가지는 LSC와 18,722bp (*M. sprengeri*) ~ 18,800bp (*Mich. odora*)의 범위를 가지는 SSC로 나뉘어 진다. 유전체 구조의 비교 분석 결과 1) *M. kobus*의 658bp, *M. liliiflora*의 *trnV-GAC* 을 포함하는 952bp의 결실과 같은 종특이적 특성과 2) LSC, SSC, 그리고 IR간의 구역 구분간의 차이를 제시하였다. 전체 엽록체 게놈을 포함하는 여러 계통 분석은 *Yulania* 아속과 관계된 종들의 사이의 새로운 계통관계를 보여주었는데 1) *M. sprengeri*은 *M. kobus*/*M. salicifolia* 계통군과 sister를 형성하고, 2) *M. liliiflora*/*M. denudata* 계통

군은 *M. kobus*/*M. salicifolia*/*M. sprengeri*의 계통군과 sister를 형성하고,  
3) *Michelia*/*M. cathcartii* 계통군은 *Yulania* 아속과 sister를 형성하였다.  
본 연구에서 결정된 목련과 식물의 11개의 엽록체 유전체와 이전의 연구  
된 2개의 엽록체 유전체의 결합 분석은 목련과 식물의 계통과 진화의 이해  
를 위한 새로운 정보를 제공해 줄 것이다.

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## I . INTRODUCTION

As a candidate basal-most angiosperm, Magnoliaceae has been the subject of keen interest from many botanists who study classical morphology (Cronquist, 1981; Takhtajan, 1997). It is believed that undifferentiated perianth and numerous and spirally arranged floral parts are characteristics of a basal-most angiosperm and this idea was supported by *Archaeanthus*, a 102 million years-old fossil from Dakota formation of central Kansas, which has magnolia-like flowers, leaves, and fruits (Dilcher and Crane, 1984; David Dilcher pers. comm.). However, recent molecular phylogenetic studies showed different views on the basal-most angiosperm. Recent innovative view on the basal-most angiosperm have suggested by Mathews and Donoghue (1999) with analyses of duplicated mitochondrial phytochrome genes (*PHYA* and *PHYC*), Qiu et al. (1999) with five nuclear, chloroplast, and mitochondrial genes, and Soltis et al. (1999) with three nuclear and chloroplast genes. These studies showed that *Amborella* is the most basal angiosperm, and Magnoliaceae is placed among the second-level basal group in the phylogenetic tree. After these studies, phylogenetic position of *Amborella* and Magnoliaceae have confirmed continuously in the various molecular phylogenetic studies (Parkinson et al., 1999; Graham and Olmstead, 2000; Hilu et al., 2003; Leebens-Mack et al., 2005; Jansen et al., 2010; Moore et al., 2010; Soltis et al., 2011). Although the basal-most position of extant angiosperms is currently *Amborella*, Magnoliaceae is still spotlighted by many botanists who seek to

understand the early-divergence of angiosperms as a second group of basal-most angiosperms as it pertains to phylogenetic, genomic, and evolutionary studies.

Magnoliaceae has approximately 220-240 species that are characterized by an androecium of numerous spirally arranged stamens, a gynoecium with many simple carpels spirally arranged on an elongated axis, and separate tepals (Law, 1984, 1996, 2004; Liu et al., 1995; Figlar and Nootboom, 2004; Xia et al., 2008). Roughly four-fifths of the species of Magnoliaceae are distributed in temperate and tropical regions of Southeast Asia and the other one-fifths occur in America, from temperate Southeast North America through tropical America as far as Brazil and Bolivia (Dandy, 1971; Thorne, 1993; Frodin and Govaerts, 1996). The distribution of the family in eastern Asia and America is the typical example of intercontinental disjunction which helps to understand the changes of morphological characters and phylogenetic positions throughout the angiosperm evolution (Kim et al., 2001; Nie et al., 2009).

Since Dandy (1927) proposed the first classification system on the Magnoliaceae, many taxonomists have been suggested different classification systems regarding the disposition of tribes, genera, and sections (Dandy, 1978; Law, 1984, 1996; Nootboom, 1985, 1993; Chen and Nootboom, 1993; Xia et al., 2008). These arguments in the taxonomy are perhaps due to inefficient morphological characters including the extensive parallelism and the homogeneity in the family (Nootboom, 2000; Kim et al., 2001).

Recent molecular phylogenetic studies of Magnoliaceae have been

progressed through accumulation of DNA sequencing data. These studies showed very different views on major clades from traditional classification systems based on morphology (Dandy, 1927, 1978; Law, 1984, 1996; Nootboom, 1985; Chen and Nootboom, 1993). The first family-level analyses of DNA sequences have performed by Azuma et al. (1999) with the regions of *trnK* intron (including *matK* gene), *psbA* ~ *trnH*, and *atpB* ~ *rbcl* intergenic spacers. However, taxon sampling was not sufficient and the tree does not show clear subgroups in the family. Kim et al. (2001) analyzed 99 chloroplast *ndhF* sequences, representing all published sections of Magnoliaceae. This study recognized eleven major clades in the family and these clades were quite different from the classical classification systems based on morphology (Dandy, 1978; Law, 1984, 1996; Nootboom, 1985, 1993; Chen and Nootboom, 1993; Xia et al., 2008): *Magnolia* was a paraphyletic group: *Michelia*, *Manglietia*, *Elmerrillia*, *Pachylarmax*, and *Kmeria* were grouped together with sections of *Magnolia*. Although most of clades were highly supported, some clades had low bootstrap values and the relationships among these clades were unclear. Especially, the sister group of subgen. *Yulania*, a deciduous group, was ambiguous relationships among the species of *Michelia*-*Yulania*-*Manglietiastrum* clade (Fig. 1A). Furthermore, the relationships among sublineages of subgen. *Yulania* were also unclear although these sublineages were highly supported. Recently, Nie et al. (2009) analyzed 37 sequences with three nuclear (*PHYA*, *LFY*, and *GALI*) and three chloroplast (*ndhF*, *trnK*, and *trnL* ~ *trnF*) DNA regions. This study showed that the basal group in the subfamily Magnolioideae is different from that of Kim et al (2001): sect. *Talauma*, Central American taxa

of *Magnolia* placed at the base of the subfamily Magnolioideae. *Michelia* was a sister to subgen. *Yulania* in this study, however, the bootstrap supporting value was very low (58%). Although this study suggested some relationships among sublineages of subgen. *Yulania*, it was failed to show entire picture of phylogeny and evolution of subgen. *Yulania* because this study included only five taxa of subgen. *Yulania* (Fig. 1B).

The main focus of current study is recognizing the unresolved phylogenetic relationships among major lineages of Magnoliaceae such as recognition of a sister group of subgen. *Yulania* and relationships among sublineages of subgen. *Yulania*. To address better phylogenetic relationships among these taxa, more DNA regions in the genome have to be added and eventually entire genomes have to be analyzed.

Chloroplast genome (cp DNA) in vascular plants is highly conserved in terms of its gene contents and genome structures more than any other genome throughout evolution (Palmer, 1991; Raubeson and Jansen, 2005). Most cp DNA of angiosperms are distributed between 100 and 200 kb in length and composed of four parts: single circular DNA chromosome structure consisting of a large-single-copy region (LSC) of 80 ~ 90 kb and a small-single-copy region (SSC) of 20 ~ 30 kb are separated by two copies of inverted repeats (IRs) of 10 ~ 30 kb. It also has high copy number as many as 1000 per cell (Raubeson and Jansen, 2005). The land plant cp DNA is made up 110 ~ 130 different genes of encode ~80 unique proteins for photosynthesis or gene expression, ~30 tRNAs, or ~4 rRNAs (Raubeson and Jansen, 2005; Chumley et al., 2006). Nevertheless, cp DNA appears much of the variation mutation in length of the genomes. It is caused by the

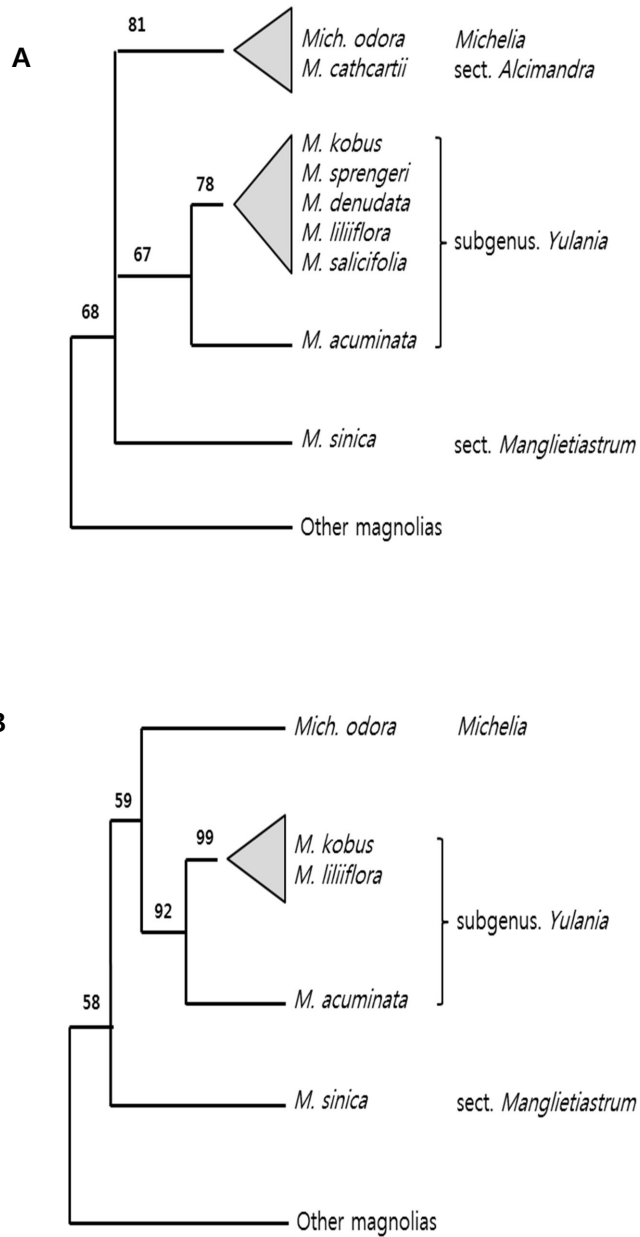


Fig. 1. Summary of phylogenetic relationships among the species of *Michelia*-*Yulania*-*Manglietiastrum* clade in the previous studies. The trees were redrawn with taxa included in this study. A, Kim et al. (2001) with chloroplast *ndhF*; B, Nie et al. (2009) with three nuclear (*PHYA*, *LFY*, and *GALI*) and three chloroplast (*ndhF*, *trnK*, and *trnL ~ trnF*) DNA regions.

expansion or contraction of the IR, gain or loss of genes, variation in length of sequences such as inverted repeat depending on divergence of species (Raubeson and Jansen, 2005; Kim et al., 2006).

During the past two decades, a large numbers of cp genome with structure, expression, and evolutionary profiles are completely sequenced and our knowledge of there organization and evolution is rapidly expanding (Palmer, 1991; Raubeson and Jansen, 2005; Kahlau et al., 2006; Kim et al., 2006; Ravi et al., 2006; Moore et al., 2006, 2007; Jansen et al., 2007; Samson et al., 2007; Saski et al., 2007; Mardanove et al., 2008; Kim et al., 2009; Hu et al., 2011). Since the cp DNA of *Nicotiana tabacum* was first determined (Shinozaki et al., 1986), one hundred and sixty-eight cp genome of angiosperms have been published (data from the GenBank organelle genome resources as of March 2012). These include 168 angiosperms representing six taxa in the ANITA clade, five magnoliids, 42 monocotyledons, and 115 eudicotyledons (Table 1). However, research of cp genome whole sequencing is virtually focused on the model plant and profitable plants like crop or medicine. Some efforts to understand evolution of early-diverging angiosperms leads the determination of cp whole genome of candidater of basal-most angiosperms, as non-model plants and non-profitable plants. As basal angiosperms, cp genomes of *Amborella trichopoda* (Amborellales; Goremykin et al., 2003), *Nuphar advena* (Nymphaeales; Raubeson et al., 2007), *Nymphaea alba* (Nymphaeales; Goremykin et al., 2004), and five taxa of Magnoliids have completely sequenced: *Drimys granadensis* (Canellales; Cai et al., 2006), *Calycanthus floridus* var. *glaucus* (Laurales; Goremykin et al., 2003),

*Liriodendron tulipifera* (Magnoliales; Cai et al., 2006), *Magnolia kwangsiensis* (= *Kmeria septentripnalis*; Magnoliales; Kuang et al., 2011), and *Piper cenocladum* (Piperales; Cai et al., 2006). Generally, data from entire cp genomes gave us upgraded phylogenetic information. For example, Cai et al. (2006) reported whole sequencing of 3 species cp DNA of *Liriodendron tulipifera*, *Drimys granadensis*, and *Piper cenocladum* and analyzed 35 taxa including 33 angiosperms and two gymnosperm outgroups (*Pinus* and *Ginkgo*). In this study, cp whole genome data provided fully resolved relationship among the taxa of Magnoliids, which was one of critical polytomies in the phylogeny of angiosperms. As a taxon of magnoliids, more recently, Kuang et al. (2011) has determined whole cp DNA of *Magnolia kwangsiensis* using New Generation Sequencing (NGS) technology called Solexa/Illumina (see below).

Over the past several years, the field of DNA sequencing technology has developed rich and diverse (Sanger, 1988; Margulies et al., 2005; Hutchison, 2007; Shendure and Ji, 2008). Since the early 1990s, DNA sequence productions have been generated through Sanger method using four fluorescent dyes and scanner read color peaks directly (Sanger et al., 1977; Swerdlow et al., 1990; Hunkapiller et al., 1991). In 2007, New Generation Sequencing (NGS) technology called second-generation DNA sequencing is begun consist of 1) 454 pyrosequencing, 2) Solexa/Illumina technology, and 3) SOLiD system. New technologies can be grouped into several categories depending on PCR method, sequencing method (sequencing by synthesis), and read length (Shendure and Ji, 2008). The first NGS developed by 454 Life Sciences Corp. (Margulies et al., 2005).

This technology enables to form a clonal colony based on emulsion PCR with a single primer-coated bead (Margulies et al., 2005; Hutchison, 2007; Shendure and Ji, 2008). With the Sanger method, sequences are synthesized by DNA polymerase in the picotiter plates made by glass fibers and a CCD imager collects the data (Margulies et al., 2005; Hutchison, 2007). Unlike 454 technology, the Solexa technology uses chain-terminating nucleotides and reads each base of a homopolymer are produced through a separate step. Therefore, it is not produced insertions and deletions as the 454 pyrosequencing (Hutchison, 2007). As an another NGS, Applied Biosystems called Supported Oligonucleotide Ligation and Detection system (SOLiD) is a parallel sequencer based on hybridization-ligation chemistry (Shendure et al., 2005) and needs to library preparation (Hutchison, 2007). While it is very similar to the 454 pyrosequencing using emulsion PCR with beads, is generated significantly higher density sequencing array (Hutchison, 2007). With the appearance of New Generation Sequencing (NGS), new approaches for genome sequencing have been steadily proposed due to their high-throughput, time-saving, and low-cost advantages (Moore et al., 2006; Cronn et al., 2008; Yang et al., 2010; Tangphatsornruang et al., 2010). Nowadays, as pioneer works, the Solexa/Illumina system is started to use to determine whole chloroplast genome (Kuang et al., 2011). With efficient method to generate massive sequences, phylogenetic analyses of genome data will be a popular approach in plant systematics field in the near future.

As a series of molecular phylogenetic studies on the Magnoliaceae, this study provides 1) the chloroplast whole genome of *Magnolia kobus*, a

member of subgen. *Yulania*, using Solexa/Illumina technology, and 2) additional 10 chloroplast genomes included in the subgen. *Yulania* and related taxa using a conventional PCR-based method. Structural analyses of genomes and phylogenetic analyses of these data and previously published magnolia cp genomes will provide new insight to understand phylogeny and evolution of subgen. *Yulania*, a deciduous magnolias, in the family.

Table 1. Number and species of taxa for each group of tracheophytes of which chloroplast genomes are searchable in GenBank as of March, 2012

	Number	Taxa name
<b>FERNS</b>	<b>10</b>	<i>Adiantum capillus-veneris</i> , <i>Alsophila spinulosa</i> , <i>Angiopteris evecta</i> , <i>Cheilanthes lindheimeri</i> , <i>Equisetum arvense</i> , <i>Huperzia lucidula</i> , <i>Isoetes flaccida</i> , <i>Psilotum nudum</i> , <i>Pteridium aquilinum</i> subsp. <i>aquilinum</i> , <i>Selaginella moellendorffii</i>
<b>GYMNOSPERMS</b>	<b>23</b>	<i>Cathaya argyrophylla</i> , <i>Cedrus deodara</i> , <i>Cephalotaxus wilsoniana</i> , <i>Cryptomeria japonica</i> , <i>Cycas taitungensis</i> , <i>Ephedra equisetina</i> , <i>Gnetum parvifolium</i> , <i>Keteleeria davidiana</i> , <i>Larix decidua</i> , <i>Picea morrisonicola</i> , <i>Picea sitchensis</i> , <i>Pinus contorta</i> , <i>Pinus gerardiana</i> , <i>Pinus koraiensis</i> , <i>Pinus krempfii</i> , <i>Pinus lambertiana</i> , <i>Pinus longaeva</i> , <i>Pinus monophylla</i> , <i>Pinus nelsonii</i> , <i>Pinus thunbergii</i> , <i>Pseudotsuga sinensis</i> var. <i>wilsoniana</i> , <i>Taiwania cryptomerioides</i> , <i>Welwitschia mirabilis</i>
<b>ANGIOSPERMS</b>	<b>168</b>	
		Amborellales 1 <i>Amborella trichopoda</i>
		Nymphaeales 2 <i>Nuphar advena</i> , <i>Nymphaea alba</i>
		Austrobaileyales 1 <i>Illicium oligandrum</i>
		Ceratophyllales 1 <i>Ceratophyllum demersum</i>
		Chloranthales 1 <i>Chloranthus spicatus</i>
<b>MAGNOLIIDS</b>	<b>5</b>	
		Canellales 1 <i>Drimys granadensis</i>
		Laurales 1 <i>Calycanthus floridus</i> var. <i>glaucus</i>
		Magnoliales 2 <i>Liriodendron tulipifera</i> , <i>Magnolia kwangsiensis</i>
		Piperales 1 <i>Piper cenocladum</i>
<b>MONOCOTS</b>	<b>42</b>	
		Acorales 2 <i>Acorus americanus</i> , <i>Acorus calamus</i>
		Alismatales 5 <i>Colocasia esculenta</i> , <i>Lemna minor</i> , <i>Spirodela polyrhiza</i> , <i>Wolffia australiana</i> , <i>Wolffiella lingulata</i>
		Asparagales 4 <i>Neottia nidus-avis</i> , <i>Oncidium Gower Ramsey</i> , <i>Phalaenopsis aphrodite</i> subsp. <i>formosana</i> , <i>Rhizanthella gardneri</i>
		Dioscoreales 1 <i>Dioscorea elephantipes</i>
		Liliales 0

Table 1. Continued.

	Number	Taxa name
<b>COMMELINIDS</b>	<b>30</b>	
Arecales	1	<i>Phoenix dactylifera</i>
Commelinales	0	
Poales	29	<i>Acidosasa purpurea, Agrostis stolonifera, Anomochloa marantoidea, Bambusa emeiensis, Bambusa oldhamii, Brachypodium distachyon, Coix lacryma-jobi, Dendrocalamus latiflorus, Ferrocalamus rimosivaginus, Festuca arundinacea, Hordeum vulgare</i> subsp. <i>vulgare, Indocalamus longiauritus, Leersia tisserantii, Lolium perenne, Oryza meridionalis, Oryza nivara, Oryza sativa</i> var. <i>indica, Oryza sativa</i> var. <i>japonica, Panicum virgatum, Phyllostachys edulis, Phyllostachys nigra</i> var. <i>henonis, Phyllostachys propinqua, Rhynchoryza subulata, Saccharum hybrid cultivar NCo 310, Saccharum hybrid cultivar SP80-3280, Sorghum bicolor, Triticum aestivum, Typha latifolia, Zea mays</i>
Zingiberales	0	
<b>EUDICOTS</b>	<b>115</b>	
Buxaceae	1	<i>Buxus microphylla</i>
Proteales	3	<i>Nelumbo lutea, Nelumbo nucifera, Platanus occidentalis</i>
Ranunculales	3	<i>Megaleranthis saniculifolia, Nandina domestica, Ranunculus macranthus</i>
<b>CORE EUDICOTS</b>	<b>108</b>	
Gunnerales	0	
Caryophyllales	6	<i>Fagopyrum esculentum</i> subsp. <i>ancestrale, Silene conica, Silene latifolia, Silene noctiflora, Silene vulgaris, Spinacia oleracea</i>
Santalales	0	
Saxifragales	0	
<b>ROSIDS</b>	<b>63</b>	
Vitaceae	1	<i>Vitis vinifera</i>
Crossosomatales	0	
Geraniales	5	<i>Erodium carvifolium, Erodium texanum, Geranium palmatum, Monsonia speciosa, Pelargonium x hortorum</i>
Myrtales	7	<i>Eucalyptus globulus</i> subsp. <i>globulus, Eucalyptus grandis, Oenothera argillicola, Oenothera biennis, Oenothera elata</i> subsp. <i>hookeri, Oenothera glazioviana, Oenothera parviflora</i>

Table 1. Continued.

	Number	Taxa name
<b>EUROSIDS I</b>	<b>25</b>	
<b>(FABIDS)</b>		
Celastrales	0	
Cucurbitales	3	<i>Corynocarpus laevigata</i> , <i>Cucumis melo</i> subsp. <i>melo</i> , <i>Cucumis sativus</i>
Fabales	10	<i>Cicer arietinum</i> , <i>Glycine max</i> , <i>Lathyrus sativus</i> , <i>Lotus japonicus</i> , <i>Medicago truncatula</i> , <i>Millettia pinnata</i> , <i>Phaseolus vulgaris</i> , <i>Pisum sativum</i> , <i>Trifolium subterraneum</i> , <i>Vigna radiata</i>
Fagales	1	<i>Castanea mollissima</i>
Malpighiales	6	<i>Hevea brasiliensis</i> , <i>Jatropha curcas</i> , <i>Manihot esculenta</i> , <i>Ricinus communis</i> , <i>Populus alba</i> , <i>Populus trichocarpa</i>
Oxalidales	0	
Rosales	5	<i>Fragaria vesca</i> subsp. <i>vesca</i> , <i>Morus indica</i> , <i>Pentactina rupicola</i> , <i>Prunus persica</i> , <i>Pyrus pyrifolia</i>
<b>EUROSIDS II</b>	<b>25</b>	
<b>(MALVIDS)</b>		
Brassicales	14	<i>Aethionema cordifolium</i> , <i>Aethionema grandiflorum</i> , <i>Arabidopsis thaliana</i> , <i>Arabis hirsuta</i> , <i>Barbarea verna</i> , <i>Brassica rapa</i> subsp. <i>Pekinensis</i> , <i>Capsella bursa-pastoris</i> , <i>Carica papaya</i> , <i>Crucihimalaya wallichii</i> , <i>Draba nemorosa</i> , <i>Lepidium virginicum</i> , <i>Lobularia maritima</i> , <i>Nasturtium officinale</i> , <i>Olimarabidopsis pumila</i>
Malvales	10	<i>Gossypium arboreum</i> , <i>Gossypium barbadense</i> , <i>Gossypium darwinii</i> , <i>Gossypium herbaceum</i> subsp. <i>africanum</i> , <i>Gossypium hirsutum</i> , <i>Gossypium mustelinum</i> , <i>Gossypium raimondii</i> , <i>Gossypium thurberi</i> , <i>Gossypium tomentosum</i> , <i>Theobroma cacao</i>
Sapindales	1	<i>Citrus sinensis</i>
<b>ASTERIDS</b>	<b>39</b>	
Cornales	0	
Ericales	1	<i>Arbutus unedo</i>
<b>EUASTERIDS I</b>	<b>23</b>	
<b>(LAMIIDS)</b>		
Garryales	0	
Gentianales	1	<i>Coffea arabica</i>

Table 1. Continued.

	Number	Taxa name
Lamiales	9	<i>Boea hygrometrica</i> , <i>Epifagus virginiana</i> , <i>Jasminum nudiflorum</i> , <i>Olea europaea</i> , <i>Olea europaea</i> subsp. <i>cuspidata</i> , <i>Olea europaea</i> subsp. <i>europaea</i> , <i>Olea europaea</i> subsp. <i>maroccana</i> , <i>Olea woodiana</i> subsp. <i>woodiana</i> , <i>Sesamum indicum</i>
Solanales	13	<i>Atropa belladonna</i> , <i>Cuscuta exaltata</i> , <i>Cuscuta gronovii</i> , <i>Cuscuta obtusiflora</i> , <i>Cuscuta reflexa</i> , <i>Ipomoea purpurea</i> , <i>Nicotiana sylvestris</i> , <i>Nicotiana tabacum</i> , <i>Nicotiana tomentosiformis</i> , <i>Nicotiana undulata</i> , <i>Solanum bulbocastanum</i> , <i>Solanum lycopersicum</i> , <i>Solanum tuberosum</i>
<b>EUASTERIDS II</b>	<b>15</b>	
<b>(CAMPANULIIDS)</b>		
Apiales	8	<i>Anthriscus cerefolium</i> , <i>Crithmum maritimum</i> , <i>Daucus carota</i> , <i>Eleutherococcus senticosus</i> , <i>Hydrocotyle</i> sp., <i>Oxypolis greenmanii</i> , <i>Panax ginseng</i> , <i>Petroselinum crispum</i>
Aquifoliales	0	
Asterales	7	<i>Ageratina adenophora</i> , <i>Guizotia abyssinica</i> , <i>Helianthus annuus</i> , <i>Jacobaea vulgaris</i> , <i>Lactuca sativa</i> , <i>Parthenium argentatum</i> , <i>Trachelium caeruleum</i>
Dipsacales	0	

## II. MATERIALS AND METHODS

### 1. Materials and DNA extraction

Based on the previous result of molecular phylogenetic studies on the Magnoliaceae (Kim et al., 2001; Nie et al., 2009), eleven taxa representing deciduous magnolia (subgen. *Yulania*) and related taxa were selected. Plant materials used in this study were extracted DNA used in Kim et al. (2001; Table 2) except *M. kobus* (see below). For *M. kobus*, approximately 2 grams of grounded leaves using liquid nitrogen is used for DNA extraction. The DNA extraction have completed with the commercial extraction kit based on DNA binding filter (GeneAll, Seoul, Korea).

### 2. Chloroplast genome sequencing

#### 1) New generation sequencing

*M. kobus* is a common garden tree in Korea. However, it is distributed only in Jeju island as an endangered species in Korean Nature. A sample collected in Jeju island is used to show successful quick determination of cp genome with an efficient new sequencing system, which is Solexa/Illumina technology (Margulies et al., 2005). The sample was randomly sheared 150 ~ 200 bp size and two unique Solexa/Illumina oligonucleotide adapters were ligated to the fragments. The ligation fragments were formed the clusters using PCR. Genome sequencing has performed using GA2 system (Solexa/Illumina, San Diego, USA). NGS reads were matched and filtered against the cp genome of *Liriodendron tulififera*

(Cai et al., 2006). These were respectively *de novo* assembly through three assemblers of NGS Cell (Ver. 3.1.0; CLCBio, Denmark), ABySS (Ver. 1.2.5; Simpson et al., 2009), and Velvet (Ver. 1.0.15; Zerbino and Birney, 2008). All contigs generated from three assemblers were combined using Sequencer 4.9 (Gene Code Corporation, Ann Arbor, USA) with *L. tulififera* sequences as reference. For verifying dubious regions, about 10 kbp (6.2%) of sequences including IR borders are confirmed by conventional Sanger method using primers designed with cp genome of *L. tulififera* (Cai et al., 2006; Fig. 2; Table 3).

## 2) PCR-based method

For the sequencing of 10 magnolia genomes, 250 primers including previously reported 35 primers (Kim et al., 2001) were designed from the cp genome of *M. kobus* which is determined by NGS method (Fig. 3; Table 4). These primers are searched against the cp genome of *L. tulififera* (Cai et al., 2006) and all of them were well matched. Each pair of primers produced 1.2 ~ 1.5 kbp of DNA fragment and the products were used for the sequencing of both directions. The polymerase chain reaction (PCR) have performed with 20µl of reaction volumes containing 1µl template DNA, 10µl 2X AmpMaster Taq (GeneAll, Seoul, Korea), 1µl of each primer, and 7µl of distilled water using a S1000 Thermal cycler (BioRad, Hercules, USA). The reaction cycle involves 10 min at 95°C for pre-denaturation, 30 cycles consisting of 1 min at 95°C for denaturation, 1min at 55°C for annealing, and 3 min at 72°C for extension, with a final extension of 7 min at 72°C. The PCR products were checked in 1.5% polyacrylamide gel with

0.001% ethidium bromide under the UV light of Gel Doc XR+ System (BioRad, Hercules, USA). For sequencing, PCR products were purified with commercial PCR product purification kit (PCR SV; GeneAll, Seoul, Korea) and then sequenced with ABI 3700 sequencer (ABI, Carlsbad, USA).

Table 2. Species of Magnoliaceae included in this study, and information of their voucher and Genbank accession number

Taxa	Voucher information	Genbank accession
Family Magnoliaceae		
Subfamily Magnolioideae		
Tribe Magnolioideae		
Genus <i>Magnolia</i>		
Subgen. <i>Magnolia</i>		
Section <i>Rytidospermum</i>		
<i>dealbata</i>	<i>S. Kim 1008</i> NPRI	JX280393
<i>fraseri</i> var. <i>pyramidata</i>	<i>S. Kim 1011</i> NPRI	JX280395
Section <i>Alcimandra</i>		
<i>cathcartii</i>	<i>S. Kim 1091</i> NPRI	JX280392
Subgen. <i>Yulania</i>		
Section <i>Yulania</i>		
<i>denudata</i>	<i>S. Kim 1010</i> NPRI	JX280394
<i>sprengeri</i>	<i>S. Kim 1023</i> NPRI	JX280401
Section <i>Buergeria</i>		
<i>kobus</i>	<i>S. Kim 2011371</i> SWU	JX280396
<i>salicifolia</i>	<i>S. Kim 1019</i> NPRI	JX280399
Section <i>Tulipastrum</i>		
<i>acuminata</i>	<i>S. Kim 1001</i> NPRI	JX280391
<i>liliflora</i>	<i>S. Kim 1014</i> NPRI	JX280397
Subgen. <i>Talauma</i>		
Section <i>Manglietiastrum</i>		
<i>sinica</i>	<i>S. Kim 1098</i> NPRI	JX280400
Genus <i>Kmeria</i>		
<i>kwangsiensis</i>	-	NC_015892 <sup>a</sup>
Tribe Micheliaceae		
Genus <i>Michelia</i>		
<i>odora</i>	<i>S. Kim 1099</i> NPRI	JX280398
Subfamily Liriodendroideae		
Genus <i>Liriodendron</i>		
<i>tulipifera</i>	-	DQ899947 <sup>b</sup>

<sup>a</sup> Kuang et al. (2011); <sup>b</sup> Cai et al. (2006)

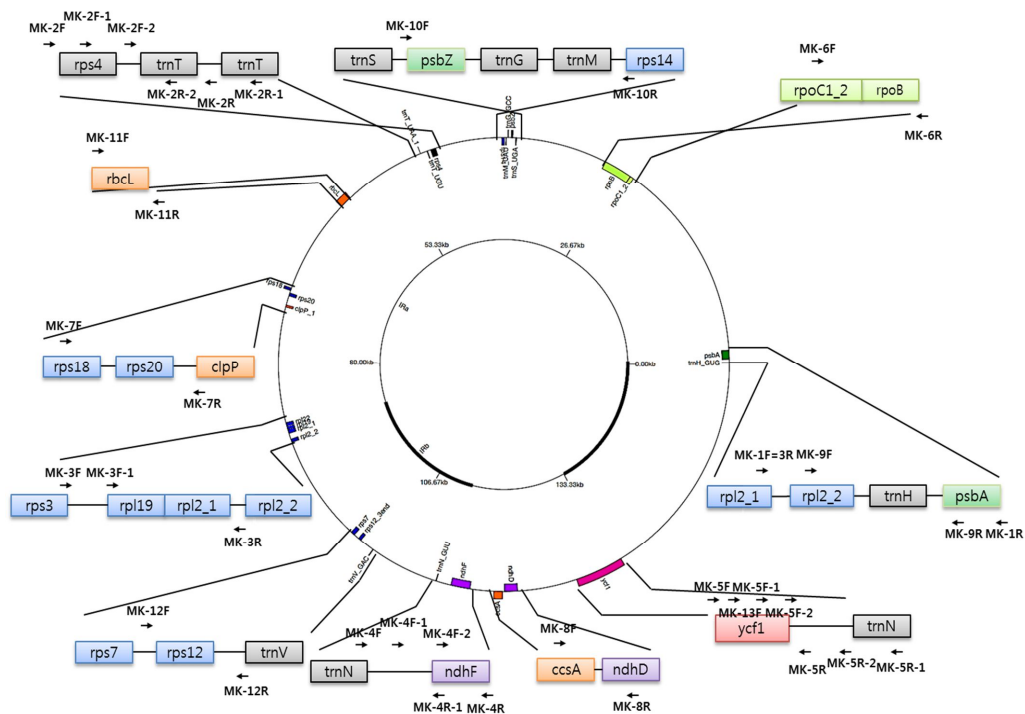


Fig. 2. Primer positions used for the confirmation of NGS result in the *M. kobus*.

Table 3. Primer sequences used for the confirmation of NGS result in the *M. kobus*

Primer name	Primer sequences	Primer name	Primer sequences
MK-1F	ATAAGCCAGATGACGGAACG	MK 5F-2	TTTACCGATTCTCTATTCCA
MK-1R	AGCACAAGCCTATGGGGTC	MK-5R	TTGGATTGGTATTATTCTGG
MK-2F	TCCAAACGCATCTCAAGTAG	MK-6F	CGACCCCGCATTGTTAC
MK-2F-1	TGTTGACCCCTTGGCCTTTC	MK-6R	GGTGGTGAACCTGCTTTGGG
MK-2F-2	ATCTTTGTTTCATCTCTCGTAGT	MK-7F	ATTTATTAGTGAACAGGG
MK-2R	CCCATCTTCTTTCATCTCTATC	MK-7R	AGAAGCAGAAAAGATTATG
MK-2R-1	TGCTCCTATTCTTACCCATC	MK-8F	GTGCCCTGCTTACAATCC
MK-2R-2	CAATCCAATCAAGTCCGTAG	MK-8R	TTTTCTCCCTGGTTGATG
MK-3F	CAGGTGGGTCTTTTATCGG	MK-9F	GGTAAGCGTCTGTAGTAAGAG
MK-3F-1	CTCATAGGAACGCCACG	MK-9R	GCCCTTGGATTGCTGTTG
MK-3R	ATAAGCCAGATGACGGAACG	MK-10F	ATCTCTATTTTATCCCCCG
MK-4F	TTGGATTGGTATTATTCTGG	MK-10R	TTCGTCCATTAGTTCAGTTC
MK-4F-1	TAGGCGTAGAGGAACACAC	MK-11F	CACCAAAAACAGAGACTAAAGC
MK-4F-2	TACCGTCGCCTATTGTAC	MK-11R	CCATACCTCACAAAGCAGCAG
MK-4R	GTCCTATTTACTTTGTTTGTG	MK-12F	CACTTATTTTGGCTTTTTGACC
MK-4R-1	ACTTACTTTTGAGGGGCATT	MK-12R	TGGGATAGGGATAGAGGAAGAG
MK-5F	TTTTCCATTATTATTCC	MK-13F	ATTCTTCTCATTCTTCTGCTC
MK-5F-1	TTCCATTATTATTCCAG		

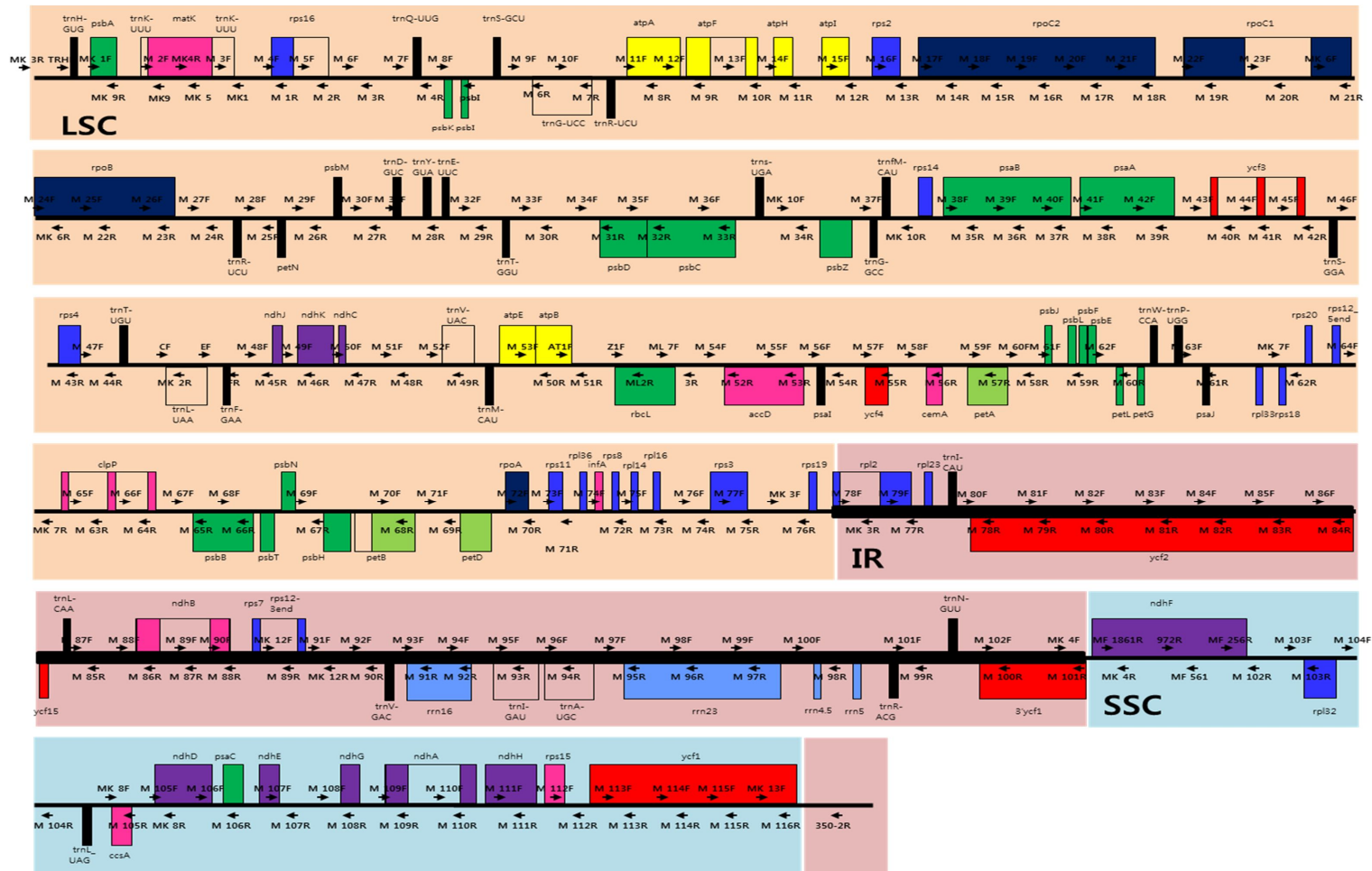


Fig. 3. Primer positions used for PCR-based chloroplast genome determination.

Table 4. Primers sequences used for PCR-based chloroplast genome determination

Primer name	Primer sequences	Primer name	Primer sequences
MK-3R*	ATAAGCCAGATGACGGAACG	MK-9R*	GCCCTTGGATTGCTGTG
TRHF**	CGCATGGTGGATTACAATC	MK-9R*	GCCCTTGGATTGCTGTG
M 1F	AGGCATACCATCAGAGAAGC	MK9**	CTTCGACTTTCGTGTGCTAG
M 2F	ATCCAAATACCAAATCCGTT	MK5**	CACTGCTGGATACAAGATGC
MK4R**	TTTACGGAGAAACACTAATACG	MK1**	ACGAATGTGTAGAAGAAACGG
M 3F	CCTCTCTCTTCCATCCAAT	M 1R	GGGGGCATTGTTCACTA
M 4F	AAGAGATTGGATTGCCCTAC	M 2R	AGGGTTAGTGCCAGTCAATA
M 5F	GCCGTCTCTAACCTCTTTTG	M 3R	CGACTTGTGATTGATTGATT
M 6F	CGGAAAAGTCGCAAGTGA	M 4R	GGTTTTGGTCCCCTTACT
M 7F	CACCCAGCTTTAGGAGC	M 5R	CAAACAAGGGCTAAGAGAAA
M 8F	TTACCCGAGGCTTATGCT	M 6R	CGAAAGACCCCTAACTATT
M 9F	TATGTTCCGACTTCAATGGC	M 7R	GTTTCATTGGCTCCTTTAT
M 10F	CTCCCTTTTTCCATACATCG	M 8R	GCTTATCGCCAAATGTCTCT
M 11F	GGAGACGGAAATACCCACAT	M 9R	CGAGTTACATTTACGCACCA
M 12F	TTCCCTGCCATTACTTC	M 10R	GAGTGTGTGCGAGTTGTGATT
M 13F	GCGAGACACCCATTTTTTC	M 11R	GCTTGCTTCTATTGGACCTG
M 14F	CCATAAAAGCCAGACTAAGC	M 12R	CAACCAACCCCAATACTTTTAC
M 15F	AATCCCGCTTGTGAATAATC	M 13R	GCAGGAGTTCATTTTGGTCA
M 16F	TTCCCGCTCTTTTGTTC	M 14R	GAAAAGAGGATTGAAGGTTG
M 17F	GATGCCCTCGTTATTCCC	M 15R	GGAATCAAAAAATGGAAAAAT
M 18F	GGCATTCTTATTCTATTTCAG	M 16R	GAAAGAACTAATGCCCCG
M 19F	CGGGAATGAAAAAATCG	M 17R	CTGTAGATTATGTTATGGTCGG
M 20F	GCGAATCTCAGCAATCACTT	M 18R	GCCACTGCTACATCCAT TTC
M 21F	TGTTGTTGAGCATCTTGGAC	M 19R	CATTTGTCATTCGTGGTCTA
M 22F	GGTGGGTGCTCTATTTCAG	M 20R	ATTAGCCATTCCATTCTTTTA
M 23F	ACACCAAATAAAGAAAGGGG	M 21R	GGAGAAGTGACAAAACCTTA
MK-6F*	CGACCCCGCATTGTTTAC	MK-6R*	CGAACACGAGGGAAAGAT
M 24F	ATGCGGTATTTCTGTAGTGA	M 22R	ATTGGCTCTGGTTCGTTTAG
M 25F	TTGAGATAAAGGGGTAGGC	M 23R	GATGGAAATGAGGGAATGTCTA
M 26F	CAATGAACCTACAAAATCCCTC	M 24R	CCAAAACAAAAGAAATCCC
M 27F	TTTTGGATTCTGTAAGTGA	M 25R	CATTCTTGGCGGGGTTAC
M 28F	ATTGGATGGGTGATTGGC	M 26R	TCCATTTGTATTGATTCCGA
M 29F	TACAATGAGGAGCAACCAAC	M 27R	TTTCTTCCTATTTACCCCATC
M 30F	CTCATTTCCACTCTTTCTTTTC	M 28R	GTCTACGCTGGTTCAAATCC
M 31F	GTGCTCTGACCGATTGAACT	M 29R	TAGGGGGCTCATTCAAGA
M 32F	AACTCGTAAATCTGGGAAGG	M 30R	CTTTCTCGCATTGCTCT
M 33F	TTTATCCGAGTCACAAGAGC	M 31R	GCGAAATAAGCACAAGGAAA
M 34F	TTCGGAAATGGTTGAAGTAG	M 32R	TGATAAGTCGGGCATTCC
M 35F	CGGTTTATGGATGAGTGCTA	M 33R	GCGATGAAACCAAAGACAGA
M 36F	GGGGAGAAGGATGGATTG	M 34R	ATTCCCACTTTATTTTATTTCG
MK-10F*	ATCTCTATTTTATCCCCCG	MK-10R*	TTCGTCCATTAGTCTCAGTTC
M 37F	CCTCCTCTTTTCTCCCA	M 35R	CTTGTTTGGGCTACTGGATT
M 38F	GTAGAGGCAATCAAGAAAGC	M 36R	ATCACCAATACATCGCAGGA
M 39F	GAACCCAGAAACAGGCT	M 37R	CAATCGGCTTACGCACTA
M 40F	TCGGCATTTTTGAACAC	M 38R	GCAGTCAGATGTTTGGGG
M 41F	CACCCAGGAAAAAAGGC	M 39R	GCTTTTTGTGTTGGTT
M 42F	CTCGGCAAACTGGGATA	M 40R	ATTGACCCACCTATTCCG
M 43F	TACCAGATGAGATAGAACGATG	M 41R	CAACGGAGAACATACGAAGG
M 44F	TCGGCTCGTATGAAGTCTCT	M 42R	GAGATGGTGCAGTTGATTTC
M 45F	GGGATACACGACAGAAGGAA	M 43R	GACTTTTCACTCATCCCAAT
M 46F	CGGAAAAGAGTGAAAAAGAAT	M 44R	ACAGAACAATCAAGAAAAGGA
M 47F	CTGAACTAAACGATAAACGAAG	MK-2R-2*	CAATCCAATCAAGTCCGTAG

Table 4. Continued.

Primer name	Primer sequences	Primer name	Primer sequences
CF**	CGAAATCGGTAGACGCTACG	FR**	ATTGAACTGGTGACACGAG
EF**	GGTTCAAGTCCCTCTATCCC	M 45R	GGGCTAATAAAAAGAAAGGGG
M 48F	TTTCTATTTCTTTACTCCCTCC	M 46R	TGGGTCTCAACAGGAAAATC
M 49F	CACAAACACACCCCTGCCT	M 47R	ATGACCCACAGCAAACAAAC
M 50F	AATGCCAAAATAGGAATAACAC	M 48R	GAATCCCCCAACTCATCACT
M 51F	GGTTAGGCTTCGTGACAATA	M 49R	GTGCCAAATAGAACCATCA
M 52F	TTGACAGGAAGATAACGAGATG	M 50R	GATGGTCTTCCCGAGCAG
M 53F	TACGGCTGTGGCAATAGG	M 51R	TACCAACGAAATCAAGCG
AT1**	AGAACCAGAAGTAGTAGGAT	ML2R**	TTCAATTATCTCTCTCAACTTGG
Z1**	ATGTCACCACAACAGAACTAAAGCAAGT	3'**	CGGCTCAACCTTTTAGTAAAGATTGGGCCG
ML7*	GGAGGAACTTTAGGACACCC	M 52R	TCCCTGACACCTAAAAAATGAT
M 54F	CAAATAGGGGGCAGGAAG	M 53R	GTTGTAGGAGATGTAAGGATTG
M 55F	GGTGTGTGCTTCTGGAGGAG	M 54R	CGTTCGGATTGCCAGTTC
M 56F	TTACCCTCTATTTTGTGCC	M 55R	CGAGTCAAGGGAATGGCT
M 57F	GTGTGTATTTTCTGTGGGG	M 56R	TTATCATTTCTGCCAACAGG
M 58F	GATTCAAAGTGCCAAAAAAG	M 57R	ACAGTATCAGGAAGCACAGC
M 59F	TGGGTAAGGAACAGATGAC	M 58R	TATTCTCCTCTACTTATGCCT
M 60F	TGTTTTGCTTGCTTTGTTA	M 59R	ACCCGAACGAACAAAATG
M 61F	CTATCAGCCAAAGAGGAATC	M 60R	TGCTCAGACCAATCAATAGA
M 62F	GTTCTCCCGTGCTCCAG	M 61R	AAAGACCCAAACCATAGAGTAG
M 63F	ATCCCTGTCTTGTTCAC	M 62R	CGAACAAAACATCAATCAATCT
MK7F-1	CTTTTCGTAGGCGTTTGC	MK-7R*	AGAAGCAGAAAAGATTATG
M 64F	CACACTCTTTGGCTCTACCC	M 63R	CCTTTTTGCTTCCACACC
M 65F	GACAAATAGAATCCATCAGACC	M 64R	GTCGTAGCAAAAAGAAAGTGG
M 66F	TTTTGACTTGACTTGCTTCC	M 65R	ACAGAAAGCAACCGACCCG
M 67F	CTGCTTCTCTTTGTTCCTACGA	M 66R	AATAATCCCCCTTTCGCC
M 68F	GCTTTCGTTGTTGCTGGA	M 67R	ATAGAGCCATTGCGACAC
M 69F	CGAACTATTACAGGGGATTT	M 68R	AAAAAGTCATAGCAAAACCG
M 70F	CGAGATTCAGGCGATTGC	M 69R	AGCCTCCGTTCTTCCTTA
M 71F	GAGGATAGGCTGGTTCGC	M 70R	TGCGGAGGAACAGGACAT
M 72F	CTAAGGAAGAACGGAGGC	M 71R	GGACACCATTGCTGCTC
M 73F	CGTCTTTTTTAGGAGTCT	M 72R	TTGGAGGAGAAGTTTTGTGT
M 74F	TTTTGTTCTTTCATTCCAGG	M 73R	GAAATGGGCGGAGTATCG
M 75F	AATGGGCTGAGGTTGAATC	M 74R	AAAAGGCAGTGTGATAAAGC
M 76F	TTGGTTCCTGTTGGTTC	M 75R	GCAAAAACCTTATGGACAACC
M 77F	CCTTTTGATCCGCTTGTTT	M 76R	GGAGAAGGTGGAAGAAGGTC
MK-3F-1*	CTCATAGGAACGCCACG	MK-3R*	ATAAGCCAGATGACGGAACG
M 78F	ATCAATAAAAACCCCTTCCC	M 77R	ATCATTACGCTTCAACCG
M 79F	CGACCTTTACCACAATGATG	M 78R	CCCCAGTTAGATTACGGC
M 80F	TTTGATGGGGCTTCTTCC	M 79R	TGTCAGAGAAAAAGAACGAAT
M 81F	CAAACGGAACGAACAGAG	M 80R	CCCGATACTCACAAGAAAA
M 82F	CCGTTTTCAAGTAGTGTCCG	M 81R	AGCACTATCTCGTTGAAAGG
M 83F	ACTTATTGTCAGCCTCTTTCAG	M 82R	TCTCTTTCTTCATCATCAATCG
M 84F	CATACCAAATCCCATCAATC	M 83R	GCAACGCCCTTCTTATC
M 85F	GGCTTCTATTCCACAACAA	M 84R	TCGGATGGAGTATTAGAACG
M 86F	CCCTTTGTCTCTGTGTTTTT	M 85R	GTTTTAGGGATTGGCGAC
M 87F	TGGATTCTCTTTCGGATAGG	M 86R	CGAAACCAAGAAATAACCCC
M 88F	CATAACCCAGCCCATTC	M 87R	TTTCTGACTTGTCTCTACGG
M 89F	GACTTTCATCTCGCACGG	M 88R	CCGATGGAGAGAAGAACCTA
M 90F	AGGTAGGAGCATAAACTGAAAC	M 89R	AAAAGGAGGGAAACGGATAC
MK-12F*	CACTTATTTTGGCTTTTGGACC	MK-12R*	TGGGATAGGGATAGAGGAAGAG
M 91F	TTACCAAAAATGTGCGGAT	M 90R	GAAGCAGAACCAAGTCAAGA
M 92F	AGGCAAGAGGATAGCAAGTTAC	M 91R	GCCGTGTCTCAGTCCCAG
M 93F	GGACGGGAAGTGGTGTTT	M 92R	CGGGTTTTTGGAGTTAGC

Table 4. Continued.

Primer name	Primer sequences	Primer name	Primer sequences
M 94F	TCGTGCCGTAAGGTGTTG	M 93R	CCGTCACCCAGATAAAAAG
M 95F	TCAGGAGGATAGATGGGG	M 94R	CCGCCGACTCCAACATC
M 96F	GCGATTACGGGTTGGATG	M 95R	GGTTGTCTTTGCCTGCC
M 97F	CCTTCCATTTAGCAGCAC	M 96R	GCATTTTACATCCCACAGC
M 98F	GAGACGATGGGGGATAAG	M 97R	CGCCCCATAGAAACTGTC
M 99F	GTAAGTCCGACCCGCAC	M 98R	TAGAGAGGGAGGGCAGAG
M 100F	GGGATGGAGCGACAGAAG	M 99R	GAATCACCGTCAATACCTCG
M 101F	TTTGTGTTTTACTCCCCG	M 100R	AGAAATGAAACAAAAGATACGG
M 102F	CGGACTTATTATGGATTTCTG	M 101R	CGAAAAGAAGAGTCAACAAGAGG
MK-4F-2*	TACCGTCGCCTATTGTCAC	MK-4R*	GTCTATTTACTTTGTTTGTG
MF1861R**	TGAAAAGATGAATAAACAGACCC	MF561**	TGGTTTATTATTAGGAATCTTAGG
972R**	CATAATATAACCCAATTGAGAC	M 102R	ATCGCCGTAATAGTGGAATG
MF256R**	TGGGTCGATCAAGTGGCC	M 103R	TCTACGAATACGCTTTTTTG
M 103F	GTAGCGGACCTCATAGACATAG	M 104R	GTGTGAGGATTTACCGAACC
M 104F	GACTTTGCTTTGTAACCTCTCCG	M 105R	GACTAATGACACGATAACTCCA
MK-8F*	GTGCCTGCTCTACAATCC	MK-8R*	TTTTCTCCCTGGTTGATG
M 105F	CCATTGAGTCCCGTATCG	M 106R	TGCTCCTGCTCCAAGAAC
M 106F	ACCAAGGAAAATAACTCGTG	M 107R	GCCGTGTTTTGTTCTGTGTT
M 107F	CCGATAGAAAATAAATAGGCAC	M 108R	GGATAACCCCTTGATTC
M 108F	ATCCCGCTTTTGTATCCG	M 109R	CTTTACTTGGGCGGATGG
M 109F	ATAGGAATGAACAGGAACAAAT	M 110R	AGTAAACATAAGCAGTGGAAC
M 110F	CGTTCCCGATAGTCATTTCT	M 111R	AATGGCAAAAAGAAGGAGAC
M 111F	TCCTTTTGGGGCTTCTACTC	M 112R	TGACTGGCATTATTATTATCC
M 112F	CCAAATGTGAAGTAAGTCTCCG	M 113R	CACGAAACCGACAAAAAG
M 113F	ATCCATTGTCCATCCCAT	M 114R	TGATGAAAGAAATAAAGAAGGA
M 114F	CTCTATTTCCGCAATTTTTGC	M 115R	GAGGATTGGAAGGAGTGG
M 115F	TTTTTCCTTTCTTTTTCATTCCG	M 116R	TCAGAAAATCAAACGAAATG
MK-13F*	ATTCTTCCTATTTCTTGCTC	350-2R**	GGAAGAAAAGGAGGATCCGG

\* primers used for the confirmation of NGS result in the *M. kobus*; \*\* primers from Taberlet et al. (1991), Morgan and Soltis (1993), Olmstead and Sweere (1994), Plunkett et al. (1996), Sang et al. (1997), Kim et al. (2001), and Tobacco cp DNA

### **3. Chloroplast gene annotation and sequence statistics of cp genome**

DNA sequence fragments obtained from individual sequencing are edited using SEQUENCER 4.9 (Gene Code Corporation, Ann Arbor, USA). Sequence alignment is performed using CLUSTALX (Thompson et al., 1994) with default options and finally adjusted by eyes. The aligned FASTA file is then transformed into MEGA file (MEGA5, ver. 5.05) to address GC contents of each taxon and pairwise distances. For sequence comparison, the genes, introns, and intergenic space regions of cp genomes have been sorted out by DOGMA (Wyman et al., 2004) and GenomeVx (Conanat and Wolfe, 2008) have used to draw the gene map of the cp genome of Magnoliaceae. Also, sequence comparison of entire genomes between *L. tulipifera* and other magnolias used in this study have performed using the mVISTA (Mayor et al., 2000). For the detection of simple sequence repeats (SSRs), Msatfinder v.2.0 (<http://www.genomics.ceh.ac.uk/msatfinder/>) have used with thresholds of eight repeat units for mononucleotide SSRs; four repeat units for dinucleotide; three repeat units for tri-, tetra-, penta-, and hexanucleotide SSRs.

### **4. Phylogenetic analyses**

To address unresolved evolutionary relationships in the family phylogenetic analyses of eleven cp genomes determined in this study (Table 2) and previously reported *M. kwangsiensis* (Kuang et al., 2011) in the subgenus Magnolioideae was included in the matrix with *L. tulipifera* (Cai et al., 2006) as an outgroup. *L. tulipifera* was an independent subfamily in the all previous taxonomic and phylogenetic studies (Dandy,

1927, 1950, 1978; Nooteboom, 1985; Chen and Nooteboom, 1993; Kim et al., 2001; Nie et al., 2009). Three different methods of phylogenetic analyses have performed using MEGA5 (Ver. 5.05; Tamura et al., 2011): Maximum parsimony (MP), neighbor joining (NJ), and Maximum likelihood (ML). In the MP analysis, the CNI (Close-Neighbor-Interchange) searching method with potions of 10 initial trees and complete deletion of gaps is used and 500 replications of bootstrap have applied with same condition. In the NJ analysis, *p*-distance substitution model have used with default options. Five-hundred replicates of bootstrap analyses are followed in this analysis. The general time reversible+Gamma distribution with Invariant sites (GTR+G+I) model is selected as the best model in the model test module in the MEGA5. However, it is impossible to apply the best model (GTR+G+I), the second model (GTR+G), and the third model (HKY+G+I) in the ML analysis because of insufficient memory in the PC (8G). Therefore, the fourth model, GTR+I, have used to perform ML analysis with Nearest-Neighbor-Interchange (NNI) option.

### III. RESULTS

#### 1. Determination of cp DNA in *M. kobus* using NGS technique

About 168 million reads containing 24.53 billion bps are generated by Genome Analyzer II (Solexa/Illumina, San Diego, USA). It is about 28.86X coverage of the genome based on previously reported genome size of *M. kobus* (0.88Gbp; Olszewska and Osiecka, 1984). The blast search of cp genome of *L. tulipifera* against these reads, which is previously reported cp genome in Magnoliaceae (Cai et al., 2006), generated about 500X coverage of sequences compare to expected the cp genome size of *M. kobus* (Fig. 4). Filtered reads were assembled using three different assemblers: NGS Cell (Ver. 3.1.0; CLCBio, Denmark) generated 49 contigs (181,310bp in total; N50=10,885bp), ABySS (Ver. 1.2.5; Simpson et al., 2009) generated 49 contigs (181,310bp in total; N50=3,822bp), and Velvet (Ver. 1.0.15; Zerbino and Birney, 2008) generated 8 contigs (120,049bp in total; N50=34,890bp). With these contigs from three assemblers the hybrid assembly has performed using SEQUENCER (Ver. 4.8; Gene Code Corporation, Ann Arbor, USA) with the frame of cp genome sequences of *L. tulipifera* (without the second IR). We confirmed that some long contigs have assembly errors containing non-chloroplast sequences especially in the result from NGS Cell. Assembled super-contigs with SEQUENCER covers entire reference genome region. However, some parts have two different parallel sequences: one well matched with reference sequences but another one is completely different from the reference sequences. To clarify the true cp sequences in

the region having different two sequences, about 10 kbp (6.2%) of sequences were confirmed by conventional Sanger sequencing. When we chose a well-aligned sequence in the two parallel sequences, the sequences were completely matched with the sequences from the result of Sanger sequencing (an error rate of zero). As a result, the chloroplast genome of *M. kobus* is 159,443bp long and has four subregions: 87,484bp of large single copy and 18,783bp of small single copy regions are separated by 26,588bp of inverted repeat regions (Fig. 5).

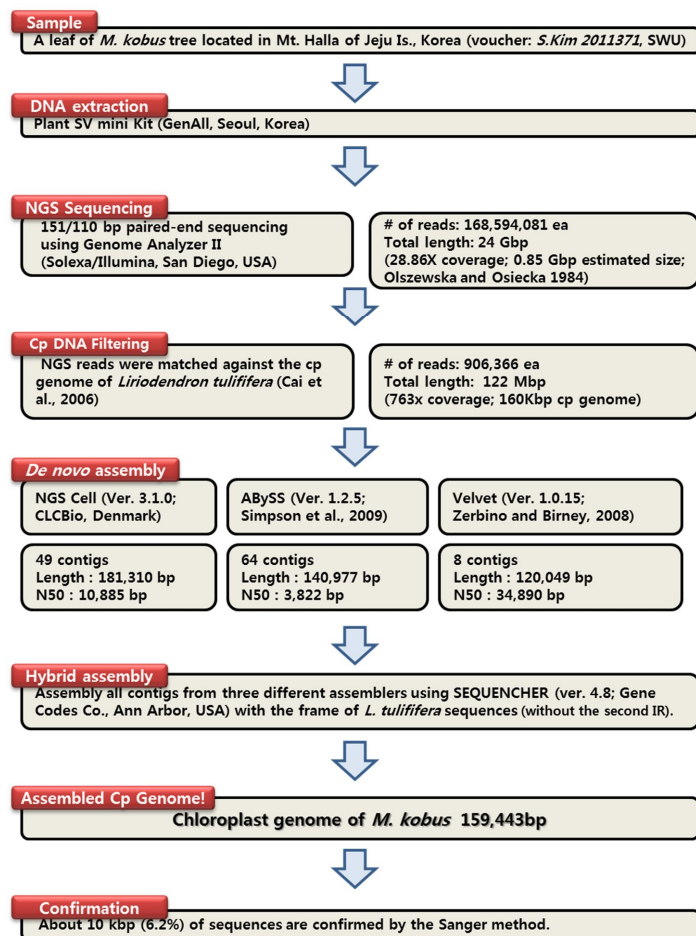


Fig. 4. The quick determination procedure of the chloroplast whole genome and the sequence analyses of *M. kobus*.

## 2. The general features of cp genomes in Magnoliaceae and their structure analyses

Ten cp genomes representing subgen. *Yulania* and related taxa in the family have determined using conventional PCR and Sanger sequencing method and the size of genomes are ranged between 158,177bp ~ 160,093bp (Fig. 5; Table 5). Eleven cp genomes including *M. kobus* showed typical circular chromosome with a quadripartite structure including IR regions ranging from 25,651bp (*M. liliiflora*) to 26,602bp (*M. acuminata*) separated by LSC regions ranging from 87,484bp (*M. kobus*) to 88,156bp (*M. sinica*), and SSC regions ranging from 18,722bp (*M. sprengeri*) to 18,800bp (*Mich. odora*).

The gene annotations of 11 genomes determined in this study are performed by DOGMA (Wyman et al., 2004). These results and two of previously reported genomes in Magnoliaceae (*L. tulipifera*, Cai et al., 2006; *M. kwangsiensis*, Kuang et al., 2011) showed that the overall gene number is 112 in the Magnoliaceae (78 unique genes, 4rRNAs, and 30tRNAs) except *M. liliiflora* (111 genes; 29tRNAs) (Table 6). For ten cp genomes except *M. liliiflora*, the LSC contains 60 protein coding genes and 22 tRNA genes and 12 protein coding genes and one tRNA gene are located in the SSC; the IR region contains 6 protein coding genes and 11 tRNA genes. In *M. liliiflora*, *trnV-GAC* located in IR is deleted. The size of coding regions are 57.09% (*M. sprengeri*) ~ 57.89% (*M. liliiflora*) and RNA are 7.06% (*M. liliiflora*) ~ 7.41% (*M. kobus*) (in both IRs) of the cp genome, respectively (Table 7). These are similar to previously reported Magnoliids: 57.5% in *Drimys granadensis*, 55.7% in *Piper cenocladum*, 57.5% in *L. tulipifera*,

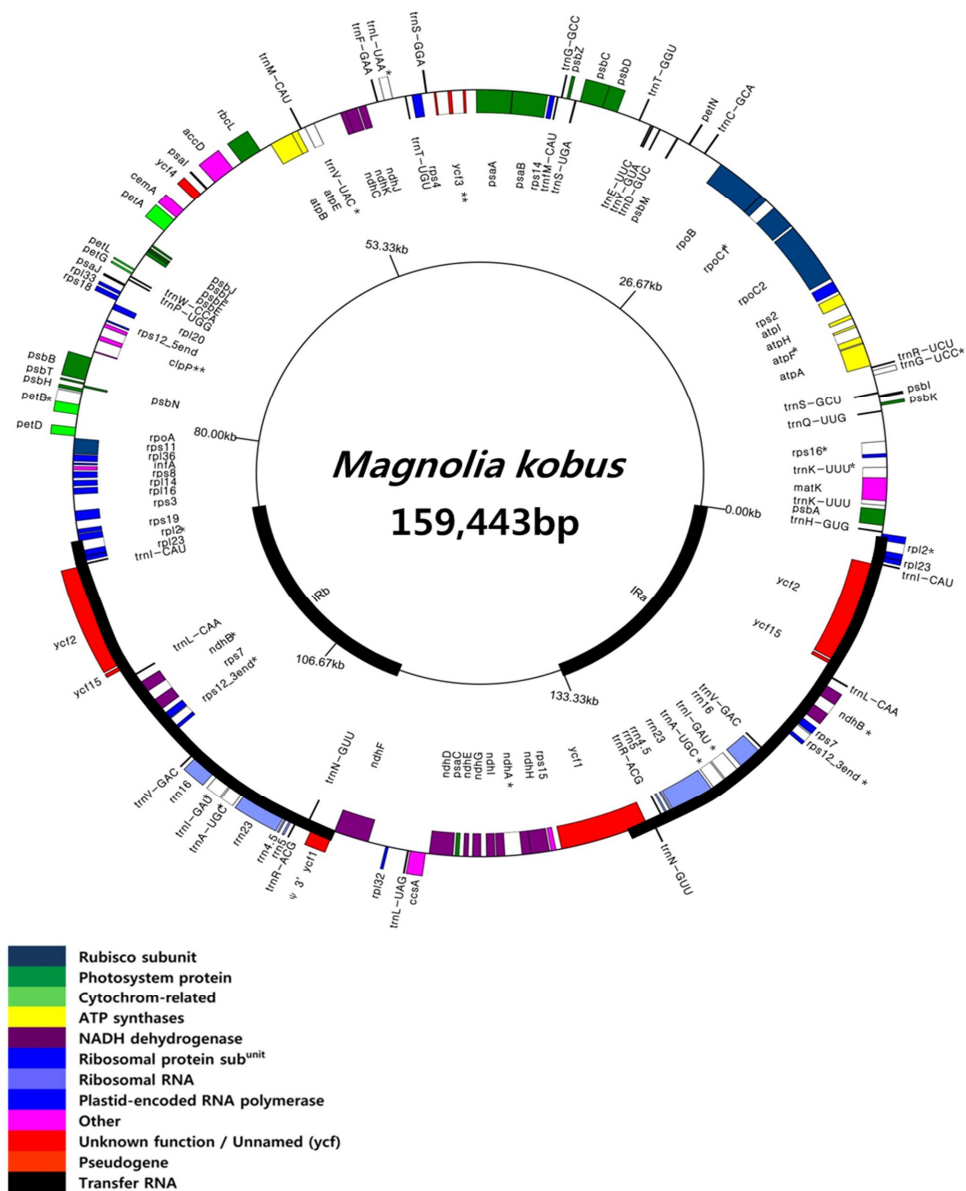


Fig. 5. The gene maps of 11 chloroplast genomes in Magnoliaceae. A pair of thick lines at the inside circle illustrates the large single-copy (LSC), the inverted repeats (IRb and IRa), small single copy (SSC) and IRa regions. Genes drawn outside of the outer circle are transcribed in a clockwise direction, while inside of the outer circle are transcribed in a counterclockwise direction. Each group of genes is represented by a different color. \*, genes containing one intron; \*\*, genes containing two introns; Ψ, a pseudogene; #, unique deletion of *trnV-GAC* in *M. liliiflora*.



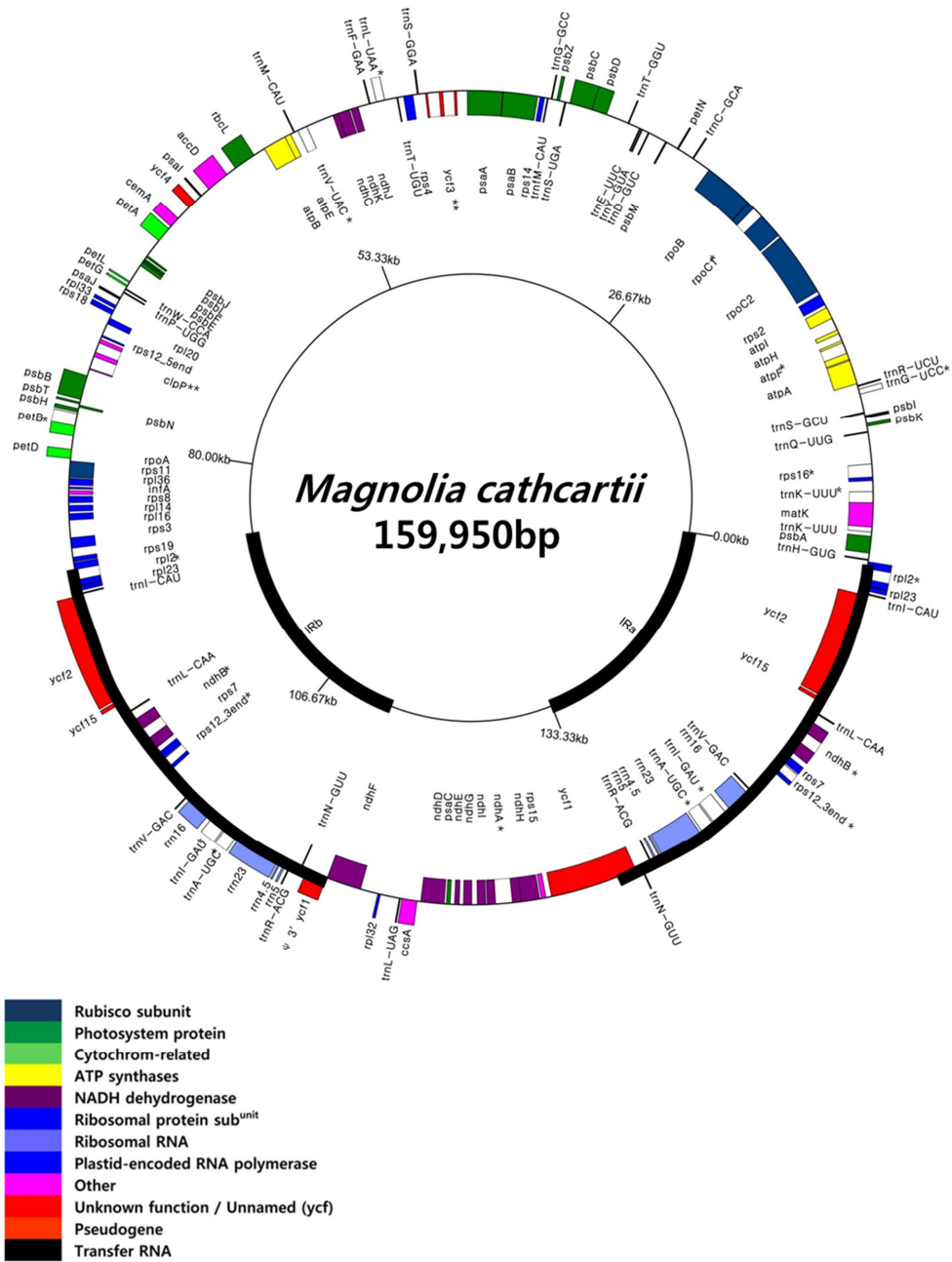


Fig. 5. Continued.

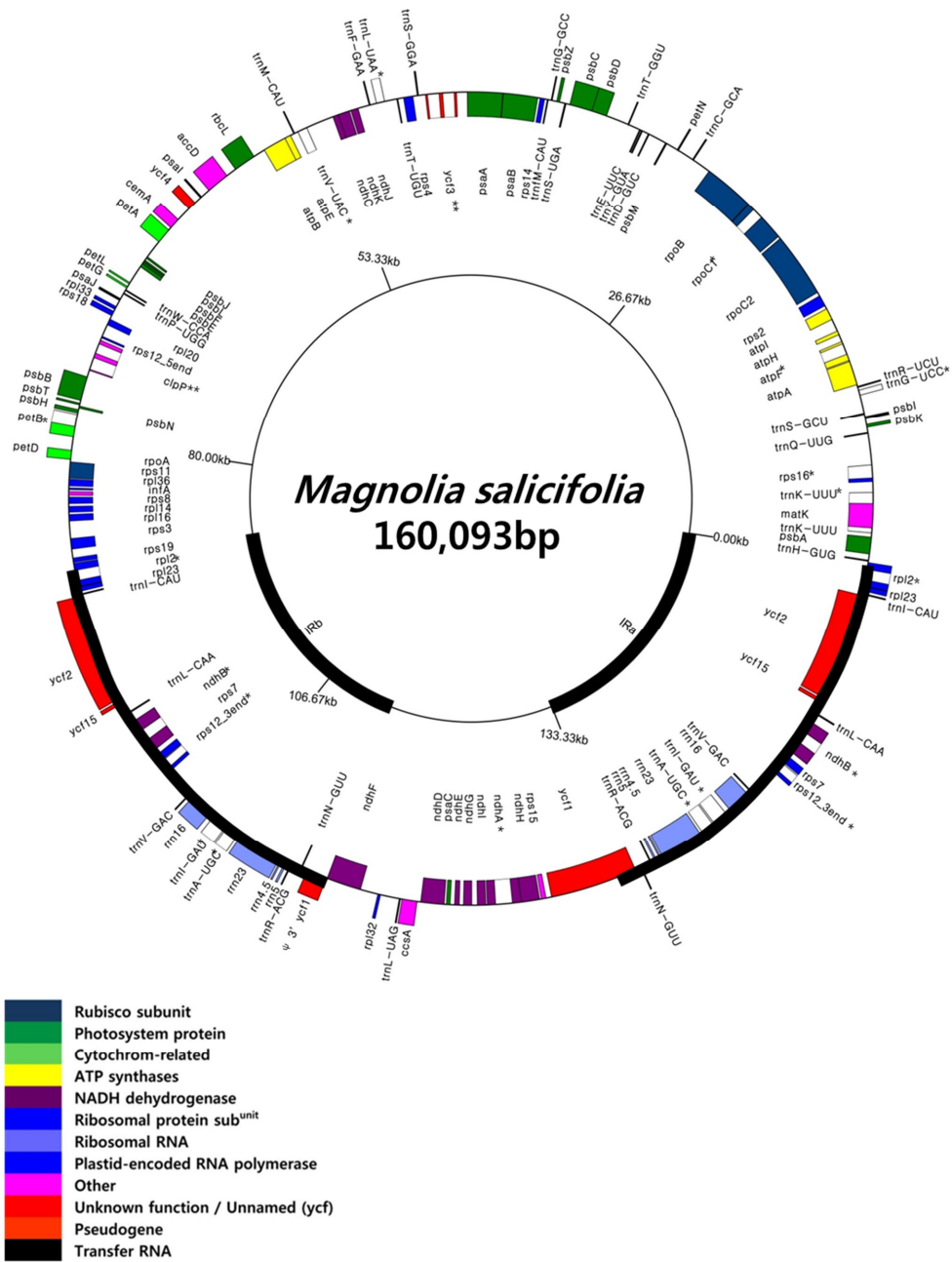


Fig. 5. Continued.

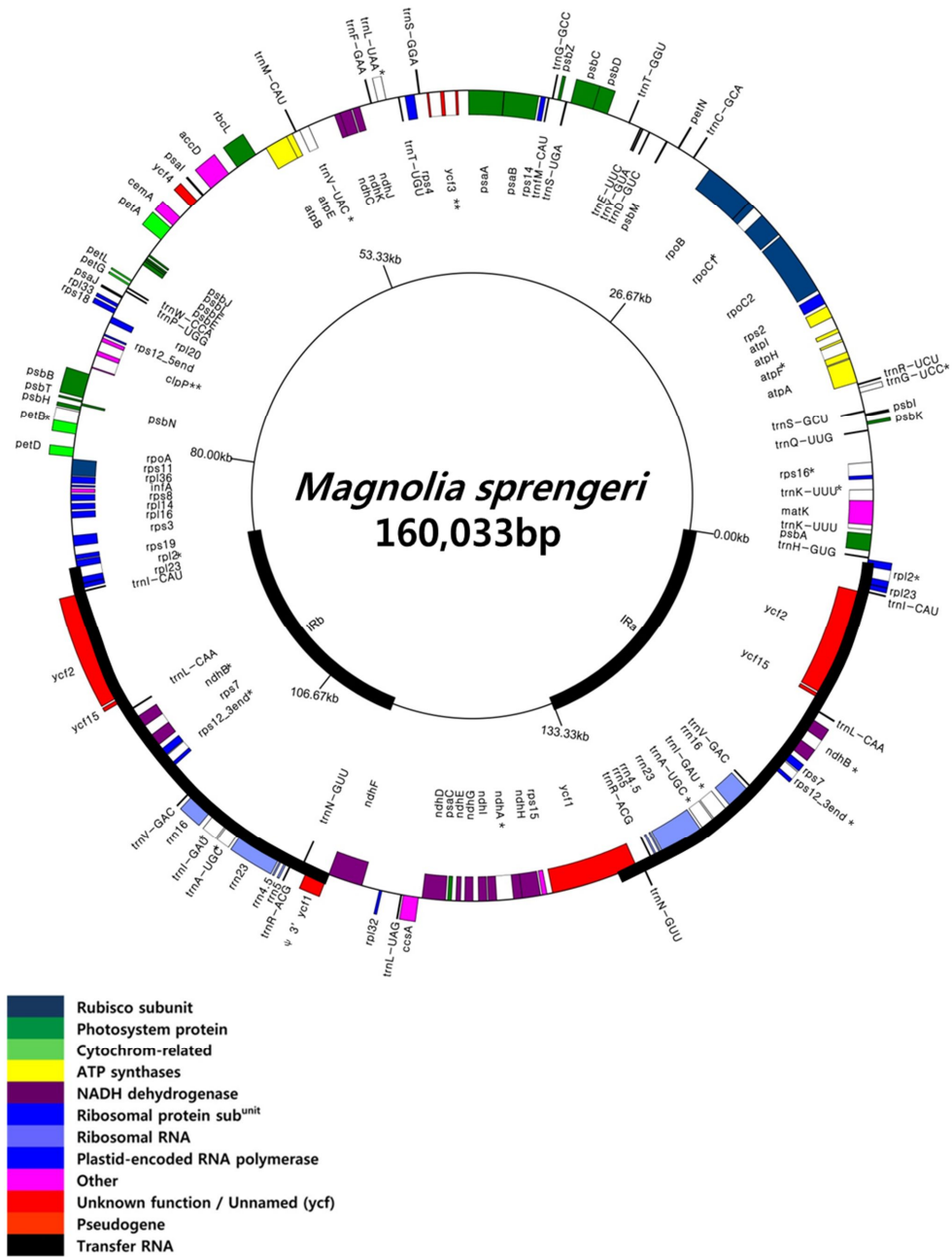


Fig. 5. Continued.

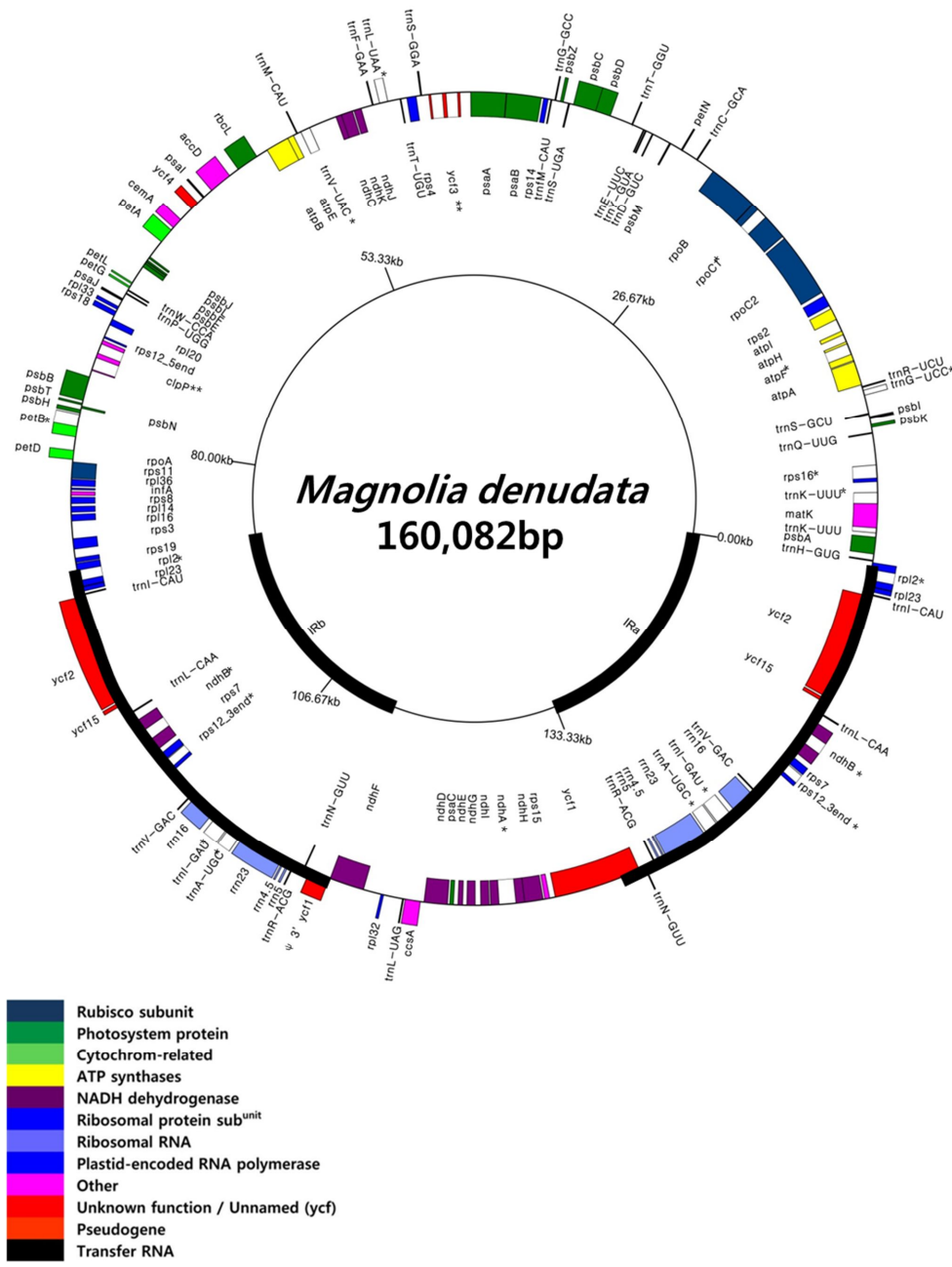


Fig. 5. Continued.

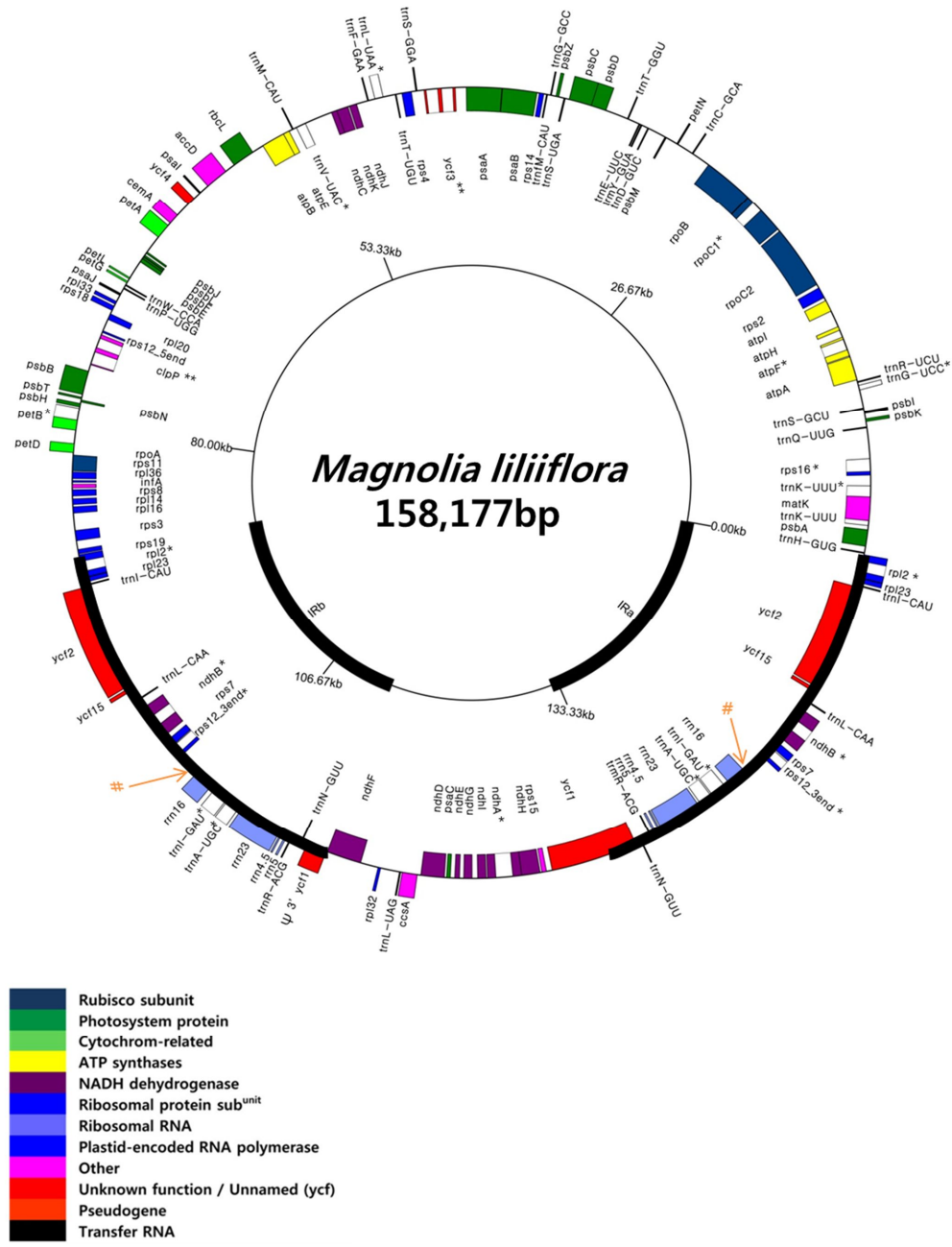


Fig. 5. Continued.

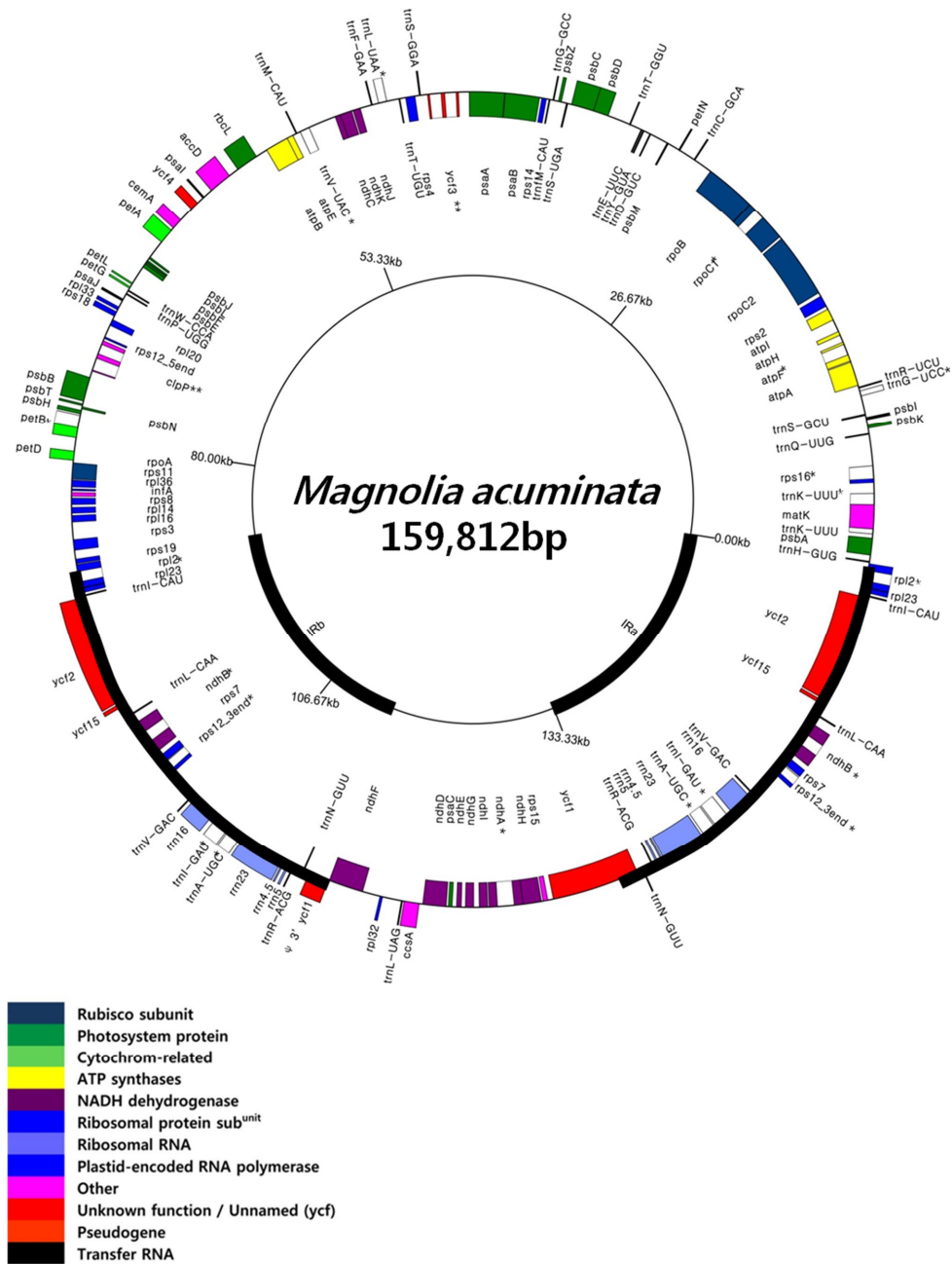


Fig. 5. Continued.

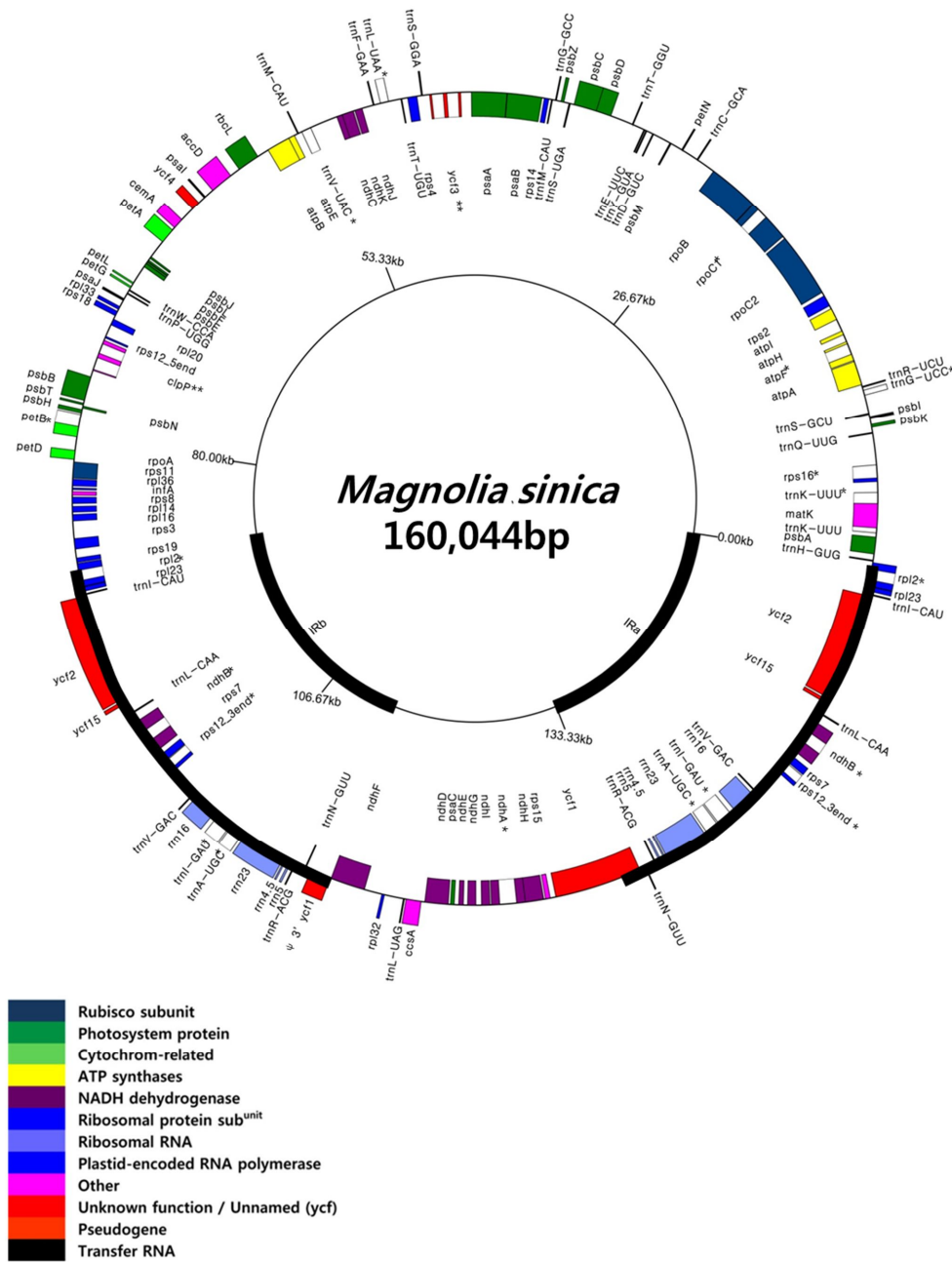


Fig. 5. Continued.

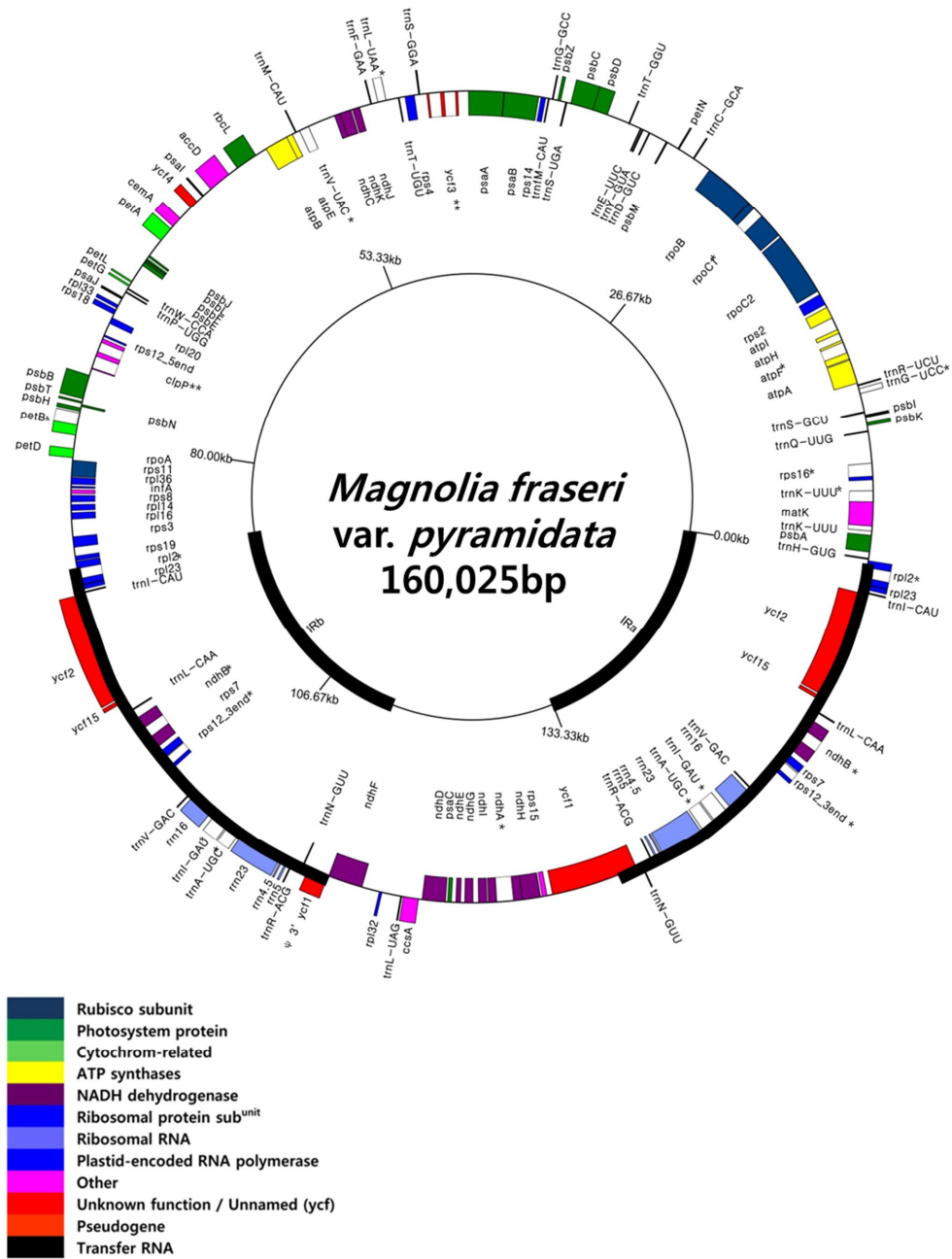


Fig. 5. Continued.

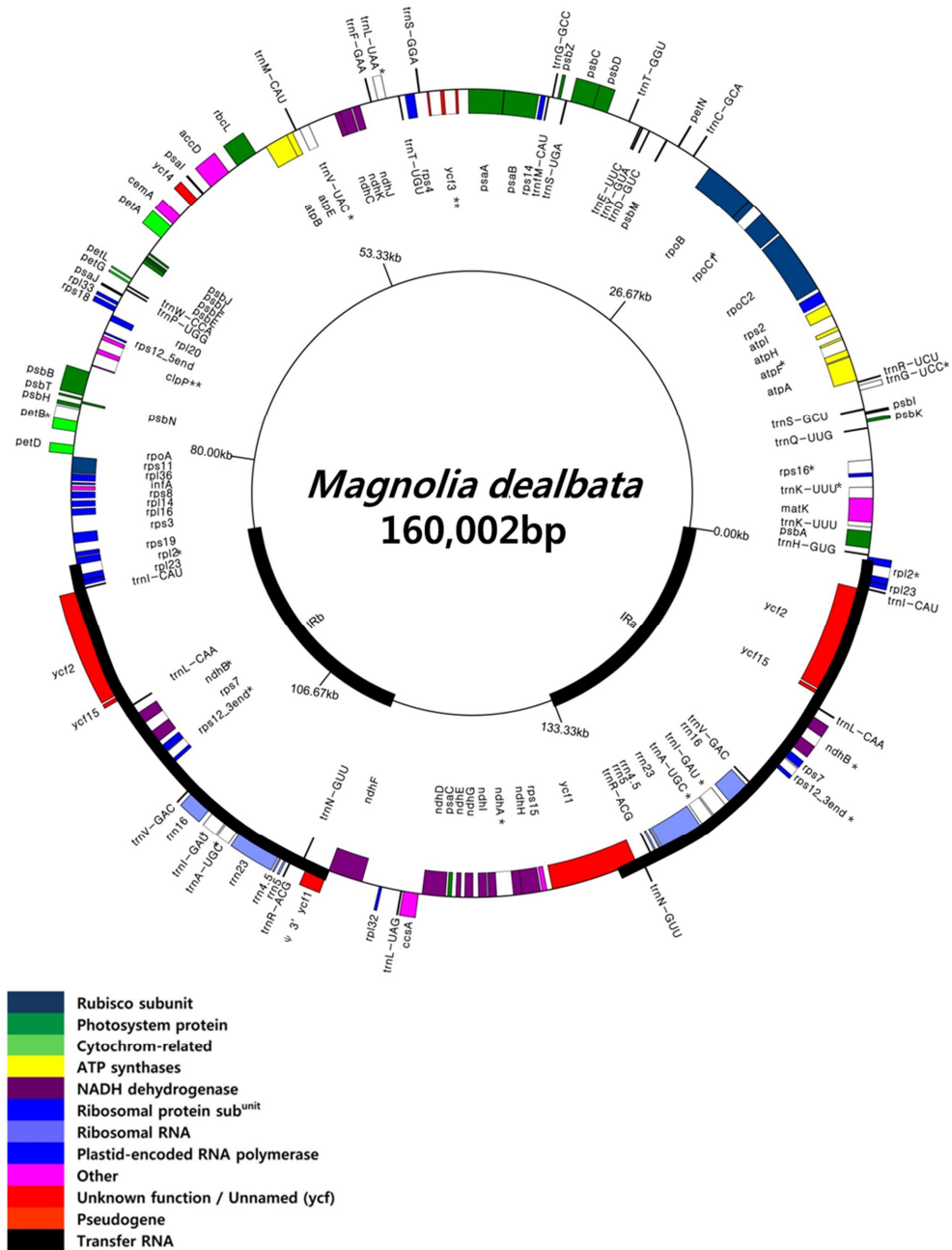


Fig. 5. Continued.

Table 5. Length and base composition of 11 chloroplast genomes in Magnoliaceae

		T(U) (%)	C (%)	A (%)	G (%)	No. Sequence (bp)
<i>Mich. odora</i>	LSC	31.72	19.42	30.33	18.53	88098
	IR	28.24	20.89	28.56	22.31	26586
	SSC	32.86	18.08	32.86	16.19	18800
	Total	30.75	19.99	29.99	19.27	160070
<i>M. cathartii</i>	LSC	31.74	19.40	30.34	18.51	88142
	IR	28.26	20.90	28.54	22.30	26509
	SSC	32.87	18.05	33.00	16.08	18790
	Total	30.77	19.97	30.01	19.25	159950
<i>M. kobus</i>	LSC	31.69	19.47	30.31	18.53	87484
	IR	28.27	20.86	28.56	22.32	26588
	SSC	32.94	18.04	32.90	16.10	18783
	Total	30.75	20.01	29.98	19.27	159443
<i>M. salicifolia</i>	LSC	31.70	19.43	30.32	18.55	88143
	IR	28.25	20.85	28.59	22.31	26591
	SSC	32.88	18.09	32.89	16.14	18768
	Total	30.75	19.99	29.99	19.27	160093
<i>M. sprengeri</i>	LSC	31.73	19.42	30.32	18.53	88133
	IR	28.25	20.85	28.58	22.31	26589
	SSC	32.91	18.07	32.90	16.13	18722
	Total	30.76	19.98	29.99	19.26	160033
<i>M. denudata</i>	LSC	31.73	19.43	30.32	18.52	88124
	IR	28.24	20.87	28.58	22.31	26589
	SSC	32.95	18.06	32.78	16.21	18780
	Total	30.77	19.99	29.98	19.27	160082
<i>M. liliiflora</i>	LSC	31.73	19.41	30.34	18.52	88133
	IR	28.31	20.88	28.66	22.14	25651
	SSC	32.93	18.07	32.84	16.17	18742
	Total	30.82	19.94	30.03	19.21	158177
<i>M. acuminata</i>	LSC	31.72	19.43	30.32	18.53	87838
	IR	28.23	20.86	28.57	22.34	26602
	SSC	32.95	18.04	32.85	16.16	18770
	Total	30.76	19.99	29.98	19.27	159812
<i>M. sinica</i>	LSC	31.73	19.44	30.32	18.51	88156
	IR	28.25	20.87	28.56	22.32	26571
	SSC	32.86	18.11	32.84	16.20	18746
	Total	30.76	20.00	29.98	19.26	160044
<i>M. kwangsiensis</i>	LSC	31.71	19.42	30.13	18.50	88030
	IR	29.89	20.90	28.60	21.11	26484
	SSC	32.78	18.19	32.83	16.20	18669
	Total	30.75	20.00	29.99	19.26	159667
<i>M. fraseri</i> var. <i>pyramidata</i>	LSC	31.75	19.37	30.38	18.50	88043
	IR	28.25	20.85	28.59	22.31	26597
	SSC	32.92	18.06	32.87	16.14	18788
	Total	30.78	19.95	30.02	19.25	160025
<i>M. dealbata</i>	LSC	31.74	19.44	30.34	18.49	88088
	IR	28.23	20.87	28.59	22.28	26587
	SSC	32.77	18.14	32.93	16.16	18740
	Total	30.75	20.00	30.00	19.25	160002
<i>L. tulipifera</i>	LSC	31.81	19.37	30.41	18.41	88150
	IR	28.27	20.88	28.53	22.33	26373
	SSC	32.98	18.14	32.72	16.16	18990
	Total	30.82	19.96	30.02	19.19	159886

Table 6. Genes contained in the 11 newly sequenced cp genomes in this study

Genes categories	Group of genes	Name of genes
Self replication	Ribosomal RNA genes	<i>rrn16x2, rrn23x2, rrn4.5x2, rrn5x2</i>
	Transfer RNA genes	<i>trnH-GUG, trnK-UUU*, trnQ-UUG, trnS-GCU, trnG-UCC*, trnR-UCU, trnC-GCA, trnD-GUC, trnY-GUA, trnE-UUC, trnT-GGU, trnS-UGA, trnG-GCC, trnM-CAU, trnS-GGA, trnT-UGU, trnL-UAA*, trnF-GAA, trnV-UAC*, trnM-CAU, trnW-CCA, trnP-UGG, trnI-CAUx2, trnL-CAAx2, trnV-GAC***x2, trnI-GAU*x2, trnA-UGC*x2, trnR-ACGx2, trnN-GUUX2, trnL-UAG</i>
	Small subunits of ribosomal protein genes	<i>rps16*, rps2, rps14, rps4, rps18, rps12*x2, rps11, rps8, rps3, rps19, rps7x2, rps15</i>
	Large subunit of ribosomal protein genes	<i>rpl33, rpl20, rpl36, rpl14, rpl16, rpl2*x2, rpl23x2, rpl32</i>
	DNA dependent RNA polymerase genes	<i>rpoC2, rpoC1*, rpoB, rpoA</i>
Genes for photosynthesis	Subunits of photosystem I genes	<i>psaB, psaA, psaI, psaJ, psaC</i>
	Subunits of photosystem II genes	<i>psbA, psbK, psbI, psbM, psbZ, psbJ, psbL, psbF, psbE, psbB, psbT, psbM, psbH, psbD, psbC</i>
	Subunits of Cytochrome genes	<i>petN, petA, petL, petG, petB*, petD</i>
	Subunits of ATP-synthases genes	<i>atpA, atpF*, atpH, atpI, atpE, atpB</i>
	RuBisCo large subunit gene	<i>rbcL</i>
	Subunits of NADH dehydrogenase genes	<i>ndhJ, ndhK, ndhC, ndhB*x2, ndhF, ndhD, ndhE, ndhG, ndhI, ndhA*, ndhH</i>
Other genes	Maturase gene	<i>matK</i>
	Protease gene	<i>clpP**</i>
	Envelope membrane protein gene	<i>cemA</i>
	Subunit of Acetyl-CoA-carboxylase gene	<i>accD</i>
	C-type cytochrome synthesis gene	<i>ccsA</i>
Unknown function	Conserved open reading frames	<i>ycf3**, ycf2x2, ycf1, ycf15, ycf4</i>
Pseudogenes	Pseudogene	<i>ψycf1</i>

\*, genes contain one intron; \*\*, genes contain two introns; \*\*\*, a gene deleted only *M. liliiflora*; x2, duplicate genes; ψ, a pseudogene.

Table 7. Percentage of coding region and non-coding region of 11 chloroplast genomes in Magnoliaceae

Taxa	Coding region (protein+RNA genes) (%)	Non-coding region (%)
<i>Mich. odora</i>	57.48 (50.09+7.39)	42.52
<i>M. cathcartii</i>	57.49 (50.10+7.39)	42.51
<i>M. kobus</i>	57.31 (49.89+7.41)	42.69
<i>M. salicifolia</i>	57.13 (49.74+7.39)	42.87
<i>M. sprengeri</i>	57.09 (49.70+7.39)	42.91
<i>M. denudata</i>	57.15 (49.75+7.40)	42.85
<i>M. liliiflora</i>	57.89 (50.83+7.06)	42.11
<i>M. acuminata</i>	57.74 (50.35+7.39)	42.26
<i>M. sinica</i>	57.48 (50.09+7.39)	42.52
<i>M. kwangsiensis</i>	57.68 (50.27+7.41)	42.32
<i>M. fraseri</i> var. <i>pyramidata</i>	57.51 (50.12+7.39)	42.49
<i>M. dealbata</i>	57.48 (50.09+7.39)	42.52
<i>L. tulipifera</i>	57.50 (50.18+7.32)	42.50

and 57.68% in *M. kwangsiensis* (Cai et al., 2006; Kuang et al., 2011). However, *M. liliiflora* has the highest % of coding regions while it has minimum % of coding regions of RNA genes due to the deletion of *trnV-GAC* in IR. The highest % of the protein coding region is found in *M. liliiflora* in the 13 Magnoliaceae species included in this study.

The intron and exon sizes of splitted genes in the cp genome are indicated in Table 8. Sixteen genes have one (13 genes) or two (three genes) introns. Among them, the *rps12* has a unique splitted structure: one exon is located in LSC region and another exon and intron are located in IR regions. *TrnG-GCC* was found the range from 11,082 to 11,118 in the aligned matrix by DOGMA. But, it was replaced by *trnG-UCC* through comparing with location of *L. tulipifera* and *M. kwangsiensis*. *TrnG-UCC* is composed with an exon1 range from 10,256 to 10,279 and an exon2 range from 11,072 to 11,119 in the aligned matrix, respectively. It was lost in the *Drimys granadensis* and *Piper cenocladum*, but was found in Laurales (*Calycanthus floridus* var. *glaucus*) and Magnoliales (*L. tulipifera*, *M. kwangsiensis*, and 11 species in this study). This result supports the previously reported phylogenetic relationships among Magnoliids (Cai et al., 2006): recognition of Canellales/Piperales and Laurales/Magnoliales clades. While the *petD* and *rpl16* genes in three lineages of Magnoliids (Canellales, Piperales, and Laurales) were composed of two exons, Magnoliales lineage (*L. tulipifera*, *M. kwangsiensis*, and 11 species in this study) lost one of exons (short one in the 5'end). It supports phylogenetic relationship of Laurales and Magnoliales in the tree of Magnoliids by Kuang et al. (2011). The genes were divided into introns and exons in 11 species cp DNA are

Table 8. List of genes having introns and length of introns/exons in 11 cp genomes of

Magnoliaceae

Taxa	Genes	Exon I	Intron I	Exon II	Intron II	Exon III
<i>Mich. odora</i>	<i>trnK-UUU</i>	37	2489	35		
	<i>rps16</i>	42	824	219		
	<i>trnG-UCC</i>	24	792	48		
	<i>atpF</i>	144	697	411		
	<i>rpoC1</i>	434	722	1624		
	<i>ycf3</i>	126	739	228	730	153
	<i>trnL-UAA</i>	50	490	35		
	<i>trnV-UAC</i>	39	583	37		
	<i>rps12</i>	114	*	232	535	26
	<i>clpP</i>	69	784	291	626	246
	<i>petB</i>	6	783	642		
	<i>rpl2</i>	387	657	432		
	<i>ndhB</i>	777	699	756		
	<i>trnI-GAU</i>	42	935	35		
	<i>trnA-UGC</i>	38	798	35		
	<i>ndhA</i>	552	1076	540		
<i>M. cathcartii</i>	<i>trnK-UUU</i>	37	2487	35		
	<i>rps16</i>	42	826	219		
	<i>trnG-UCC</i>	24	792	48		
	<i>atpF</i>	144	708	411		
	<i>rpoC1</i>	431	721	1624		
	<i>ycf3</i>	126	736	228	727	153
	<i>trnL-UAA</i>	50	490	35		
	<i>trnV-UAC</i>	39	583	37		
	<i>rps12</i>	114	*	232	535	26
	<i>clpP</i>	69	782	291	630	246
	<i>petB</i>	6	783	642		
	<i>rpl2</i>	387	657	432		
	<i>ndhB</i>	777	699	756		
	<i>trnI-GAU</i>	42	935	35		
	<i>trnA-UGC</i>	38	798	35		
	<i>ndhA</i>	552	1076	540		
<i>M. kobus</i>	<i>trnK-UUU</i>	37	2488	35		
	<i>rps16</i>	42	823	219		
	<i>trnG-UCC</i>	24	792	48		
	<i>atpF</i>	144	707	411		
	<i>rpoC1</i>	434	721	1624		
	<i>ycf3</i>	126	741	228	730	153
	<i>trnL-UAA</i>	50	494	35		
	<i>trnV-UAC</i>	39	583	37		
	<i>rps12</i>	114	*	231	536	30
	<i>clpP</i>	69	780	291	626	246
	<i>petB</i>	6	783	642		
	<i>rpl2</i>	387	658	432		
	<i>ndhB</i>	777	699	756		
	<i>trnI-GAU</i>	42	935	35		
	<i>trnA-UGC</i>	38	798	35		
	<i>ndhA</i>	552	1076	540		
<i>M. salicifolia</i>	<i>trnK-UUU</i>	37	2489	35		
	<i>rps16</i>	43	823	218		
	<i>trnG-UCC</i>	24	792	48		
	<i>atpF</i>	144	710	411		
	<i>rpoC1</i>	434	721	1624		
	<i>ycf3</i>	126	737	228	730	153
	<i>trnL-UAA</i>	50	490	35		
	<i>trnV-UAC</i>	39	583	37		
	<i>rps12</i>	114	*	232	535	26
	<i>clpP</i>	69	779	291	631	246

\* the *rps12* gene is divided: the 5'-*rps12* is located on the LSC regions and 3'-*rps12* included two exons is located on the IR region.

Table 8. Continued.

Taxa	Genes	Exon I	Intron I	Exon II	Intron II	Exon III
	<i>petB</i>	6	783	642		
	<i>rpl2</i>	387	657	432		
	<i>ndhB</i>	777	699	756		
	<i>trnI-GAU</i>	42	935	35		
	<i>trnA-UGC</i>	38	798	35		
	<i>ndhA</i>	552	1076	540		
<i>M. sprengeri</i>	<i>trnK-UUU</i>	37	2488	35		
	<i>rps16</i>	43	816	228		
	<i>trnG-UCC</i>	24	792	48		
	<i>atpF</i>	144	707	411		
	<i>rpoC1</i>	434	721	1624		
	<i>ycf3</i>	126	737	228	727	153
	<i>trnL-UAA</i>	50	490	35		
	<i>trnV-UAC</i>	39	584	37		
	<i>rps12</i>	114	*	232	535	26
	<i>clpP</i>	69	779	291	626	246
	<i>petB</i>	6	784	642		
	<i>rpl2</i>	387	657	432		
	<i>ndhB</i>	777	699	756		
	<i>trnI-GAU</i>	42	935	35		
	<i>trnA-UGC</i>	38	798	35		
	<i>ndhA</i>	552	1077	540		
<i>M. denudata</i>	<i>trnK-UUU</i>	37	2490	35		
	<i>rps16</i>	42	823	219		
	<i>trnG-UCC</i>	24	792	48		
	<i>atpF</i>	144	709	401		
	<i>rpoC1</i>	434	721	1624		
	<i>ycf3</i>	126	737	228	728	153
	<i>trnL-UAA</i>	50	490	35		
	<i>trnV-UAC</i>	39	583	37		
	<i>rps12</i>	113	*	232	535	26
	<i>clpP</i>	69	784	291	629	246
	<i>petB</i>	6	783	642		
	<i>rpl2</i>	387	657	432		
	<i>ndhB</i>	777	699	756		
	<i>trnI-GAU</i>	42	935	35		
	<i>trnA-UGC</i>	38	798	35		
	<i>ndhA</i>	552	1075	540		
<i>M. liliiflora</i>	<i>trnK-UUU</i>	37	2488	35		
	<i>rps16</i>	40	818	225		
	<i>trnG-UCC</i>	24	792	48		
	<i>atpF</i>	144	709	411		
	<i>rpoC1</i>	434	721	1624		
	<i>ycf3</i>	126	738	228	727	153
	<i>trnL-UAA</i>	50	490	35		
	<i>trnV-UAC</i>	39	583	37		
	<i>rps12</i>	114	*	232	535	26
	<i>clpP</i>	69	784	291	633	246
	<i>petB</i>	6	771	654		
	<i>rpl2</i>	387	657	432		
	<i>ndhB</i>	777	699	756		
	<i>trnI-GAU</i>	42	935	35		
	<i>trnA-UGC</i>	38	798	35		
	<i>ndhA</i>	552	1075	540		
<i>M. acuminata</i>	<i>trnK-UUU</i>	37	2488	35		
	<i>rps16</i>	42	821	228		
	<i>trnG-UCC</i>	24	792	48		
	<i>atpF</i>	144	705	411		
	<i>rpoC1</i>	434	721	1624		
	<i>ycf3</i>	126	736	228	729	153
	<i>trnL-UAA</i>	50	489	35		
	<i>trnV-UAC</i>	39	584	37		
	<i>rps12</i>	114	*	232	535	26

Table 8. Continued.

Taxa	Genes	Exon I	Intron I	Exon II	Intron II	Exon III
	<i>clpP</i>	69	777	291	629	247
	<i>petB</i>	6	771	654		
	<i>rpl2</i>	387	657	432		
	<i>ndhB</i>	777	699	756		
	<i>trnI-GAU</i>	42	935	35		
	<i>trnA-UGC</i>	38	798	35		
	<i>ndhA</i>	552	1068	540		
<i>M. sinica</i>	<i>trnK-UUU</i>	37	2489	35		
	<i>rps16</i>	42	826	219		
	<i>trnG-UCC</i>	24	792	48		
	<i>atpF</i>	144	706	411		
	<i>rpoC1</i>	434	721	1624		
	<i>ycf3</i>	126	735	228	727	153
	<i>trnL-UAA</i>	50	490	35		
	<i>trnV-UAC</i>	39	583	37		
	<i>rps12</i>	114	*	232	535	26
	<i>clpP</i>	69	778	291	627	246
	<i>petB</i>	6	783	642		
	<i>rpl2</i>	387	657	432		
	<i>ndhB</i>	777	701	756		
	<i>trnI-GAU</i>	42	934	35		
	<i>trnA-UGC</i>	38	798	35		
	<i>ndhA</i>	552	1075	540		
<i>M. kwangsiensis</i>	<i>trnK-UUU</i>	37	2487	35		
	<i>rps16</i>	42	824	252		
	<i>trnG-UCC</i>	24	766	48		
	<i>atpF</i>	144	705	411		
	<i>rpoC1</i>	432	733	1614		
	<i>ycf3</i>	126	732	228	730	153
	<i>trnL-UAA</i>	50	483	35		
	<i>trnV-UAC</i>	39	583	37		
	<i>rps12</i>	114	*	231	536	30
	<i>clpP</i>	69	782	291	629	246
	<i>petB</i>	6	783	642		
	<i>rpl2</i>	384	660	432		
	<i>ndhB</i>	777	699	756		
	<i>trnI-GAU</i>	42	927	35		
	<i>trnA-UGC</i>	38	779	36		
	<i>ndhA</i>	552	1069	540		
<i>M. fraseri</i> var. <i>pyramidata</i>	<i>trnK-UUU</i>	37	2489	35		
	<i>rps16</i>	42	824	219		
	<i>trnG-UCC</i>	24	792	48		
	<i>atpF</i>	144	727	411		
	<i>rpoC1</i>	434	753	1588		
	<i>ycf3</i>	126	736	228	728	153
	<i>trnL-UAA</i>	50	490	35		
	<i>trnV-UAC</i>	39	583	37		
	<i>rps12</i>	114	*	232	535	26
	<i>clpP</i>	69	779	291	629	246
	<i>petB</i>	6	783	642		
	<i>rpl2</i>	387	657	432		
	<i>ndhB</i>	777	699	756		
	<i>trnI-GAU</i>	42	935	35		
	<i>trnA-UGC</i>	38	798	35		
	<i>ndhA</i>	552	1074	540		
<i>M. dealbata</i>	<i>trnK-UUU</i>	37	2490	35		
	<i>rps16</i>	42	821	219		
	<i>trnG-UCC</i>	24	792	48		
	<i>atpF</i>	144	705	411		
	<i>rpoC1</i>	434	723	1624		
	<i>ycf3</i>	126	740	228	730	153
	<i>trnL-UAA</i>	50	490	35		
	<i>trnV-UAC</i>	39	583	37		
	<i>rps12</i>	114	*	232	535	26

Table 8. Continued.

Taxa	Genes	Exon I	Intron I	Exon II	Intron II	Exon III
	<i>clpP</i>	69	779	291	627	246
	<i>petB</i>	6	783	642		
	<i>rpl2</i>	387	657	432		
	<i>ndhB</i>	777	699	756		
	<i>trnI-GAU</i>	42	937	35		
	<i>trnA-UGC</i>	38	798	35		
	<i>ndhA</i>	552	1070	540		
<i>L. tulipitera</i>	<i>trnK-UUU</i>	37	2485	35		
	<i>rps16</i>	43	819	218		
	<i>trnG-UCC</i>	24	774	48		
	<i>atpF</i>	145	725	410		
	<i>rpoC1</i>	432	728	1614		
	<i>ycf3</i>	126	745	228	727	153
	<i>trnL-UAA</i>	50	489	35		
	<i>trnV-UAC</i>	39	588	35		
	<i>rps12</i>	113	*	242		
	<i>clpP</i>	70	779	293	626	246
	<i>petB</i>	6	788	642		
	<i>rpl2</i>	391	699	431		
	<i>ndhB</i>	777	699	756		
	<i>trnI-GAU</i>	37	945	35		
	<i>trnA-UGC</i>	38	798	35		
	<i>ndhA</i>	553	1076	539		

little changes in lengths. However, unlike other 12 cp genomes in Magnoliaceae, the *rpoC1* gene of *M. fraseri* var. *pyramidata* has 40bp of unique deletion.

The overall GC contents of Magnoliaceae are in the range of 39.15% (*M. liliiflora*) ~ 39.28% (*M. kobus*). The GC content is little bit higher than *Drimys granadensis* (38.79%) and *Piper cenocladum* (38.31%), and similar to *L. tulipifera* (39.16%) and *M. kwangsiensis* (39.26%) of Magnoliaceae. The overall GC contents in the IR regions are the highest among four cp genome regions because of the high number of C and G bases in the rRNA genes (55.30%). The range of GC contents of IR region is 43.02% (*M. liliiflora*) ~ 43.20% (the others species), whereas the SSC region showed the lowest value ranging 34.13% (*M. cathcartii*) ~ 34.31% (*M. sinica*). The nucleotide frequencies of 11 species cp DNA are provided in the Table 5.

Chloroplast simple sequence repeat (cpSSR) numbers in Magnoliaceae showed large variation. Based on the analyses using Msafinder, 230 (*M. liliiflora*) ~ 248 (*M. cathcartii*) cpSSRs were identified with the condition described in the materials and methods (Table 9; APPENDIX 1). These numbers are similar to those of previously reported in Magnoliaceae, *L. tulipifera* (241) and *M. kwangsiensis* (237). However, maximum variations were found in *M. liliiflora* and *M. denudata* having fewer numbers than other 9 species of Magnoliaceae caused by lacks of mononucleotide, trinucleotide, and hexanucleotide units. Pentanucleotide units are present only in *M. cathcartii* and *M. sprengeri*. *M. denudata* does not have hexanucleotide units in Magnoliaceae. The longest polyA among

Table 9. Statistics of simple sequence repeats (SSRs) in 11 chloroplast genomes in Magnoliaceae

Taxa	No. SSRs (%)						Total	Lengths of the longest SSRs		No. SSRs of mono-nucleotides				No. >9bp monos SSRs (%)			No. SSRs in each region		
	Mono	Di	Tri	Tetra	Penta	Hexa		A	T	A	T	C	G	8	9	10	LSC	IR	SSC
<i>Mich. odora</i>	115 (48%)	43 (18%)	72 (30%)	9 (4%)	0 (0%)	3 (1%)	242	12	17	45	65	4	1	94 (38.84%) 43 35 16			146	57	39
<i>M. cathcartii</i>	119 (48%)	43 (17%)	74 (30%)	9 (4%)	1 (0%)	2 (1%)	248	14	14	46	66	4	2	96 (38.71%) 44 33 19			152	57	39
<i>M. kobus</i>	116 (48%)	43 (18%)	71 (29%)	9 (4%)	0 (0%)	2 (1%)	241	14	14	42	67	5	2	96 (39.83%) 46 32 18			146	57	38
<i>M. salicifolia</i>	112 (47%)	42 (18%)	74 (31%)	9 (4%)	0 (0%)	3 (1%)	240	15	17	39	66	5	2	85 (35.42%) 37 31 17			144	57	39
<i>M. sprengeri</i>	117 (48%)	43 (18%)	71 (29%)	9 (4%)	1 (0%)	3 (1%)	244	14	16	42	66	7	2	102 (41.80%) 53 36 13			151	55	38
<i>M. denudata</i>	110 (47%)	43 (18%)	71 (30%)	9 (4%)	0 (0%)	0 (0%)	233	14	16	38	65	5	1	87 (37.34%) 45 26 16			140	55	38
<i>M. liliiflora</i>	109 (47%)	43 (19%)	67 (29%)	9 (4%)	0 (0%)	2 (1%)	230	13	15	37	66	5	1	88 (38.21%) 43 30 15			140	52	38
<i>M. acuminata</i>	118 (48%)	44 (18%)	72 (30%)	8 (3%)	0 (0%)	2 (1%)	244	14	12	47	66	4	1	105 (43.03%) 44 44 17			148	60	36
<i>M. sinica</i>	118 (48%)	43 (18%)	72 (30%)	9 (4%)	0 (0%)	2 (1%)	244	20	13	43	69	4	2	102 (41.80%) 47 40 15			151	55	38
<i>M. kwangsiensis</i>	113 (48%)	41 (17%)	73 (31%)	8 (3%)	0 (0%)	2 (1%)	237	15	15	43	66	2	2	93 (39.25%) 42 35 16			141	57	39
<i>M. fraseri</i> var. <i>pyramidata</i>	121 (49%)	43 (17%)	71 (29%)	9 (4%)	0 (0%)	2 (1%)	246	15	14	45	69	4	3	103 (41.87%) 48 39 16			152	58	36
<i>M. dealbata</i>	118 (49%)	43 (18%)	71 (29%)	9 (4%)	0 (0%)	2 (1%)	243	13	14	46	67	4	1	99 (40.74%) 50 32 17			148	57	38
<i>L. tulipifera</i>	114 (47%)	43 (18%)	73 (30%)	9 (4%)	0 (0%)	2 (1%)	241	20	16	40	66	5	3	76 (31.53%) 32 23 21			150	53	38

cpSSRs is found in *M. sinica* (20bp). Stretches of polyA repeating unit in 12 species except *M. sinica* are similar to polyT. Mononucleotide repeats are composed of G or C is very few compared to polyA or polyT, which is similar to other species including *M. kwangsiensis* (Raubeson et al., 2007; Kim et al., 2009; Kuang et al., 2011). Repeating units consist of >9bp mononucleotides are about 40% of total cpSSRs likes in *M. kwangsiensis* (42.62%). Among them, 10bp mononucleotides of cpSSRs are similar to the 11 species, while 8bp and 9bp of repeat units is the lowest in *M. salicifolia* and *M. denudata*, respectively. Distributions of cpSSRs in 11 species are similar to regions of SSC. CpSSRs of *M. liliiflora* and *M. denudata* in LSC and IR regions are fewer than other 9 species Magnoliaceae.

To recognize structural variations in Magnoliaceae, multiple alignment were performed with 11 cp genomes determined in this study and two previously reported cp genomes in Magnoliaceae using CLUSTALW module in the MEGA5 (version5.05). The variations of border positions in 11 species cp DNA are caused by expansions and contractions or insertions/deletions of the regions. Figure 6 shows the comparison of borders among LSC, IR, and SSC in 13 cp genomes in Magnoliaceae. In the IRa/LSC boarder, IRa boundary appears 6bp (TCAGCT) of deletion in both *M. cathcartii* and *M. sprengeri*. *M. sinica* have 5bp (CAGCT) of deletion in IRa and this sequences transferred into LSC. *M. cathcartii* has 2bp (AA) of deletion and *L. tulipifera* has 18bp of insertion (AAAAAACAACAAAGGA) in LSC regions. However, *M. cathcartii* does not have 6bp of deletion in IRa but has 5bp (GCTGA) of deletion, and one base pair is transfered into LSC (Fig. 6B). *M. sprengeri* and *L. tulipifera* showed independent contraction of

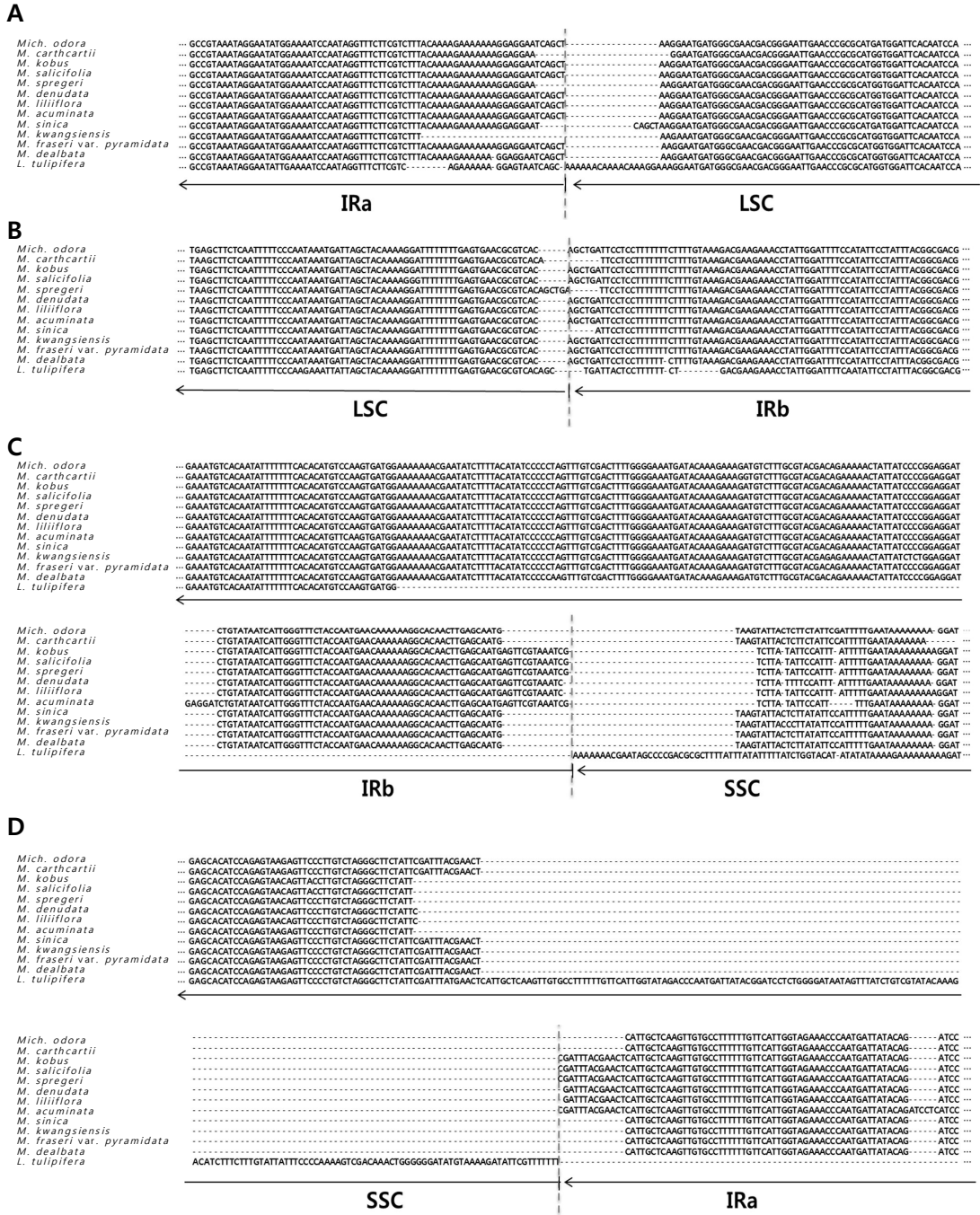


Fig. 6. Comparison of the IR/single copy (SC) boundaries among 13 species in Magnoliaceae. A, IRa/LSC boundary; B, LSC/IRb boundary; C, IRb/SSC boundary; D, SSC/IRa boundary.

IR and these sequences stretched into LSC resulting 6bp (AGCTGA) and 3bp (AGC) of insertion, respectively. In the IRb/SSC border, *L. tulipifera* showed IRb contraction due to the 170bp of deletion and 13bp (AAAAAACGAATA) of sequences are transferred into the LSC (Fig. 6C). Genomes of subgen. *Yulania* (*M. kobus*, *M. salicifolia*, *M. sprengeri*, *M. denudata*, *M. liliiflora*, and *M. acuminata*) shows variation of about 20bp in SSC and insertion of about 13bp (AGTTCGTAAATCG) in IRb. *M. denudata* and *M. liliiflora* among them indicate 1bp (G) deletion compared with other subgen. *Yulania* in IRb. However, these showed the contraction of IR through not 1bp (C) in IRb but transfer of 1bp into SSC (Fig. 6D). The SSC region of *L. tulipifera* is expanded due to 164bp of insertion.

Taxon-specific large gaps were found in several taxa (Fig. 7). *M. acuminata* have 299bp of deletion in LSC, which is positioned from 15,670bp ~ 15,968bp in the aligned matrix. It shows in intergenic spacer (IGS) between *atpH* and *atpI* (Fig. 7A). *M. kobus* also has 658bp of long deletion in LSC, which is located in intergenic spacer (IGS) between *psbE* and *petL* (Fig. 7B). *M. liliiflora* has 952bp of two gaps because deletion occurs in IR (Fig. 7C). In this long gap region, *trnV-GAC* is located in this long gap in other magnolias. Overall sequence comparison between *L. tulipifera* and other magnolias have performed using mVISTA (Fig. 8). This picks in figures are very similar except large white areas (gaps) indicating very low overall sequence variations in the subfamily Magnolioideae.

Sequence divergences among 13 cp genomes in Magnoliaceae are calculated using *p*-distance for various categories: 1) taxonomic group (Magnoliaceae, subfamily Magnolioideae, and subgen. *Yulania*), 2) LSC, SSC,

A

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Mich. odora ... CTCCTAACCGACTTTTTATGAATCTTATTGTTGTAAACTCTCTCTTTTCCCTATCATTATCCGCATGGATATAAACGAACGGAT ... TCATCATCCCCGAATCATTACATATCAATCAAGATTGGATGA
M. carthcartii ... CTCCTAACCGACTTTTTATGAATCTTATTGTTGTAAACTCTCTCTTTTCCCTATCATTATCCGCATGGATATAAACGAACGGAT ... TCATCAGCCCCGAATCATTACATATCAATCAAGATTGGATGA
M. kobus ... CTCCTAACCGACTTTTTATGAATCTTATTGTTGTAAACTCTCTCTTTTCCCTATCATTATCCGCATGGATATAAACGAACGGAT ... TCATCAGCCCCGAATCATTACATATCAATCAAGATTGGATGA
M. salicifolia ... CTCCTAACCGACTTTTTATGAATCTTATTGTTGTAAACTCTCTCTTTTCCCTATCATTATCCGCATGGATATAAACGAACGGAT ... TCATCAGCCCCGAATCATTACATATCAATCAAGATTGGATGA
M. sprengeri ... CTCCTAACCGACTTTTTATGAATCTTATTGTTGTAAACTCTCTCTTTTCCCTATCATTATCCGCATGGATATAAACGAACGGAT ... TCATCAGCCCCGAATCATTACATATCAATCAAGATTGGATGA
M. denudata ... CTCCTAACCGACTTTTTATGAATCTTATTGTTGTAAACTCTCTCTTTTCCCTATCATTATCCGCATGGATATAAACGAACGGAT ... TCATCAGCCCCGAATCATTACATATCAATCAAGATTGGATGA
M. liliiflora ... CTCCTAACCGACTTTTTATGAATCTTATTGTTGTAAACTCTCTCTTTTCCCTATCATTATCCGCATGGATATAAACGAACGGAT ... TCATCAGCCCCGAATCATTACATATCAATCAAGATTGGATGA
M. acuminata ... CTCCTAACCGACTTTTTATGAATCTTATTGTTGTAAACTCTCTCTTTTCCCTATCATTATCCGCATGGATATAAACGAACGGAT ... TCATCAGCCCCGAATCATTACATATCAATCAAGATTGGATGA
M. sinica ... CTCCTAACCGACTTTTTATGAATCTTATTGTTGTAAACTCTCTCTTTTCCCTATCATTATCCGCATGGATATAAACGAACGGAT ... TCATCAGCCCCGAATCATTACATATCAATCAAGATTGGATGA
M. kwangsiensis ... CTCCTAACCGACTTTTTATGAATCTTATTGTTGTAAACTCTCTCTTTTCCCTATCATTATCCGCATGGATATAAACGAACGGAT ... TCATCAGCCCCGAATCATTACATATCAATCAAGATTGGATGA
M. fraseri var. pyramidata ... CTCCTAACCGACTTTTTATGAATCTTATTGTTGTAAACTCTCTCTTTTCCCTATCATTATCCGCATGGATATAAACGAACGGAT ... TCATCAGCCCCGAATCATTACATATCAATCAAGATTGGATGA
M. dealbata ... CTCCTAACCGACTTTTTATGAATCTTATTGTTGTAAACTCTCTCTTTTCCCTATCATTATCCGCATGGATATAAACGAACGGAT ... TCATCAGCCCCGAATCATTACATATCAATCAAGATTGGATGA
L. tulipifera ... CTCCTAACCGACTTTTTATGAATCTTATTGTTGTAAACTCTCTCTTTTCCCTATCATTATCCGCATGGATATAAACGAACGGAT ... TCATCAGCCCCGAATCATTACATATCAATCAAGATTGGATGA

TCCAAT ... TGAATTGCAATTAAATGACATGGTCAATCGTTGAATTGAATGAATCAATGAATGGGAATGATTCATCAGTTTTCTTTTTTTT ... GTTGTAGGGCGATGTCGGAACAGCATAAACATCAAACTCTTATT
M. odora ... TGAATTGCAATTAAATGACATGGTCAATCGTTGAATTGAATGAATCAATGAATGGGAATGATTCATCAGTTTTCTTTTTTTT ... GTTGTAGGGCGATGTCGGAACAGCATAAACATCAAACTCTTATT
M. carthcartii ... TGAATTGCAATTAAATGACATGGTCAATCGTTGAATTGAATGAATCAATGAATGGGAATGATTCATCAGTTTTCTTTTTTTT ... GTTGTAGGGCGATGTCGGAACAGCATAAACATCAAACTCTTATT
M. kobus ... TGAATTGCAATTAAATGACATGGTCAATCGTTGAATTGAATGAATCAATGAATGGGAATGATTCATCAGTTTTCTTTTTTTT ... GTTGTAGGGCGATGTCGGAACAGCATAAACATCAAACTCTTATT
M. salicifolia ... TGAATTGCAATTAAATGACATGGTCAATCGTTGAATTGAATGAATCAATGAATGGGAATGATTCATCAGTTTTCTTTTTTTT ... GTTGTAGGGCGATGTCGGAACAGCATAAACATCAAACTCTTATT
M. sprengeri ... TGAATTGCAATTAAATGACATGGTCAATCGTTGAATTGAATGAATCAATGAATGGGAATGATTCATCAGTTTTCTTTTTTTT ... GTTGTAGGGCGATGTCGGAACAGCATAAACATCAAACTCTTATT
M. denudata ... TGAATTGCAATTAAATGACATGGTCAATCGTTGAATTGAATGAATCAATGAATGGGAATGATTCATCAGTTTTCTTTTTTTT ... GTTGTAGGGCGATGTCGGAACAGCATAAACATCAAACTCTTATT
M. liliiflora ... TGAATTGCAATTAAATGACATGGTCAATCGTTGAATTGAATGAATCAATGAATGGGAATGATTCATCAGTTTTCTTTTTTTT ... GTTGTAGGGCGATGTCGGAACAGCATAAACATCAAACTCTTATT
M. acuminata ... TGAATTGCAATTAAATGACATGGTCAATCGTTGAATTGAATGAATCAATGAATGGGAATGATTCATCAGTTTTCTTTTTTTT ... GTTGTAGGGCGATGTCGGAACAGCATAAACATCAAACTCTTATT
M. sinica ... TGAATTGCAATTAAATGACATGGTCAATCGTTGAATTGAATGAATCAATGAATGGGAATGATTCATCAGTTTTCTTTTTTTT ... GTTGTAGGGCGATGTCGGAACAGCATAAACATCAAACTCTTATT
M. kwangsiensis ... TGAATTGCAATTAAATGACATGGTCAATCGTTGAATTGAATGAATCAATGAATGGGAATGATTCATCAGTTTTCTTTTTTTT ... GTTGTAGGGCGATGTCGGAACAGCATAAACATCAAACTCTTATT
M. fraseri var. pyramidata ... TGAATTGCAATTAAATGACATGGTCAATCGTTGAATTGAATGAATCAATGAATGGGAATGATTCATCAGTTTTCTTTTTTTT ... GTTGTAGGGCGATGTCGGAACAGCATAAACATCAAACTCTTATT
M. dealbata ... TGAATTGCAATTAAATGACATGGTCAATCGTTGAATTGAATGAATCAATGAATGGGAATGATTCATCAGTTTTCTTTTTTTT ... GTTGTAGGGCGATGTCGGAACAGCATAAACATCAAACTCTTATT
L. tulipifera ... TGAATTGCAATTAAATGACATGGTCAATCGTTGAATTGAATGAATCAATGAATGGGAATGATTCATCAGTTTTCTTTTTTTT ... GTTGTAGGGCGATGTCGGAACAGCATAAACATCAAACTCTTATT

CAAATATGAATCGTAATTGACTGAACAATAATAAATAAGAAATATCGATTGGAGAGGATGAATAAATGGACCAAGCGGGAATCTTAGGAAAAGATCTTTAGATTAGATCTCTCCCTTTTTTTT ... ACAATTCCTAA ...
M. odora ... CAAATATGAATCGTAATTGACTGAACAATAATAAATAAGAAATATCGATTGGAGAGGATGAATAAATGGACCAAGCGGGAATCTTAGGAAAAGATCTTTAGATTAGATCTCTCCCTTTTTTTT ... ACAATTCCTAA ...
M. carthcartii ... CAAATATGAATCGTAATTGACTGAACAATAATAAATAAGAAATATCGATTGGAGAGGATGAATAAATGGACCAAGCGGGAATCTTAGGAAAAGATCTTTAGATTAGATCTCTCCCTTTTTTTT ... ACAATTCCTAA ...
M. kobus ... CAAATATGAATCGTAATTGACTGAACAATAATAAATAAGAAATATCGATTGGAGAGGATGAATAAATGGACCAAGCGGGAATCTTAGGAAAAGATCTTTAGATTAGATCTCTCCCTTTTTTTT ... ACAATTCCTAA ...
M. salicifolia ... CAAATATGAATCGTAATTGACTGAACAATAATAAATAAGAAATATCGATTGGAGAGGATGAATAAATGGACCAAGCGGGAATCTTAGGAAAAGATCTTTAGATTAGATCTCTCCCTTTTTTTT ... ACAATTCCTAA ...
M. sprengeri ... CAAATATGAATCGTAATTGACTGAACAATAATAAATAAGAAATATCGATTGGAGAGGATGAATAAATGGACCAAGCGGGAATCTTAGGAAAAGATCTTTAGATTAGATCTCTCCCTTTTTTTT ... ACAATTCCTAA ...
M. denudata ... CAAATATGAATCGTAATTGACTGAACAATAATAAATAAGAAATATCGATTGGAGAGGATGAATAAATGGACCAAGCGGGAATCTTAGGAAAAGATCTTTAGATTAGATCTCTCCCTTTTTTTT ... ACAATTCCTAA ...
M. liliiflora ... CAAATATGAATCGTAATTGACTGAACAATAATAAATAAGAAATATCGATTGGAGAGGATGAATAAATGGACCAAGCGGGAATCTTAGGAAAAGATCTTTAGATTAGATCTCTCCCTTTTTTTT ... ACAATTCCTAA ...
M. acuminata ... CAAATATGAATCGTAATTGACTGAACAATAATAAATAAGAAATATCGATTGGAGAGGATGAATAAATGGACCAAGCGGGAATCTTAGGAAAAGATCTTTAGATTAGATCTCTCCCTTTTTTTT ... ACAATTCCTAA ...
M. sinica ... CAAATATGAATCGTAATTGACTGAACAATAATAAATAAGAAATATCGATTGGAGAGGATGAATAAATGGACCAAGCGGGAATCTTAGGAAAAGATCTTTAGATTAGATCTCTCCCTTTTTTTT ... ACAATTCCTAA ...
M. kwangsiensis ... CAAATATGAATCGTAATTGACTGAACAATAATAAATAAGAAATATCGATTGGAGAGGATGAATAAATGGACCAAGCGGGAATCTTAGGAAAAGATCTTTAGATTAGATCTCTCCCTTTTTTTT ... ACAATTCCTAA ...
M. fraseri var. pyramidata ... CAAATATGAATCGTAATTGACTGAACAATAATAAATAAGAAATATCGATTGGAGAGGATGAATAAATGGACCAAGCGGGAATCTTAGGAAAAGATCTTTAGATTAGATCTCTCCCTTTTTTTT ... ACAATTCCTAA ...
M. dealbata ... CAAATATGAATCGTAATTGACTGAACAATAATAAATAAGAAATATCGATTGGAGAGGATGAATAAATGGACCAAGCGGGAATCTTAGGAAAAGATCTTTAGATTAGATCTCTCCCTTTTTTTT ... ACAATTCCTAA ...
L. tulipifera ... CAAATATGAATCGTAATTGACTGAACAATAATAAATAAGAAATATCGATTGGAGAGGATGAATAAATGGACCAAGCGGGAATCTTAGGAAAAGATCTTTAGATTAGATCTCTCCCTTTTTTTT ... ACAATTCCTAA ...

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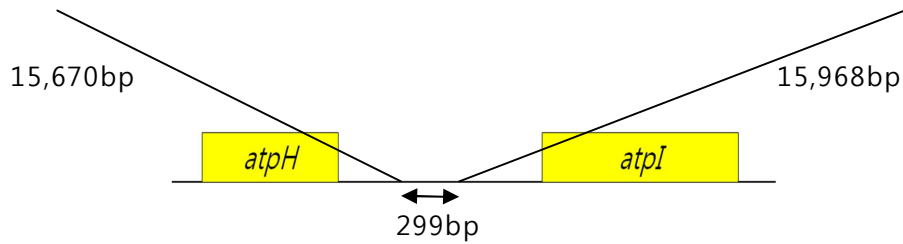


Fig. 7. Major indels found in chloroplast genomes in Magnoliaceae. A, Unique major deletions in *M. acuminata* found between *atpH*~*atpI* (299bp); B, Unique major deletions in *M. kobus* found between *psbE*~*petL* (658bp); C, Unique major deletions in *M. liliiflora* found between *rps12\_3end*~*rrn16* (952bp including *trnV*).





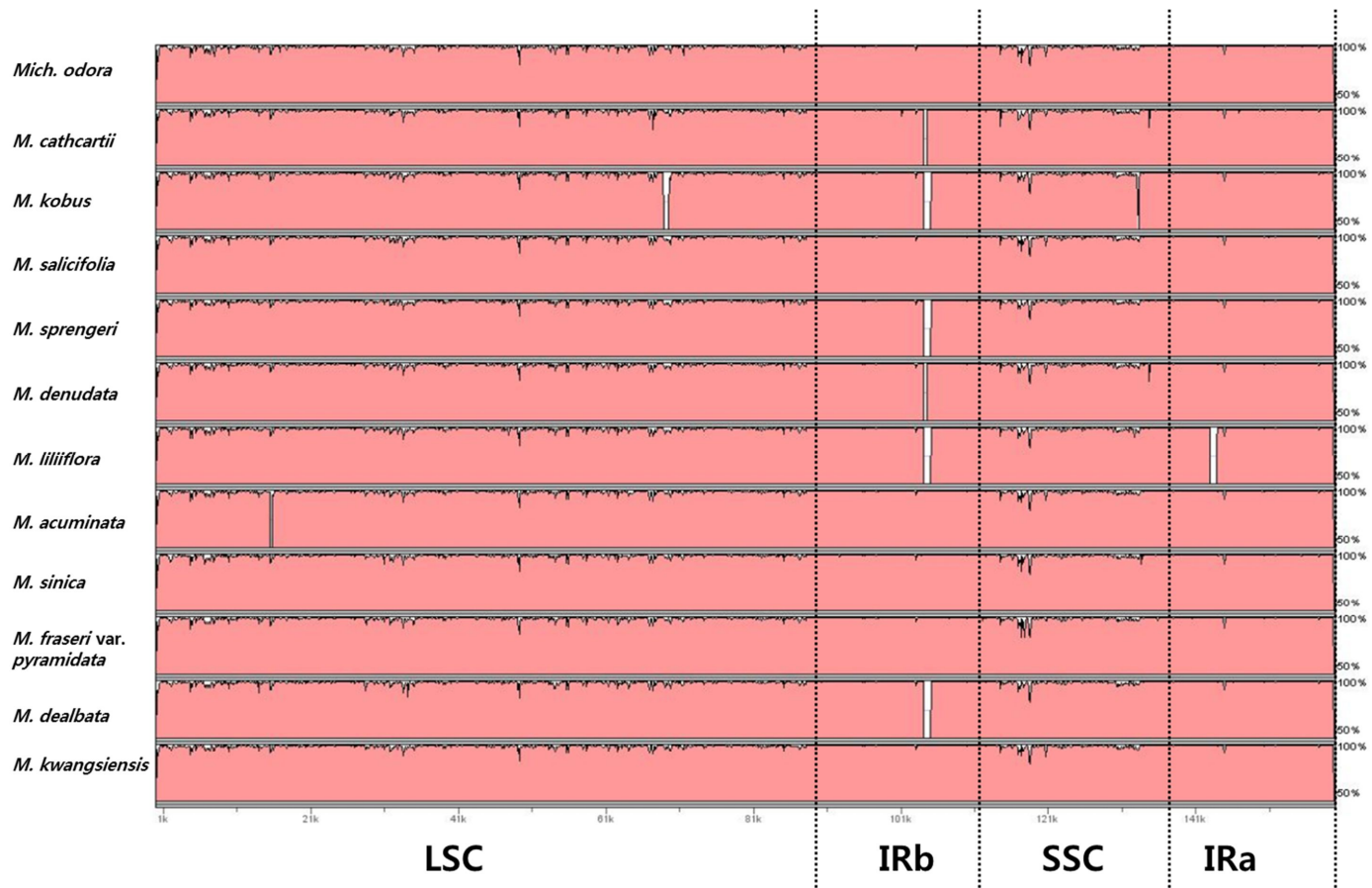


Fig. 8. Comparison of cp DNA between *L. tulipifera* and other magnolias using mVISTA. The white regions indicate rearranged regions against *L. tulipifera*.

and IR, and 3) genes and IGS/introns. Overall  $\rho$ -distance of IGS, intron, and genes (including RNA) are 0.00937, 0.00475, and 0.00407 (0.00045 in RNA), respectively. The divergence rate of IGSs is about two times higher than overall gene regions. The overall  $\rho$ -distance of LSC, IR, and SSC are 0.00642, 0.00133, and 0.00937, respectively. It shows that the IR region has 4X lower divergence than LSC and SSC. The  $\rho$ -distance between the 13 species cp DNA is represented in Table 10. This shows that variation of IGS generally is higher than intron and genes. Among subgen. *Yulania*, *M. liliiflora*/*M. denudata* and *M. kobus*/*M. salicifolia* have close relationships of phylogeny compared with other species. The  $\rho$ -distance of IR appears lower values than LSC and SSC. Comparison of sequence characteristics for genes and intergenic spacers (+introns) with three different hierarchical levels in the Magnoliaceae showed low variation of Magnoliaceae (Fig. 9; APPENDIX 2). In the genes, *ycf1* has the highest number of informative sites in three different hierarchical levels. The highest divergence values were found in *ycf1* and in *rps8* for Magnoliaceae and for Magnolioideae and subgen. *Yulania*, respectively. In the intergenic spacers (+introns), *petA* ~ *psbJ* region showed the highest number of informative characters in all different hierarchical levels. In the Magnoliaceae the highest divergence value was found in *petG* ~ *trnW-CCA*. However, that was *ccsA* ~ *ndhD* in Magnolioideae and also in subgen. *Yulania*.

### 3. Phylogenetic analyses

Three different phylogenetic analyses have performed using entire chloroplast genome regions of 11 taxa determined in this study and two

Table 10. The  $\rho$ -distance among 13 chloroplast genomes in Magnoliaceae ( $\rho$ -distancex100)

Overall (mean=0.00590)												
	1	2	3	4	5	6	7	8	9	10	11	12
1												
2	0.34											
3	0.52	0.54										
4	0.48	0.51	0.13									
5	0.52	0.53	0.25	0.22								
6	0.52	0.55	0.28	0.24	0.25							
7	0.51	0.53	0.25	0.22	0.24	0.09						
8	0.47	0.50	0.38	0.34	0.36	0.37	0.37					
9	0.47	0.49	0.45	0.42	0.44	0.45	0.44	0.41				
10	0.49	0.51	0.48	0.45	0.47	0.48	0.47	0.43	0.40			
11	0.53	0.55	0.53	0.49	0.51	0.52	0.51	0.46	0.45	0.44		
12	0.48	0.51	0.48	0.44	0.46	0.47	0.47	0.41	0.41	0.41	0.41	
13	1.51	1.53	1.50	1.48	1.49	1.51	1.49	1.45	1.43	1.42	1.43	1.40

Intergenic Spacers (mean=0.00937)												
	1	2	3	4	5	6	7	8	9	10	11	12
1												
2	0.59											
3	0.79	0.86										
4	0.73	0.83	0.19									
5	0.82	0.89	0.38	0.35								
6	0.82	0.93	0.44	0.38	0.43							
7	0.78	0.86	0.38	0.35	0.40	0.17						
8	0.73	0.83	0.59	0.52	0.59	0.59	0.59					
9	0.71	0.78	0.66	0.63	0.66	0.69	0.65	0.61				
10	0.80	0.87	0.75	0.72	0.76	0.79	0.75	0.70	0.65			
11	0.86	0.94	0.82	0.78	0.82	0.85	0.82	0.73	0.69	0.75		
12	0.73	0.85	0.73	0.66	0.72	0.73	0.73	0.63	0.61	0.67	0.66	
13	2.41	2.49	2.38	2.37	2.38	2.42	2.39	2.33	2.27	2.29	2.31	2.26

Genes (mean=0.00407)												
	1	2	3	4	5	6	7	8	9	10	11	12
1												
2	0.21											
3	0.38	0.38										
4	0.35	0.35	0.10									
5	0.36	0.35	0.17	0.14								
6	0.36	0.35	0.18	0.16	0.16							
7	0.36	0.36	0.19	0.16	0.16	0.05						
8	0.33	0.33	0.28	0.25	0.25	0.25	0.26					
9	0.32	0.32	0.33	0.31	0.32	0.32	0.32	0.29				
10	0.33	0.32	0.34	0.31	0.32	0.33	0.34	0.30	0.28			
11	0.36	0.36	0.39	0.36	0.36	0.37	0.37	0.33	0.32	0.29		
12	0.34	0.34	0.36	0.33	0.34	0.34	0.35	0.31	0.31	0.28	0.28	
13	1.03	1.03	1.03	1.01	1.02	1.03	1.03	0.99	0.98	0.96	0.98	0.95

Introns (mean=0.00475)												
	1	2	3	4	5	6	7	8	9	10	11	12
1												
2	0.30											
3	0.49	0.43										
4	0.45	0.39	0.10									
5	0.45	0.39	0.20	0.17								
6	0.47	0.41	0.26	0.22	0.20							
7	0.45	0.39	0.23	0.20	0.20	0.07						
8	0.41	0.36	0.29	0.26	0.26	0.28	0.26					
9	0.50	0.45	0.44	0.41	0.38	0.41	0.41	0.41				
10	0.44	0.39	0.38	0.35	0.33	0.34	0.33	0.35	0.35			
11	0.42	0.36	0.35	0.33	0.33	0.35	0.33	0.33	0.36	0.30		
12	0.41	0.37	0.36	0.32	0.32	0.35	0.32	0.32	0.35	0.29	0.28	
13	1.31	1.27	1.25	1.22	1.22	1.25	1.22	1.22	1.23	1.17	1.16	1.17

Table 10. Continued.

LSC region (mean=0.00642)												
	1	2	3	4	5	6	7	8	9	10	11	12
1												
2	0.38											
3	0.58	0.60										
4	0.56	0.58	0.13									
5	0.58	0.60	0.25	0.23								
6	0.60	0.61	0.29	0.27	0.26							
7	0.57	0.58	0.26	0.24	0.25	0.10						
8	0.53	0.54	0.41	0.39	0.40	0.41	0.40					
9	0.53	0.55	0.50	0.49	0.49	0.50	0.49	0.44				
10	0.54	0.56	0.52	0.50	0.51	0.52	0.51	0.45	0.44			
11	0.60	0.62	0.58	0.56	0.56	0.57	0.56	0.49	0.50	0.49		
12	0.54	0.56	0.52	0.50	0.50	0.52	0.51	0.44	0.45	0.43	0.45	
13	1.65	1.66	1.62	1.60	1.60	1.62	1.61	1.55	1.55	1.52	1.55	1.51

IR region (mean=0.00133)												
	1	2	3	4	5	6	7	8	9	10	11	12
1												
2	0.06											
3	0.11	0.13										
4	0.09	0.11	0.05									
5	0.09	0.12	0.08	0.06								
6	0.09	0.11	0.07	0.06	0.05							
7	0.09	0.12	0.08	0.06	0.06	0.01						
8	0.08	0.11	0.09	0.08	0.08	0.08	0.08					
9	0.08	0.11	0.13	0.11	0.11	0.11	0.11	0.10				
10	0.08	0.09	0.11	0.08	0.09	0.09	0.09	0.08	0.09			
11	0.08	0.09	0.11	0.09	0.09	0.09	0.09	0.08	0.09	0.07		
12	0.07	0.09	0.10	0.08	0.09	0.08	0.09	0.08	0.09	0.07	0.06	
13	0.38	0.39	0.41	0.39	0.39	0.40	0.38	0.39	0.39	0.36	0.38	0.37

SSC region (mean=0.00937)												
	1	2	3	4	5	6	7	8	9	10	11	12
1												
2	0.54											
3	0.80	0.83										
4	0.65	0.74	0.23									
5	0.78	0.80	0.43	0.35								
6	0.76	0.84	0.49	0.34	0.45							
7	0.78	0.80	0.45	0.38	0.43	0.17						
8	0.72	0.82	0.62	0.45	0.56	0.56	0.60					
9	0.68	0.71	0.65	0.55	0.61	0.68	0.66	0.65				
10	0.83	0.86	0.81	0.72	0.78	0.83	0.80	0.80	0.66			
11	0.81	0.86	0.83	0.74	0.82	0.86	0.82	0.81	0.67	0.72		
12	0.73	0.84	0.82	0.66	0.78	0.76	0.79	0.72	0.68	0.74	0.65	
13	2.35	2.44	2.39	2.35	2.39	2.46	2.40	2.41	2.26	2.34	2.27	2.25

1, *Mich. odora*; 2, *M. carthcartii*; 3, *M. kobus*; 4, *M. salicifolia*; 5, *M. sprengeri*; 6, *M. denudata*; 7, *M. liliiflora*; 8, *M. acuminata*; 9, *M. sinica*; 10, *M. kwangsiensis*; 11, *M. fraseri* var. *pyramidata*; 12, *M. dealbata*; 13, *L. tulipifera*.

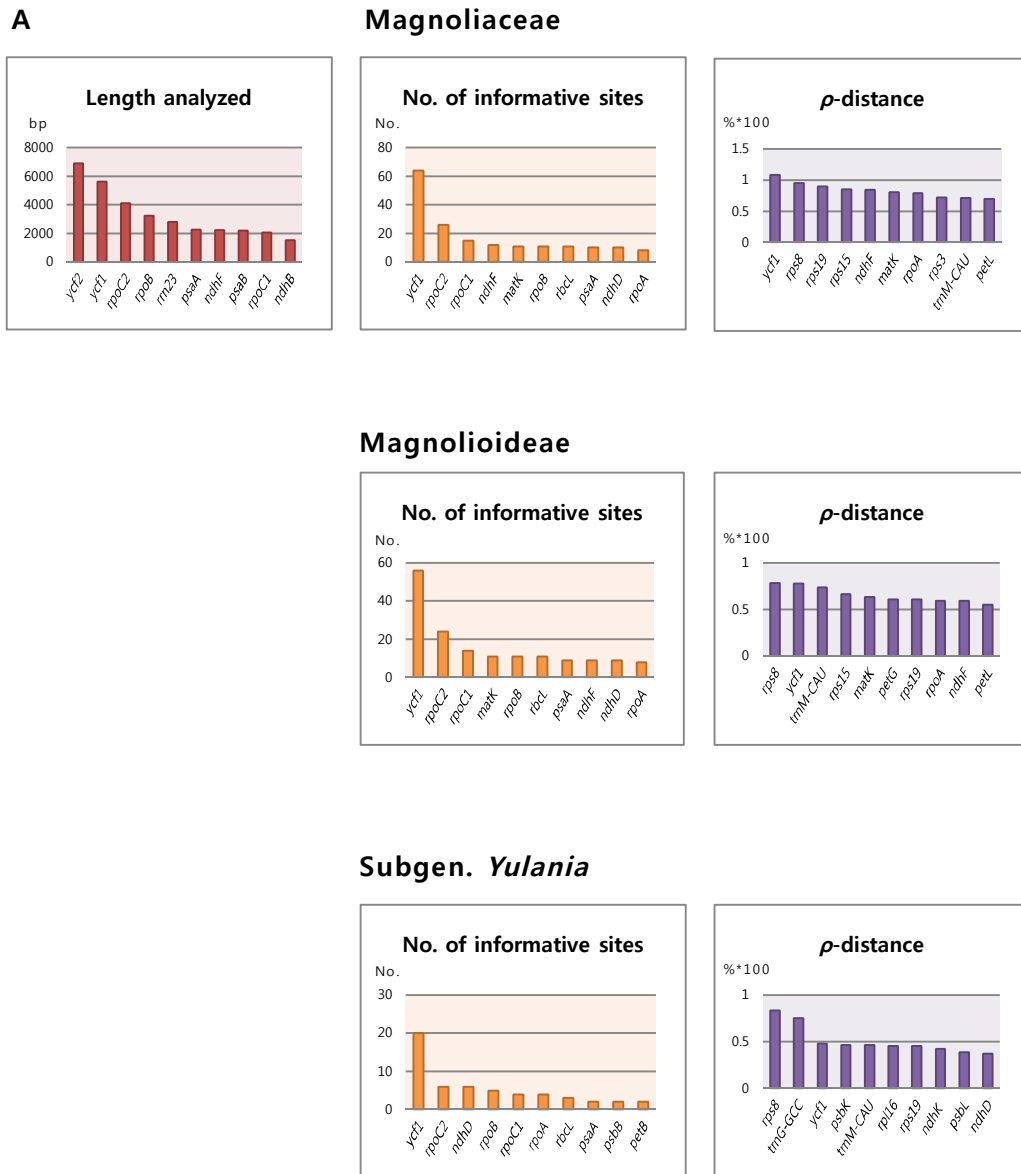
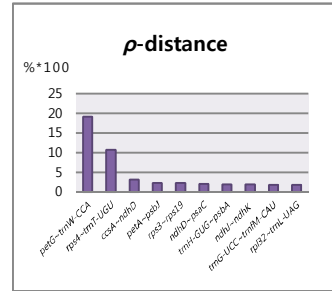
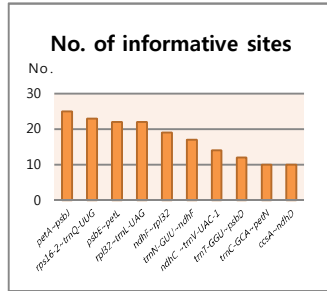
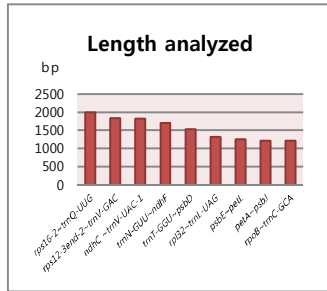


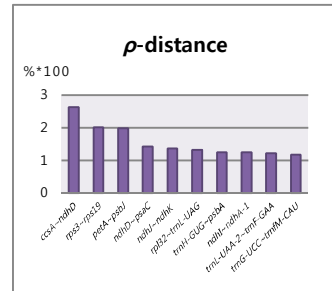
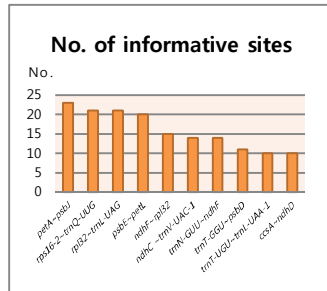
Fig. 9. Comparison of sequence characteristics for genes (A) and intergenic spacers (+introns) (B) with three different hierarchical levels in the Magnoliaceae. Only ten top-ranked genes or IGSs (+introns) have shown in the figure.

**B**

**Magnoliaceae**



**Magnolioideae**



**Subgen. Yulania**

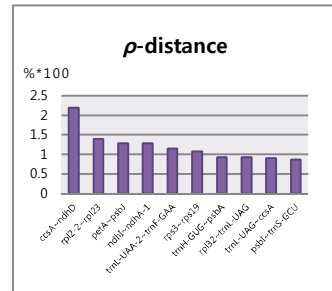
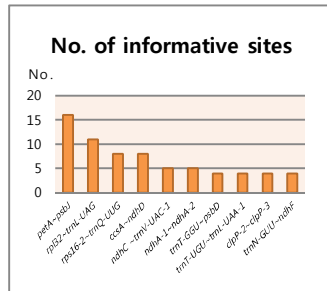


Fig. 9. Continued.

previously reported genomes (*L. tulipifera* and *M. kwangsiensis*; Cai et al., 2006; Kuang et al., 2011); the size of aligned matrix is 161526. In the MP analyses, just one MP tree with 4295 steps is found (Fig. 10). The consistency index (CI) and retention index are 0.93 and 0.81, respectively. When we used *L. tulipifera* as outgroup, *M. dealbata*, *M. fraseri* var. *pyramidata*, and *M. kwangsiensis* form a grade at the base. For the taxa of subgen. *Yulania* and related taxa, the tree was completely resolved with 100% of bootstrap values except just one clade (81%). In the subgen. *Yulania*, relationships among four sublineages recognized in the previous studies (Kim et al., 2001; Nie et al., 2008) are clarified: *M. sprengeri* is a sister to *M. kobus*/*M. salicifolia* and this clade is a sister to *M. liliiflora*/*M. denudata*. Same as previous studies, *M. acuminata*, which is the only North American species, was placed at the base of subgen. *Yulania*. Sister group of the subgen. *Yulania* is also clarified: *M. cathcartii*/*Mich. odora* clade is a sister to subgen. *Yulania* and *M. sinica* is placed at the outside of this clade.

The Neighbor Joining (NJ) tree and Maximum Likelihood (ML) tree showed almost similar topology to MP tree: the basal group of Magnolioideae was *M. dealbata*/*M. fraseri* var. *pyramidata*. Except the relationships at the base of the tree, all three trees from different analyses methods showed exactly same topology with high bootstrap values (Fig. 11; Fig. 12).

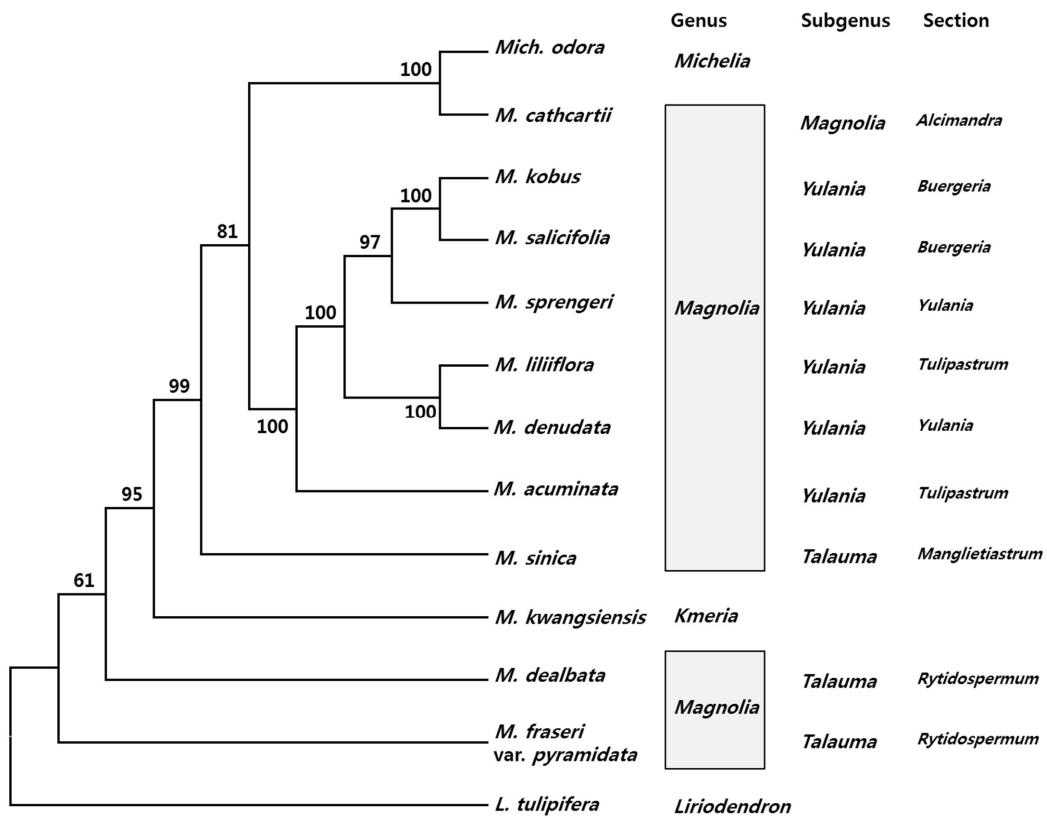


Fig. 10. A most parsimonious tree (MP). Numbers above the nodes indicate bootstrap values.

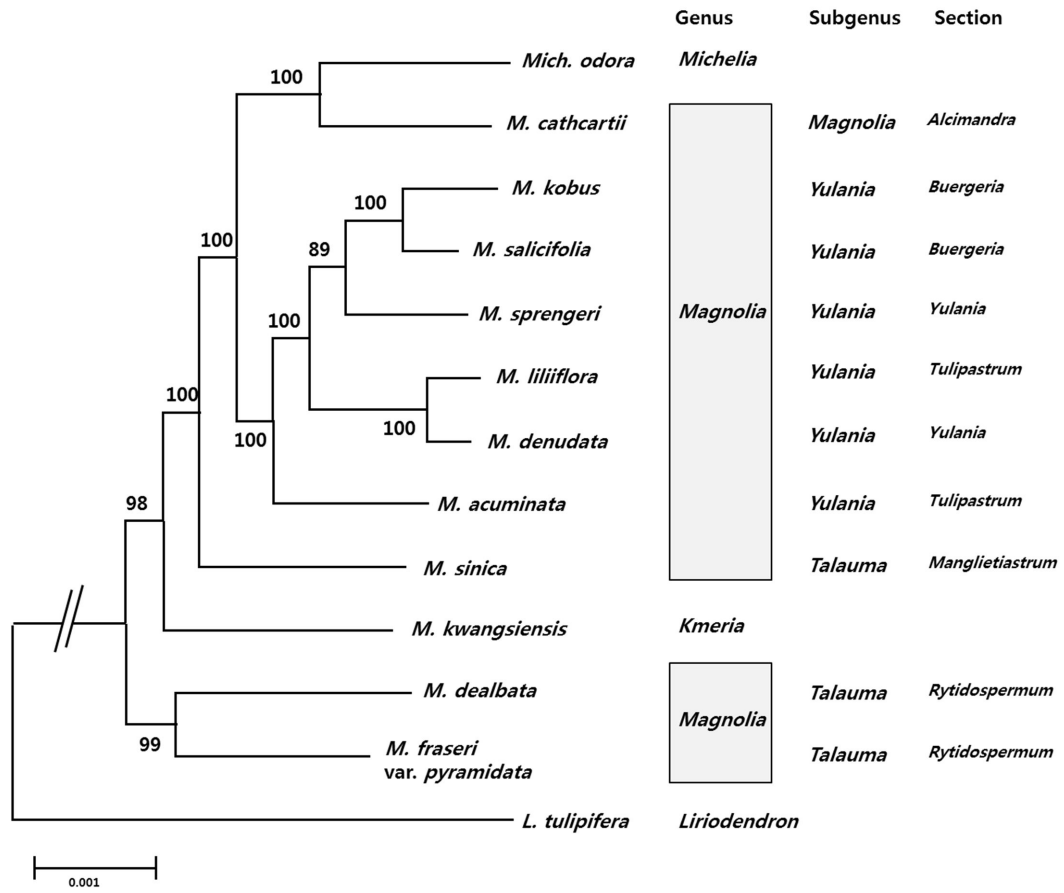


Fig. 11. Neighbor joining (NJ) tree with *p*-distance method. Numbers above the nodes indicate bootstrap values.

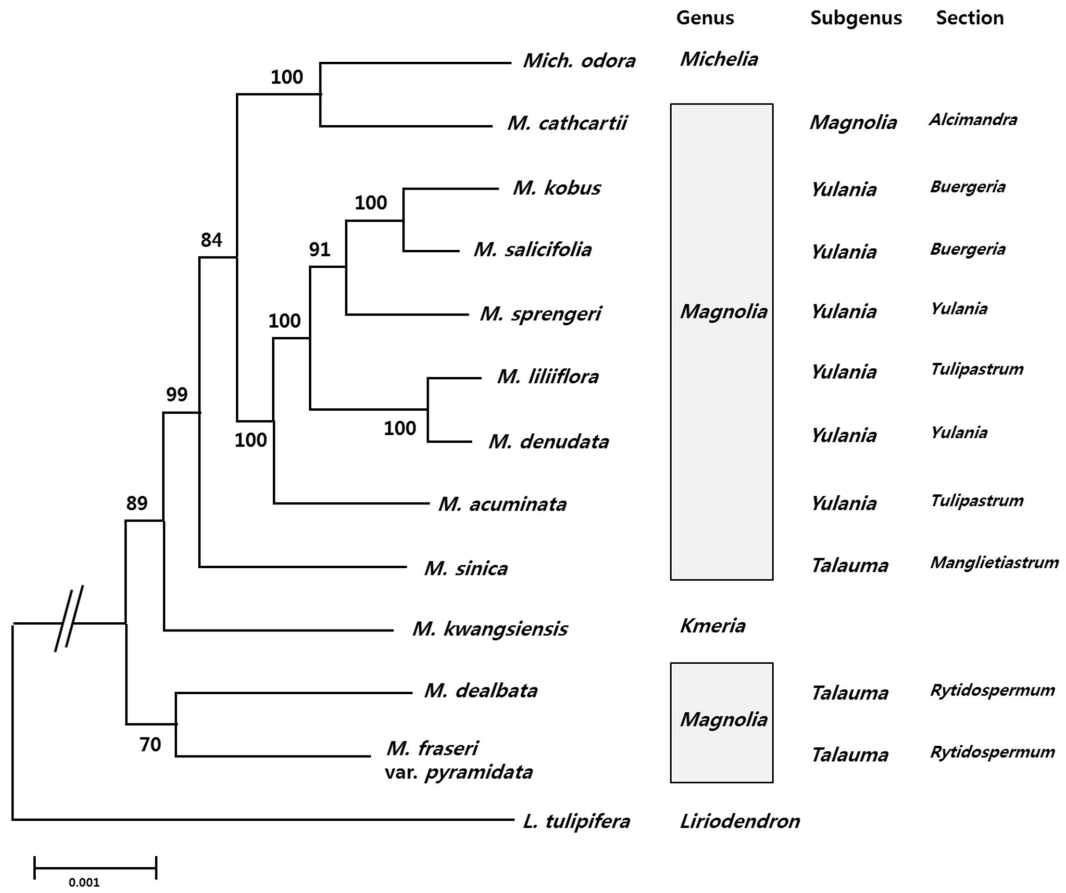


Fig. 12. Maximum Likelihood (ML) tree with GTR+I model (ln L= - 246231.40). Numbers above the nodes indicate bootstrap values.

## IV. DISCUSSION

The function and origin of cpSSRs in the chloroplast genome are not fully understood. However, SSR loci contained in the plant cp genome can provide useful information about plant population genetics (Powell et al., 1995; Echt et al., 1998). Also, it is important for understanding of the population structure, protection, propagation and phylogenetic relationship of species (Provan et al., 1997; Doyle et al., 1998; Deguilloux et al., 2004; Kuang et al., 2011). This study provides detailed cpSSRs in various magnolia species which is applicable for the studies of population genetics and conservation biology of Magnoliaceae.

The border variations among LSC, IR, and SSC include deletion, insertion, or translocation to other regions (Fig. 6). Among them, *L. tulipifera* has large variation in 4 border regions and expansion of SSC due to insertion of 164bp. This result clearly showed subfamilial delimitation in Magnoliaceae: Magnolioideae and Liriodendroideae. Inserted/deleted sequences at the borders among LSC, IR, and SSC may reflect the phylogenetic relationships among these taxa. For example, 20bp for six species of subgen. *Yulania* and 19bp of insertion (or 1bp of deletion after 20bp of insertion) for *M. liliiflora* and *M. denudata* at the border between SSC and IR.

Three different analysis methods with cp genome data in Magnoliaceae showed fully resolved phylogenetic relationship among subgen. *Yulania* and related taxa. First of all, *M. acuminata*, the only natural

North American species in the subgen. *Yulania*, was placed at the base of subgen. *Yulania* as showed in Kim et al. (2001) and Nie et al. (2008). This result supports that sect. *Tulipastrum* is clearly a paraphyletic group. In the previous classification system based on morphology, sect. *Tulipastrum* is distinguished from other magnolias having distinctive tepal color (yellow or purple) and sepaloid tepals. In the previous studies (Kim et al., 2001) the monophyly of the sect. *Buergeria* was doubted because species of sect. *Buergeria* did not form a clade as basal polytomy in the tree. However, this study showed close relationship between *M. kobus* and *M. salicifolia*. If we add more cp genome data for remaining species of sect. *Buergeria*, it is potentially form a monophyletic group and this will be one of further research of a continyation of this study. Another polytomy among *Michelia*/*M. cathcartii*, subgen. *Yulania*, and sect. *Manglietiastrum* in the previous study (Kim et al., 2001) is also clarified: *Michelia*/*M. cathcartii* was a sister to subgen. *Yulania*. This result will provide a clue to understand evolution of subgen. *Yulania*, deciduous magnolias, in the Magnoliaceae.

In summary, various phylogenetic analyses with entire cp genome region showed that strong new phylogenetic relationships among species of subgen. *Yulania* and related taxa: 1) *M. sprengeri* is a sister to *M. kobus*/*M. salicifolia* clade, 2) *M. liliiflora*/*M. denudata* clade is a sister to the clade of *M. kobus*/*M. salicifolia*/*M. sprengeri*, 3) *Michelia*/*M. cathcartii* clade is a sister to subgen. *Yulania*, which is a deciduous magnolia group.

This study includes quick determination of entire cp genome with NGS (Solexa/Illumina) with reference taxon. With intensive sampling of genome data, phylogeny of Magnoliaceae especially subgen. *Yulania* and

related taxa are clarified. As a chloroplast phylogeny, no more addition data is possible. Combined analyses of eleven chloroplast genomes and previously reported two chloroplast genomes in Magnoliaceae showed that new understanding of phylogeny and evolution of Magnoliaceae.

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## ABSTRACT

### Phylogeny of subgenus *Yulania* (Magnoliaceae) and related taxa based on chloroplast genomes

Eunji Song  
Department of Biology  
Graduate School  
Sungshin Women's University

As a candidate basal-most angiosperm, Magnoliaceae has been the subject of keen interest from many botanists who study classical morphology. Although the basal-most position of extant angiosperms is currently *Amborella*, Magnoliaceae is still spotlighted by many botanists who seek to understand the early-divergence of angiosperms as a second group of basal-most angiosperms as it pertains to phylogenetic, genomic, and evolutionary studies. Although recent molecular phylogenetic studies showed that major lineages exist in this family, the phylogenetic relationships among these lineages remain unclear due to insufficient informative characters, indicating that genome-level phylogenetic analyses are needed. As a series of molecular phylogenetic studies on the Magnoliaceae, this study provides 1) the chloroplast whole genome of *Magnolia kobus*, a member of subgen. *Yulania*, using Solexa/Illumina

technology, and 2) additional 10 chloroplast genomes included in the subgen. *Yulania* and related taxa using a conventional PCR-based method. The chloroplast genomes of Magnoliaceae are 158,177bp ~ 160,093bp in length, including IR regions ranging from 25,651bp (*M. liliiflora*) to 26,602bp (*M. acuminata*) separated by LSC regions ranging from 87,484bp (*M. kobus*) to 88,156bp (*M. sinica*), and SSC regions ranging from 18,722bp (*M. sprengeri*) to 18,800bp (*Mich. odora*). Sequence structural analyses based on an aligned matrix showed 1) taxon-specific large gaps such as the 658bp deletion in *M. kobus* and 952bp deletions including trnV-GAC in *M. liliiflora* and 2) boarder variations of LSC, SSC, and IR. Various phylogenetic analyses of the entire cp genome region showed strong new phylogenetic relationships among the species of subgen. *Yulania* and related taxa: 1) *M. sprengeri* is a sister to the *M. kobus*/*M. salicifolia* clade; 2) the *M. liliiflora*/*M. denudata* clade is a sister to the clade of *M. kobus*/*M. salicifolia*/*M. sprengeri*; and 3) the *Michelia*/*M. cathcartii* clade is a sister to subgen. *Yulania*, which is a deciduous magnolia group. Combined analyses of eleven chloroplast genomes and two previously reported chloroplast genomes in Magnoliaceae offer a new understanding of the phylogeny and evolution of Magnoliaceae.

## **APPENDIX I**

Distribution of simple sequence repeats (SSRs) in 11 chloroplast genomes  
in Magnoliaceae

Taxa	Unit	Lengths	No. SSRs	Coordinated Basepairs	
<i>Mich. odora</i>	A	8	18	9772-9779, 19886-19893, 23547-23554, 29213-29220, 31814-31821, 39216-39223, 39894-39901, 69836-69843, 72343-72350, 74231-74238, 81787-81794, 92199-92206, 114716-114723, 118576-118583, 121172-121179, 135667-135674, 141092-141099, 143969-143976	
		9	15	1726-1734, 3938-394, 4749-4757, 13858-13866, 32778-32786, 33392-33400, 34069-34077, 47158-47166, 50234-50242, 58364-58372, 63302-63310, 67334-67342, 93473-93481, 117895-117903, 130354-130362	
		10	3	19886-19893, 30577-30586, 132576-132585	
		11	4	15836-15846, 49366-49376, 64740-64750, 126149-126159	
		12	5	5392-5403, 12778-12789, 38853-38864, 46898-46909, 112093-112104	
		C	8	1	53693-53696
			9	1	48455-48463
			10	2	71228-71237, 118566-118575
		G	8	1	37003-37010
			T	8	25
		9		21	14148-14156, 30813-30821, 33501-33509, 33579-33587, 34793-34801, 34846-34854, 38746-38754, 53500-53508, 55823-55831, 62532-62540, 64353-64361, 77921-77929, 84466-84474, 86413-86421, 87055-87063, 119677-119685, 122850-122858, 130365-130373, 131661-131669, 131958-131966, 154688-154696
		10		3	5373-5382, 10383-10392, 70267-70276
		11		6	4580-4590, 17543-17553, 45365-45375, 51193-51203, 55662-55672, 84962-84972
		12		4	10277-10288, 19742-19753, 31358-31369, 136065-136076
		13		1	96865-96872
		14		2	7760-7773, 72373-72386
		15		2	15820-15834, 75479-75493
		17		1	124212-124228
	AG	8		2	99147-99154, 137766-137773
		AT		8	4
	10			2	21116-21125, 33343-33352
	CT	8		5	3751-3758, 87293-87300, 110396-110403, 128035-128042, 149015-149022
		GA		8	8
	GT			8	1
		TA		8	8
	TC			10	2
		12		2	34969-34980, 71843-71854
	AAC	8	7	32330-32337, 53693-53700, 87271-87278, 154668-154675, 156865-156872, 157867-157874, 157879-157886	
		10	2	64943-64952, 128837-128846	
	AAG	9	4	16005-16013, 72097-72105, 104949-104957, 118470-118478	
		AAT	9	3	4836-4844, 104139-104147, 153866-153874
	ACC		9	1	67173-67181
		AGA	12	1	128891-128902
	AGC		9	1	153602-153610
		AGT	9	4	72358-72366, 99442-99450, 100901-100909, 153978-153986
	ATA		9	1	126767-126775
		ATG	9	1	72753-72761
	ATT		9	2	82828-82836, 123517-123525
		CAC	12	1	57878-57889
	CAG		9	1	41280-41288
		CCT	9	2	115475-115483, 117649-117657
	CCT		9	1	154597-154605
		CTC	9	2	66394-66402, 139324-139332
	CTG		9	1	70283-70291
		CTT	9	1	70283-70291
	GAA		9	1	34202-34210
		GCA	9	2	73971-73979, 108837-108845
	GCT		9	3	88212-88220, 94295-94303, 144022-144030
		GCT	9	3	54656-54664, 96022-96030, 159948-159956
	GTA		9	1	43178-43186
		GTT	9	1	70283-70291
	GTT		9	1	34202-34210
GTT		9	2	73971-73979, 108837-108845	
	GTT	9	3	88212-88220, 94295-94303, 144022-144030	
GTT		9	3	54656-54664, 96022-96030, 159948-159956	
	GTT	9	1	43178-43186	
GTT		9	1	60004-60012	
	GTT	9	1	83928-83936	
GTT		9	1	94559-94567	
	GTT	9	1	34988-34996	
GTT		9	1	93564-93572	
	GTT	9	2	54184-54192, 143212-143220	

Taxa	Unit	Lengths	No. SSRs	Coordinated Basepairs
	TAA	9	2	38874-38882, 116399-116407
	TAT	9	3	39781-39789, 49113-4912, 119491-119499
	TCA	9	3	64449-64457, 152820-152828, 153360-153368
	TCC	9	2	24279-24287, 122178-122186
	TCT	9	2	94183-94191, 148719-148727
	TGA	9	2	94801-94809, 95341-95349
	TGC	9	1	87546-87554
	TGT	9	1	17886-17894
	TTA	9	4	14231-14239, 16801-16809, 88002-88010, 131228-131236
	TTT	9	4	23360-23368, 68894-68902, 147259-147267, 152139-152147
	TTG	9	3	42885-42893, 118454-118462, 134276-134284
	AAAT	12	1	117042-117053
		16	1	4392-4407
	AATC	12	1	126461-126472
	AATG	12	1	65553-65564
	AATT	12	1	115069-115080
	ACAT	12	1	87677-87688
	ATAA	12	1	73117-73128
	CATT	12	1	131006-131017
	TAAA	12	1	46886-46897
	AACCGA	18	1	7031-7048
	ACTAAT	18	1	144533-144550
	TATTAG	18	1	103618-103635
<i>M. cathartii</i>	A	8	16	1726-1733, 9742-9749, 13423-13430, 19890-19897, 23551-23558, 29213-29220, 31818-31825, 39915-39922, 67369-67376, 67404-67411, 92237-92244, 114683-114690, 121153-121160, 135556-135563, 140984-140991, 143861-143868, 4748-4756, 30576-30584, 33391-33399, 34070-34078, 34973-34981, 38876-38884, 39236-39244, 46916-46924, 47173-47181, 49389-49397, 50255-50263, 58383-58391, 67351-67359, 72390-72398, 93511-93519, 117882-117890, 130329-130337
		9	17	3935-3944, 4387-4396, 32770-32779, 64759-64768, 69870-69879, 132551-132560
		10	6	13843-13853, 126125-126135
		11	2	209-220, 5395-5406, 15827-15838
		12	3	12753-12765
		13	1	112126-112139
		14	1	48466-48473
	C	8	1	118554-118562
		9	1	7167-7177
		11	1	71264-71277
	G	8	1	37021-37028
		10	1	47808-47817
	T	8	26	1762-1769, 2040-2047, 10679-10686, 15506-15513, 15681-15688, 15818-15825, 27414-27421, 34822-34829, 38770-38777, 45386-45393, 49188-49195, 53521-53528, 54971-54978, 55898-55905, 63566-63573, 64173-64180, 87328-87335, 88119-88126, 107102-107109, 112530-112537, 117930-117937, 119651-119658, 128823-128830, 129588-129595, 131324-131331, 155849-155856, 14135-14143, 33580-33588, 34874-34882, 47347-47355, 53511-53519, 55842-55850, 72421-72429, 87098-87106, 118572-118580, 119664-119672, 122831-122839, 130340-130348, 131636-131644, 131933-131941, 154574-154582, 4977-4986, 10247-10256, 10351-10360, 13453-13462, 30811-30820, 55682-55691, 62551-62560, 64371-64380, 70303-70312, 71278-71287, 84508-84517, 86455-86464
		9	15	17548-17558, 31363-31373, 33500-33510, 51214-51224, 85005-85015
		10	12	5374-5385, 7746-7757, 19746-19757, 74542-74553, 77960-77971, 124193-124204
		11	5	4578-4590, 75527-75539
		12	2	135954-135967
		13	2	99185-99192, 137657-137664
	AG	8	2	21030-21037, 87397-87404, 118725-118732, 149638-149645
	AT	8	4	21120-21129, 33342-33351
		10	2	3751-3758, 87336-87343, 110429-110436, 128011-128018, 148901-148908
	CT	8	5	9235-9242, 38107-38114, 49453-49460, 59129-59136, 90321-90328, 90333-90340, 91335-91342, 93532-93539
	GA	8	8	129110-129117
		8	8	48614-48621, 49228-49235, 53780-53787, 85720-85727, 85744-85751, 96903-96910, 98447-98454, 151183-151190
		10	3	9429-9438, 34998-35007, 60224-60233
		14	1	71888-71901
	TC	8	7	32334-32341, 53713-53720, 87314-87321, 154554-154561, 156751-156758, 157753-157760, 157765-157772
		10	2	64961-64970, 128813-128822
	AAC	9	5	15997-16005, 72144-72152, 104981-104989, 118458-118466, 153488-153496
	AAG	9	3	4835-4843, 104171-104179, 153752-153760
	AAT	9	1	67191-67199
		12	1	128867-128878
	AGA	9	4	72406-72414, 99480-99488, 100939-100947, 153864-153872
	AGC	9	1	126743-126751
	AGT	9	1	72796-72804
	ATA	9	2	82870-82878, 123498-123506

Taxa	Unit	Lengths	No. SSRs	Coordinated Basepairs
<i>M. kobus</i>	ATA	9	2	82870-82878, 123498-123506
		12	1	57897-57908
	ATG	9	1	41301-41309
	CAA	9	2	113879-113887, 117755-117763
	CAC	9	1	154483-154491
	CAG	9	2	66412-66420, 139215-139223
	CCT	9	1	70319-70327
	CAG	9	2	66412-66420, 139215-139223
	CCT	9	1	70319-70327
	CTC	9	1	34203-34211
	CTG	9	2	74015-74023, 108870-108878
	CTT	9	3	88250-88258, 94333-94341, 143914-143922
	GAA	9	2	54676-54684, 96060-96068
	GCA	9	1	43199-43207
	GCT	9	1	60023-60031
	GGA	9	1	83970-83978
	GGT	9	1	94597-94605
	GTA	9	1	35015-35023
	GTG	9	1	93602-93610
	GTT	9	2	54204-54212, 143104-143112
	TAA	9	2	38894-38902, 116359-116367
	TAT	9	4	39802-39810, 49131-49139, 67765-67773, 119478-119486
	TCA	9	3	64468-64476, 152706-152714, 153246-153254
	TCC	9	2	24282-24290, 122159-122167
	TCT	9	2	94221-94229, 148605-148613
	TGA	9	2	94839-94847, 95379-95387
	TGC	9	1	87589-87597
	TGT	9	1	17890-17898
	TTA	9	6	14218-14226, 16806-16814, 49153-49161, 88045-88053, 115433-115441, 131203-131211
	TTC	9	5	23364-23372, 68929-68937, 71583-71594, 147145-147153, 152025-152033
	TTG	9	3	42906-42914, 118442-118450, 134206-134214
	TCT	9	2	94221-94229, 148605-148613
	TGA	9	2	94839-94847, 95379-95387
	TGC	9	1	87589-87597
	TGT	9	1	17890-17898
	TTA	9	6	14218-14226, 16806-16814, 49153-49161, 88045-88053, 115433-115441, 131203-131211
	TTC	9	5	23364-23372, 68929-68937, 71583-71594, 147145-147153, 152025-152033
	TTG	9	3	42906-42914, 118442-118450, 134206-134214
	AAAT	12	1	117002-117013
	AATC	12	1	126437-126448
	AATG	12	1	65571-65582
	AATT	12	1	115029-115040
	ACAT	12	1	87720-87731
	ATAA	12	1	73161-73172
	CATT	12	1	130981-130992
	TAAA	12	2	4397-4408, 46904-46915
	TTTAT	15	1	123530-123544
	ACTAAT	18	1	144425-144442
	TATTAG	18	1	103650-103667
	A	8	15	13411-13418, 19867-19874, 23528-23535, 31808-31815, 33382-33389, 39923-39930, 47189-47196, 50274-50281, 58407-58414, 71727-71734, 91586-91593, 117274-117281, 120546-120553, 140283-140290, 143155-143162, 211-219, 3941-3949, 4751-4759, 5398-5406, 9737-9745, 29193-29201, 34960-34968, 46932-46940, 49404-49412, 92860-92868, 111463-111471, 114093-114101, 129715-129723
		9	13	1725-1734, 13831-13840, 15806-15815, 29230-29239, 30568-30577, 49859-49868, 64794-64803, 69225-69234, 131937-131946, 125516-125526, 134858-134868
		10	9	1725-1734, 13831-13840, 15806-15815, 29230-29239, 30568-30577, 49859-49868, 64794-64803, 69225-69234, 131937-131946, 125516-125526, 134858-134868
		11	2	125516-125526, 134858-134868
		12	1	117975-117986
		13	1	38875-38887
		14	1	12740-12753
	C	8	2	5927-5934, 70618-70625
		10	1	7176-7185
		11	1	29219-29229
		12	1	117963-117974
G	8	2	4823-4830, 37019-37026	
T	8	27	2043-2050, 10666-10673, 15493-15500, 27394-27401, 33490-33497, 34434-34441, 49203-49210, 54994-55001, 55922-55929, 62582-62589, 62993-63000, 63594-63601, 64207-64214, 69082-69089, 73882-73889, 85794-85801, 86665-86672, 87462-87469, 106440-106447, 117322-117329, 119046-119053, 119059-119066, 122224-122231, 128216-128223, 128974-128981, 130710-130717, 155137-155144	
	9	19	4981-4989, 14122-14130, 15796-15804, 30804-30812, 34808-34816, 34861-34869, 38768-38776, 47362-47370, 48492-48500, 51232-51240, 69658-69666, 70626-70634, 77301-77309, 86435-86443, 129726-129734, 131022-131030, 131319-131327, 135259-135267, 153862-153870	

Taxa	Unit	Lengths	No. SSRs	Coordinated Basepairs
		10	8	10351-10360, 13441-13450, 31354-31363, 53542-53551, 55705-55714, 55865-55874, 71755-71764, 83846-83855
		11	8	1763-1773, 4583-4593, 17525-17535, 45394-45404, 64405-64415, 84343-84353, 111862-111872, 123585-123595
		12	3	5377-5388, 19723-19734, 74863-74874
		13	1	7753-7765
		14	1	10243-10256
	AG	8	2	98527-98534, 136957-136964
	AT	8	4	21007-21014, 86740-86747, 118120-118127, 148933-148940
		10	2	21097-21106, 33333-33342
	CT	8	5	3754-3761, 86673-86680, 109766-109773, 127402-127409, 148196-148203
	GA	8	8	9257-9264, 38105-38112, 49468-49475, 59152-59159, 89670-89677, 89682-89689, 90684-90691, 92881-92888
	GT	8	1	128496-128503
	TA	8	8	48631-48638, 49243-49250, 53803-53810, 85059-85066, 85083-85090, 96252-96259, 97789-97796, 150471-150478
		10	2	9424-9433, 60255-60264
		12	2	34985-34996, 71227-71238
	TC	8	7	32324-32331, 53736-53743, 86651-86658, 153842-153849, 156039-156046, 157041-157048, 157053-157060
		10	1	64996-65005
		12	1	128204-128215
	AAC	9	3	15974-15982, 71481-71489, 104324-104332
	ACC	9	1	152776-152784
	AAG	9	3	4839-4847, 103514-103522, 153040-153048
	AAT	9	1	67226-67234
		12	1	128260-128271
	AGA	9	4	71742-71750, 98822-98830, 100281-100289, 153152-153160
	AGC	9	1	126134-126142
	AGT	9	1	72131-72139
	ATA	9	2	82208-82216, 122890-122898
		12	1	57921-57932
	ATG	9	1	41309-41317
	ATT	9	1	117028-117036
	CAA	9	2	113255-113263, 117147-117155
	CAC	9	1	153771-153779
	CAG	9	2	66447-66455, 138515-138523
	CCT	9	1	69673-69681
	CTC	9	1	34189-34197
	CTG	9	2	73355-73363, 108207-108215
	CTT	9	3	87598-87606, 93682-93690, 143208-143216
	GAA	9	2	54699-54707, 95409-95417, 159123-159131
	GCA	9	1	43207-43215
	GCT	9	1	60054-60062
	GGA	9	1	83308-83316
	GGT	9	1	93946-93954
	GTA	9	1	35004-35012
	GTG	9	1	92951-92959
	GTT	9	1	54227-54235, 142398-142406
	TAA	9	2	38902-38910, 115777-115785
	TAT	9	3	39810-39818, 49146-49154, 118873-118881
	TCA	9	3	64503-64511, 151994-152002, 152534-152542
	TCC	9	2	24259-24267, 121552-121560
	TCT	9	2	93570-93578, 147900-147908
	TGA	9	2	94188-94196, 94728-94736
	TGC	9	1	86932-86940
	TGT	9	1	17867-17875
	TTA	9	5	14205-14213, 16783-16791, 87388-87396, 114851-114859, 130589-130597
	TTC	9	4	23341-23349, 70922-70933, 146440-146448, 151313-151321
	TTG	9	3	42914-42922, 117834-117842, 133467-133475
	AAAT	12	1	116420-116431
		16	1	4395-4410
	AATC	12	1	125828-125839
	AATG	12	1	65606-65617
	AATT	12	1	114447-114458
	ACAT	12	1	87063-87074
	ATAA	12	1	72496-72507
	CATT	12	1	130367-130378
	TAAA	12	1	46920-46931
	ACTAAT	18	1	143719-143736
	TATTAG	18	1	102993-103010
<i>M. salicifolia</i>	A	8	13	19871-19878, 23532-23539, 31816-31823, 33385-33392, 39926-39933, 58405-58412, 72382-72389, 92244-92251, 114760-114767, 117940-117947, 121192-121199, 141122-141129, 143999-144006
		9	9	211-219, 9741-9749, 29237-29245, 47188-47196, 49405-49413, 93518-93526, 118623-118631, 130367-130375, 135698-135706
		10	7	1725-1734, 4754-4763, 34963-34972, 46930-46939, 69874-69883, 112131-112140, 132589-132598

Taxa	Unit	Lengths	No. SSRs	Coordinated Basepairs
		11	5	3943-3953, 5401-5411, 29197-29207, 30574-30584, 126168-126178
		12	1	64784-64795
		13	1	15814-15826
		14	2	12745-12758, 13835-13848
		15	1	38881-38895
	C	8	1	5932-5939
		12	1	7179-7190
		13	2	29224-29236, 71267-71279
		14	1	118609-118622
	G	8	1	37023-37030
		9	1	4827-4835
	T	8	22	2045-2052, 10671-10678, 14130-14137, 27398-27405, 30812-30819, 49204-49211, 54993-55000, 55920-55927, 62984-62991, 63585-63592, 86453-86460, 87324-87331, 88121-88128, 107108-107115, 117988-117995, 119692-119699, 119705-119712, 122870-122877, 128868-128875, 129626-129633, 131362-131369, 155986-155993
		9	21	4986-4994, 10351-10359, 15500-15508, 15804-15812, 33493-33501, 34811-34819, 34864-34872, 48493-48501, 55704-55712, 62572-62580, 64198-64206, 64397-64405, 69730-69738, 70307-70315, 77956-77964, 87094-87102, 112531-112539, 130378-130386, 131674-131682, 131971-131979, 154711-154719
		10	10	4587-4596, 5382-5391, 10247-10256, 13445-13454, 17536-17545, 31362-31371, 47362-47371, 55863-55872, 71280-71289, 136097-136106
		11	6	38772-38782, 45397-45407, 53540-53550, 72410-72420, 75519-75529, 84501-84511
		12	3	7758-7769, 19727-19738, 51228-51239
		13	1	74533-74545
		14	2	1763-1776, 84999-85012
		17	1	124231-124247
	AG	8	2	99185-99192, 137796-137803
	AT	8	3	21011-21018, 118765-118772, 149782-149789
		10	2	21101-21110, 33336-33345
	CT	8	5	3756-3763, 87332-87339, 110434-110441, 128054-128061, 149045-149052
	GA	8	8	9261-9268, 38109-38116, 49469-49476, 59150-59157, 90328-90335, 90340-90347, 91342-91349, 93539-93546
	GT	8	1	129148-129155
	TA	8	8	48632-48639, 49244-49251, 53802-53809, 85718-85725, 85742-85749, 96910-96917, 98447-98454, 151320-151327
		10	2	9428-9437, 60245-60254
		12	2	34989-35000, 71882-71893
	TC	8	7	32332-32339, 53735-53742, 87310-87317, 154691-154698, 156888-156895, 157890-157897, 157902-157909
		10	1	64988-64997
		12	1	128856-128867
	AAC	9	4	15985-15993, 72136-72144, 104987-104995, 118513-118521
	AAG	9	3	4844-4852, 104177-104185, 153889-153897
	AAT	9	1	67218-67226
		12	1	128912-128923
	ACC	9	1	153625-153633
	AGA	9	4	72397-72405, 99480-99488, 100939-100947, 154001-154009
	AGC	9	1	126786-126794
	AGT	9	1	72787-72795
	ATA	9	4	47493-47501, 82863-82871, 123536-123544, 125859-125867
		12	1	57919-57930
	ATG	9	1	41312-41320
	ATT	9	1	117694-117702
	CAA	9	2	113922-113930, 117813-117821
	CAC	9	1	154620-154628
	CAG	9	2	66439-66447, 139354-139362
	CCT	9	1	70322-70330
	CTC	9	1	34193-34201
	CTG	9	2	74006-74014, 108875-108883
	CTT	9	3	88257-88265, 94340-94348, 144052-144060
	GAA	9	3	54698-54706, 96067-96075, 159971-159979
	GCA	9	1	43210-43218
	GCT	9	1	60044-60052
	GGA	9	1	83963-83971
	GGT	9	1	94604-94612
	GTA	9	1	35008-35016
	GTG	9	1	93609-93617
	GTT	9	2	54226-54234, 143242-143250
	TAA	9	2	38905-38913, 116443-116451
	TAT	9	3	39813-39821, 49147-49155, 119519-119527
	TCA	9	3	64493-64501, 152843-152851, 153383-153391
	TCC	9	2	24263-24271, 122198-122206
	TCT	9	2	94228-94236, 148749-148757
	TGA	9	2	94846-94854, 95386-95394
	TGC	9	1	87591-87599
	TGT	9	1	17877-17885

Taxa	Unit	Lengths	No. SSRs	Coordinated Basepairs
<i>M. sprengeri</i>	TTA	9	5	14212-14220, 16794-16802, 88047-88055, 115517-115525, 131241-131249
	TTC	9	4	23345-23353, 68931-68939, 147289-147297, 152162-152170
		12	1	71577-71588
	TTG	9	3	42917-42925, 134307-134315
	AAAT	12	1	117086-117097
		16	1	4399-4414
	AATC	12	1	126480-126491
	AATG	12	1	65598-65609
	AATT	12	1	115113-115124
	ACAT	12	1	87722-87733
	ATAA	12	1	73152-73163
	CATT	12	1	131019-131030
	TAAA	12	1	46918-46929
	ACTAAT	18	1	144563-144580
	TATAAC	18	1	49061-49078
	TATTAG	18	1	103656-103673
	A	8	19	13406-13413, 19864-19871, 23525-23532, 29190-29197, 29224-29231, 31801-31808, 32766-32773, 33380-33387, 34958-34965, 39913-39920, 50258-50265, 58391-58398, 69865-69872, 92228-92235, 114748-114755, 121173-121180, 130345-130352, 141061-141068, 143938-143945, 211-219, 1725-1733, 3941-3949, 4750-4758, 5396-5404, 38872-38880, 39174-39182, 49392-49400, 72368-72376, 93502-93510, 112122-112130, 117927-117935, 118603-118611
		9	13	9723-9732, 15799-15808, 30560-30569, 46914-46923, 47172-47181, 132567-132576
		10	6	13825-13835
		11	1	126145-126156
		12	1	12735-12748, 64773-64786
		14	2	5925-5932, 32443-32450, 48472-48479
	C	8	3	7164-7172, 29215-29223, 71256-71264, 118594-118602
		9	4	37016-37023, 58399-58406
	G	8	2	10656-10663, 14117-14124, 27391-27398, 34433-34440, 34807-34814, 45384-45391, 48480-48487, 49191-49198, 51216-51223, 54977-54984, 5906-5913, 62559-62566, 62973-62980, 63574-63581, 64187-64194, 69722-69729, 74520-74527, 86437-86444, 87308-87315, 88105-88112, 107099-107106, 117976-117983, 118614-118621, 119672-119679, 122851-122858, 128846-128853, 129604-129611, 131340-131347, 155932-155939
		9	19	2042-2050, 4979-4987, 10334-10342, 13436-13444, 15487-15495, 30796-30804, 33488-33496, 34859-34867, 38765-38773, 70296-70304, 84492-84500, 84988-84996, 87078-87086, 119685-119693, 130356-130364, 131652-131660, 131949-131957, 136037-136045, 154657-154665
		10	7	4583-4592, 10230-10239, 31347-31356, 53525-53534, 55689-55698, 55849-55858, 64385-64394
		11	6	7742-7752, 47347-47357, 71265-71275, 72397-72407, 75501-75511, 77938-77948
		12	3	1762-1773, 5375-5386, 19720-19731
		13	1	124212-124224
		16	1	17518-17533
	AG	8	2	99176-99183, 137735-137742
	AT	8	4	21004-21011, 87383-87390, 118746-118753, 149721-149728
		10	2	21094-21103, 33331-33340
	CT	8	5	3754-3761, 87316-87323, 110425-110432, 128032-128039, 148984-148991
	GA	8	8	9243-9250, 38102-38109, 49456-49463, 59137-59144, 90312-90319, 90324-90331, 91326-91333, 93523-93530
	GT	8	1	129126-129133
	TA	8	8	48619-48626, 49231-49238, 53786-53793, 85702-85709, 85726-85733, 96894-96901, 98438-98445, 151266-151273
		10	2	9410-9419, 60232-60241
		12	2	34982-34993, 71868-71879
	TC	8	7	32320-32327, 53719-53726, 87294-87301, 154637-154644, 156834-156841, 157836-157843, 157848-157855
		10	1	64979-64988
		12	1	128834-128845
	AAC	9	3	15967-15975, 72122-72130, 104978-104986
	AAG	9	3	4837-4845, 104168-104176, 153835-153843
	AAT	9	1	67209-67217
		12	1	128890-128901
ACC	9	1	153571-153579	
AGA	9	4	72384-72392, 99471-99479, 100930-100938, 153947-153955	
AGC	9	1	126764-126772	
AGT	9	1	72774-72782	
ATA	9	2	82848-82856, 123517-123525	
	12	1	57905-57916	
ATG	9	1	41299-41307	
ATT	9	1	117681-117689	
CAA	9	2	113910-113918, 117800-117808	
CAG	9	2	66430-66438, 139293-139301	
CAC	9	1	154566-154574	
CCT	9	1	70311-70319	

Taxa	Unit	Lengths	No. SSRs	Coordinated Basepairs
	CTC	9	1	34188-34196
	CTG	9	2	73993-74001, 108866-108874
	CTT	9	3	88241-88249, 94324-94332, 143991-143999
	GAA	9	3	54682-54690, 96051-96059, 159917-159925
	GCA	9	1	43197-43205
	GCT	9	1	60031-60039
	GGA	9	1	83954-83962
	GGT	9	1	94588-94596
	GTA	9	1	35001-35009
	GTG	9	1	93593-93601
	GTT	9	2	54210-54218, 143181-143189
	TAA	9	2	38890-38898, 116431-116439
	TAT	9	3	39800-39808, 49134-49142, 119499-119507
	TCA	9	3	64482-64490, 152789-152797, 153329-153337
	TCC	9	2	24256-24264, 122179-122187
	TCT	9	2	94212-94220, 148688-148696
	TGA	9	2	94830-94838, 95370-95378
	TGC	9	1	87575-87583
	TGT	9	1	17864-17872
	TTA	9	5	14199-14207, 16776-16784, 88031-88039, 115505-115513, 131219-131227
	TTC	9	4	23338-23346, 68923-68931, 147228-147236, 152108-152116
		12	1	71563-71574
	TTG	9	2	42904-42912, 134249-134257
	AAAT	12	1	117074-117085
		16	1	4395-4410
	AATC	12	1	126458-126469
	AATG	12	1	65589-65600
	AATT	12	1	115101-115112
	ACAT	12	1	87706-87717
	ATAA	12	1	73139-73150
	CATT	12	1	130997-131008t
	TAAA	12	1	46902-46913
	AAAGA	18	1	10959-10973
	ACTAAT	18	1	144502-144519
	TATAAC	18	1	49048-49065
	TATTAG	18	1	103647-103664
<i>M. denudata</i>	A	8	17	4761-4768, 5405-5412, 13417-13424, 19871-19878, 23532-23539, 31813-31820, 33380-33387, 50251-50258, 58383-58390, 72362-72369, 92225-92232, 114739-114746, 118597-118604, 121168-121175, 135688-135695, 141111-141118, 143988-143995
		9	7	9740-9748, 29232-29240, 38872-38880, 93499-93507, 117919-117927, 130339-130347, 132561-132569,
		10	6	1730-1739, 3949-3958, 30569-30578, 46912-46921, 112112-112121, 126143-126152
		11	2	47170-47180, 69853-69863
		12	3	12748-12759, 49382-49393, 64765-64776
		13	2	13836-13848, 29193-29205
		14	1	211-224
	C	8	1	48469-48476
		9	2	29223-29231, 118588-118596
		10	1	7172-7181
		11	1	71249-71259
	G	8	1	37017-37024
	T	8	25	2051-2058, 10674-10681, 14130-14137, 15500-15507, 27398-27405, 34434-34441, 34808-34815, 38766-38773, 48477-48484, 49181-49188, 54970-54977, 55898-55905, 62550-62557, 62964-62971, 63565-63572, 64178-64185, 87305-87312, 88102-88109, 107089-107096, 112512-112519, 119666-119673, 128840-128847, 129598-129605, 131334-131341, 132055-132062, 155975-155982
		9	17	13447-13455, 15803-15811, 34860-34868, 45381-45389, 47346-47354, 51209-51217, 72390-72398, 77937-77945, 84487-84495, 86433-86441, 87075-87083, 118607-118615, 122846-122854, 130350-130358, 131646-131654, 131943-131951, 154700-154708
		10	9	17529-17538, 33488-33497, 53513-53522, 55681-55690, 55841-55850, 71260-71269, 84983-84992, 119679-119688, 136086-136095
		11	6	10352-10362, 30805-30815, 31358-31368, 64376-64386, 70287-70297, 74511-74521
		12	4	4592-4603, 10246-10257, 19727-19738, 75499-75510
		13	3	1768-1780, 5383-5395, 7756-7768
		16	1	124207-124222
	AG	8	2	99173-99180, 137785-137792
	AT	8	4	21011-21018, 87380-87387, 118740-118747, 149764-149771
		10	2	21101-21110, 33331-33340
	CT	8	5	3762-3769, 87313-87320, 110415-110422, 128028-128035, 149027-149034
	GA	8	8	9260-9267, 38103-38110, 49449-49456, 59128-59135, 90309-90316, 90321-90328, 91323-91330, 93520-93527
		8	1	129120-129127
	GT	8	1	129120-129127
	TA	8	8	48615-48622, 49221-49228, 53774-53781, 85698-85705, 85722-85729, 96891-96898, 98435-98442, 151309-151316

Taxa	Unit	Lengths	No. SSRs	Coordinated Basepairs	
<i>M. liliiflora</i>		10	2	9427-9436, 60223-60232	
		12	1	71862-71873	
		14	1	34982-34995	
		TC	8	7	32321-32328, 53707-53714, 87291-87298, 154680-154687, 156877-156884, 157879-157886, 157891-157898
			10	2	64969-64978, 128830-128839
		AAC	9	3	15978-15986, 72116-72124, 104968-104976
		AAG	9	3	4847-4855, 104158-104166, 153878-153886
		AAT	9	1	67199-67207
			12	1	128884-128895
		ACC	9	1	153614-153622
		AGA	9	4	72377-72385, 99468-99476, 100927-100935, 153990-153998
		AGC	9	1	126760-126768
		AGT	9	1	72765-72773
		ATA	9	2	82844-82852, 123512-123520
			12	1	57897-57908
		ATG	9	1	41296-41304
		ATT	9	1	117673-117681
		CAA	9	2	113902-113910, 117792-117800
		CAC	9	1	154609-154617
		CAG	9	2	66420-66428, 139343-139351
		CCT	9	1	70304-70312
		CTC	9	1	34189-34197
		CTG	9	2	73984-73992, 108856-108864
		CTT	9	3	88238-88246, 94321-94329, 144041-144049
		GAA	9	3	54675-54683, 96048-96056, 159960-159968
		GCA	9	1	43194-43202
		GCT	9	1	60022-60030
		GGA	9	1	83949-83957
		GGT	9	1	94585-94593
		GTA	9	1	35003-35011
		GTG	9	1	93590-93598
		GTT	9	2	54203-54211, 143231-143239
		TAA	9	2	38890-38898, 116422-116430
		TAT	9	3	39798-39806, 49124-49132, 119493-119501
		TCA	9	3	64474-64482, 152832-152840, 153372-153380
		TCC	9	2	24263-24271, 122174-122182
		TCT	9	2	94209-94217, 148731-148739
		TGA	9	2	94827-94835, 95367-95375
		TGC	9	1	87572-87580
		TGT	9	1	17871-17879
		TTA	9	4	14212-14220, 16787-16795, 88028-88036, 115496-11550
		TTT	9	3	23345-23353, 68912-68920, 147271-147279, 152151-152159
			12	1	71557-71568
		TTG	9	3	42901-42909, 118479-118487, 134297-134305
		AAAT	12	1	117065-117076
			16	1	4404-4419
		AATC	12	1	126454-126465
		AATG	12	1	65579-65590
		AATT	12	1	115092-115103
		ATAA	12	1	73130-73141
		CATT	12	1	130991-131002
		TAAA	12	1	46900-46911
		A	8	14	3946-3953, 4757-4764, 13404-13411, 19859-19866, 23520-23527, 29213-29220, 31786-31793, 33361-33368, 50258-50265, 58381-58388, 92234-92241, 120241-120248, 134721-134728, 140145-140152
			9	9	1728-1736, 5405-5413, 9732-9740, 38853-38861, 93508-93516, 113810-113818, 116990-116998, 117669-117677, 129408-129416
			10	4	29176-29185, 30545-30554, 125212-125221, 131630-131639
			11	5	46913-46923, 49390-49400, 64763-64773, 72360-72370, 111182-111192
			12	2	211-222, 47172-47183
			13	3	12734-12746, 13823-13835, 69850-69862
		C	8	2	5934-5941, 7174-7181
			10	2	29203-29212, 117659-117668
			12	1	71246-71257
		G	8	1	36998-37005
		T	8	26	2048-2055, 10341-10348, 10660-10667, 14117-14124, 15487-15494, 27386-27393, 34415-34422, 38747-38754, 45383-45390, 49189-49196, 54970-54977, 55896-55903, 62960-62967, 63563-63570, 64176-64183, 86443-86450, 87314-87321, 88111-88118, 106159-106166, 111583-111590, 118739-118746, 127909-127916, 128667-128674, 130403-130410, 131124-131131, 154070-154077
			9	21	10238-10246, 13434-13442, 30781-30789, 34843-34851, 47349-47357, 48484-48492, 51208-51216, 55681-55689, 55840-55848, 62548-62556, 70286-70294, 72391-72399, 77947-77955, 84497-84505, 87084-87092, 117680-117688, 121920-121928, 129419-129427, 130715-130723, 131012-131020, 152795-152803
			10	9	15790-15799, 17517-17526, 31332-31341, 33469-33478, 34789-34798, 53518-53527, 71258-71267, 84993-85002, 118752-118761

Taxa	Unit	Lengths	No. SSRs	Coordinated Basepairs
		11	4	5385-5395, 64374-64384, 123281-123291, 135119-135129
		12	3	7749-7760, 19715-19726, 75504-75515
		13	2	1765-1777, 4587-4599
		15	1	74512-74526
	AG	8	2	99182-99189, 136819-136826
	AT	8	4	20999-21006, 87389-87396, 117813-117820, 147859-147866
		10	2	21089-21098, 33312-33321
	CT	8	5	3759-3766, 87322-87329, 109485-109492, 127097-127104, 147122-147129
	GA	8	8	9252-9259, 38084-38091, 49456-49463, 59126-59133, 90318-90325, 90330-90337, 91332-91339, 93529-93536
	GT	8	1	128189-128196
	TA	8	8	48623-48630, 49229-49236, 53779-53786, 85708-85715, 85732-85739, 96900-96907, 98444-98451, 149404-149411
		10	2	9419-9428, 60221-60230
		12	2	34965-34976, 71860-71871
	TC	8	7	32302-32309, 53712-53719, 87300-87307, 152775-152782, 154972-154979, 155974-155981, 155986-155993
		10	2	64966-64975, 127899-127908
	AAC	9	2	15966-15974, 72114-72122
	AAG	9	1	4849-4857, 151973-151981
	AAT	9	1	67196-67204
		12	1	127953-127964
	ACC	9	1	151709-151717
	AGA	9	3	72378-72386, 99477-99485, 100936-100944, 152085-152093
	AGC	9	1	125829-125837
	AGT	9	1	72766-72774
	ATA	9	2	82854-82862, 122586-122594
		12	1	57895-57906
	ATG	9	1	41277-41285
	ATT	9	1	116744-116752
	CAA	9	2	112973-112981, 116863-116871
	CAC	9	1	152704-152712
	CAG	9	1	66417-66425, 138377-138385
	CCT	9	1	70301-70309
	CTC	9	1	34170-34178
	CTG	9	2	73985-73993, 107926-107934
	CTT	9	2	88247-88255, 94330-94338
	GAA	9	2	54675-54683, 96057-96065, 158055-158063
	GCA	9	1	43175-43183
	GCT	9	1	60020-60028
	GGA	9	1	83959-83967
	GGT	9	1	94594-94602
	GTA	9	1	34984-34992
	GTG	9	1	93599-93607
	GTT	9	1	54203-54211
	TAA	9	2	38871-38879, 115494-115502
	TAT	9	3	39779-39787, 49132-49140, 118566-118574
	TCA	9	3	64472-64480, 150927-150935, 151467-151475
	TCC	9	2	24251-24259, 121247-121255
	TCT	9	1	94218-94226, 146826-146834
	TGA	9	2	94836-94844, 95376-95384
	TGC	9	1	87581-87589
	TGT	9	1	17859-17867
	TTA	9	4	14199-14207, 16775-16783, 88037-88045, 114568-114576
	TTC	9	4	23333-23341, 68909-68917, 145366-145374, 150246-150254
		12	1	71555-71566
	TTG	9	3	42882-42890, 117550-117558, 133330-133338
	AAAT	12	1	116137-116148
		16	1	4399-4414
	AATC	12	1	125523-125534
	AATG	12	1	65576-65587
	AATT	12	1	114164-114175
	ACAT	12	1	87712-87723
	ATAA	12	1	73131-73142
	CATT	12	1	130060-130071
	TAAA	12	1	46901-46912
	ACTAAT	18	1	142640-142657
	TATTAG	18	1	103653-103670
<i>M. acuminata</i>	A	8	21	3941-3948, 13412-13419, 19575-19582, 23236-23243, 31516-31523, 34669-34676, 39622-39629, 49100-49107, 49965-49972, 58097-58104, 63034-63041, 63879-63886, 67079-67086, 72082-72089, 81522-81529, 91939-91946, 114462-114469, 120903-120910, 135412-135419, 140834-140841, 143711-143718
		9	16	211-219, 4752-4760, 5394-5402, 13831-13839, 17247-17255, 28908-28916, 30273-30281, 32474-32482, 33089-33097, 33767-33775, 46625-46633, 69579-69587, 93213-93221, 111833-111841, 117641-117649, 130063-130071
		10	7	1725-1734, 9735-9744, 38582-38591, 46882-46891, 118325-118334, 125865-125874, 132279-132288
		11	1	67089-67099

Taxa	Unit	Lengths	No. SSRs	Coordinated Basepairs
		12	1	64484-64495
		14	1	12741-12754
	C	8	1	48180-48187
		9	1	7178-7186
		10	1	70970-70979
		17	1	118308-118324
	G	8	1	36727-36734
	T	8	21	2043-2050, 15382-15389, 27102-27109, 33276-33283, 34145-34152, 38476-38483, 63279-63286, 67191-67198, 69436-69443, 70011-70018, 86148-86155, 87019-87026, 87816-87823, 106810-106817, 112232-112239, 119396-119403, 122581-122588, 128564-128571, 129322-129329, 131058-131065, 155705-155712, 4979-4987, 10346-10354, 10666-10674, 13442-13450, 17238-17246, 30509-30517, 34570-34578, 47057-47065, 48188-48196, 48898-48906, 50923-50931, 53233-53241, 54684-54692, 55396-55404, 55555-55563, 55611-55619, 62264-62272, 63896-63904, 75218-75226, 86789-86797, 118337-118345, 119409-119417, 130074-130082, 131364-131372, 131661-131669, 135810-135818, 154430-154438
		9	27	
		10	9	1763-1772, 5375-5384, 7754-7763, 10242-10251, 14121-14130, 15511-15520, 45093-45102, 70980-70989, 84201-84210
		11	4	64095-64105, 74234-74244, 77653-77663, 84698-84708
		12	5	4583-4594, 19431-19442, 31060-31071, 72110-72121, 123942-123953, 15511-15520
	AG	8	2	98887-98894, 137508-137515
	AT	8	4	20715-20722, 87094-87101, 118470-118477, 149494-149501
		10	2	20805-20814, 33040-33049
	CT	8	4	3754-3761, 110136-110143, 127752-127759, 148757-148764
		12	1	87027-87038
	GA	8	8	9255-9262, 37813-37820, 49163-49170, 58842-58849, 90023-90030, 90035-90042, 91037-91044, 93234-93241
	GT	8	1	128844-128851
	TA	8	10	9422-9429, 28955-28962, 48327-48334, 48939-48946, 53493-53500, 85414-85421, 85438-85445, 96605-96612, 98149-98156, 151039-151046
		10	1	59937-59946
		12	2	34693-34704, 71582-71593
	TC	8	3	32026-32033, 53426-53433, 87005-87012, 154410-154417, 156607-156614, 157609-157616, 157621-157628
		10	2	64688-64697, 128554-128563
	AAC	9	4	15687-15695, 71836-71844, 104689-104697, 118212-118220
	AAG	9	3	4839-4847, 103879-103887, 153608-153616
	AAT	9	1	66918-66926
		12	1	128608-128619
	ACC	9	1	153344-153352
	AGA	9	4	72097-72105, 99182-99190, 100641-100649, 153720-153728
	AGC	9	1	126484-126492
	AGT	9	1	72488-72496
	ATA	9	2	82563-82571, 123247-123255
		12	1	57611-57622
	ATG	9	1	41008-41016
	ATT	9	1	117395-117403
	CAA	9	1	113622-113630
	CAC	9	1	154339-154347
	CAG	9	1	66139-66147, 139066-139074
	CCT	9	1	70025-70033
	CTC	9	1	33900-33908
	CTG	9	2	73707-73715, 108577-108585
	CTT	9	3	87952-87960, 94035-94043, 143764-143772
	GAA	9	3	54389-54397, 95762-95770, 159690-159698
	GCA	9	1	42906-42914
	GCT	9	1	59736-59744
	GGA	9	1	83663-83671
	GGT	9	1	94299-94307
	GTA	9	1	34712-34720
	GTG	9	1	93304-93312
	GTT	9	2	53917-53925, 142954-142962
	TAA	9	2	38601-38609, 116145-116153
	TAT	9	3	39509-39517, 48836-48844, 119223-119231
	TCA	9	3	64193-64201, 152562-152570, 153102-153110
	TCC	9	2	23967-23975, 121909-121917
	TCT	9	2	94035-94043, 148461-148469
	TGA	9	2	94541-94549, 95081-95089
	TGC	9	1	87286-87294
	TGT	9	1	17581-17589
	TTA	9	6	14205-14213, 16496-16504, 48863-48871, 87742-87750, 115219-115227, 130937-130945
	TTC	9	4	23049-23057, 68637-68645, 147001-147009, 151881-151889
		12	1	71277-71288
	TTG	9	2	42613-42621, 134021-134029
	AAAT	12	1	116788-116799

Taxa	Unit	Lengths	No. SSRs	Coodinated Basepairs
<i>M. sinica</i>		16	1	4395-4410
	AATC	12	1	126178-126189
	AATG	12	1	65298-65309
	AATT	12	1	114815-114826
	ACAT	12	1	87417-87428
	ATAA	12	1	72853-72864
	TAAA	12	1	46613-46624
	ACTAAT	18	1	144275-144292
	TATTAG	18	1	103358-103375
	A	8	17	216-223, 13468-13475, 19923-19930, 23584-23591, 31857-31864, 33430-33437, 34107-34114, 35037-35044, 39990-39997, 46970-46977, 67412-67419, 67422-67429, 72411-72418, 92252-92259, 114759-114766, 121189-121196, 143948-143955
		9	14	1729-1737, 3941-3949, 4745-4753, 9780-9788, 32815-32823, 34132-34140, 49436-49444, 50303-50311, 58439-58447, 69909-69917, 93526-93534, 117939-117947, 130361-130369, 135647-135655
		10	8	13887-13896, 15864-15873, 29249-29258, 30619-30628, 38949-38958, 47226-47235, 126162-126171, 132583-132592
		11	2	64818-64828, 112145-112155
		14	1	5388-5401
		20	1	12791-12810
	C	8	2	29276-29283, 118608-118615
		10	1	71297-71306
		11	1	7192-7202
	G	8	1	37093-37100
		9	1	4815-4823
	T	8	27	2043-2050, 4974-4981, 10717-10724, 15549-15556, 27450-27457, 30855-30862, 33616-33623, 34511-34518, 45440-45447, 48531-48538, 49235-49242, 55026-55033, 63019-63026, 63620-63627, 64233-64240, 67521-67528, 69766-69773, 86472-86479, 87343-87350, 88134-88141, 117987-117994, 118624-118631, 119682-119689, 128861-128868, 129626-129633, 131356-131363, 155942-155949
		9	25	10390-10398, 13498-13506, 14178-14186, 33538-33546, 34885-34893, 34938-34946, 38842-38850, 47401-47409, 51151-51159, 53575-53583, 55737-55745, 55953-55961, 62606-62614, 64431-64439, 74562-74570, 77980-77988, 84525-84533, 87113-87121, 112546-112554, 119695-119703, 122867-122875, 130372-130380, 131668-131676, 131965-131973, 154667-154675
		10	6	4578-4587, 5369-5378, 10286-10295, 55896-55905, 70341-70350, 75544-75553
		11	6	15852-15862, 17583-17593, 51263-51273, 72439-72449, 85021-85031, 136046-136056
		12	3	7770-7781, 19779-19790, 71307-71318
		13	2	31400-31412, 124229-124241
	AG	8	2	99200-99207, 137746-137753
	AT	8	4	21063-21070, 51003-51010, 118756-118763, 149731-149738
		10	2	21153-21162, 33381-33390
	CT	8	4	3754-3761, 110448-110455, 128047-128054, 148994-149001
		14	1	87351-87364
	GA	8	8	9273-9280, 38179-38186, 49500-49507, 59184-59191, 90336-90343, 90348-90355, 91350-91357, 93547-93554
	GT	8	1	129148-129155
	TA	8	8	48669-48676, 49275-49282, 53835-53842, 85737-85744, 85761-85768, 96918-96925, 98462-98469, 151276-151283
		10	3	9467-9476, 35061-35070, 60279-60288
		12	1	71911-71922
	TC	8	7	32367-32374, 53768-53775, 87329-87336, 154647-154654, 156844-156851, 157846-157853, 157858-157865
		10	1	65021-65030
		12	1	128849-128860
	AAC	9	4	16032-16040, 72165-72173, 105002-105010, 118512-118520
	AAG	9	3	4832-4840, 104192-104200, 153845-153853
	AAT	9	1	67251-67259
		12	1	128905-128916
	ACC	9	1	153581-153589
	AGA	9	4	72426-72434, 99495-99503, 100954-100962, 153957-153965
	AGC	9	1	126779-126787
	AGT	9	1	72816-72824
	ATA	9	2	82887-82895, 123534-123542
		12	1	57953-57964
	ATG	9	1	41376-41384
	ATT	9	1	117693-117701
	CAA	9	2	113937-113945, 117812-117820
CAC	9	1	154576-154584	
CAG	9	2	66472-66480, 139304-139312	
CCT	9	1	70357-70365	
CTC	9	1	34265-34273	
CTG	9	2	74035-74043, 108889-108897	
CTT	9	3	88265-88273, 94348-94356, 144001-144009	
GAA	9	2	54731-54739, 96075-96083, 159927-159935	

Taxa	Unit	Lengths	No. SSRs	Coordinated Basepairs
	GCA	9	1	43274-43282
	GCT	9	1	60078-60086
	GGA	9	1	83987-83995
	GGT	9	1	94612-94620
	GTA	9	1	35078-35086
	GTG	9	1	93617-93625
	GTT	9	2	54259-54267, 143191-143199
	TAA	9	2	38968-38976, 116442-116450
	TAT	9	3	39877-39885, 49178-49186, 119509-119517
	TCA	9	3	64527-64535, 152799-152807, 153339-153347
	TCC	9	2	24315-24323, 122195-122203
	TCT	9	2	94236-94244, 148698-148706
	TGA	9	2	94854-94862, 95394-95402
	TGC	9	1	87604-87612
	TTA	9	6	14261-14269, 16841-16849, 49200-49208, 88060-88068, 115516-115524, 131235-131243
	TTC	9	4	23397-23405, 68967-68975, 147238-147246, 152118-152126
		12	1	71606-71617
	TTG	9	2	42981-42989, 134256-134264
	AAAT	12	2	4394-4405, 117085-117096
	AATC	12	1	126473-126484
	AATG	12	1	65631-65642
	AATT	12	1	115112-115123
	ACAT	12	1	87735-87746
	ATAA	12	1	73181-73192
	CATT	12	1	131013-131024
	TAAA	12	1	46958-46969
	TATTAG	18	1	103671-103688
<i>M. fraseri</i> var. <i>pyramidata</i>	A	8	20	206-213, 4382-4389, 4743-4750, 13413-13420, 19887-19894, 23548-23555, 31811-31818, 32764-32771, 39203-39210, 39941-39948, 50295-50302, 63242-63249, 67287-67294, 69789-69796, 72294-72301, 92144-92151, 114672-114679, 121106-121113, 135611-135618, 141041-141048, 1719-1727, 3932-3940, 15830-15838, 29202-29210, 33378-33386, 46923-46931, 49429-49437, 93418-93426, 117854-117862, 126076-126084, 130280-130288, 143923-143931
		9	12	
		10	6	30567-30576, 34056-34065, 38900-38909, 58314-58323, 75879-75888, 132502-132511
		11	2	5390-5400, 112050-112060
		12	4	13851-13862, 47180-47191, 64684-64695, 67297-67308
		15	1	12741-12755
	C	8	3	5921-5928, 48480-48487, 71182-71189
		9	1	7177-7185
	G	8	1	37045-37052
		13	1	4812-4824
		14	1	58324-58337
	T	8	24	1756-1763, 2034-2041, 10667-10674, 15515-15522, 27410-27417, 34419-34426, 34836-34843, 48488-48495, 49228-49235, 54902-54909, 62490-62497, 63487-63494, 64099-64106, 67400-67407, 69646-69653, 88021-88028, 104139-104146, 107021-107028, 112451-112458, 118539-118546, 128774-128781, 129539-129546, 131275-131282, 155918-155925
		9	26	4975-4983, 7759-7767, 13443-13451, 14144-14152, 30803-30811, 38793-38801, 45392-45400, 47357-47365, 51253-51261, 53558-53566, 55613-55621, 55772-55780, 55828-55836, 64298-64306, 77868-77876, 86360-86368, 87002-87010, 117902-117910, 119598-119606, 119612-119620, 122784-122792, 124147-124155, 130291-130299, 131587-131595, 131884-131892, 154643-154651
		10	10	5371-5380, 10238-10247, 17548-17557, 33487-33496, 34888-34897, 66873-66882, 71190-71199, 72324-72333, 84413-84422, 84910-84919
		11	6	5371-5380, 4575-4585, 15818-15828, 70220-70230, 74446-74456, 75430-75440
		12	1	19743-19754
		13	1	31354-31366
		14	1	10342-10355
	AG	8	2	99092-99099, 137709-137716
	AT	8	5	8157-8164, 21027-21034, 87299-87306, 118671-118678, 149707-149714
		10	2	21117-21126, 33329-33338
	CT	8	4	3745-3752, 110353-110360, 127960-127967, 148970-148977
	GA	8	8	9260-9267, 38131-38138, 49493-49500, 59068-59075, 90228-90235, 90240-90247, 91242-91249, 93439-93446
	GT	8	1	129061-129068
	TA	8	8	48626-48633, 49268-49275, 53818-53825, 85625-85632, 85649-85656, 96810-96817, 98354-98361, 151252-151259
		10	2	9427-9436, 60163-60172,
		12	2	35011-35022, 71794-71805
	TC	8	6	32328-32335, 53751-53758, 154623-154630, 156820-156827, 157822-157829, 157834-157841
		10	2	64888-64897, 87213-87224
		12	1	128762-128773
	AAC	9	4	15997-16005, 72048-72056, 104900-104908, 118428-118436
	AAG	9	3	4833-4841, 104084-104092, 153821-153829

Taxa	Unit	Lengths	No. SSRs	Coordinated Basepairs
	AAT	9	1	67121-67129
		12	1	128818-128829
	ACC	9	1	153557-153565
	AGA	9	4	72309-72317, 99387-99395, 100846-100854, 153933-153941
	AGC	9	1	126692-126700
	ATA	9	2	82775-82783, 123452-123460
		12	1	57828-57839
	ATG	9	1	41327-41335
	ATT	9	1	117608-117616
	CAA	9	2	113841-113849, 117727-117735
	CAC	9	1	154552-154560
	CAG	9	2	66339-66347, 139273-139281
	CCT	9	1	70237-70245
	CTC	9	1	34174-34182
	CTG	9	2	73919-73927, 108788-108796
	CTT	9	3	88157-88165, 94240-94248, 143977-143985
	GAA	9	3	54607-54615, 95967-95975, 159903-159911
	GCA	9	1	43225-43233
	GCT	9	1	59962-59970
	GGA	9	1	83875-83883
	GGT	9	1	94504-94512
	GTA	9	1	35030-35038
	GTG	9	1	93509-93517
	GTT	9	2	54234-54242, 143161-143169
	TAA	9	2	38919-38927, 116355-116363
	TAT	9	3	39828-39836, 49171-49179, 119425-119433
	TCA	9	3	64394-64402, 152775-152783, 153315-153323
	TCC	9	2	24275-24283, 122112-122120
	TCT	9	2	94128-94136, 148674-148682
	TGA	9	2	94746-94754, 95286-95294
	TGC	9	1	87491-87499
	TGT	9	1	17887-17895
	TTA	9	5	14227-14235, 16806-16814, 49193-49201, 87947-87955, 115429-115437
	TTC	9	4	23361-23369, 68846-68854, 147214-147222, 152094-152102
		12	1	71489-71500
	TTG	9	2	42932-42940, 134220-134228
	AAAT	12	1	116998-117009
	AATC	12	1	126386-126397
	AATG	12	1	65498-65509
	AATT	12	1	115025-115036
	ACAT	12	1	87622-87633
	ATAA	12	1	73065-73076
	CATT	12	1	130932-130943
	TAAA	12	1	46911-46922
		16	1	4390-4405
	ACTAAT	18	1	144488-144505
	TATTAG	18	1	103563-103580
<i>M. dealbata</i>	A	8	20	13421-13428, 19877-19884, 31806-31813, 32770-32777, 34061-34068, 34988-34995, 39943-39950, 49401-49408, 50266-50273, 58404-58411, 63346-63353, 67406-67413, 72334-72341, 92188-92195, 114707-114714, 117884-117891, 121134-121141, 135598-135605, 141025-141032, 143902-143909, 1726-1734, 5395-5403, 9744-9752, 13840-13848, 30562-30570, 33384-33392, 34086-34094, 67383-67391, 69905-69913, 93462-93470, 118561-118569, 126101-126109, 130303-130311
		9	13	211-220, 3942-3951, 4750-4759, 29197-29206, 67416-67425, 132525-132534
		10	6	12753-12763, 15816-15826
		11	2	46927-46938, 47188-47199, 112084-112095
		12	3	4394-4406, 64787-64799
		13	2	7189-7196, 48488-48495
	C	8	2	118552-118560
		9	1	71300-71313
		14	1	37046-37053
	G	8	1	2044-2051, 10679-10686, 15501-15508, 27404-27411, 34463-34470, 34837-34844, 48496-48503, 49200-49207, 54990-54997, 62578-62585, 63591-63598, 64204-64211, 64402-64409, 67517-67524, 69762-69769, 77904-77911, 87269-87276, 88066-88073, 107059-107066, 112486-112493, 117931-117938, 118572-118579, 119623-119630, 128797-128804, 129562-129569, 131298-131305, 155896-155903
	T	8	27	4584-4592, 7770-7778, 13451-13459, 14130-14138, 30797-30805, 34889-34897, 38795-38803, 47365-47373, 51224-51232, 55861-55869, 71314-71322, 72364-72372, 74485-74493, 87039-87047, 130314-130322, 131610-131618, 131907-131915, 154621-154629
		9	18	5376-5385, 10250-10259, 33493-33502, 55701-55710, 55917-55926, 75468-75477, 84448-84457, 84945-84954, 119636-119645, 122812-122821, 124175-124184
		10	11	1763-1773, 15804-15814, 17536-17546, 45393-45403, 53536-53546, 86395-86405
		11	6	19733-19744, 70337-70348, 135996-136007
		12	3	10354-10367, 31348-31361
		14	2	99136-99143, 137697-137704
	AG	8	2	

Taxa	Unit	Lengths	No. SSRs	Coordinated Basepairs
	AT	8	4	21017-21024, 87344-87351, 118704-118711, 149685-149692
		10	2	21107-21116, 33335-33344
	CT	8	4	3755-3762, 110387-110394, 127985-127992, 148948-148955
		14	1	87277-87290
	GA	8	7	9264-9271, 38132-38139, 59156-59163, 90272-90279, 90284-90291, 91286-91293, 93483-93490
		14	1	49458-49471
	GT	8	1	129084-129091
	TA	8	8	48634-48641, 49240-49247, 53798-53805, 85660-85667, 85684-85691, 96854-96861, 98398-98405, 151230-151237
		10	2	9431-9440, 60251-60260
		12	2	35012-35023, 71906-71917
	TC	8	7	32322-32329, 53731-53738, 87255-87262, 154601-154608, 156798-156805, 157800-157807, 157812-157819
		10	2	64992-65001, 128787-128796
	AAC	9	4	15985-15993, 72088-72096, 104938-104946, 118456-118464
	AAG	9	3	4838-4846, 104128-104136, 153799-153807
	AAT	9	1	67222-67230
		12	1	128841-128852
	ACC	9	1	153535-153543
	AGA	9	4	72349-72357, 99431-99439, 100890-100898, 153911-153919
	AGC	9	1	126717-126725
	AGT	9	1	72739-72747
	ATA	9	2	82810-82818, 123480-123488
		12	1	57918-57929
	ATG	9	1	41329-41337
	ATT	9	1	117638-117646
	CAA	9	2	113876-113884, 117757-117765
	CAC	9	1	154530-154538
	CAG	9	2	66443-66451, 139255-139263
	CCT	9	1	70355-70363
	CTC	9	1	34219-34227
	CTG	9	2	73958-73966, 108828-108836
	CTT	9	3	88201-88209, 94284-94292, 143955-143963
	GAA	9	3	54695-54703, 96011-96019, 159881-159889
	GCA	9	1	43227-43235
	GCT	9	1	60050-60058
	GGA	9	1	83910-83918
	GGT	9	1	94548-94556
	GTA	9	1	35031-35039
	GTG	9	1	93553-93561
	GTT	9	2	54223-54231, 143145-143153
	TAA	9	2	38922-38930, 116390-116398
	TAT	9	3	39830-39838, 49143-49151, 119450-119458
	TCA	9	3	64497-64505, 152753-152761, 153293-153301
	TCC	9	2	24269-24277, 122140-122148
	TCT	9	2	94172-94180, 148652-148660
	TGA	9	2	94790-94798, 95330-95338
	TGC	9	1	87536-87544
	TGT	9	1	17877-17885
	TTA	9	5	14213-14221, 16794-16802, 49165-49173, 87992-88000, 115464-115472
	TTC	9	4	23351-23359, 68963-68971, 147192-147200, 152072-152080
	TTG	9	2	42934-42942, 134207-134215
	AAAT	12	1	117033-117044
	AATC	12	1	126411-126422
	AATG	12	1	65602-65613
	AATT	12	1	115060-115071
	ACAT	12	1	87667-87678
	ATAA	12	1	73104-73115
	CATT	12	1	130955-130966
	TAAA	12	1	46915-46926
	ACTAAT	18	1	144466-144483
	TATTAG	18	1	103607-103624

## **APPENDIX II**

Sequence characteristics for genes and intergenic spacers (+introns)  
in Magnoliaceae

Gene	Size (bp)	Magnoliaceae			Magnolioideae			Yulania		
		$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)	$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)	$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)
<i>trnH-GUG</i>	75	0.00581	2(2.67)	1(1.33)	0.00040	1(1.33)	1(1.33)	0.00000	0(0)	0(0)
<i>psbA</i>	1062	0.00252	14(1.32)	3(0.28)	0.00150	7(0.66)	2(0.19)	0.00138	3(0.28)	1(0.09)
<i>trnK-UUU</i>	72	0.00214	1(1.39)	0(0)	0.00231	1(1.39)	0(0)	0.00000	0(0)	0(0)
<i>matK</i>	1524	0.00804	63(4.13)	11(0.72)	0.00633	45(2.95)	11(0.72)	0.00363	16(1.05)	1(0.07)
<i>rps16</i>	296	0.00348	5(1.69)	2(0.68)	0.00222	4(1.35)	1(0.34)	0.00232	2(0.68)	0(0)
<i>trnQ-UUG</i>	73	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>psbK</i>	186	0.00317	3(1.61)	1(0.54)	0.00253	2(1.08)	1(0.54)	0.00466	2(1.08)	1(0.54)
<i>psbI</i>	157	0.00099	1(0.64)	0(0)	0.00107	1(0.64)	0(0)	0.00000	0(0)	0(0)
<i>trnS-GCU</i>	88	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>trnG-UCC</i>	72	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>trnR-UCU</i>	72	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>atpA</i>	1524	0.00449	31(2.03)	8(0.52)	0.00316	17(1.12)	7(0.46)	0.00144	6(0.39)	1(0.07)
<i>atpF</i>	555	0.00591	19(3.42)	1(0.18)	0.00358	10(1.8)	1(0.18)	0.00120	2(0.36)	0(0)
<i>atpH</i>	246	0.00125	2(0.81)	0(0)	0.00136	2(0.81)	0(0)	0.00000	0(0)	0(0)
<i>atpI</i>	745	0.00226	10(1.34)	0(0)	0.00132	5(0.67)	0(0)	0.00045	1(0.13)	0(0)
<i>rps2</i>	711	0.00397	16(2.25)	1(0.14)	0.00239	8(1.13)	1(0.14)	0.00188	4(0.56)	0(0)
<i>rpoC2</i>	4149	0.00578	118(2.84)	26(0.63)	0.00427	74(1.78)	24(0.58)	0.00216	23(0.55)	6(0.14)
<i>rpoC1</i>	2047	0.00490	41(2)	15(0.73)	0.00419	31(1.51)	14(0.68)	0.00300	16(0.78)	4(0.2)
<i>rpoB</i>	3239	0.00382	63(1.95)	11(0.34)	0.00321	47(1.45)	11(0.34)	0.00250	21(0.65)	5(0.15)
<i>trnC-GCA</i>	81	0.00190	1(1.23)	0(0)	0.00206	1(1.23)	0(0)	0.00000	0(0)	0(0)
<i>petN</i>	90	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>psbM</i>	105	0.00147	1(0.95)	0(0)	0.00159	1(0.95)	0(0)	0.00317	1(0.95)	0(0)
<i>trnD-GUC</i>	74	0.00381	1(1.35)	1(1.35)	0.00410	1(1.35)	1(1.35)	0.00000	0(0)	0(0)
<i>trnY-GUA</i>	84	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>trnE-UUC</i>	73	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>trnT-GGU</i>	73	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>psbD</i>	1062	0.00208	12(1.13)	1(0.09)	0.00160	8(0.75)	1(0.09)	0.00157	5(0.47)	0(0)
<i>psbC</i>	1422	0.00274	22(1.55)	2(0.14)	0.00189	13(0.91)	2(0.14)	0.00155	6(0.42)	1(0.07)
<i>trnS-UGA</i>	93	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>psbZ</i>	189	0.00163	2(1.06)	0(0)	0.00088	1(0.53)	0(0)	0.00176	1(0.53)	0(0)
<i>trnG-UCC</i>	71	0.00397	1(1.41)	1(1.41)	0.00427	1(1.41)	1(1.41)	0.00751	1(1.41)	1(1.41)
<i>trnM-CAU</i>	74	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>rps14</i>	303	0.04490	4(1.32)	2(0.66)	0.00410	3(0.99)	2(0.66)	0.00110	1(0.33)	0(0)
<i>psaB</i>	2205	0.00208	25(1.13)	4(0.18)	0.00142	15(0.68)	3(0.14)	0.00100	6(0.27)	1(0.05)
<i>psaA</i>	2253	0.00355	36(1.6)	10(0.44)	0.00266	23(1.02)	9(0.4)	0.00151	9(0.4)	2(0.09)
<i>ycf3</i>	507	0.00152	5(0.99)	0(0)	0.00131	4(0.79)	0(0)	0.00131	2(0.39)	0(0)
<i>trnS-GGA</i>	87	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>rps4</i>	606	0.00372	13(2.15)	2(0.33)	0.00210	6(0.99)	2(0.33)	0.00110	2(0.33)	0(0)
<i>trnT-UGU</i>	73	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>trnL-UAA</i>	85	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>trnF-GAA</i>	73	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>ndhJ</i>	477	0.00435	11(2.31)	1(0.21)	0.00289	6(1.26)	1(0.21)	0.00000	0(0)	0(0)
<i>ndhK</i>	678	0.00433	15(2.21)	2(0.29)	0.00393	13(1.92)	2(0.29)	0.00423	8(1.18)	1(0.15)
<i>ndhC</i>	363	0.00127	3(0.83)	0(0)	0.00138	3(0.83)	0(0)	0.00000	0(0)	0(0)
<i>trnV-UAC</i>	76	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>trnM-CAU</i>	72	0.00712	1(1.39)	1(1.39)	0.00737	1(1.39)	1(1.39)	0.00463	1(1.39)	0(0)
<i>atpE</i>	405	0.00317	6(1.48)	1(0.25)	0.00254	4(0.99)	1(0.25)	0.00247	3(0.74)	0(0)

Gene	Size (bp)	Magnoliaceae			Magnolioideae			Yulania		
		$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)	$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)	$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)
<i>atpB</i>	1497	0.00190	16(1.07)	1(0.07)	0.00103	7(0.47)	1(0.07)	0.00045	2(0.13)	0(0)
<i>rbcL</i>	1439	0.00690	44(3.06)	11(0.76)	0.00422	20(1.39)	11(0.76)	0.00299	11(0.76)	3(0.21)
<i>accD</i>	1467	0.00547	43(2.93)	4(0.27)	0.00343	24(1.64)	3(0.2)	0.00130	5(0.34)	1(0.07)
<i>psaI</i>	111	0.00139	1(0.9)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>ycf4</i>	555	0.00670	21(3.78)	2(0.36)	0.00541	15(2.7)	2(0.36)	0.00276	4(0.72)	1(0.18)
<i>cemA</i>	690	0.00392	15(2.17)	2(0.29)	0.00259	9(1.3)	1(0.14)	0.00222	4(0.58)	1(0.14)
<i>petA</i>	963	0.00394	18(1.87)	4(0.42)	0.00283	11(1.14)	3(0.31)	0.00069	2(0.21)	0(0)
<i>psbJ</i>	123	0.00125	1(0.81)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>psbL</i>	86	0.00358	2(2.33)	0(0)	0.00194	1(1.16)	0(0)	0.00388	1(1.16)	0(0)
<i>psbF</i>	120	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>psbE</i>	252	0.00244	4(1.59)	0(0)	0.00265	4(1.59)	0(0)	0.00132	1(0.4)	0(0)
<i>petL</i>	96	0.00694	2(2.08)	1(1.04)	0.00552	1(1.04)	1(1.04)	0.00347	1(1.04)	0(0)
<i>petG</i>	114	0.00585	2(1.75)	1(0.88)	0.00611	2(1.75)	1(0.88)	0.00292	1(0.88)	0(0)
<i>trnW-CCA</i>	74	0.00208	1(1.35)	0(0)	0.00225	1(1.35)	0(0)	0.00000	0(0)	0(0)
<i>trnP-UGG</i>	74	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>psaJ</i>	135	0.00228	2(1.48)	0(0)	0.00247	2(1.48)	0(0)	0.00000	0(0)	0(0)
<i>rpl33</i>	201	0.00370	4(1.99)	1(0.5)	0.00317	3(1.49)	1(0.5)	0.00332	2(1)	0(0)
<i>rps18</i>	306	0.00066	1(0.33)	0(0)	0.00071	1(0.33)	0(0)	0.00000	0(0)	0(0)
<i>rpl20</i>	353	0.00392	9(2.55)	0(0)	0.00236	5(1.42)	0(0)	0.00094	1(0.28)	0(0)
<i>rps12</i>	392	0.00190	4(1.02)	1(0.26)	0.00205	4(1.02)	1(0.26)	0.00085	1(0.26)	0(0)
<i>clpP</i>	606	0.00351	11(1.82)	2(0.33)	0.00310	9(1.49)	2(0.33)	0.00275	5(0.83)	0(0)
<i>psbB</i>	1527	0.00349	33(2.16)	2(0.13)	0.00258	22(1.44)	2(0.13)	0.00201	8(0.52)	2(0.13)
<i>psbT</i>	117	0.00394	3(2.56)	0(0)	0.00142	1(0.85)	0(0)	0.00000	0(0)	0(0)
<i>psbN</i>	132	0.00233	2(1.52)	0(0)	0.00253	2(1.52)	0(0)	0.00253	1(0.76)	0(0)
<i>psbH</i>	222	0.00127	1(0.45)	1(0.45)	0.00137	1(0.45)	1(0.45)	0.00240	1(0.45)	0(0)
<i>petB</i>	648	0.00356	11(1.7)	3(0.46)	0.00330	9(1.39)	3(0.46)	0.00319	5(0.77)	2(0.31)
<i>petD</i>	477	0.00398	10(2.1)	2(0.42)	0.00267	6(1.26)	2(0.42)	0.00070	1(0.21)	0(0)
<i>rpoA</i>	1014	0.00794	39(3.85)	8(0.79)	0.00596	25(2.47)	8(0.79)	0.00316	7(0.69)	4(0.39)
<i>rps11</i>	417	0.00221	6(1.44)	0(0)	0.00120	3(0.72)	0(0)	0.00000	0(0)	0(0)
<i>rpl36</i>	114	0.00270	2(1.75)	0(0)	0.00146	1(0.88)	0(0)	0.00292	1(0.88)	0(0)
<i>infA</i>	234	0.00197	3(1.28)	0(0)	0.00142	2(0.85)	0(0)	0.00142	1(0.43)	0(0)
<i>rps8</i>	399	0.00954	23(5.76)	1(0.25)	0.00782	17(4.26)	1(0.25)	0.00835	10(2.51)	0(0)
<i>rpl14</i>	369	0.00292	7(1.9)	0(0)	0.00271	6(1.63)	0(0)	0.00090	1(0.27)	0(0)
<i>rpl16</i>	411	0.00533	10(2.43)	2(0.49)	0.00446	8(1.95)	2(0.49)	0.00454	5(1.22)	1(0.24)
<i>rps3</i>	660	0.00719	23(3.48)	6(0.91)	0.00480	12(1.82)	6(0.91)	0.00182	3(0.45)	1(0.15)
<i>rps19</i>	279	0.00901	14(5.02)	1(0.36)	0.00608	8(2.87)	1(0.36)	0.00454	3(1.08)	1(0.36)
<i>rpl2</i>	816	0.00091	4(0.49)	1(0.12)	0.00061	3(0.37)	0(0)	0.00082	2(0.25)	0(0)
<i>rpl23</i>	285	0.00055	1(0.35)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>trnI-CAU</i>	74	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>ycf2</i>	6906	0.00090	35(0.51)	4(0.06)	0.00062	21(0.3)	4(0.06)	0.00032	6(0.09)	1(0.01)
<i>ycf15</i>	234	0.00285	2(0.85)	1(0.43)	0.00298	2(0.85)	1(0.43)	0.00142	1(0.43)	0(0)
<i>trnL-CAA</i>	81	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>ndhB</i>	1533	0.00047	3(0.2)	2(0.13)	0.00050	3(0.2)	2(0.13)	0.00022	1(0.07)	0(0)
<i>rps7</i>	468	0.00066	2(0.43)	0(0)	0.00036	1(0.21)	0(0)	0.00000	0(0)	0(0)
<i>trnV-GAC</i>	72	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>rrn16</i>	1491	0.00025	2(0.13)	0(0)	0.00027	2(0.13)	0(0)	0.00000	0(0)	0(0)

Gene	Size (bp)	Magnoliaceae			Magnolioideae			Yulania		
		$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)	$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)	$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)
<i>trnI-GAU</i>	77	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>trnA-UGC</i>	73	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>rrn23</i>	2811	0.00016	3(0.11)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>rrn4.5</i>	103	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>rrn5</i>	121	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>trnR-ACG</i>	74	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>trnN-GUU</i>	72	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>ndhF</i>	2226	0.00845	102(4.58)	12(0.54)	0.00592	64(2.88)	9(0.4)	0.00365	23(1.03)	1(0.04)
<i>rpl32</i>	174	0.00648	5(2.87)	1(0.57)	0.00401	2(1.15)	1(0.57)	0.00192	1(0.57)	0(0)
<i>trnL-UAG</i>	80	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>ccsA</i>	966	0.00519	30(3.11)	2(0.21)	0.00342	19(1.97)	1(0.1)	0.00207	6(0.62)	0(0)
<i>ndhD</i>	1479	0.00581	43(2.91)	10(0.68)	0.00466	32(2.16)	9(0.61)	0.00374	13(0.88)	6(0.41)
<i>psaC</i>	246	0.00188	3(1.22)	0(0)	0.00068	1(0.41)	0(0)	0.00000	0(0)	0(0)
<i>ndhE</i>	306	0.00218	2(0.65)	1(0.33)	0.00173	1(0.33)	1(0.33)	0.00109	1(0.33)	0(0)
<i>ndhG</i>	531	0.00604	16(3.01)	2(0.38)	0.00422	9(1.69)	2(0.38)	0.00188	3(0.56)	0(0)
<i>ndhI</i>	543	0.00548	12(2.21)	5(0.92)	0.00480	9(1.66)	5(0.92)	0.00221	3(0.55)	1(0.18)
<i>ndhA</i>	1092	0.00355	21(1.92)	3(0.27)	0.00246	13(1.19)	2(0.18)	0.00153	5(0.46)	0(0)
<i>ndhH</i>	1182	0.00405	18(1.52)	3(0.25)	0.00286	18(1.52)	1(0.08)	0.00169	6(0.51)	0(0)
<i>rps15</i>	273	0.00855	11(4.03)	3(1.1)	0.00666	7(2.56)	3(1.1)	0.00000	0(0)	0(0)
<i>ycf1</i>	5627	0.01084	298(5.3)	64(1.14)	0.00780	118(2.1)	56(1)	0.00479	68(1.21)	20(0.36)

IGS (+introns)	Size (bp)	Magnoliaceae			Magnolioideae			Yulania		
		$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)	$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)	$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)
		<i>trnH-GUG~psbA</i>	409	0.01909	33(8.07)	8(1.96)	0.01253	18(4.4)	8(1.96)	0.00925
<i>psbA~trnK-UUU-1</i>	272	0.01652	19(6.99)	6(2.21)	0.01138	11(4.04)	5(1.84)	0.00252	2(0.74)	0(0)
<i>trnK-UUU1~matK</i>	270	0.01183	20(7.41)	1(0.37)	0.00625	9(3.33)	1(0.37)	0.00250	2(0.74)	0(0)
<i>matK~trnK-UUU-2</i>	707	0.00733	28(3.96)	5(0.71)	0.00523	17(2.4)	5(0.71)	0.00421	7(0.99)	3(0.42)
<i>trnK-UUU2~rps16-1</i>	656	0.01090	35(5.34)	9(1.37)	0.00719	19(2.9)	8(1.22)	0.00305	5(0.76)	1(0.15)
<i>rps16-1~rps16-2</i>	836	0.00660	29(3.47)	3(0.36)	0.00424	17(2.03)	3(0.36)	0.00187	4(0.48)	1(0.12)
<i>rps16-2~trnQ-UUG</i>	1999	0.01185	120(6)	23(1.15)	0.00931	76(3.8)	21(1.05)	0.00582	28(1.4)	8(0.4)
<i>trnQ-UUG~psbK</i>	334	0.00471	11(3.3)	2(0.6)	0.00250	5(1.5)	2(0.6)	0.00000	0(0)	0(0)
<i>psbK~psbI</i>	365	0.01337	21(5.75)	6(1.64)	0.01115	15(4.11)	6(1.64)	0.00375	4(1.1)	0(0)
<i>psbI~trnS-GCU</i>	139	0.01096	9(6.47)	1(0.72)	0.00698	5(3.6)	1(0.72)	0.00863	3(2.16)	1(0.72)
<i>trnS-GCU~trnG-UCC-1</i>	698	0.01045	41(5.87)	4(0.57)	0.00696	21(3.01)	4(0.57)	0.00289	5(0.72)	1(0.14)
<i>trnG-UCC-1~trnG-UCC-2</i>	792	0.00553	28(3.54)	3(0.38)	0.00380	19(2.4)	2(0.25)	0.00264	6(0.76)	0(0)
<i>trnG-UCC-2~trnR-UCU</i>	152	0.00762	7(4.61)	1(0.66)	0.00660	5(3.29)	1(0.66)	0.00590	2(1.32)	1(0.66)
<i>trnR-UCU~atpA</i>	111	0.00601	2(1.8)	1(0.9)	0.00628	2(1.8)	1(0.9)	0.00300	1(0.9)	0(0)
<i>atpA~atpF-1</i>	79	0.00918	4(5.06)	0(0)	0.00249	1(1.27)	0(0)	0.00000	0(0)	0(0)
<i>atpF-1~atpF-2</i>	747	0.00327	17(2.28)	1(0.13)	0.00215	9(1.2)	0(0)	0.00142	3(0.4)	0(0)
<i>atpF-2~atpH</i>	482	0.00751	15(3.11)	3(0.62)	0.00553	9(1.87)	3(0.62)	0.00212	3(0.62)	0(0)
<i>atpH~atpI</i>	1115	0.00825	53(4.75)	7(0.63)	0.00596	31(2.78)	7(0.63)	0.00370	8(0.72)	3(0.27)
<i>atpI~rps2</i>	210	0.00950	9(4.29)	2(0.95)	0.00761	6(2.86)	2(0.95)	0.00571	3(1.43)	1(0.48)
<i>rps2~rpoC2</i>	232	0.00568	9(3.88)	2(0.86)	0.00349	7(3.02)	1(0.43)	0.00155	4(1.72)	0(0)
<i>rpoC2~rpoC1-1</i>	149	0.00516	5(3.36)	0(0)	0.00224	2(1.34)	0(0)	0.00447	2(1.34)	0(0)
<i>rpoC1-1~rpoC1-2</i>	735	0.00597	24(3.27)	5(0.68)	0.00412	14(1.9)	5(0.68)	0.00400	7(0.95)	3(0.41)
<i>rpoC1-2~rpoB</i>	26	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>rpoB~trnC-GCA</i>	1229	0.00628	56(4.56)	4(0.33)	0.00469	31(2.52)	4(0.33)	0.00367	13(1.06)	2(0.16)
<i>trnC-GCA~petN</i>	1057	0.00910	49(4.64)	10(0.95)	0.00637	29(2.74)	9(0.85)	0.00445	12(1.14)	3(0.28)
<i>petN~psbM</i>	767	0.00856	34(4.43)	4(0.52)	0.00489	17(2.22)	3(0.39)	0.00404	7(0.91)	2(0.26)
<i>psbM~trnD-GUC</i>	1192	0.00728	45(3.78)	6(0.5)	0.00437	22(1.85)	6(0.5)	0.00222	7(0.59)	1(0.08)
<i>trnD-GUC~trnY-GUA</i>	398	0.01485	28(7.04)	6(1.51)	0.00794	11(2.76)	5(1.26)	0.00306	3(0.75)	1(0.25)
<i>trnY-GUA~trnE-UUC</i>	59	0.00261	1(1.69)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>trnE-UUC~trnT-GGU</i>	754	0.00786	32(4.24)	3(0.4)	0.00436	16(2.12)	3(0.4)	0.00383	7(0.93)	2(0.27)
<i>trnT-GGU~psbD</i>	1528	0.01031	84(5.5)	12(0.79)	0.00714	49(3.21)	11(0.72)	0.00485	18(1.18)	4(0.26)
<i>psbC~trnS-UGA</i>	235	0.00927	11(4.68)	2(0.85)	0.00858	9(3.83)	2(0.85)	0.00738	4(1.7)	1(0.43)
<i>trnS-UGA~psbZ</i>	337	0.01228	17(5.04)	8(2.37)	0.01053	12(3.56)	8(2.37)	0.00356	3(0.89)	1(0.3)
<i>psbZ~trnG-UCC</i>	299	0.00957	17(5.69)	2(0.67)	0.00742	12(4.01)	2(0.67)	0.00491	3(1)	2(0.67)
<i>trnG-UCC~trnM-CAU</i>	173	0.01810	16(9.25)	5(2.89)	0.01168	9(5.2)	4(2.31)	0.00390	2(1.16)	0(0)
<i>trnM-CAU~rps14</i>	164	0.00661	6(3.66)	0(0)	0.00515	4(2.44)	0(0)	0.00210	1(0.61)	0(0)
<i>rps14~psaB</i>	144	0.00557	5(3.47)	0(0)	0.00242	2(1.39)	0(0)	0.00242	1(0.69)	0(0)
<i>psaB~psaA</i>	25	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>psaA~ycf3-1</i>	703	0.00726	24(3.41)	4(0.57)	0.00608	17(2.42)	4(0.57)	0.00470	9(1.28)	1(0.14)
<i>ycf3-1~ycf3-2</i>	731	0.00683	26(3.56)	4(0.55)	0.00406	12(1.64)	4(0.55)	0.00137	3(0.41)	0(0)
<i>ycf3-2~ycf3-3</i>	752	0.00427	17(2.26)	2(0.27)	0.00318	11(1.46)	2(0.27)	0.00136	3(0.4)	0(0)
<i>ycf3-3~trnS-GGA</i>	443	0.01169	29(6.55)	3(0.68)	0.00932	17(3.84)	3(0.68)	0.00520	6(1.35)	1(0.23)
<i>trnS-GGA~rps4</i>	282	0.00612	9(3.19)	2(0.71)	0.00404	5(1.77)	2(0.71)	0.00195	1(0.35)	1(0.35)
<i>rps4~trnT-UGU</i>	367	0.10650	24(6.54)	3(0.82)	0.00881	16(4.36)	3(0.82)	0.00863	8(2.18)	2(0.54)
<i>trnT-UGU~trnL-UAA-1</i>	852	0.01324	52(6.1)	10(1.17)	0.01028	33(3.87)	10(1.17)	0.00579	12(1.41)	4(0.47)
<i>trnL-UAA-1~trnL-UAA-2</i>	495	0.00557	11(2.22)	3(0.61)	0.00357	5(1.01)	3(0.61)	0.00068	1(0.2)	0(0)

IGS (+introns)	Size (bp)	Magnoliaceae			Magnolioideae			Yulania		
		$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)	$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)	$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)
<i>trnL-UAA-2~trnF-GAA</i>	362	0.01452	24(6.63)	6(1.66)	0.01221	17(4.7)	6(1.66)	0.01146	11(3.04)	2(0.55)
<i>trnF-GAA~ndhJ</i>	736	0.00705	33(4.48)	4(0.54)	0.00597	23(3.13)	4(0.54)	0.00350	7(0.95)	1(0.14)
<i>ndhJ~ndhK</i>	99	0.01865	8(8.08)	3(3.03)	0.01362	5(5.05)	3(3.03)	0.00539	1(1.01)	1(1.01)
<i>ndhK~ndhC</i>	59	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>ndhC~trnV-UAC-1</i>	1818	0.00940	89(4.9)	14(0.77)	0.00737	59(3.25)	14(0.77)	0.00437	22(1.21)	5(0.28)
<i>trnV-UAC-1~trnV-UAC-2</i>	588	0.00391	14(2.38)	1(0.17)	0.00309	10(1.7)	1(0.17)	0.00205	3(0.51)	1(0.17)
<i>trnV-UAC-2~trnM-CAU</i>	178	0.00182	2(1.12)	0(0)	0.00095	1(0.56)	0(0)	0.00189	1(0.56)	0(0)
<i>trnM-CAU~atpE</i>	222	0.00817	9(4.05)	1(0.45)	0.00214	2(0.9)	1(0.45)	0.00000	0(0)	0(0)
<i>atpE~rbcl</i>	785	0.00457	19(2.42)	1(0.13)	0.00248	9(1.15)	1(0.13)	0.00132	3(0.38)	0(0)
<i>rbcl~accD</i>	663	0.00960	32(4.83)	7(1.06)	0.00801	24(3.62)	6(0.9)	0.00564	10(1.51)	2(0.3)
<i>accD~psaI</i>	653	0.01206	46(7.04)	5(0.77)	0.00808	29(4.44)	4(0.61)	0.00258	5(0.77)	0(0)
<i>psaI~ycf4</i>	458	0.01047	25(5.46)	3(0.66)	0.00676	13(2.84)	3(0.66)	0.00485	6(1.31)	1(0.22)
<i>ycf4~cemA</i>	962	0.00996	53(5.51)	2(0.21)	0.00644	32(3.33)	2(0.21)	0.00526	15(1.56)	0(0)
<i>cemA~petA</i>	203	0.00303	4(1.97)	0(0)	0.00082	1(0.49)	0(0)	0.00164	1(0.49)	0(0)
<i>petA~psbJ</i>	1220	0.02269	135(11.07)	25(2.05)	0.01980	104(8.52)	23(1.89)	0.01283	34(2.79)	16(1.31)
<i>psbJ~psbL</i>	126	0.00123	1(0.79)	0(0)	0.00133	1(0.79)	0(0)	0.00000	0(0)	0(0)
<i>psbL~psbF</i>	53	0.00290	1(1.89)	0(0)	0.00314	1(1.89)	0(0)	0.00000	0(0)	0(0)
<i>psbF~psbE</i>	9	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>psbE~petL</i>	1256	0.01131	90(7.17)	22(1.75)	0.00941	65(5.18)	20(1.59)	0.00639	28(2.23)	3(0.24)
<i>petL~petG</i>	177	0.01536	16(9.04)	2(1.13)	0.01113	11(6.21)	1(0.56)	0.00565	3(1.69)	0(0)
<i>petG~trnW-CCA</i>	121	0.19120	10(8.26)	2(1.65)	0.01010	4(3.31)	2(1.65)	0.00000	0(0)	0(0)
<i>trnW-CCA~trnP-UGG</i>	160	0.00926	7(4.38)	1(0.63)	0.00665	4(2.5)	1(0.63)	0.00625	3(1.88)	0(0)
<i>trnP-UGG~psaI</i>	349	0.01010	18(5.16)	3(0.86)	0.00847	13(3.72)	3(0.86)	0.00592	5(1.43)	2(0.57)
<i>psaI~rpl33</i>	442	0.00901	27(6.11)	4(0.9)	0.00547	16(3.62)	4(0.9)	0.00079	2(0.45)	0(0)
<i>rpl33~rps18</i>	133	0.00470	4(3.01)	0(0)	0.00254	2(1.5)	0(0)	0.00000	0(0)	0(0)
<i>rps18~rpl20</i>	277	0.00692	12(4.33)	1(0.36)	0.00570	9(3.25)	0(0)	0.00499	4(1.44)	0(0)
<i>rpl20~rps12-5end</i>	784	0.00616	21(2.68)	7(0.89)	0.00513	15(1.91)	7(0.89)	0.00349	7(0.89)	2(0.26)
<i>rps12-5end~clpP-1</i>	136	0.00712	6(4.41)	2(1.47)	0.01018	5(3.68)	2(1.47)	0.00509	2(1.47)	0(0)
<i>clpP-1~clpP-2</i>	634	0.00691	24(3.79)	3(0.47)	0.00420	2(0.32)	3(0.47)	0.00245	4(0.63)	1(0.16)
<i>clpP-2~clpP-3</i>	787	0.00406	15(1.91)	5(0.64)	0.00389	12(1.52)	5(0.64)	0.00368	6(0.76)	4(0.51)
<i>clpP-3~psbB</i>	437	0.00364	10(2.29)	0(0)	0.00354	9(2.06)	0(0)	0.00000	0(0)	0(0)
<i>psbB~psbT</i>	173	0.00641	7(4.05)	0(0)	0.00493	5(2.89)	0(0)	0.00392	2(1.16)	0(0)
<i>psbT~psbN</i>	59	0.00869	1(1.69)	1(1.69)	0.00899	1(1.69)	1(1.69)	0.00565	1(1.69)	0(0)
<i>psbN~psbH</i>	107	0.00288	2(1.87)	0(0)	0.00156	1(0.93)	0(0)	0.00000	0(0)	0(0)
<i>psbH~petB-1</i>	120	0.00535	4(3.33)	0(0)	0.00435	3(2.5)	0(0)	0.00278	1(0.83)	0(0)
<i>petB-1~petB-2</i>	790	0.00638	26(3.29)	5(0.63)	0.00535	19(2.41)	5(0.63)	0.00162	3(0.38)	1(0.13)
<i>petB-2~petD</i>	906	0.00516	23(2.54)	6(0.66)	0.00387	14(1.55)	6(0.66)	0.00309	7(0.77)	2(0.22)
<i>petD~rpoA</i>	191	0.01154	8(4.19)	4(2.09)	0.00917	6(3.14)	2(1.05)	0.00351	2(1.05)	0(0)
<i>rpoA~rps11</i>	77	0.00679	3(3.9)	0(0)	0.00490	2(2.6)	0(0)	0.00000	0(0)	0(0)
<i>rps11~rpl36</i>	124	0.01086	6(4.84)	1(0.81)	0.00732	3(2.42)	1(0.81)	0.00282	1(0.81)	0(0)
<i>rpl36~infA</i>	121	0.00840	4(3.31)	2(1.65)	0.00496	2(1.65)	1(0.83)	0.00000	0(0)	0(0)
<i>infA~rps8</i>	117	0.00833	4(3.42)	1(0.85)	0.00596	2(1.71)	1(0.85)	0.00570	2(1.71)	0(0)
<i>rps8~rpl14</i>	183	0.01195	12(6.56)	2(1.09)	0.01007	9(4.92)	2(1.09)	0.00189	1(0.55)	0(0)
<i>rpl14~rpl16</i>	134	0.01547	13(9.7)	2(1.49)	0.00627	5(3.73)	1(0.75)	0.00258	1(0.75)	0(0)
<i>rpl16~rps3</i>	1105	0.00678	37(3.35)	6(0.54)	0.00523	24(2.17)	6(0.54)	0.00200	6(0.54)	1(0.09)
<i>rps3~rps19</i>	563	0.02265	73(12.97)	8(1.42)	0.02009	61(10.83)	8(1.42)	0.01078	17(3.02)	2(0.36)

IGS (+introns)	Size (bp)	Magnoliaceae			Magnolioideae			Yulania		
		$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)	$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)	$\rho$ -distance	No. of variable sites (%)	No. of informative sites (%)
<i>rps19~rpl2-1</i>	61	0.00655	2(3.28)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>rpl2-1~rpl2-2</i>	662	0.00163	7(1.06)	0(0)	0.00126	5(0.76)	0(0)	0.00252	5(0.76)	0(0)
<i>rpl2-2~rpl23</i>	24	0.00641	1(4.17)	0(0)	0.00694	1(4.17)	0(0)	0.01389	1(4.17)	0(0)
<i>rpl23~trnI-CAU</i>	157	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>trnI-CAU~ycf2</i>	68	0.00452	2(2.94)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>ycf2~ycf15</i>	115	0.00245	1(0.87)	1(0.87)	0.00264	1(0.87)	1(0.87)	0.00464	1(0.87)	1(0.87)
<i>ycf15~trnL-CAA</i>	673	0.00261	7(1.04)	3(0.45)	0.00226	5(0.74)	3(0.45)	0.00180	3(0.45)	1(0.15)
<i>trnL-CAA~ndhB-1</i>	560	0.00110	4(0.71)	0(0)	0.00030	1(0.18)	0(0)	0.00000	0(0)	0(0)
<i>ndhB-1~ndhB-2</i>	700	0.00022	1(0.14)	0(0)	0.00024	1(0.14)	0(0)	0.00000	0(0)	0(0)
<i>ndhB-2~rps7</i>	327	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>rps7~rps12-3end-1</i>	58	0.00265	1(1.72)	0(0)	0.00287	1(1.72)	0(0)	0.00000	0(0)	0(0)
<i>rps12-3end-1~rps12-3end-2</i>	536	0.00057	2(0.37)	0(0)	0.00031	1(0.19)	0(0)	0.00000	0(0)	0(0)
<i>rps12-3end-2~trnV-GAC</i>	1840	0.00445	39(2.12)	5(0.27)	0.00245	16(0.87)	5(0.27)	0.00175	7(0.38)	1(0.05)
<i>trnV-GAC~rm16</i>	232	0.00073	1(0.43)	0(0)	0.00080	1(0.43)	0(0)	0.00176	1(0.43)	0(0)
<i>rm16~trnI-GAU-1</i>	293	0.00053	1(0.34)	0(0)	0.00058	1(0.34)	0(0)	0.00000	0(0)	0(0)
<i>trnI-GAU-1~trnI-GAU-2</i>	943	0.00080	4(0.42)	1(0.11)	0.00069	3(0.32)	1(0.11)	0.00093	2(0.21)	1(0.11)
<i>trnI-GAU-2~trnA-UGC-1</i>	64	0.00441	1(1.56)	1(1.56)	0.00473	1(1.56)	1(1.56)	0.00000	0(0)	0(0)
<i>trnA-UGC-1~trnA-UGC-2</i>	799	0.00104	3(0.38)	1(0.13)	0.00110	3(0.38)	1(0.13)	0.00042	1(0.13)	0(0)
<i>trnA-UGC-2~rrn23</i>	152	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>rrn23~rrn4.5</i>	105	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>rrn4.5~rrn5</i>	218	0.00075	1(0.46)	0(0)	0.00076	1(0.46)	0(0)	0.00153	1(0.46)	0(0)
<i>rrn5~trnR-ACG</i>	250	0.00480	6(2.4)	1(0.4)	0.00443	5(2)	1(0.4)	0.00139	1(0.4)	0(0)
<i>trnR-ACG~trnN-GUU</i>	618	0.00448	13(2.1)	2(0.32)	0.00338	9(1.46)	2(0.32)	0.00164	3(0.49)	0(0)
<i>trnN-GUU~ndhF</i>	1707	0.00473	58(3.4)	17(1)	0.00570	33(1.93)	14(0.82)	0.00307	13(0.76)	4(0.23)
<i>ndhF~rpl32</i>	1190	0.01675	99(8.32)	19(1.6)	0.01093	62(5.21)	15(1.26)	0.00748	23(1.93)	4(0.34)
<i>rpl32~trnL-UAG</i>	1314	0.01762	118(8.98)	22(1.67)	0.01316	72(5.48)	21(1.6)	0.00923	28(2.13)	11(0.84)
<i>trnL-UAG~ccsA</i>	99	0.01416	8(8.08)	1(1.01)	0.01010	5(5.05)	1(1.01)	0.00903	2(2.02)	1(1.01)
<i>ccsA~ndhD</i>	230	0.03135	23(10)	10(4.35)	0.02635	15(6.52)	10(4.35)	0.02191	8(3.48)	8(3.48)
<i>ndhD~psaC</i>	146	0.02020	11(7.53)	8(5.48)	0.01422	10(6.85)	2(1.37)	0.00365	1(0.68)	1(0.68)
<i>psaC~ndhE</i>	287	0.01296	19(6.62)	3(1.05)	0.00826	11(3.83)	2(0.7)	0.00361	3(1.05)	0(0)
<i>ndhE~ndhG</i>	265	0.01226	17(6.42)	2(0.75)	0.00819	10(3.77)	2(0.75)	0.00721	5(1.89)	1(0.38)
<i>ndhG~ndhI</i>	354	0.00697	14(3.95)	2(0.56)	0.00464	8(2.26)	2(0.56)	0.00249	2(0.56)	1(0.28)
<i>ndhI~ndhA-1</i>	78	0.01151	5(6.41)	1(1.28)	0.01243	5(6.41)	1(1.28)	0.01282	3(3.85)	0(0)
<i>ndhA-1~ndhA-2</i>	1084	0.00982	54(4.98)	9(0.83)	0.00740	35(3.23)	9(0.83)	0.00500	13(1.2)	5(0.46)
<i>ndhA-2~ndhH</i>	1	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)	0.00000	0(0)	0(0)
<i>ndhH~rps15</i>	104	0.00444	3(2.88)	0(0)	0.00321	2(1.92)	0(0)	0.00641	2(1.92)	0(0)
<i>rps15~ycf1</i>	382	0.01064	21(5.5)	5(1.31)	0.00719	13(3.4)	2(0.52)	0.00502	5(1.31)	1(0.26)