J. Plant Biol. (2013) 56:290-305
DOI 10.1007/s12374-013-0111-9

ORIGINAL ARTICLE

Phylogeny of Magnoliaceae Based on Ten Chloroplast DNA Regions

Sangtae Kim[1] and Youngbae Suh[2]*

[1]*School of Biological Sciences and Chemistry, Sungshin University, Seoul 142-732, Korea*
[2]*Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 151-742, Korea*

Received: March 21, 2013 / Accepted: June 19, 2013

Abstract Phylogenetic analyses of ten chloroplast DNA regions, *ndhF*, *rbcL*, *matK*, ORF350, *trnL* intron, *trnL-trnF*, *trnH-psbA*, *rbcL-atpB*, *trnK* 5' intron, and *trnK* 3' intron (8,719 bp in aligned sequences) from 48 selected taxa were carried out to address phylogenetic questions in the family Magnoliaceae. The major clades in the molecular tree are considerably different from the currently suggested classification system and from the traditionally recognized subgroups in the family. Eleven major clades were recognized with strong support in the subfamily Magnolioideae: (1) MICHELIA clade: *Michelia*, *Elmerrillia*, sect. *Maingola*, sect. *Alcimandra*, and sect. *Aromadendron*, (2) YULANIA clade: subgen. *Yulania,* (3) GYNOPODIUM clade: *Pachylarnax*, sect. *Manglietiastrum*, and sect. *Gynopodium*, (4) KMERIA clade: *Kmeria*, (5) THEORHODON clade: sect. *Theorhodon sensu stricto* (excluding sect. *Splendentes*, which was recently separated from sect. *Theorhodon*) and sect. *Magnolia*, (6) GWILLIMIA clade: sect. *Gwillimia*, sect. *Lirianthe*, and sect. *Blumiana*, (7) TALAUMA clade: sect. *Talauma* and sect. *Splendentes*, (8) MANGLIETIA clade: *Manglietia*, (9) RYTIDOSPERMUM clade: sect. *Rytidospermum sensu stricto* (excluding *Magnolia fraseri*, *M. macrophylla*, and *M. dealbata*) and sect. *Oyama*, (10) FRASERI clade: *M. fraseri*, and (11) MACROPHYLLA clade: *M. macrophylla* and *M. dealbata*. The recognition of eleven major clades in the subfamily Magnolioideae in this study is in good agreement with previous molecular studies based on less sampling or fewer DNA regions. All of these eleven clades were highly supported with bootstrap values exceeding 80% in both maximum parsimony and maximum likelihood analyses and with posterior probabilities exceeding 0.98 in a Bayesian analysis. However, detailed relationships among the major clades were weakly supported. The molecular data suggest that the taxonomic circumscription of infra-familial delimitations and compositions should be reconsidered.

*Corresponding author; Youngbae Suh
Tel : +82-2-880-2486
E-mail : ysuh@snu.ac.kr

Keywords: Chloroplast genes, Classification system, *Magnolia,* Magnoliaceae, Molecular phylogeny

Introduction

Magnoliaceae Juss. contains over 223 species characterized by stipules falling in time and leaving an annular scar around each node; spirally arranged leaves, usually conspicuous; floral parts of six or more; monosulcate pollen; beetle pollination; an androecium of numerous spirally arranged stamens; a gynoecium with many simple carpels spirally arranged on an elongated axis; and separate tepals (Frodin and Govaerts 1996). All species of the family have bisexual flowers except for *Kmeria* (Pierre) Dandy and some species of *Magnolia* L. sect. *Gynopodium* (Chen and Nooteboom 1993). Carpels open mostly along dorsal or ventral sutures, sometimes circumscissile in *Magnolia* subgen. *Talauma*, and rarely indehiscent (then samaretum) in *Liriodendron* L. One or more seeds with an arilloid testa in a carpel are suspended by a funicular thread when ripening.

Four-fifths of the species of Magnoliaceae are currently distributed in temperate and tropical regions of Southeast Asia, and the remaining one-fifth is found in the Americas, from temperate southeast North America through Central America to Brazil (Dandy 1971; Thorne 1993; Frodin and Govaerts 1996). The distribution of Magnoliaceae in eastern Asia and the Americas is an outstanding example of intercontinental disjunction (Li 1952, 1972).

Magnoliaceae has attracted keen interest from many botanists because the family has played a key role in forming the concepts of the first flowers. One of the classical theories pertaining to the primitive angiosperm flower is *Magnolia*-like evergreen trees in tropical uplands that are solitary, terminal, bisexual, and actinomorphic, with numerous tepals, stamens, and carpels all spirally arranged on an elongated axis (Takhtajan 1969). A fossil record of *Archaeanthus* Dilcher & Crane from the mid-Cretaceous (uppermost Albian-

mid-Cenomanian) Dakota formation of central Kansas, which is considered to be a direct ancestor of Magnoliaceae, shows that the family has a long evolutionary history of over 100 million years (Dilcher and Crane 1984). Recent molecular studies, however, provided a different perspective on the basal-most angiosperm. Intensive molecular phylogenetic studies continue to infer to the phylogenetic history of angiosperm while also attempting to find the basal group of angiosperm using various genes. Most of these studies agree that Amborellaceae Pichon is the basal-most angiosperm (Mathews and Donoghue 1999; Parkinson et al. 1999; Soltis et al. 1999; Qiu et al. 1999; Graham and Olmstead 2000; D. Soltis et al. 2011; Magallon and Sanderson 2001; Zanis et al. 2002; Borsch et al. 2003; Hilu et al. 2003; Kim et al. 2004; Nickerson and Drouin 2004; Moore et al. 2007; Jansen et al. 2007) although alternative topologies in which *Amborella* and Nymphaeaceae are sisters to each other, with this clade sister to all other extant angiosperms, has been found in some analyses (e.g., Parkinson et al. 1999; Barkman et al. 2000; Qiu et al. 2000; P. Soltis et al. 2000; Kim et al. 2004). As a member of Magnoliales, Magnoliaceae was placed among the second-level basal group of phylogenetic trees in most of these studies, after *Amborella*, Nymphaeaceae, and Austrobaileyales as the first group (e.g., Moore et al. 2007; Jansen et al. 2007; Soltis et al. 2011). Although recent molecular studies provided a different perspective on the basal-most angiosperm, Magnoliaceae still hold an important phylogenetic position when attempting to grasp the big picture of angiosperm evolution.

Recent phylogenetic analyses of 640 representatives of angiosperms based on 17 genes showed phylogenetic relationships among families in Magnoliales (Soltis et al. 2011). In this analysis, a clade of Eupomatiaceae/Annonaceae and Himantiandraceae is the sister to Degeneriaceae/Myristicaceae, and Magnoliaceae is a sister to all other families in the order. However, this basal placement of Magnoliaceae in the tree was not highly supported (bootstrap <50%).

Since Dandy (1927) proposed the first taxonomic treatment of the Magnoliaceae, many different infra-familial taxonomic schemes have been suggested by various authors based on morphology (Dandy 1978; Law 1984, 1996; Nooteboom 1985, 1993; Chen and Nooteboom 1993). Taxonomic treatment in the family is controversial regarding the disposition of tribes, genera, and sections. This is mainly due to the lack of phylogenetically useful morphological characters caused by the extensive parallelism and homogeneity in the family (Nooteboom 2000).

Early molecular studies of the phylogeny of the family (Qiu et al. 1995b; Azuma et al. 1999) produced very different results from the major groups recognized in the traditional classification systems based on morphology (Dandy 1927, 1978; Law 1984, 1996; Nooteboom 1985; Chen and Nooteboom 1993). Qiu et al. (1995a) analyzed the restriction fragment length polymorphism of cpDNA for 21 species, representing only four genera and nine sections of the family according to Nooteboom's (1985) treatment. On the other hand, Azuma et al. (1999) analyzed sequences of *trnK* intron (including *matK* gene), *psbA-trnH* spacer, and *rbcL-atpB* spacer for 26 species of three genera and 10 sections by Nooteboom (1985). The major drawback of these studies was the insufficient taxon sampling, which does not include enough subgroups of the family to demonstrate monophyly for each group. The *ndhF* analysis by Kim et al. (2001) was the first attempt to elucidate the phylogenetic relationships of the entire family from a comprehensive sampling of taxa representing all sections and genera recognized to date, amounting to 99 taxa of all seven genera and 16 sections by Nooteboom (1985). In this study, eight major clades were recognized, although some clades were not highly supported: (1) *M. macrophylla* and *M. dealbata*, North American species of *Magnolia* sect. *Rytidospermum*, which are placed at the base in the subfamily Magnolioideae; (2) a clade consisting of the three subclades of *Michelia-Elmerrillia*-sect. *Maingola*-sect. *Aromadendron*-sect. *Alcimandra*, subgen. *Yulania*, and *Pachylarnax*, sect. *Manglietiastrum*-sect. *Gynopodium*; (3) *Manglietia*; (4) sect. *Magnolia*-sect. *Theorhodon*; (5) sect. *Gwillimia*-sect. *Lirianthe*-sect. *Blumiana*; (6) sect. *Oyama*-sect. *Rytidospermum* (excluding *M. fraseri*, *M. macrophylla*, and *M. dealbata*); (7) sect. *Talauma*-sect. *Splendentes*; and (8) *Kmeria*-*M. fraseri*. Later, Azuma et al. (2001) analyzed 57 taxa using *matK* and 47 taxa based on *trnK* intron including *matK*, *psbA-trnH*, and *rbcL-atpB*. Their analyses showed major groups of Magnoliaceae similar to those of *ndhF* analyses (Kim et al. 2001) but some clades are poorly supported and the relationships among these clades were quite different from those noted in the *ndhF* analysis.

In 2004, Figlar and Nooteboom proposed a new classification system in Magnoliaceae, recognizing only two genera in Magnoliaceae (*Magnolia* and *Liriodendron*) containing three subgenera and 12 sections. Although they indicated that the system was based on the phylogeny of the chloroplast DNA and on morphological reexaminations, none of the results from previous phylogenetic analyses match their three subgenera. However, 12 sections of Figlar and Nooteboom (2004) were in relatively good agreement with the major clades in the tree from Kim et al. (2001) if three subclades of a major clade of Kim et al. (2001) are separately recognized as sections in addition to the eight major clades.

Recently, Nie et al. (2008) analyzed three nuclear genes (*PHYA*, *LFY*, and *GAL1*) and compared the result with those from chloroplast regions. The major clades of two trees were nearly identical, although the placements of some taxa in the

nuclear tree were different from those in the chloroplast tree (e.g., *M. acuminate*, *M. sieboldii*, *Kmeria*, and *Manglietia*). In the recent "Flora of China" (Xia et al., 2008), Magnoliaceae in China are summarized into 13 genera. In this classification system, *Magnolia s. l.*, a paraphyletic group in most previous molecular studies (Qiu 1995a, Kim et al. 2001; Azuma et al 2001), is divided into several genera, including the newly proposed genera of *Oyama* (Nakai) N. H. Xia & C. Y. Wu and *Houpoëa* N. H. Xia & C. Y. Wu. However, the system did not show the entire classification of Magnoliaceae because it includes only Chinese species.

In spite of the large difference in the taxon sampling size, previous molecular studies of Magnoliaceae recognized some major groups in common, which are significantly different from those traditionally perceived on the basis of morphology. Although molecular analyses have provided new insight into the phylogeny of Magnoliaceae, several problems still need to be solved, as follows: (1) some of the major clades are weakly supported; (2) the relationships among the major clades remain unresolved at deep nodes; (3) the monophylies of some clades were in conflict; and (4) basal members in the subfamily Magnolioideae were ambiguous. Because the nucleotide substitution rate in Magnoliaceae was very low in comparison to other angiosperm families (Qiu et al. 1995a; Azuma et al. 1999; Kim et al. 2001), analyses of a single or limited number of genes provided weak or ambiguous support for the major clades in Magnoliaceae. Recently, combined data analyses of multiple DNA regions have been conducted in phylogenetic studies because increasing the number of nucleotides in phylogenetic analyses as well as taxa may improve the accuracy of the estimated trees while also reducing the computational difficulty of the inference process (e.g., Olmstead and Sweere 1994; Graham and Olmstead 2000; Soltis et al. 2011). Especially in cases in which rapidly radiated taxa resist resolution, the addition of a reasonable amount of DNA sequence data must be considered (Flook et al. 1999).

To enhance the phylogenetic resolution, which was uncertain in previous molecular phylogenetic studies of Magnoliaceae, combined data sets were analyzed from 10 cpDNA regions of 8.7 kb in total, encompassing the following: the *ndhF* gene, the *rbcL* gene, ORF350 of about 450 bases 3' downstream from *ndhF*, the *trnL* intron, the *trnL-trnF* spacer, the *trnK* 5' intron from the *trnK* 5' exon to the *matK* 5' end, the *trnK* 3' intron from the *matK* 3' end to the *trnK* 3' exon, the *matK* gene, the *trnH-psbA* spacer, and the *rbcL-atpB* spacer. The purpose of this study is to provide a well-supported phylogeny of Magnoliaceae capable of resolving the controversy surrounding infra-familial groupings. Eventually, our result will become supporting evidence for the establishment of a stable classification system of Magnoliaceae.

Results

Sequence Variations and Homogeneity

8,719 sequences in total in an alignment of 10 cpDNA regions from 48 taxa were examined in this study (Table 1 and S1). Compared with the total chloroplast genome of *Liriodendron tulipifera* (Cai et al. 2006), which is about 159 kb in size, the determined sequences analyzed in this study correspond to about 1/18 of the total chloroplast genome,

Table 1. Summary of statistics for each data set and the combined data sets (matrix I)

Region	No. of characters examined	Size of region	No. of variable sites (%)	Maximum sequence divergence (Kimura K × 100)				GC contents (%)
				No. of informative Sites (%)	Family Magnoli-aceae	Subfamily Magnoli-oideae	Subfamily Liriodend-oideae	
*ndh*F*	2199	2196-2199	154 (7.00)	97 (4.41)	2.45	1.01	0.73	34.47-34.83
*rbc*L*	1368	1365-1368	65 (4.75)	51 (3.73)	2.46	1.03	0.37	45.18-46.18
*mat*K	1524	1524	114 (7.48)	73 (4.79)	2.57	1.61	0.72	34.82-36.16
*trn*L intron	500	489-500	22 (4.40)	14 (2.80)	2.05	1.01	0.41	35.48-37.37
*trn*L-*trn*F	379	362-371	35 (9.23)	17 (4.49)	3.94	2.79	0.82	36.44-38.08
*rbc*L-*atp*B*	817	785-803	38 (4.65)	18 (2.20)	2.18	0.89	0.50	32.25-33.33
*trn*H-*psb*A*	457	419-451	60 (13.13)	35 (7.66)	6.38	3.40	2.50	31.71-33.41
*trn*K 5' intron*	720	708-713	50 (6.94)	26 (3.61)	2.31	1.43	0.71	38.82-39.97
*trn*K 3' intron*	260	251-259	23 (8.85)	14 (5.38)	6.20	2.79	1.21	36.26-38.25
ORF350*	445	282-428	77 (17.34)	33 (7.43)	18.70	3.37	5.58	29.69-33.88
Coding genes	5091		337 (6.22)	223 (4.38)	2.38	0.99	0.63	37.42-38.07
Non-coding regions	3628		305 (8.53)	157 (4.39)	4.24	1.17	1.30	34.98-36.76
Total	8719	8500-8618	642 (7.36)	380 (4.36)	2.89	0.95	0.88	36.61-37.63

*Partially sequenced regions in comparison with *Liriodendron tulipifera* (Cai et al. 2006)

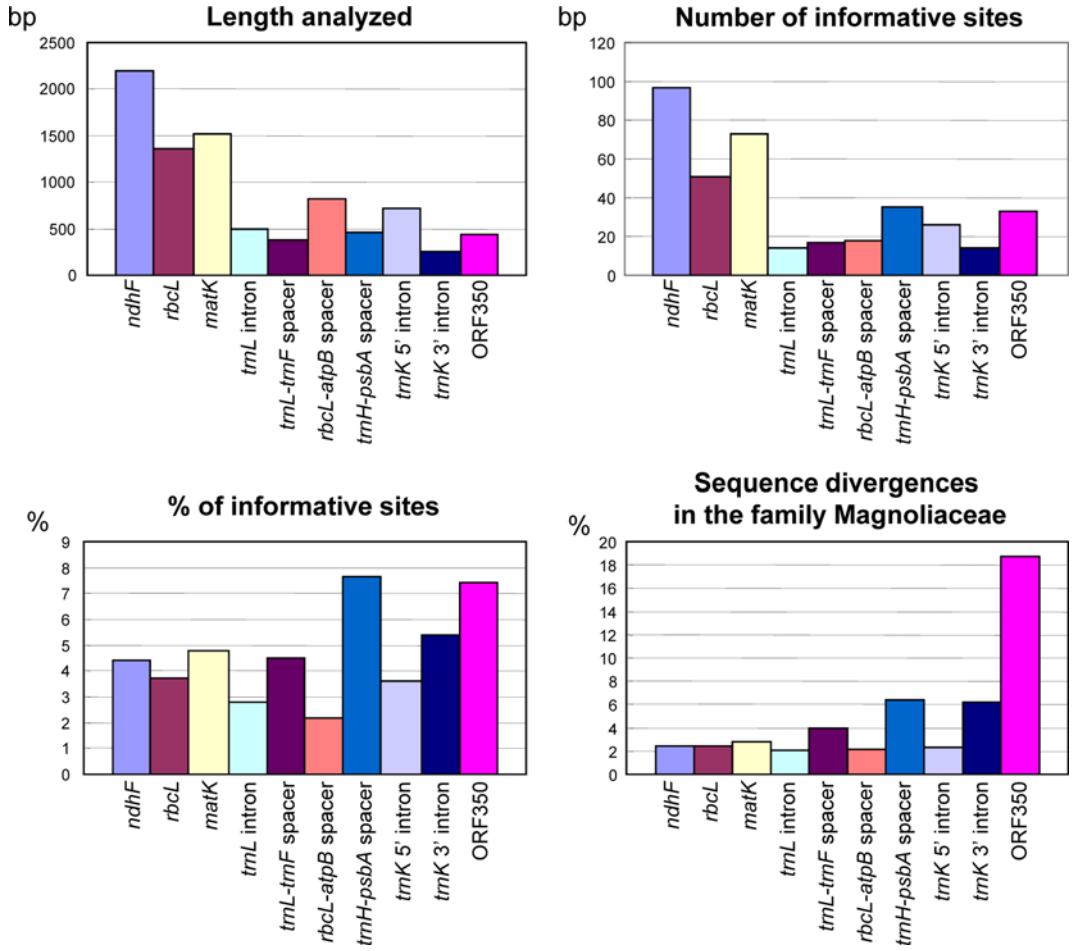


Fig. 1. Comparison of sequence characteristics among 10 cpDNA regions in Magnoliaceae.

which is about 1/15 when one inverted repeat region is excluded. Out of 8,719 sites, 642 sites (7.36%) were variable and 380 sites (4.36%) were phylogenetically informative. ORF350 showed the highest ratio of informative sites (7.43%) among the 10 regions. Although the ratio of informative sites of the *ndhF* gene (4.41%) was lower than that of the *matK* gene (4.79%), the number of informative sites of *ndhF* (97 sites) was higher than that of *matK* (73 sites; Table 1; Fig. 1). This indicates that the *ndhF* gene may be the most useful from among the three genes included in this study (*ndhF*, *matK*, and *rbcL*) to reconstruct the phylogeny of Magnoliaceae. The maximum sequence divergence (Kimura *K*×100; Kimura, 1980) in the combined data set of 10 regions was 2.94% in the family Magnoliaceae, 0.95% in the subfamily Magnolioideae, and 0.89% in the subfamily Liriodendroideae (Tables 1). Sequence divergence was relatively low (2.45-3.94%) in *ndhF, rbcL, matK*, the *trnL* intron, the *trnL-trnF* spacer, the *rbcL-atpB* spacer, and the *trnK* 5' intron for the family Magnoliaceae. Relatively high values of sequence divergence ranging from 6.20 to 18.7% were observed in the *trnH-psbA* spacer, *trnK* 3' intron, and ORF350 region. Sequence divergence was the highest in the ORF350 region (18.7%) mainly due to the high divergence between the subfamily Magnolioideae and subfamily Liriodendroideae. The maximum sequence divergence of ORF350, which is positioned on the border between the small single-copy region (SSR) and the inverted-repeat region (IR), was 3.37% in the subfamily Magnolioideae and 5.58% in the subfamily Liriodendroideae (Table 1). The GC contents of all regions except *rbcL* ranged from 29.69% to 39.97% (Table 1). The GC content was considerably high in the *rbcL* gene, ranging from 45.18 to 46.18%.

The values of skewness (g1) ranged from -0.96 (*trnH-psbA* spacer) to -4.05 (*rbcL-atpB* spacer) and from -0.63 (*ndhF* gene) to -1.82 (*trnK* 5' intron) when outgroup taxa were excluded (Table 2). With the exclusion of outgroup taxa, the skewness was substantially decreased in *ndhF, rbcL,* the *rbcL-atpB* spacer, the *trnK* 3' intron, ORF350, and the combined data set. This indicates that these data sets use large amount of phylogenetic information to distinguish ingroups from outgroups. In particular, the most substantial

Table 2. The result of parsimony analyses and g1 values for each region and combined regions

	Number of trees	Steps	CI	$CI_{excluding\ informative\ sites}$	RI	g1	$g1_{excluding}$ outgroup
*ndh*F	2	192	0.88	0.82	0.90	-1.71	-0.63
*rbc*L	366	88	0.76	0.72	0.88	-1.31	-0.55
*mat*K	1340	121	0.90	0.85	0.93	-1.30	-0.95
*trn*L intron	11	24	0.92	0.88	0.95	-1.64	-1.20
*trn*L-*trn*F IGS	1	36	1.00	1.00	1.00	-1.77	-1.59
*rbc*L-*atp*B IGS	280	42	0.93	0.86	0.91	-4.50	-1.26
*trn*H-*psb*A IGS	218	68	0.91	0.86	0.94	-0.96	-0.70
*trn*K intron 5' partial	40	53	0.96	0.93	0.96	-1.62	-1.45
*trn*K intron 3' partial	450	24	1.00	1.00	1.00	-3.66	-1.82
ORF350	>5000	89	0.94	0.88	0.94	-2.01	-1.32
Coding genes	188	422	0.82	0.75	0.86	-2.00	-0.79
Non-coding regions	>5000	353	0.90	0.82	0.90	-2.47	-0.90
Total	68	780	0.85	0.77	0.87	-2.43	-0.81

Table 3. *P*-values from the partition homogeneity (PH) test for several data partitions. *P*-values of 0.05 or more indicate that the partition of data sets is random, indicating congruence between the data sets. Each test done with 100 replicates of 10 random additions TBR searches and with Maxtree = 5000

Data partitions	*P*-value
*ndh*F gene vs. the rest	0.99
ORF350 vs. the rest	0.01
*rbc*L gene vs. the rest	0.01
*mat*K gene vs. the rest	0.03
*trn*L intron vs. the rest	1.00
*trn*L-*trn*F spacer vs. the rest	1.00
*trn*K 5' intron vs. the rest	0.30
*trn*K 3' intron vs. the rest	0.84
*trn*H-*psb*A spacer vs. the rest	0.30
*rbc*L-*atp*B spacer vs. the rest	0.93
Coding vs. noncoding	0.14

change was observed in the *rbcL-atpB* spacer. The g1 value was -4.50 including outgroups and -1.26 excluding outgroups. Outgroup removal had less of an effect on the *matK*, *trnL* spacer, *trnL* intron, *trnL-trnF* spacer, *trnH-psbA* spacer, and *trnK* 5' intron data sets.

In the examination of the degree of congruence between the data sets, ORF350, *rbcL*, and *matK* failed to demonstrate homogeneity against the remaining nine cpDNA regions in the partition homogeneity test ($P<0.05$; Table 3). However, the analyses of each data partition and combined data sets showed high Consistency Index (CI) values. This implies that these regions may contribute to improving the resolution of the tree, while un-rejected regions contribute to enhancing the supporting values of major clades. Homogeneity between coding regions and non-coding regions was not rejected despite the fact that the *P*-value was relatively low ($P = 0.14$).

Phylogenetic Analyses

To address the phylogeny of Magnoliaceae, we first focused on the phylogeny of the subfamily Magnolioideae and used the subfamily Liriodendroideae as an outgroup (matrix I) because all previous morphological and molecular studies (e.g., Nooteboom 1985; Azuma 1999; Kim et al 2001; Nie et al. 2008; Xia et al., 2008) agreed that there are two major subgroups are recognized in Magnoliaceae: the subfamily Magnolioideae and the subfamily Liriodendroideae. In the Maximum Parsimony (MP) analyses of matrix I, CI values for each data partition and combined data sets were relatively very high and ranged from 0.72 to 1.00 excluding uninformative sites (Table 2). All bootstrap values of the major clades recognized in the analyses of the combined data set significantly increased compared to those recognized in the analyses of each cpDNA region. All major clades recognized in the molecular analyses were supported by bootstrap values of 82-100% (Table 3). This demonstrates that the integration of data partitions positively affects the recognition of the major clades in the combined analysis.

In the combined data set of all regions, the parsimony analysis generated 68 equally parsimonious trees with 826 steps (Table 2; Fig. S1). The CI was 0.77 excluding uninformative sites, and the Retention Index (RI) was 0.86. The major clades recognized in the parsimony analysis were very different from the traditional classification system of Magnoliaceae suggested by Nooteboom (1985). Eleven major clades with high supporting values were recognized in the subfamily Magnolioideae: (1) MICHELIA clade: *Michelia*, *Elmerrillia*, sect. *Maingola*, sect. *Alcimandra*, and sect. *Aromadendron*, (2) YULANIA clade: subgen. *Yulania,* (3) GYNOPODIUM clade: *Pachylarnax*, sect. *Manglietiastrum*, and sect. *Gynopodium*, (4) KMERIA clade: *Kmeria*, (5)

THEORHODON clade: sect. *Theorhodon sensu stricto* (excluding sect. *Splendentes* which was recently separated from sect. *Theorhodon*) and sect. *Magnolia*, (6) GWILLIMIA clade: sect. *Gwillimia*, sect. *Lirianthe*, and sect. *Blumiana*, (7) TALAUMA clade: sect. *Talauma* and sect. *Splendentes*, (8) MANGLIETIA clade: *Manglietia*, (9) RYTIDOSPERMUM clade: sect. *Rytidospermum sensu stricto* (excluding *M. fraseri*, *M. macrophylla*, and *M. dealbata*) and sect. *Oyama*, (10) FRASERI clade: *M. fraseri*, and (11) MACROPHYLLA clade: *M. macrophylla* and *M. dealbata*. These major clades were similar to those recognized in a previous study of *ndhF* (Kim et al. 2001). However, the supporting values (bootstrap values and decay index) were significantly increased. All 11 major clades were supported with bootstrap values higher than 80% and decay indices of more than three additional steps. The difference among the 68 shortest trees was noted in the relationships among clades MICHELIA/YULANIA/GYNOPODIUM, KMERIA, and THEORHODON (Fig. S1). *Magnolia macrophylla* and *M. dealbata* were placed at the base of the subfamily Magnolioideae with low support. The bootstrap value to place them as basal members of the subfamily Magnolioideae was 33% and only one additional step was required to collapse the node. Supporting character classified according to cpDNA region (Fig. S2) were plotted on the branches. A large portion of supporting characters (143 changes) was used to separate the subfamily Magnolioideae from the subfamily Liriodendroideae. The *ndhF* gene was the only gene of which the characters were used to separate all major clades without any ambiguity (characters with CI = 1). In the MICHELIA clade, *Michelia*, *Magnolia* sect. *Maingola*, and *Elmerrillia* formed a robust subclade supported with a bootstrap value of 98%. Section *Alcimandra* and sect.

Table 4. Summary of supporting values for the clades in each analysis: "–" indicates bootstrap values <50% or posterior provability <0.70

Clade name	Taxa included	Subfamily Magnolioideae with subfamily Liriodendroideae as an outgroup (Matrix I)				Magnoliaceae with other four magnoliid taxa as an outgroup (Matrix II)		
		MP bootstrap 500 rep.	ML bootstrap 500 rep.	Posterior probability	Compartmentalization MP bootstrap 500 rep.	MP bootstrap 500 rep.	ML bootstrap 500 rep.	Posterior probability
A. MICHELIA	*Michelia* *Elmerrillia* sect. *Maingola* sect. *Alcimandra* sect. *Aromadendron*	86	82	1.00		80	93	1.00
B. YULANIA	subgen. *Yulania*	95	97	1.00		95	97	1.00
C. GYNOPODIUM	*Pachylarnax* sect. *Manglietiastrum* sect. *Gynopodium*	100	100	1.00		100	100	1.00
D. GWILLIMIA	sect. *Gwillimia* sect. *Lirianthe* sect. *Blumiana*	100	100	1.00		100	100	1.00
E. TALAUMA	sect. *Talauma* sect. *Splendentes*	82	100	1.00		80	82	0.98
F. THEORHODON	sect. *Theorhodon*[#] sect. *Magnolia*	100	100	1.00		100	100	1.00
G. KMERIA	*Kmeria*	100	100	1.00		100	100	1.00
H. MANGLIETIA	*Manglietia*	100	100	1.00		100	100	1.00
I. RYTIDOSPERMUM	sect. *Rytidospermum*[#] sect. *Oyama*	92	92	1.00		92	91	1.00
J. FRASERI	*M. fraseri*	100	100	1.00		100	100	1.00
K. MACROPHYLLA	*M. macrophylla* *M. dealbata*	100	100	1.00		100	100	
A+B+C		77	70	0.70	90	83	82	0.88
I+J+K		-	-	-	-	51	72	-
D+E		-	-	-	70	-	-	-
A+B+C+F+G+H+I+J+K		-	-	-	-	63	73	-

[#]*sensu stricto*

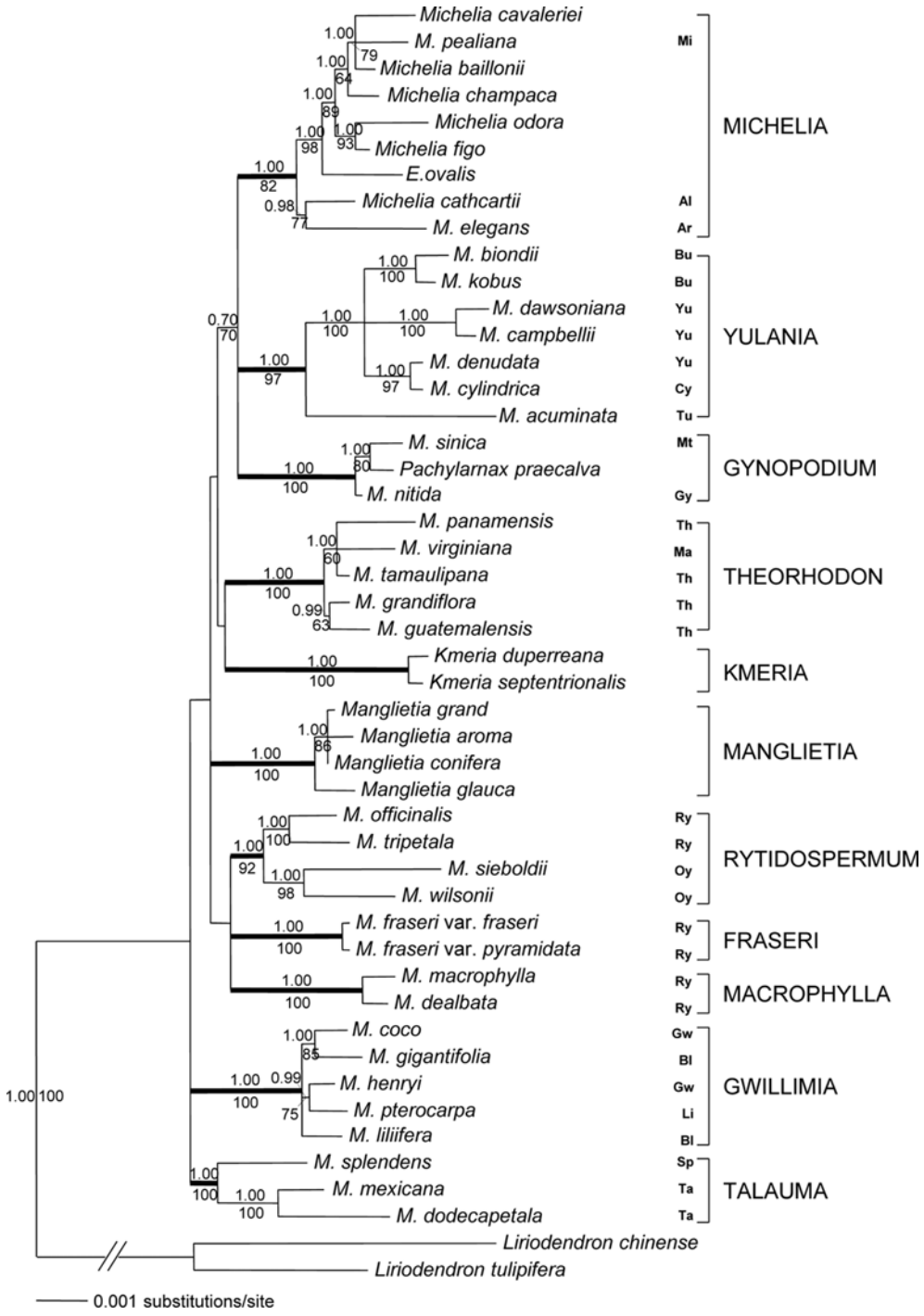


Fig. 2. Maximum likelihood tree of subgen. Magnolioideae (matrix I). Subgenus *Liriodendroideae* is used as an outgroup. Numbers above the node indicate bootstrap values (500 replicates) and those below the node indicate posterior probabilities from the Bayesian analysis. Only values above 50% (bootstrap) and 0.9 (posterior probability) are indicated. Mi; sect. *Maingola*; Al, sect. *Alcimandra*; Ar, sect. *Aromadendron*; Bu, sect. *Buergeria*; Yu, sect. *Yulania*; Cy, sect. *Cylindrica*; Tu, sect. *Tulipastrum*; Mt, sect. *Manglietiastrum*; Gy, sect. *Gynopodium*; Th, sect. *Theorhodon*; Ma, sect. *Magnolia*; Gw, sect. *Gwillimia*; Li, sect. *Lirianthe*; Bl, sect. *Blumiana*; Ta, sect. *Talauma*; Sp, sect. *Splendentes*; Ry, sect. *Rytidospermum*; Oy; sect. *Oyama*.

Aromadendron were a sister group of *Michelia- Elmerrillia*- sect. *Maingola* in the MICHELIA clade. Members of *Magnolia* subgen. *Yulania* formed a well-defined clade. *Magnolia acuminata,* the sole North American species in *Magnolia* subgen. *Yulania*, was located at the base of the YULANIA clade. In the remaining taxa of the YULANIA clade, three subclades were clearly recognized with 100% bootstrap values. Section *Cylindrica* of subgen. *Yulania*

(Spongberg 1998) was strongly tied to *M. denudata*, which belongs to sect. *Yulania* of subgen. *Yulania*. Section *Splendentes*, recently described by Vázquez-G. (1994), was positioned as a sister group of sect. *Talauma*. Section *Rytidospermum sensu stricto* excluding *M. macrophylla, M. dealbata*, and *M. fraseri* showed close affinity with sect. *Oyama* to form a robust clade. *Magnolia macrophylla* and *M. dealbata*, which are clearly separated from other species of sect. *Rytidospermum*, were linked as a highly supported group with a 100% bootstrap value and placed at the base of the subfamily Magnolioideae. Two varieties of *M. fraseri* were also separated from the remaining species of sect. *Rytidospermum*, and these form a distinctive clade.

Maximum Likelihood (ML) analysis generated a tree having similar topology to that of the MP trees (Fig. 2). Same as MP analysis, eleven major clades of subfamily Magnolioideae

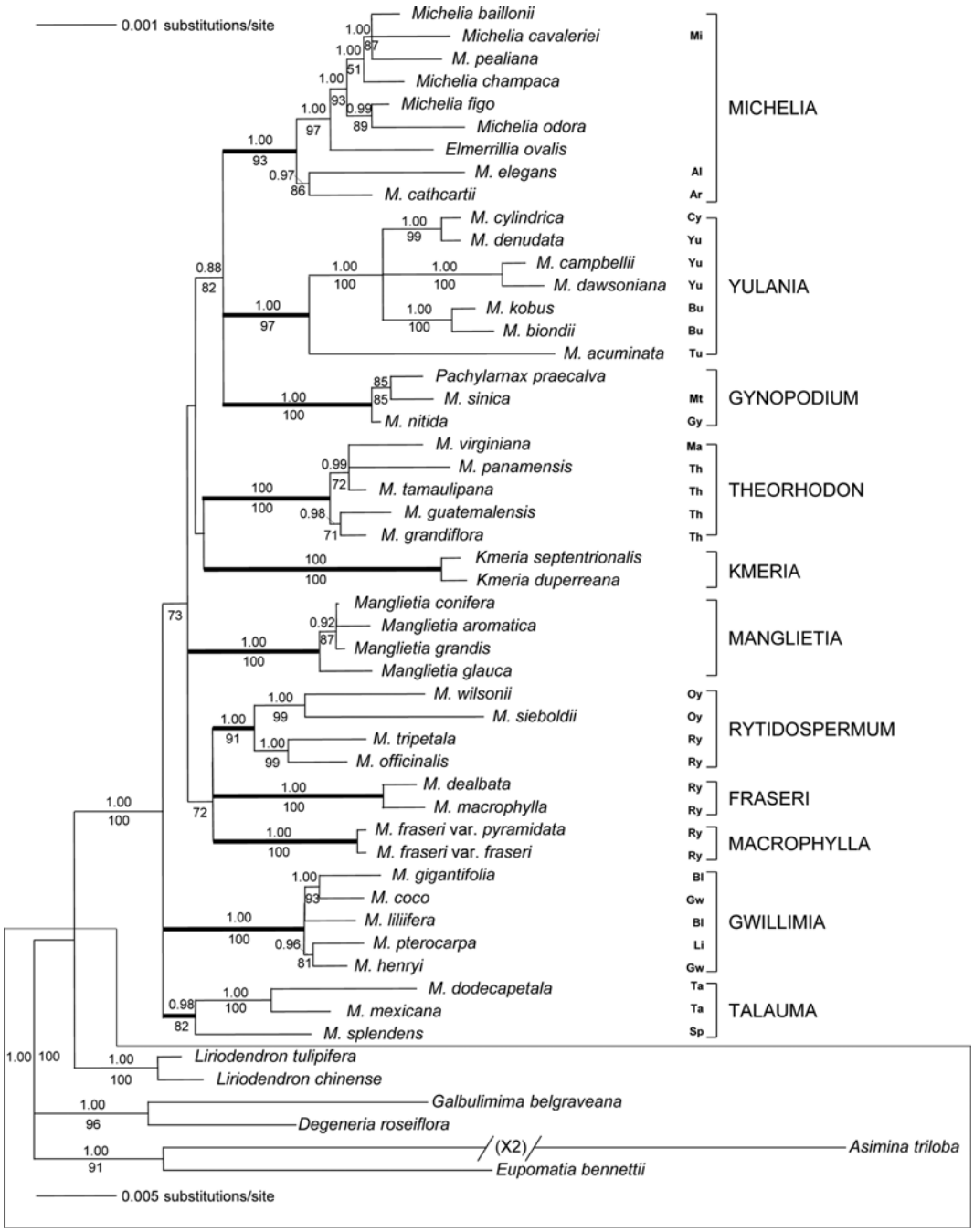


Fig. 3. Maximum likelihood tree of Magnoliaceae (matrix II). Four other magnoliid taxa are used as an outgroup. Numbers above the node indicate bootstrap values (500 replicates) and those below the node indicate posterior probabilities from the Bayesian analysis. Only values above 50% (bootstrap) and 0.9 (posterior probability) are indicated. Abbreviations are identical to those in Fig. 3.

were recognized with bootstrap values of 82%~100% for each sublineage (Table 4). The relationships among these major clades were slightly different: 1) The GWILLIMIA and TALAUMA clades were both sisters to all other sublineages of the subfamily Magnolioideae, and 2) RYTIDOSPERMUM, FRASERI, and MACROPHYLLA form a clade. However, these relationships among the major clades in the ML tree were not strongly supported. All of the relationships which differed from those in the MP analysis received less than 50% support.

The posterior probabilities from the Bayesian analysis were well matched to the bootstrap values from the MP and ML analyses (Table 4; Fig. 2). All of the eleven major clades received a posterior probability score of 1.00. Regarding the relationships among the major clades, only the clade of MICHELIA, YULANIA, and GYNOPODIUM received a posterior probability score of 0.7, while the other relationships recognized in the MP and ML analyses were assigned very low posterior probabilities.

We also tested the effect of the outgroup with the second matrix containing four other magnoliid taxa as an outgroup: *Degeneria roseiflora* (Degeneriaceae), *Asimina triloba* (Annonaceae), *Galbulimima belgraveana* (Himantandraceae), and *Eupomatia bennettii* (Eupomatiaceae) (matrix II). The result equally showed eleven major clades in the subfamily Magnolioideae in the MP, ML, and Bayesian analyses with high supporting values (Table 4; Figs. 3 and S3). Like the result from matrix I, the clade of MICHELIA/YULANIA/GYNOPODIUM received relatively high supporting values of 83% and 82% bootstrap values in the MP and ML analyses, respectively, and a posterior probability value of 0.88. Additional relationships among the major clades were suggested in the analyses of this matrix, although the supporting values of the MP and ML analyses are not high enough, as follows: 1) the clade of RYTIDOSPERMUM, FRASERI, and MACROPHYLLA (51% in MP and 72% in ML) and 2) the clade of subfamily Magnolioideae except GWILLIMIA and TALAUMA (63% in MP and 73% in ML). However, both clades were given posterior probabilities of less than 0.7 in the Bayesian analysis (Table 4).

Compartmentalization

To determine the relationships among the major clades which were poorly resolved in the analysis, a compartmentalization analysis was performed (Mishler 1994; Mishler et al. 1998; Soltis et al. 2000) with matrix I. Two equally parsimonious trees were generated in the global MP analysis by means of an exhaustive search for compartmentalization (CI = 0.61 excluding uninformative sites, with RI = 0.67). Trees generated by compartmentalization differed only in the relationships among the MICHELIA, YULANIA, and GYNOPODIUM clades (Fig. S4). Significant increases of the bootstrap supporting value were observed for the MICHELIA/YULANIA/GYNOPODIUM clade and the GWILLIMIA/TALAUMA clade in comparison with a normal parsimony analysis; the values increased from 77% to 90% for the MICHELIA/YULANIA/GYNOPODIUM clade and from 45% to 70% for the GWILLIMIA/TALAUMA clade. The bootstrap value which supports the basal placement of *M. macrophylla* and *M. dealbata* in the subfamily Magnolioideae increased slightly (from 33% to 46%) but remained at less than 50%. A local analysis of the MICHELIA clade with the YULANIA/GYNOPODIUM outgroup generated a different local tree of the MICHELIA clade from the parsimony analysis. *Magnolia elegans*, a member of sect. *Aromadendron*, was clearly positioned at the base of *Michelia*-*Elmerrillia*-sect. *Maingola*-sect. *Alcimandra* with a 95% bootstrap value. Local analyses of other clades produced a topology and supporting values similar to those in the parsimony analysis.

Discussion

Phylogeny of Magnoliaceae

Phylogenetic analyses of 10 chloroplast DNA regions with various inference methods confirmed that there are eleven major clades in the subfamily Magnolioideae. This result is in good agreement with the findings of previous molecular studies based on matrices containing fewer samples or fewer DNA regions (Kim et al. 2001; Azuma et al. 2001; Nie et al. 2008). However, the supporting values for these clades in this study are dramatically increased in comparison to those from the previous studies. Each of these eleven clades was highly supported with bootstrap values that exceeded 80% in both MP and ML analyses and with posterior probabilities exceeding 0.98 for Bayesian interference. Regarding the relationships among the major clades, the clade of MICHELLIA, YULANIA, and GYNOMODIUM gained a high supporting value. However, other relationships among the major clades were weakly supported. Potential close relationships among 1) TALAUMA and GWILLIMIA and 2) RYTIDOSPERMUM, FREASERI, and MACROPHYLLA were suggested by a compartmentalization analysis of matrix I and a ML analysis of matrix II, respectively, although the supporting values for these clades were not high enough (summarized in the Table 4). Species included in MACROPHYLLA were placed at the base of the subfamily Magnolioideae in a previous *ndhF* analysis (Kim et al. 2001). However, in this study, the basal groups were GWILLIMIA and TALAUMA or a clade of GWILLIMIA + TAMALUMA. It appears that the phylogenetic placement of MACROPHYLLA in the *ndhF* tree occurred due to the long-branch attraction (Felsenstein 1978) between

MACROPHYLLA and *Liriodendron*.

Reconsideration of Selected Morphological Characters

Molecular phylogenetic trees produced by extensive DNA analyses have challenged the traditional classification system based on morphology (Dandy 1927; Law 1984, 1996; Nooteboom 1985; Chen and Nooteboom 1993). Difficulties in reconstructing phylogenies in the family Magnoliaceae are due to lack of synapomorphic changes shared by major groupings, although several characters were proposed for delimitating tribes, genera, and subgenera, including axillary flowers in the tribe Michelieae, capsule fruits in *Pachylarnax*, unisexual flowers in *Kmeria*, four or more of ovules per carpel in *Manglietia*, concrescence of the carpel in subgen. *Talauma*, and latrorse anther dehiscence in subgen. *Yulania*.

The genus *Pachylarnax* has been considered as a separate genus in taxonomic systems based on morphology (Dandy 1927; Law 1984, 1996; Nooteboom 1985; Chen and Nooteboom 1993; Xia et al. 2008) because its fruits were capsule type, which is a unique feature in Magnoliaceae. This term was erroneously used for *Pachylarnax* in previous descriptions (Dandy 1927; Law 1984; Nooteboom 1985). The capsular fruit, which was first described by de Candolle (1813), is defined by Spjut as a type of rhexocarpic fruit which derives from only one pistil and always from one flower (1994). Although the fruit of *Pachylarnax* was not included in his study, Spjut (1994) defined the fruits of all Magnoliaceae as multiple fruits which derived from more than one pistil. Because *Pachylarnax* clearly has many pistils in a flower, the fruits should be multiple fruits. In the molecular tree, *Pachylarnax* was strongly tied with *M. sinica*, which is monotypic species of the sect. *Manglietiastrum* of *Magnolia*. A careful examination confirmed that the fruits of *Pachylarnax* resemble the upper portion of *Manglietiastrum* fruits and that their carpels dehisce ventrally. The carpels of all other taxa in Magnoliaceae dehisce dorsally or circumscissily (except for *Kmeria duperreana*, of which the carpel opens ventrally).

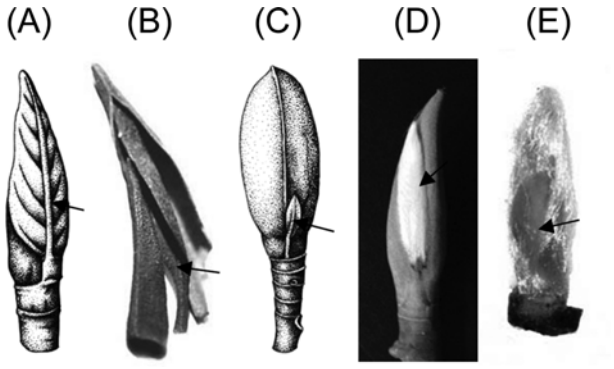


Fig. 4. Open (A-C) and conduplicate (D-E) prefoliation in Magnoliaceae. (A) *Parakmeria yunnanensis* (GYNOPODIUM clade; sect. *Gynopodium*), redrawn from Law (1996). (B) *Pachylarnax praecalva* (GYNOPODIUM clade). (C) *M. sinica* (GYNOPODIUM clade; sect. *Manglietiastrum*), redrawn from Law (1996). (D) *M. obovata* (RYTIDOSPERMUM clade; sect. *Rytidospermum*). (E) *M. kobus* (YULANIA clade; sect. *Yulania*). Arrows indicate the midrib of prefoliation.

Open leaf prefoliation (Fig. 4) is another morphological feature that supports the close affinity among *Pachylarnax*, sect. *Manglietiastrum*, and sect. *Gynopodium* (Figlar and Nooteboom 2004), which constitutes the GYNOPODIUM clade in all molecular trees generated in this study (Figs. 2, 3, S1, and S3) and in previous molecular studies (Azuma et al 1999; Kim et al 2001; Xia et al. 2008). All other members of the family show conduplicate leaf prefoliation (Figlar and Nooteboom 2004; Fig. 4). This indicates that open prefoliation serves as a prominent synapomorphic character state that defines the GYNOPODIUM clade.

The concrescence of carpels was considered as an important character state to define subgen. *Talauma* in previous studies (Law 1984; Nooteboom 1985; Chen and Nooteboom 1993). However, the subgen. *Talauma* was not recognized in the molecular trees (Figs. 2, 3, S1, and S3). Sections in the subgen. *Talauma*, sects. *Aromadendron*, *Manglietiastrum*, *Blumiana*, and *Talauma*, were disposed in various lineages. Therefore, the concrescence of carpels no longer serves as a synapomorphic character state to define the subgen. *Talauma*. It is sometimes difficult to recognize connate carpels in Magnoliaceae because carpels are free in a flower and become connate as the fruit ripens (Fig. 5). As a similar circumscription, the shape of the fruits has been classified as cylindrical or elliptic without a clear distinction. In contrast to these obscure characters, the shape of the fruit can be judged by the axis of the fruit. A thickened and less elongated axis indicates fruit with an elliptic or ovoid shape because many carpels are located in the central part of the axis; this type of fruit sometimes becomes connate. A thin and elongated axis makes the fruit cylindrical in shape. The interpretation of the fruit shape given this concept is in good agreement with molecular data. All members of the MICHELIA/YULANIA/GYNOPODIUM clade have cylindrical fruit except for sect. *Aromadendron*, of which the fruits are connate and elliptic or ovoid in shape. However, the axis of the *Aromadendron* fruits is very thin in comparison to other groups of Magnoliaceae having connate carpels (Fig. 5). Therefore, the fruit of *Aromadendron* should be regarded as cylindrical because it has a thin axis, like the other members in the MICHELIA clade. The fruits of sect. *Oyama* have been described as cylindrical (Chen and Nooteboom 1993). However, their axes are not very elongated and the number of carpels is relatively low. Therefore, the fruits of sect. *Oyama* should be considered

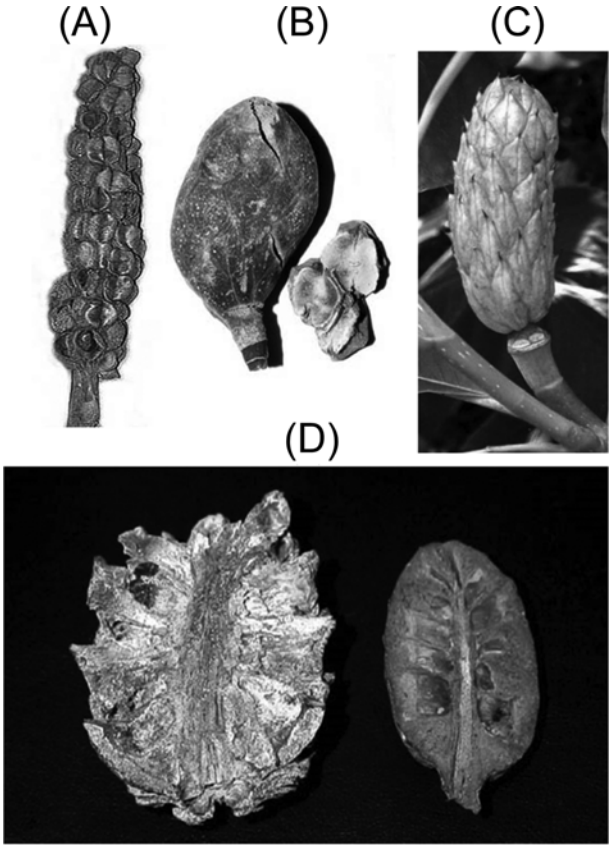


Fig. 5. Fruit shape in the subfamily Magnolioideae. (A) Cylindrical fruit of *M. griffithii* (MICHELIA clade; sect. *Maingola* of subgenus *Yulania*). (B) Ovoidal shape; however, basically cylindrical fruit of *M. elegans* (MICHELIA clade; sect. *Aromadendron*). (C) Ellipsoidal fruit of *M. dealbata* (MACROPHYLLA clade; sect. *Rytidospermum*). (D) Longitudinal section of fruits: *M. macrophylla* (left; MACROPHYLLA clade; sect. *Rytidospermum*) having a thickened axis and *M. elegans* (right; MICHELIA clade; sect. *Aromadendron*) having a thin axis.

as ellipsoid when interpreted based on the axis.

The position of the flower in the twig (terminal/axillary) is considered to be an important character, like the anther states of dehiscence (introrse/lateral) from a traditional point of view (Dandy 1927; Law 1984, 1996; Nooteboom 1985; Chen and Nooteboom 1993). Axillary flowers serve as a key character state to distinguish the tribe Michelieae and laterally dehisce for subgen. *Yulania* of the genus *Magnolia*. However, Nooteboom (1985) adopted a different view regarding the position of the flowers; flowering buds of *Michelia* and *Elmerrillia* are brachyblasts and their flowers are terminal on the brachyblasts in the axils. Therefore, the position of the flower has lost its importance due to his different viewpoint regarding this character. Proleptic growth serves as a synapomorphic character state for the MICHELIA clade and this character is evidence that supports the combining of *Michelia* with *Magnolia* (Figlar 2000; Figlar and Nooteboom 2004).

Although *Manglietia* and *Kmeria* have been recognized as distinct genera because *Manglietia* has four or more ovules in each carpel while *Kmeria* has a unisexual flower, many exceptions have been reported (Nooteboom 1985; Chen and Nooteboom 1993; Law 1996). Four or more ovules in each carpel, used to define *Manglietia*, is a characteristic also found in *Pachylarnax* (Nooteboom 1985), some species of *Michelia* such as *Mich. odora* (Chen and Nooteboom 1993) and *Mich. baillonii* (Law 1996), and sect. *Gynopodium* (Chen and Nooteboom 1993). Given that sect. *Gynopodium* of *Magnolia* is known to have androdioecious flowers, the importance of unisexual flowers should be emphasized less as a unique character state which supports *Kmeria* as a distinct genus.

Classification of Magnoliaceae

We clearly recognized 11 major clades in the subfamily Magnolioideae based on multiple cpDNA regions using samples representing all published genera and sections of Magnoliaceae. If we recognize 11 major clades in the subfamily Magnolioideae without recognizing the relationships among them, we may consider two possible classification systems (proposed as system I and II in Fig. 6). The categorical ranks themselves are only mental constructs and they have only relative meanings (Judd et al. 2008). Therefore, these clades can be either sections or genera as a primary rank under the subfamily Magnolioideae. The recent classification system devised by Figlar and Nooteboom (2004) recognized three subgenera and eleven sections. These sections precisely match major clades, similar to the proposed system I. However, molecular data does not support these three subgenera; their subgen. *Yulania* is a paraphyletic group and the subgen. *Magnolia* is a polyphyletic group in the molecular phylogenetic tree of this study and in earlier studies (Kim et al. 2001; Azuma et al. 2001; Nie et al. 2008). The classification system proposed for the flora of China (Xia and Liu 2008) recognized 13 genera distributed in China. This system is comparable to the proposed system II (Fig. 6). However, it appears that the recognition of only one genus (*Magnolia*) in Magnolioideae is more feasible than the recognition of several independent genera because 1) the basal group of subfamily Magnolioideae is a member of the genus *Magnolia* (of Nooteboom 1984) in most molecular phylogenetic studies; 2) genera *Michelia*, *Elmerrillia*, and *Pachylarnax* in the past classification system were not clearly recognized as independent major clades in the molecular tree but were included in each of their respective major groups; 3) two subfamilies in Magnoliaceae have nearly identical genetic divergence rates, although *Kmeria* and *Manglietia* serve as one of the major

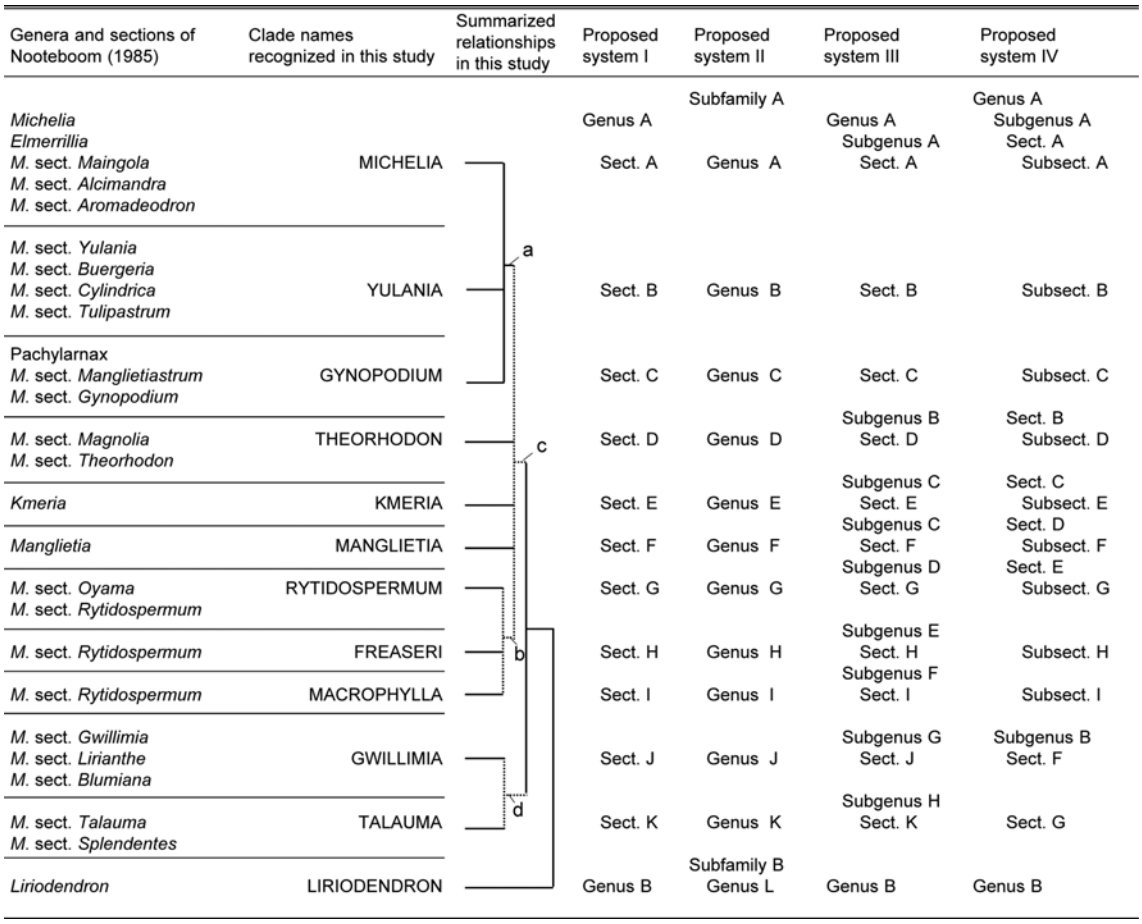


Fig. 6. Taxonomic structures of possible classification systems based on the summary of phylogenetic analyses conducted as part of this study. a-d, potential relationships among major clades. a, a relatively highly supported clade from all analyses in this study; b and c, clades recognized from the ML analysis of matrix II (with bootstrap values of 72% and 73%, respectively); d, a clade recognized from the compartmentalization analysis of matrix I (bootstrap value of 70%).

clades in each respective case (Liriodendroideae and Magnolioideae have overall sequence divergence rates of 0.88 and 0.95 in ten chloroplast regions: Table 2); and 4) the taxonomic importance of the key characters defining genera in the previous systems has diminished due to the reinterpretation of the morphological characters and the finding of new taxonomic evidence.

Although phylogenetic relationships among 11 major clades are not fully resolved in this study, we have provided potential molecular evidence of new classification systems, such as the proposed system III or system IV (Fig. 6), which reflect the phylogenetic relationships among the 11 major clades in the family. This classification system serves better if the taxonomic structure of the system reflects the phylogenetic relationships. Phylogenetic relationships in the family Magnoliaceae will be further confirmed by genome-level data such as a comparison of whole chloroplast genomes and/or a massive comparison of nuclear single-copy genes.

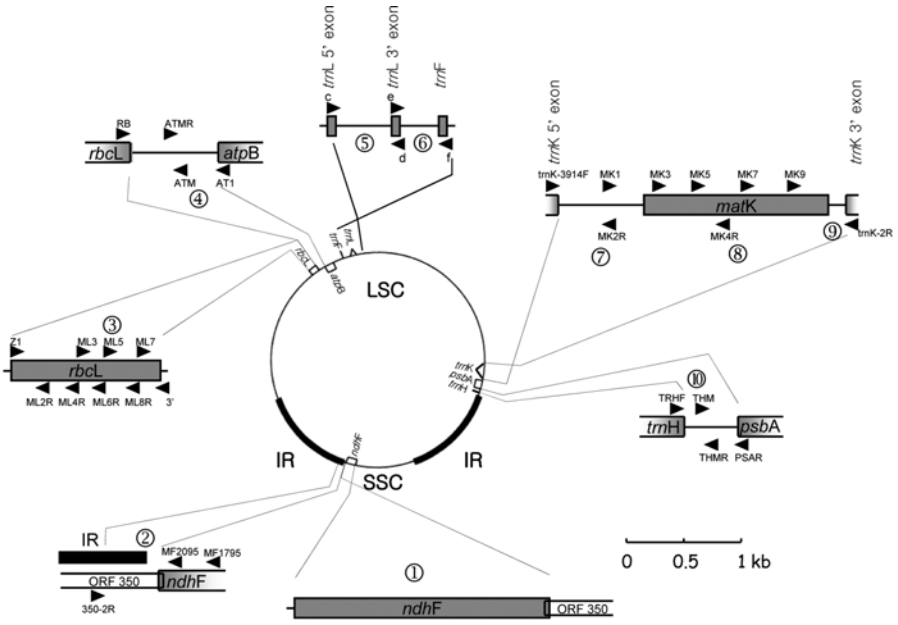
Materials and Methods

Taxa Sampling

Forty-eight taxa were carefully chosen to represent 1) all genera and sections of Magnoliaceae according to Nooteboom (1985); 2) two recently recognized sections of the genus *Magnolia*, sect. *Splendentes* (Vázquez-G 1994) and sect. *Cylindrica* (Spongberg 1998); and 3) all major clades generated by a previous *ndhF* analysis (Kim et al. 2001). Each major lineage of the family was represented by at least two terminal taxa (Table S1). In this study, we adopted the scientific names listed in the bibliographic checklist of Magnoliaceae by Frodin and Govaerts (1996) and the classification system by Nooteboom (1985) because the recognition of genera in the recent classification systems (e.g., Figlar and Nooteboom 2004; Xia et al. 2008) remains controversial.

Choice of chloroplast DNA Regions

Ten cpDNA regions were selected for this study (Fig. 7). In addition to *ndhF* and *matK*, which have been frequently used for infra-familial phylogenetic relationships (Johnson and Soltis 1994, 1995; Olmstead and Palmer 1994; Steele and Vilgalys 1994; Kim and Jansen 1995;



MF1795	5'-GTGACAAATGCAATTTATTCA-3'	Kim et al. (2001)
MK2095	5'-AATCAAATATGTGGGGGGC-3'	*
350-2R	5'-GGAAGAAAAGGAGGATCCGG-3'	Kim et al. (2001)
Z1	5'-ATGTCACCACAAACAGAAACTAAAGCAAGT-3'	Morgan and Soltis (1993)
ML3	5'-CCAGGGAATTGGGAGTTC-3'	*
ML5	5'-GGGGAACGGGACATCACT-3'	*
ML7	5'-GGAGGAACTTTAGGACACCC-3'	*
ML2R	5'-TTCAATTTATCTCTCTCAACTTGG-3'	*
ML4R	5'-GAACTCCCAATTCCCTGG-3'	*
ML6R	5'-AGTGATGTCCCGTTCCCC-3'	*
ML8R	5'-GGGTGTCCTAAAGTTCCTCC-3'	*
3'	5'-CGGCTCAATCCTTTTAGTAAAAGATTGGGCCGAG-3'	Morgan and Soltis (1993)
trnK-3914F	5'-GGGGTTGCTAACTCAACGG-3'	Plunkett et al. (1996)
MK1	5'-ACGAATGTGTAGAAGAAACGG-3'	*
MK3	5'-CGATTTTTTACGAACCTATGG-3'	*
MK5	5'-CACTGCTGGATACAAGATGC-3'	*
MK7	5'-AAATGGAAATCTCACCTTGTC-3'	*

MK9	5'-CTTCGACTTTCGTGTGCTAG-3'	*
MK2R	5'-GATCCACTCACAGGAGAACC-3'	*
MK4R	5'-TTTACGGAGAAACACTAATACG-3'	*
trnK-2R	5'-AACTAGTCGGATGGAGTAG-3'	Plunkett et al. (1996)
c	5'-CGAAATCGGTAGACGCTACG-3'	Taberlet et al.(1991)
e	5'-GGTTCAAGTCCCTCTATCCC-3'	Taberlet et al.(1991)
d	5'-GGGGATAGAGGGACTTGAAC-3'	Taberlet et al.(1991)
f	5'-ATTGAACTGGTGACACGAG-3'	Taberlet et al.(1991)
trnL4	5'-KAATTTTGARCCACTGAC-3'	*
TRHF	5'-CGCATGGTGGATTCACAATC-3'	Sang et al. (1997)
THM	5'-CAATAATGTTTTCTTAAGTTGAAAT-3'	*
THMR	5'-CTTTATTTCAACTTAAGAAAACATT-3'	*
PSAR	5'-AGACCTAGCTGCTATCGAAG-3'	Tobacco cpDNA
AT1	5'-AGAACCAGAAGTAGTAGGAT-3'	Tobacco cpDNA
ATM	5'-CATGGATGAATTCTGCCTA-3'	Azuma et al. (1999)
ATMR	5'-AGTGATATATGTTGTATATGTAAATCC-3'	*
RB	5'-ACACCACTTTGAATCCAAC-3'	Tobacco cpDNA

Fig. 7. Positions and sequences of primers used for PCR and the sequencing of the 10 cpDNA regions used in this study. Arrows indicate the relative position of primers. j *ndhF*; k ORF350 (partial); l *rbcL*; m *rbc*L-*atp*B spacer; n *trnL* intron; o *trnL-trnF* spacer; p *trnK* 5' intron; q *matK*; r *matK* 3' intron; s *trnH-psbA* spacer; *primers newly designed in this study; LSC, large single copy; SSC, small single copy; IR, inverted repeat.

Clark et al. 1995; Olmstead and Reeves 1995; Scotland et al. 1995; Johnson et al. 1996; Soltis et al. 1996; Bohs and Olmstead 1997; Oxelman et al. 1999), *rbcL* was chosen because it is the most widely used gene in angiosperms (Chase et al. 1993; Clegg 1993; Palmer et al. 1988). The vast number of taxa in the *rbcL* analysis enables us to compare the rates of nucleotide substitution in Magnoliaceae with those previously reported from various angiosperm families. Because molecular studies of Magnoliaceae have demonstrated that the rates of nucleotide substitution in the family are significantly low in comparison to those in other angiosperm families (Qiu et al. 1995b; Azuma et al. 1999; Kim et al. 2001), the *trnL* intron, *trnL-F* spacer, *rbcL-atpB* spacer, *trnH-psbA* spacer, *trnK* 5' intron, and *trnK* 3' intron were added because introns and spacers are generally known to change more rapidly than coding regions (Taberlet et al. 1991; Fragan et al, 1994; Gielly and Taberlet 1996; Gielly et al. 1996; Kim et al. 1996; Azuma et al. 1999; Richardson et al. 2000). ORF 350 adjacent to the 3' downstream region of *ndhF* was also included because high variation of the sequences in this family was observed in the analysis conducted in the *ndhF* study (Kim et al. 2001).

DNA Extraction, PCR Amplification and Sequencing

Total genomic DNA was isolated from leaves, either fresh, dried with silica gel, or from herbarium specimens using a standard hexadecyltrimethylammonium bromide (CTAB) extraction method (Doyle and Doyle 1987) or using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). Total DNA extracted by CTAB was further purified with Geneclean Kit II (BIO101, Carlsbad, California, USA) for a polymerase chain reaction (PCR). All DNA samples were extracted from the same materials used in the previous *ndhF* study (Kim et al. 2001). DNA amplifications by the polymerase chain reaction (PCR) method were performed using a 9600 Thermal Cycler (Perkin Elmer, Norwalk, Connecticut, USA) as described in the *ndhF* analysis (Kim et al. 2001). The primer pairs used for PCRs were MF1795 and ORF-2R for ORF350; Z1, ML6R, ML3, and 3' for the *rbcL* gene; RB and AT1 for the *rbcL-atpB* spacer; 49317 (c) and 50272R (f) for the *trnL* intron and *trnL-trnF* spacer; *trnK*-3914F, MK4R, MK5, and *trnK*-3R for the *trnK* intron (including *matK* gene); and TRHF and PSAR for the *trnH-psbA* spacer (Fig. 1). PCR products were purified by precipitation using a 20% PEG/2.5 mol/L NaCl solution, followed by washing in 80% and 95% ethanol (Soltis and Soltis 1997). Finally, DNA pellets were dissolved in distilled H_2O. Purified PCR DNA was used in cyclic sequencing reactions that were conducted using the ABI PRISM BigDye Terminator Cyclic Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). Conditions for cyclic sequencing and the protocol of ethanol precipitation for PCR product purification followed the recommendations in the manufacturer's manual. In addition to PCR primers, internal primers were used to

determine the sequences in both directions (Fig. 7). For degraded DNA extracted from old herbarium specimens, target DNA regions were divided into several overlapping segments and amplified by different combinations of internal primers to obtain complete sequences.

Sequence Alignment and Phylogenetic Analyses

Proofreading and editing of each sequence were performed using Sequencher 4.9 (Gene Codes Corporation, Ann Arbor, Michigan, USA). The sequence boundaries of all regions were determined by a comparison with recently published whole cp genomes of Magnoliaceae (*Liriodendron tulipifera*: Cai et al. 2006). Sequences were aligned using the Clustal X program (Thompson et al. 1997) with default settings, and then finally adjusted using the naked eye. No serious aligning problems arose in any region due to the relatively low base substitution rates.

Four inversions between inverted repeated sequences (positions 247, 606, and 1519 from the 5' end of the *matK* gene, and position 373 from the 5' end of the *trnH-psbA* spacer) were substituted for their complementary sequences. These inversions were not coded as binary characters in this study because they frequently occur in parallel regardless of the lineage (Azuma et al. 1999). In particular, similar inversions were found heterogeneously even at the infra-specific level in a study of *Fagopyrum* (Osako and Ohnishi 2000). The *ndhF* sequences of 48 taxa from the previous study (Kim et al. 2001) and the *trnK* 5' intron, *matK* gene, *trnK* 3' intron, *trnH-psbA* spacer, and *rbcL-atpB* sequences of 17 taxa from the study of Azuma et al. (1999) were adopted for the combined analysis (Table S1).

For both of the matrices (matrix I and II; see results), the maximum parsimony (MP), maximum likelihood (ML), and Bayesian analysis (Huelsenbeck 2001) were performed to reconstruct the phylogeny of Magnoliaceae. MP was performed using PAUP* ver. 4.01b10 (Swofford 2001) for various combinations of data sets: each partition of 10 DNA regions, a combination of coding genes (excluding ORF350), noncoding regions, and combined data set of all regions. To find the shortest tree, a heuristic search algorithm with MULPARS, COLLAPSE zero-length branches, tree bisection-reconnection (TBR) branch swapping, and 1000 random additions to search for multiple islands of trees (Maddison 1991) while saving all of the most parsimonious trees was adopted with ACCTRAN optimization and a setting of maxtrees = 5000. Bootstrapping (Felsenstein 1985) was performed in order to assess the degree of support of each node. A bootstrap analysis was carried out for 500 replicates by heuristic searches with 10 random addition and TBR branch-swapping options. Various statistics were compiled for each data partition and for the combined data using PAUP* ver. 4.01b10 (Swofford 2001). Included were the number of characters examined, the percentage of variable sites, the percentage of informative sites, the maximum sequence divergence (Kimura's $K\times100$), the GC content, the number of most parsimonious trees, the associated tree lengths, consistency indexes (CI), retention indexes (RI), and skewness (g1) in one hundred thousand random trees. Calculations of skewness (g1) were repeated with the exclusion of outgroup taxa (two species of *Liriodendron*).

For the ML analysis, MODELTEST (version 3.06; Posada and Crandall 1998) was used to determine the appropriate model of sequence evolution for this data set. The chosen model (GTR + I + Γ) by the Akaike Information Criterion (AIC) was applied to the data matrix using PAUP* ver. 4.01b10 (Swofford 2001). The ML analysis was conducted using the parameter values suggested in MODELTEST (-lnL = 17866.72; A:C:G:T = 0.3030:0.1774:0.1902:0.3293; P_inv = 0.6756; Shape = 0.9992) and the 68 most parsimonious trees as starting trees in a heuristic search with TBR branch swapping. The Bayesian analysis was performed using MrBayes 4.0 (Heulsenbeck, 2001). We ran four chains of Markov Chain Monte Carlo (MCMC), sampling every 1000 generations for 1,000,000 generations, starting with a random tree. Stationarity was reached at approximately generation 40,000; thus, the first 40 trees were the "burn in" of the chain, and phylogenetic inferences are based on those trees sampled after generation 40,000.

A compartmentalization analysis was performed (Mishler 1994; Mishler et al. 1998; Soltis et al. 2000) with the following steps using MP: (1) recognizing well-supported major clades using a normal analysis (heuristic search; see options of above normal analysis), (2) reconstructing the hypothetical taxonomic units (HTUs) of each major clade using the 'state for internal nodes' option for the described tree in the PAUP*b4a program, (3) creating a data matrix using HTUs, (4) analyzing the HTU matrix using a more intensive search method (an exhaustive search) than a normal analysis, (5) performing a bootstrap analysis using 500 replicates with branch and bound searches, (6) performing local analyses within the compartment using branch and bound searches (except for clades A, B, and C, for which a heuristic search was used to obtain the tree topology) and 500 replicates of bootstrap analyses with heuristic searches with the TBR option, and (7) pasting the results of the analysis within each clade onto the backbone of the HTU analysis. As an estimate of the states of the most recent common ancestor of all local operational taxonomic units (OTUs), HTU is likely to have a much shorter terminal branch with respect to the global analysis, which in turn can have a beneficial effect globally by reducing long-branch attraction (Mishler et al. 1998). In addition to these advantages of compartmentalization at the global level, local analyses will be better because these enable us to perform more intensive searches with reduced numbers of taxa. Moreover, this method suppresses homoplasy, which can change the local topology due to long-branch attractions with distant outgroups (Mishler et al. 1998).

Supporting Information

Additional Supporting Information is in the online version of this article:

Fig. S1. One of the 68 most parsimonious trees based on the combined data set of 10 cpDNA regions of the subgen. Magnolioideae (matrix I).
Fig. S2. Summary of the strict consensus tree from 68 equally parsimonious trees (matrix I).
Fig. S3. One of the 253 most parsimonious trees based on the combined data set of 10 cpDNA regions of Magnoliaceae (matrix II).
Fig. S4. The phylogenetic tree generated by compartmentalization analyses of 10 cpDNA sequences of Magnoliaceae.
Table S1. Species included in the combined analyses of 10 cpDNA regions in the Magnoliaceae and outgroup taxa.

Acknowledgements

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF 2010-0008996). We thank Hans P. Nooteboom and Richard B. Figlar for the valuable discussions of the taxonomic implications of morphological characters. We also thank Kyounghyeon Kim for illustrating some of the figures.

Author's Contributions

SK determined the sequences of target DNA regions and conducted phylogenetic analyses of DNA sequences with the supports from YS. SK wrote and revised the manuscript according to the sugesstions

made by YS. Both authors agreed on the contents of the paper and post no conflicting interest.

References

Azuma HL, Thien B, Kawano S (1999) Molecular phylogeny of Magnolia (Magnoliaceae) inferred from cpDNA sequences and evolutionary divergence of floral scents. J Plant Res 112:291–306

Azuma H, Garcia-Franco JG, Rico-Gray V, Thien LB (2001) Molecular phylogeny of Magnoliaceae, the biogeography of tropical and temperate disjunctions. Amer J Bot 88:2275–2285

Barkman TJ, Chenery JG, McNeal JR, Lyons-Weile J, Ellisens WJ, Moore G, Wolfe AD, dePamphilis CW (2000) Independent and combined analyses of sequences from all three genomic compartments converge on the root of flowering plant phylogeny. Proc Natl Acad Sci USA 97:13166–13171

Bohs L, Olmstead RG (1997) Phylogenetic relationships in *Solanum* (Solanaceae) based on *ndhF* sequences. Syst Bot 22:5–17

Borsch T, Hilu KW, Quandt D, Wilde V, Neinhuis C, Barthlott W (2003) Non-coding plastid *trnT-trnF* sequences reveal a highly supported phylogeny of basal angiosperms. J Evol Biol 15:558–567

Cai Z, Penaflor C, Kuehl JV, Leebens-Mack J, Carlson J, dePamphilis CW, Boore JL, Jansen RK (2006) Complete chloroplast genome sequences of *Drimys, Liriodendron*, and *Piper*: Implications for the phylogeny of magnoliids and the evolution of GC content. BMC Evol Biol 6:77

de Candolle AP (1813) Théorie élémentaire de la botanique, Chez Déterville, Paris

Chase MW and 31 authors (1993) Phylogenetics of seed plants: An analysis of nucleotide sequences from the plastid gene *rbcL*. Ann Missouri Bot Gard 80:528–580

Chen BL, Nooteboom HP (1993) Notes on Magnoliaceae III: The Magnoliaceae of China. Ann Missouri Bot Gard 80:999–1104

Clark LG, Zhang W, Wendel JF (1995) A phylogeny of the grass family (Poaceae) based on *ndhF* sequence data. Syst Bot 20:436–460

Clegg MT (1993) Chloroplast gene sequences and the study of plant evolution. Proc Natl Acad Sci USA 90:363–367

Dandy JE (1927) The genera of Magnoliaceae. Kew Bulletin 1927:275–264

Dandy JE (1971) The classification of the Magnoliaceae. Newsletter of the American Magnolia Society 8:3–6

Dandy JE (1978) Revised survey of the genus *Magnolia* together with *Manglietia* and *Michelia*, In NG Treseder, Magnolias. Faber and Faber, London, pp 29–37

Dilcher DL, Crane PR (1984) *Archaeanthus*: An early angiosperm from the Cenomanian of the western interior of North America. Ann Missouri Bot Gard 71:351–383

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19:11–15

Felsenstein J (1978) Case in which parsimony and compatibility methods will be positively misleading. Syst Zool 27:401–410

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Figlar RB (2000) Proleptic branch initiation in *Michelia* and *Magnolia* subgenus-*Yulania* provides basis for combinations in subfamily Magnoliaceae, In Y Liu, H Fan, Z Chen, Q Wu, Q. Zeng, eds, Proceedings of the International Symposium on the Family Magnoliaceae, Science Press, Beijing, pp 14–26

Figlar RB, Nooteboom HP (2004) Notes on Magnoliaceae IV. Blumea 49:87–100

Flook PK, Klee S Rowell HF (1999) Combined molecular phylogenetic analysis of the Orthoptera (Arthropoda, Insecta) and implications for their higher systematics. Syst Biol 48:233–253

Fragan BM, Stedje B, Stabbetorp OE, Jensen ES, Jakobsen KS (1994) A general approach for PCR-amplification and sequencing of chloroplast DNA from crude vascular plant and algal tissue. BioTechniques 16:484–494

Frodin DG, Govaerts R (1996) World checklist and bibliography of Magnoliaceae, Kew Publishing, Richmond

Graham SW, Olmstead RG (2000) Utility of 17 chloroplast genes for inferring the phylogeny of the basal angiosperms. Amer J Bot 87:1712–1730

Hilu KW, Borsch T, Muller K, Soltis DE, Soltis PE, Savolainen V, Chase M, Powell M. Alice L, Evans R, Sauquet H, Neinhuis C, Slotta T, Rohwer J, Chatrou L (2003) Inference of angiosperm phylogeny based on *matK* sequence information. Amer J Bot 90:758–1776

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755

Jansen RK, Cai Z, Raubeson LA, Daniell H, dePamphilis CW, Leebens-Mack J, Müller KF, Guisinger-Bellian M, Haberle RC, Hansen AK, Chumley TW, Lee S-B, Peery R, McNeal JR, Kuehl JV, Boore JL (2007) Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns. Proc Natl Acad Sci USA 49:19369–19374

Johnson LA, Soltis DE (1994) matK DNA sequences and phylogenetic reconstruction in Saxifragaceae *s. str.* Syst Bot 19:143-156

Johnson LA, Soltis DE (1995) Phylogenetic inference in Saxifragaceae *sensu stricto* and *Gilia* (Polemoniaceae) using *matK* sequences. Ann Missouri Bot Gard 82:149–175

Johnson LA, Schultz JL, Soltis DE, Soltis PS (1996) Monophyly and generic relationships of Polemoniaceae based on *matK* sequences. Amer J Bot 83:1207–1224

Judd WD, Campbell CS, Kellogg EA, Stevens PF, Donoghue MJ (2008) Plant systematics: a phylogenetic approach. Sinauer, Sunderland

Kim JH, Hart HT, Mes HM (1996) The phylogenetic position of East Asian *Sedum* species (Crassulaceae) based on chloroplast DNA *trnL* (UAA)-*trnF* (GAA) intergenic spacer sequence variation. Acta Botanica Neerlandica 45:309–321

Kim K-J, Jansen RK (1995) *ndhF* sequence evolution and the major clades in the sunflower family. Proc Natl Acad Sci USA 92:10379–10383

Kim S, Park J-W, Kim Y-D, Suh Y (2001) Phylogenetic relationships in family Magnoliaceae inferred from *ndhF* sequences. Amer J Bot 91:2101–2004

Kim S, Yoo M-J, Albert VA, Farris JS, Soltis PS, Soltis DE (2004) Phylogeny and diversification of B-function MADS-box genes in angiosperms: evolution and functional implications of a 260-million-year-old duplication. Amer J Bot 91:2102–2118

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparable studies of nucleotide sequences. J Mol Evol 16:111–120

Law YW (1984) A preliminary study on the taxonomy of the family Magnoliaceae. Acta Phytotax Sinica 22:80–109

Law YW (1996) Magnoliaceae, In Flora Reipublicae Popularis Sinicae. 30(1). Science Press, Beijing, China

Li HL (1952) Floristic relationships between eastern Asia and eastern North America. Transactions of the American Philosophical Society 42:371–429

Li HL (1972) Eastern Asia-eastern North America species-pairs in wide ranging genera, In A Graham ed, Floristics and paleofloristics of Asia and eastern North America, Elsevier, Amsterdam, pp 65–78

Maddison DR (1991) The discovery and importance of multiple islands of most-parsimonious trees. Syst Zoo 40:315–328

Magallón S, Sanderson MJ (2001) Absolute diversification rates in angiosperm clades. Evolution 55:1762-1780

Mathews S, Donoghue MJ (1999) The root of angiosperm phylogeny inferred from duplicate phytochrome genes. Science 268:947–950

Mishler BD (1994) Cladistic analysis of molecular and morphological data. American Journal of Physical Anthropology 94:143–156

Mishler BD, Soltis PS, Soltis DE (1998) Compartmentalization in phylogeny reconstruction: philosophy and practice, DIMACS Report, Princeton

Moore MJ, Bell CD, Soltis PS, Soltis DE (2007) Using plastid genome-scale data to resolve enigmatic relationships among basal angiosperms. Proc Natl Acad Sci USA 104:19363–19368

Morgan D, Soltis DE (1993) Phylogenetic relationships among members of Saxifragaceae *sensu lato* based on *rbc*L sequence data. Ann Missouri Bot Gard 80:631–660

Nickerson J, Drouin G (2004) The sequence of the largest subunit of RNA polymerase II is a useful marker for inferring seed plant phylogeny. Mol Phylogenet Evol 31:403–415

Nie Z-L, Wen J, Zauma H, Qiu Y-L, Sun H, Meng, Y, Sun W-B, Zimmer EA (2008) Phylogenetic and biogeographic complexity of Magnoliaceae in the Northern Hemisphere inferred from three nuclear data sets. Mol Phylogenet Evol 48:1027–1040

Nooteboom HP (1985) Notes on Magnoliaceae, with a revision of *Pachylarnax* and *Elmerrillia* and the Malesian species of *Manglietia* and *Michelia*. Blumea 31:65–12

Nooteboom HP (1993) Magnoliaceae, In K Kubitzki, JG Rohwer, V Bittrich, eds, The families and genera of vascular plants, vol. II, Springer-Verlag, New York, pp 391–401

Nooteboom HP (2000) Different looks at the classification of the Magnoliaceae. In Y Liu, H Fan, Z Chen, Q Wu, Q Zeng eds, Proceedings of the International Symposium on the Family Magnoliaceae. Science Press, Beijing, pp 26–38

Olmstead RG, Sweere JA (1994) Combining data in phylogenetic systematics: An empirical approach using three molecular data sets in the Solanaceae. Syst Biol 43:467–481

Olmstead RG, Palmer JD (1994) Chloroplast DNA systematics: A review of method and data analysis. Amer J Bot 81:1205–1224

Olmstead RG, Reeves PA (1995) Evidence for the polyphyly of the Scrophulariaceae based in chloroplast *rbcL* and *ndhF* sequences. Annals of the Missouri Botanical Garden 82:176–193

Osako T, Ohnishi O (2000) Intra- and interspecific phylogeny of wild *Fagopyrum* (Polygonaceae) species based on nucleotide sequences of noncoding regions in chloroplast DNA. Amer J Bot 87:573–582

Oxelman B, Backlund BM, Bremer B (1999) Relationships of Buddlejaceae *s. l.* Investigated using parsimony jackknife and branch support analysis of chloroplast *ndhF* and *rbcL* sequence data. Syst Bot 24:164–182

Palmer JD, Jansen RK, Michaels HJ, Chase MW, Manhart JR (1988) Chloroplast DNA variation and plant phylogeny. Ann Missouri Bot Gard 75:1180–1206

Parkinson CL, Adams KL, Palmer JD (1999) Multigene analyses identify the three earliest lineages of extant flowering plants. Curr Biol 9:1485–1488

Plunkett GM, Soltis DE, Soltis PS (1996) Evolutionary pattern in Apiaceae: inferences based on *mat*K sequence data. Syst Bot 21:477–495

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818

Qiu YL, Parks CR, Chase MW (1995a) Molecular divergence in the eastern Asian-eastern North America disjunct section Rytidospermum of Magnolia (Magnoliaceae). Amer J Bot 82:1589–1598

Qiu YL, Chase MW, Parks CR (1995b) A chloroplast DNA phylogenetic study of the eastern Asia-eastern North America disjunct section *Rytidospermum* of *Magnolia* (Magnoliaceae). Amer J Bot 82:1582–1588

Qiu YL, Lee J, Lu Y-L, Bernasconi-q F, Soltis DE, Soltis PS, Zanis M, Zimmer EA, Chen Z, Savolainen V, Chase MW (1999) The earliest angiosperms: Evidence from mitochondrial, plastid and nuclear genomes. Nature 402:404–407

Qiu YL, Lee J, Bernasconi-Quadroni F, Soltis DE, Soltis PS, Zanis M, Zimmer EA, Chen Z, Savolainen V, Chase MW (2000) Phylogeny of basal angiosperms: analyses of five genes from three genomes. Int J Plant Sci 161:S3–S27

Richardson JE, Fay MF, Cronk QCB, Bowman D, Chase MW (2000) A phylogenetic analysis of Rhamnaceae using *rbcL* and *trn*L-F plastid DNA sequences. Amer J Bot 87:1309–1324

Sang T, Crawford DJ, Stuessy TF (1997) Chloroplast DNA phylogeny, reticulate evolution, and biogeography of Paeonia (Paeoniaceae). Amer J Bot 84:1120–1136

Scotland RW, Sweere JA, Reeves PA, Olmstead RG (1995) Higher-level systematics of Acanthaceae determined by chloroplast DNA sequences. Amer J Bot 82:266–275

Soltis DE, Soltis PS (1997) Phylogenetic relationships in Saxifragaceae *sensu lato*: a comparison of topologies based on 18S rDNA and *rbcL* sequences. Amer J Bot 84:504–522

Soltis DE, Kuzoff RK, Conti E, Gornall R, Ferguson K (1996) *matK* and *rbcL* gene sequence data indicate that *Saxifraga* (Saxifragaceae) is polyphyletic. Amer J Bot 83:371–382

Soltis DE, Smith SA, Cellinese N, Wurdack KJ, Tank DC, Brockington SF, Refulio-Rodriguez NF, Walker JB, Moore MJ, Carlsward BS, Bell CD, Latvis M, Crawley S, Black C, Diouf D, Zi Z, Rushworth CA, Gitzendanner MA, Sytsma, KJ, Qiu Y-L, Hilu KW, Davis CC, Sanderson MJ, Beaman, RS, Olmstead RG, Judd WS, Donoghue MJ, Soltis PS (2011) Angiosperm phylogeny: 17 genes, 640 taxa. Amer J Bot 98:704–730.

Soltis PS, Soltis DE, Chase MW (1999) Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. Nature 402:402–404

Soltis PS, Soltis DE, Zanis M, Kim S (2000) Basal lineages of angiosperms: relationships and implications for floral evolution. Int J Plant Sci 161:97–107

Spongberg SA (1998) Magnoliaceae hardy in cooler temperate regions, In D Hunt ed, *Magnolias* and their allies, David Hunt, Milborne Port, pp 81–144

Spjut RW (1994) A systematic treatment of fruit types, Memoirs of the New York botanical garden vol. 70, The New York botanical garden, New York

Steel KP, Vilgalys R (1994) Phylogenetic analyses of Polemoniaceae using nucleotide sequences of the plastid gene *matK*. Syst Bot 19:126–142

Swofford DL (2001) PAUP* 4.0b10: Phylogenetic analysis using parsimony (*and other methods), version 4, Sinauer, Sunderland

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 17:1105–1109

Takhtajan A (1969) Diversity and classification of flowering plants, Columbia University Press, New York

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl Acids Res 25:4876–4882

Thorne RF (1993) Magnoliaceae, In VH Heywood, ed, Flowering plants of the world, Oxford University Press, New York, pp 27–28

Vázquez-Garcia JA (1994) Magnolia (Magnoliaceae) in Mexico and Central America: a synopsis. Brittonia 46:1–23

Xia N-H, Liu Y-H, Nooteboom HP (2008) Magnoliaceae, In Flora of China, Vol 7, Science Press & Missouri Botanical Garden Press, Beijing and St. Louis

Zanis MJ, Soltis DE, Soltis PE, Mathews S, and Donoghue MJ (2002) The root of the angiosperms revisited. Proc Natl Acad Sci USA 99:6848–6853