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Cover Illustration: The magnificent blossoms of magnolias have been highly prized in landscaping for centuries. Molecular analysis of the magnolia family provides new evidence for phylogenetic relationships previously undetected by systematists. See Kim et al.: Phylogenetic relationships in family Magnoliaceae inferred from *ndhF* sequences, pp. 718–730 in this issue. From top left: *Michelia champaca; Mich. figo; Mich. maudiae; Magnolia biondii; Mag. zenii; Mag. kobus; Mag. stellata; Mag. campbellii; Mag. sprengeri; Mag. denudata; Mag. cylindrica; Mag. salicifolia; Mag. lilliflora; Mag. acuminata; Manglietia moto; Mang. insignis; Mag. grandiflora; Mag. sharpii; Mag. virginiana; Mag. henryi; Mag. delavayi; Mag. coco; Mag. sieboldii; Mag. wilsonii; Mag. obovata; Mag. officinalis; Mag. tripetala; Mag. splendens; Mag. fraseri; Kmeria septentrionalis; Mag. macrophylla; Liriodendron tulipifera.* Photo credit: Sangtae Kim, Richard Figlar, Dorothy Callaway, Munyong Chong, Kihun Song, Holly Forbes, Kenneth Durio, and Junghee Lee. Design: Sangtae Kim.

In this issue . . .

Magnoliaceae phylogeny

Kim et al. make important contributions to our understanding of the phylogeny of the Magnoliaceae, a group that constitutes one of the key lineages of living basal angiosperms. Using *ndhF* data, they demonstrate that many of the major lineages within the family are not monophyletic and they establish some intriguing and previously unrecognized relationships. The separated clades in the molecular tree are considerably different from traditional taxonomic dispositions in the family.

(see p. 717)

Chilling effects: enhanced frost sensitivity

Beerling et al.'s study of effects of simulated global change on leaf ice nucleation temperatures adds to the global climate change literature and is especially relevant for predicting future vegetation shifts in temperate and subarctic regions. The authors show that both increased UV-B radiation (due to the thinning of the ozone layer) and CO_2 enrichment increase the frost sensitivity of leaves of natural stands of dwarf woody shrubs in northern Sweden by raising the temperatures of ice crystal formation. The mechanism is undetermined, but the progressively higher flux of UV-B radiation for terrestrial vegetation at high latitudes in the Northern hemisphere is well documented.

(see p. 628)

Internal pressurization in *Pragmites* culms

Arkebauer et al. made careful field measurements of the internal pressurization of gases in culms of *Phragmites australis* over two seasons and were able to correlate convective transport with environmental conditions, including wind speed, relative humidity, incident radiation, and air temperature. *Phragmites* is an increasingly important wetland species capable of contributing significant quantities of methane to the atmosphere via convective throughflow. Novel findings were that large pressure changes can occur over short time periods and that there is low pressure at high wind speeds.

(see p. 653)

The "modern" *Archaeopteris*

Fairon-Demaret and Leponce address the fascinating issue of photosynthetic display in the progymnosperm *Archaeopteris roemeriana,* a plant that has become a crucial taxon for interpretations of lignophyte biology. Their detailed study of compression fossils from Belgium shows clearly that the leaves are dimorphic (suggested from previous anatomical work) and demonstrates the presence of a new leaf type in *Archaeopteris.* The combination of anisophylly and shoot dorsiventrality to optimize light interception may be the earliest such evidence in the fossil record. *Archaeopteris* is progressively being revealed to be astonishingly "modern" in organization.

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PHYLOGENETIC RELATIONSHIPS IN FAMILY MAGNOLIACEAE INFERRED FROM *NDHF* SEQUENCES[1]

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The *ndhF* sequences of 99 taxa, representing all sections in extant Magnoliaceae, were analyzed to address phylogenetic questions in the family. *Magnolia macrophylla* and *M. dealbata*, North American species of *Magnolia* section *Rytidospermum*, are placed at the base in the subfamily Magnolioideae although its supporting value is low. In the remaining taxa, several distinctive lineages are recognized: (1) *Magnolia*, the biggest genus in the family, is not monophyletic; (2) *Michelia*, including section *Maingola* of *Magnolia* subgenus *Magnolia*, is closely related with *Elmerrillia* and sections *Alcimandra* and *Aromadendron* of *Magnolia* subgenus *Magnolia;* (3) the associates of *Michelia* are grouped with *Magnolia* subgenus *Yulania* and section *Gynopodium* of *Magnolia* subgenus *Magnolia;* (4) *Pachylarnax* forms a clade with sections *Manglietiastrum* and *Gynopodium* of *Magnolia;* (5) a well-supported *Manglietia* clade is recognized; (6) Caribbean species of section *Theorhodon* of *Magnolia* subgenus *Magnolia*, which are section *Splendentes* sensu Vázquez-Garcia, are closely allied with New World members of *Magnolia* subgenus *Talauma;* and (7) section *Rytidospermum* of *Magnolia* subgenus *Magnolia* and subgenus *Talauma* are polyphyletic. The separated clades in the molecular tree are considerably different from traditional taxonomic dispositions in the family. The molecular data strongly suggest that a taxonomic realignment of infrafamilial delimitations and compositions should be considered.

Key words: Magnoliaceae; molecular phylogeny; *ndhF*; sequences.

Magnoliaceae has attracted keen interest from many botanists. The family has been considered to be one of the earliest flowering plants and played a key role in forming concepts of the first flowers, even though recent molecular evidences have significantly changed our views on angiosperm phylogeny (Cronquist, 1981; Mathews and Donoghue, 1999; Parkinson, Adams, and Palmer, 1999; Qiu et al., 1999; Soltis, Soltis, and Chase, 1999; Graham and Olmstead, 2000). A fossil record also shows that the family has a long evolutionary history of over 100 million years (Dilcher and Crane, 1984). The family is a well-defined group of trees and shrubs with over 230 species characterized by an androecium of numerous spirally arranged stamens, a gynoecium with many simple carpels spirally arranged on an elongated axis and separate tepals. All species of the family have bisexual flowers except for *Kmeria* and some species of *Magnolia* section *Gynopodium* (Chen and Nooteboom, 1993). Four-fifths of the species are currently distributed in temperate and tropical regions of Southeast Asia, and the remaining one-fifth is found in America, from temperate southeast North America through tropical America to Brazil (Dandy, 1971; Thorne, 1993; Frodin and Govaerts, 1996). The distribution of Magnoliaceae in eastern Asia and America is an outstanding example of intercontinental disjunction (Li, 1952, 1972). The heterogeneous pattern of molecular divergence between several Asian and North American *Magnolia* species pairs suggests that the current distribution of *Magnolia* was attained by multiple migrations via both Bering and North Atlantic land bridges (Qiu, Parks, and Chase, 1995).

Since Dandy (1927) proposed the first comprehensive taxonomic treatment of the Magnoliaceae, many different infrafamilial taxonomic schemes have been suggested by various authors (Dandy, 1978; Law, 1984; Nooteboom, 1985, 1993; Chen and Nooteboom, 1993; Law, 1996). Taxonomic treatment in the family has been controversial regarding the disposition of tribes, genera, and sections (Fig. 1). Dandy (1927, 1978) recognized ten genera disposed in two tribes: *Liriodendron* in the tribe Liriodendreae, and *Magnolia*, *Talauma*, *Aromadendron*, *Kmeria*, *Alcimandra*, *Manglietia*, *Pachylarnax*, *Elmerrillia*, and *Michelia* in the tribe Magnolieae. Hutchinson (1959) accepted two additional Chinese genera, *Paramichelia* H. H. Hu (1940) and *Tsoongiodendron* W. Y. Chun (1963), in the tribe Magnolieae.

Law (1984) proposed a view slightly different from the previous taxonomic treatments (Dandy, 1927, 1978; Hutchinson, 1959). He divided the family Magnoliaceae into two subfamilies, Magnolioideae and Liriodendroideae. Two tribes, four subtribes, and 14 genera were recognized in the former and the sole genus *Liriodendron* in the latter (Law, 1984; Fig. 1). He accepted the genus *Parakmeria*, which was established from a Chinese species by Hu and Cheng (1951), including species of *Magnolia* section *Gynopodium* in *Parakmeria*. He also recognized *Manglietiastrum* as a distinctive genus (Law, 1979, 1984).

In his comprehensive study of Magnoliaceae, Nooteboom (1985, 1987, 1993, 1998) agreed with Law (1984) on dividing the Magnoliaceae into two subfamilies, Magnolioideae and Liriodendroideae. He also recognized a sole genus *Liriodendron* in the subfamily Liriodendroideae, but subdivided the subfamily Magnolioideae into two tribes, Magnolieae and

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[5] Author for reprint requests.

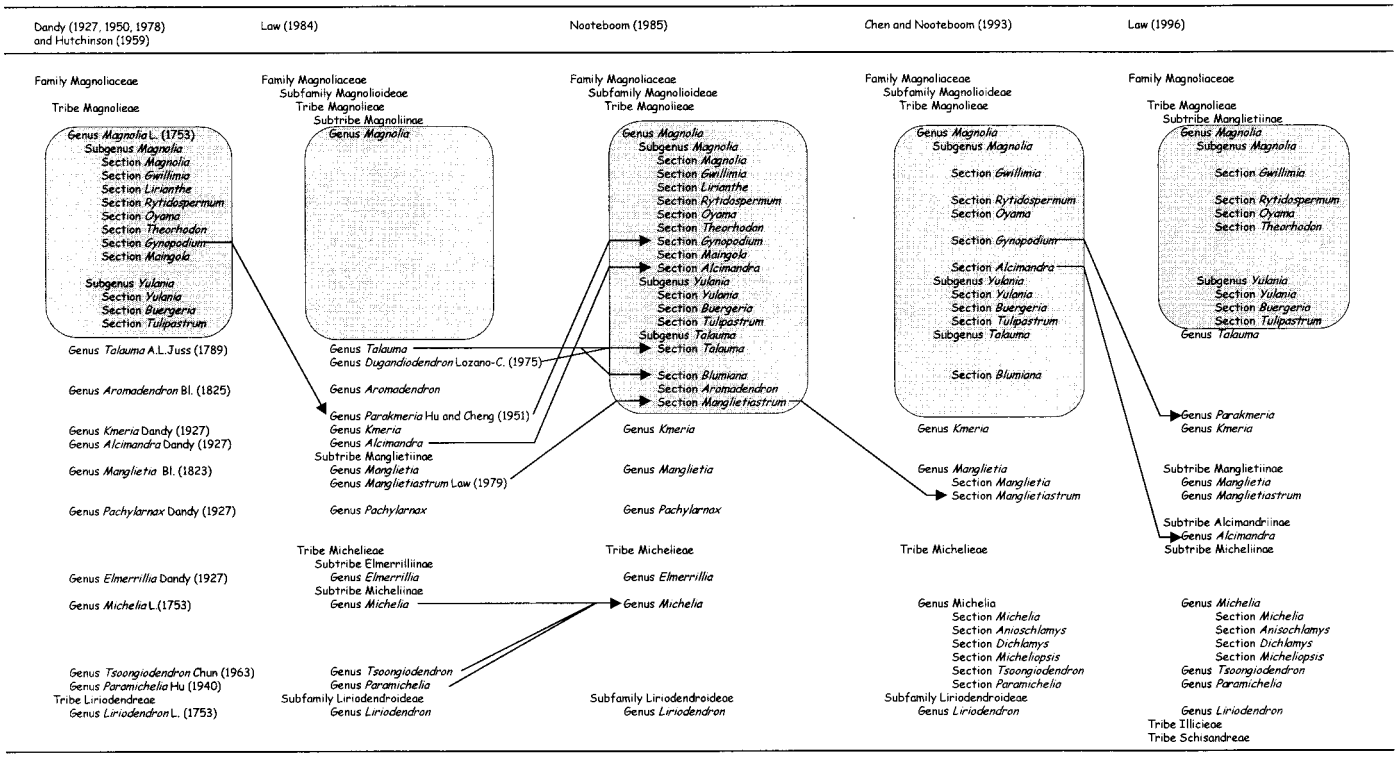


Fig. 1. Taxonomic treatments of the Magnoliaceae by different authors. Arrows indicate taxa whose taxonomic positions have been frequently changed. Gray boxes indicate species of the genus *Magnolia* by Nooteboom (1985). Chen and Nooteboom (1993) and Law (1996) treated only Chinese taxa including cultivated species in China.

Michelieae (Nooteboom, 1985). Thus the tribe Magnolieae consisted of four genera, *Magnolia*, *Manglietia*, *Pachylarnax*, and *Kmeria*, and the tribe Michelieae contained two genera, *Elmerrillia* and *Michelia*. In the genus *Magnolia*, he recognized three subgenera and 16 sections (Nooteboom, 1985). *Talauma*, *Dugandiodendron*, *Aromadendron*, *Alcimandra*, *Manglietiastrum*, *Tsoongiodendron*, and *Paramichelia*, which were recognized as separate genera by previous authors (Dandy, 1927, 1978; Hutchinson, 1959; Law, 1984), were merged as sections into the genus *Magnolia* and *Michelia* (Nooteboom, 1985).

In their revision of Chinese Magnoliaceae, Chen and Nooteboom (1993) adopted the classification system of Nooteboom (1985) in broad outline. However, the section *Manglietiastrum* was transferred from the genus *Magnolia* to the genus *Manglietia*. In the treatment of Chinese Magnoliaceae, Law (1996) proposed a very different classification system of Magnoliaceae from previous works (Dandy, 1927, 1950, 1978; Hutchinson, 1959; Law, 1984; Nooteboom, 1985; Chen and Nooteboom, 1993). The most unusual feature of his classification was the inclusion of Illiciaceae and Schisandraceae in Magnoliaceae. These have generally been recognized as distinct families (Fig. 1). The inclusion of these families failed to gain general consent and is rejected by recent molecular phylogenetic analyses (Soltis et al., 1998; Soltis, Soltis, and Chase, 1999). Law (1996) recognized the section *Alcimandra* as a separate genus in the subtribe Alcimandriinae of the tribe Magnolieae and included the genus *Liriodendron* in the tribe Michelieae (Fig. 1).

The controversies surrounding the taxonomy of Magnoliaceae are due to a paucity of phylogenetically useful characters caused by the extensive homogeneity in the family (Qiu, Chase, and Parks, 1995; Nooteboom, 1998). The variation of *ndhF* gene is second only to *matK* among coding genes in chloroplast DNA longer than 1000 bp (31% between rice and tobacco) (Olmstead and Palmer, 1994). The *ndhF* gene has therefore been frequently used for phylogenetic studies at infrafamilial level (Olmstead and Palmer, 1994; Clark, Zhang, and Wendel, 1995; Kim and Jansen, 1995; Olmstead and Reeves, 1995; Scotland et al., 1995; Bohs and Olmstead, 1997; Oxelman, Backlund, and Bremer, 1999). Comparative analysis of *ndhF* sequences of rice and tobacco demonstrates that the nucleotide substitution rate of *ndhF* is about two times higher than that of *rbcL* (Olmstead and Reeves, 1995).

We examined the *ndhF* sequences to address phylogenetic questions in Magnoliaceae. The purpose of this study is to provide a well-supported phylogeny of Magnoliaceae capable of resolving controversies on infrafamilial groupings proposed by previous authors (Dandy, 1927, 1978; Law, 1984, 1996; Nooteboom, 1985; Chen and Nooteboom, 1993).

MATERIALS AND METHODS

Taxon sampling—The *ndhF* sequences were determined for 99 taxa, representing all genera and sections of the classification system of Magnoliaceae proposed by Nooteboom (1985; Table 1), which we follow here. For the purpose of specific recognition, we adopted the scientific names listed in the recent bibliographic checklist of the Magnoliaceae by Frodin and Govaerts (1996).

DNA extraction and amplification—Total genomic DNAs were isolated from leaves, either fresh, dried with silica gel, or from herbarium specimens using standard CTAB (hexadecyltrimethylammonium bromide) extraction

method (Doyle and Doyle, 1987) or using DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). Total DNA extracted by CTAB was further purified with Geneclean Kit II (BIO101, Carlsbad, California, USA) for polymerase chain reaction (PCR). For species not available from other sources, extracted total DNAs were obtained from the DNA bank of the Royal Botanical Gardens Kew (Table 1).

For DNAs extracted from fresh materials, the entire *ndhF* gene was amplified using the primer pair of primer 1 developed by Olmstead and Sweere (1994) and primer 14 by Jansen (1992) (Fig. 2). For more degraded DNAs extracted from herbarium specimens, the *ndhF* gene was amplified in overlapping segments using the following pairs of primers: primer 1 by Olmstead and Sweere (1994) and MF1165R designed by S. Kim, primer 972 and primer 2110R developed by Olmstead and Sweere (1994), and MF1795 designed by S. Kim and primer 14 developed by Jansen (1992) (Fig. 2). Since the 3′ primer, primer 14 developed by Jansen (1992), failed to work for certain taxa, the primer ORF-R (designed by S. Kim) was used for the amplification. Polymerase chain reaction was carried out in 100 μL final volume containing 0.5 ng template DNA, 2.5 units of Gold Taq polymerase (PE Applied Biosystems, Foster City, California, USA), 10 mmol/L Tris, pH 8.3, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 0.001% gelatin, 200 μmol/L for each dNTP, and 0.5 μmol/L of each primer. Amplification reactions involved 10 min at 95°C for predenaturation, 30 cycles consisting of 1 min at 95°C for denaturation, 1 min at 55°C for annealing, and 3 min at 72°C for extension, with a final extension of 7 min at 72°C, using a Thermal Cycler 9600 (PE Applied Biosystems). The reaction was kept at 4°C after amplification.

Sequencing of ndhF—Sequences were determined by the dideoxy method (Sanger, Nicklen, and Coulson, 1977) either manually, or automatically in cycle sequencing reactions. For manual sequencing, double-stranded PCR products were directly sequenced using Sequenase PCR Product Sequencing Kit (USB, Cleveland, Ohio, USA). Electrophoresis was performed with denaturing formamide gel and glycerol tolerant buffer according to protocols for Sequenase PCR Product Sequencing Kit (USB).

For automated sequencing, double-stranded PCR products were purified with the QIAquick PCR Purification Kit (QIAGEN). The cycle sequencing reaction was carried out using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems). In addition to PCR primers, the primers MF298, MF561, MF1254, MF1795, MF1945, MF256R, MF972R, MF1165R, and MF1861R (designed by S. Kim) were used as internal primers to complete sequencing in both directions (Fig. 2). Automated sequencing was employed with 377 DNA Sequencing System (PE Applied Biosystems).

Phylogenetic analysis—DNA sequences obtained from the automated DNA sequencer were assembled and consensus sequences were generated using the computer program Sequencher (version 3.1; Gene Codes Corporation, Ann Arbor, Michigan, USA). Sequences were aligned using CLUSTAL X (Thompson et al., 1997). Phylogenetic analyses were performed by maximum parsimony (MP) and neighbor joining (NJ) using PAUP* (4.0 beta version; Swofford, 1998). Trees were rooted by defining *Liriodendron* as the sister group to all other species of Magnoliaceae. *Liriodendron* is clearly distinguished from all other genera of Magnoliaceae based on both morphology and molecular data (Dandy, 1927, 1950, 1978; Nooteboom, 1985; Chase et al., 1993; Chen and Nooteboom, 1993; Qiu et al., 1993). Searches for the MP tree, bootstrapping analysis, and decay analysis (Bremer, 1988) were conducted using a standard heuristic search with MULPARS and Tree-Bisection-Reconnection (TBR) branch swapping. All nucleotide changes were equally weighted in the MP analysis (Terry, Brown, and Olmstead, 1997). Searches for islands of MP trees (Maddison, 1991) were conducted on 1000 replicates of random order entry addition of taxa, saving all optimal trees. Five hundred replicates were performed with the option of Maxtrees = 5000 to obtain bootstrap percentage. Batch command files for decay analysis were generated using AutoDecay (version 4.0; Eriksson, 1998). The NJ tree was obtained by the distances calculated using Kimura's two-parameter method (Kimura, 1980). Rates for variable sites were assumed to be equal. Minimum evolution criterion was chosen as the objective function, and negative branch-lengths were treated as zero length. In the bootstrap analysis of NJ trees, 1000 replicates were performed.

RESULTS

Sequences of the *ndhF* gene from 99 taxa of the Magnoliaceae were completely determined except for 27 bases at the annealing site of 5′ PCR primer. The size of the *ndhF* gene was 2226 bases (including the sequences of 5′ PCR primer, Olmstead and Sweere, 1994) in species of Magnoliaceae examined, except for *Magnolia macrophylla* subsp. *macrophylla, M. macrophylla* subsp. *ashei, M. dealbata,* and *Liriodendron chinensis.* The length of the *ndhF* gene of these four taxa was three bases shorter at the 3′ end of the gene. The difference in size was caused by nucleotide changes resulting in TGA (a stop codon) substituting for AGA (arginine) at 3′ end (Fig. 3). Of 2199 sites, excluding the first 27 bases for the 5′ PCR primer, 204 sites (9.3%) were variable and 124 sites (5.6%) phylogenetically informative. Variable sites in the *ndhF* gene were more densely distributed in the 3′ end than at the 5′ portion as noted in previous studies (Olmstead and Sweere, 1994; Clark, Zhang, and Wendel, 1995; Kim and Jansen, 1995; Olmstead and Reeves, 1995; Fig. 4). The G + C content of *ndhF* in the family Magnoliaceae was 34.4–35.0%. The maximum sequence divergence of the *ndhF* gene was 2.45% in the family Magnoliaceae (Kimura's $K \times 100$; Kimura, 1980) and 1.05 and 0.73% in the subfamily Magnolioideae and the subfamily Liriodendroideae, respectively. The sequence divergence in the Magnoliaceae was considerably lower than in other angiosperm families (Olmstead and Palmer, 1994; Clark, Zhang, and Wendel, 1995; Kim and Jansen, 1995; Olmstead and Reeves, 1995; Scotland et al., 1995; Bohs and Olmstead, 1997; Oxelman, Backlund, and Bremer, 1999). The ratio of transition vs. transversion was 1.34, inferred from the MP analysis.

Phylogenetic analysis produced a single most parsimonious tree of 256 steps. The consistency index (CI) was 0.79 excluding phylogenetically uninformative sites, and the retention index (RI) was 0.93. In 100 000 random trees, g_1 and g_2 values were −0.50 and 0.97, respectively.

Magnolia macrophylla subsp. *macrophylla, M. macrophylla* subsp. *ashei,* and *M. dealbata,* North American species of the section *Rytidospermum,* constitute a robust clade (Clade A) supported by a bootstrap value of 100%. It is sister to the rest of the subfamily Magnolioideae, but this position is weakly supported with a bootstrap value of just 39% (Fig. 5). In the remaining Magnolioideae (Clade B), seven distinctive clades (Clades I–VII) were recognized, but their relationships were poorly resolved due to the lack of synapomorphic changes (Fig. 5). The first clade constitutes the tribe Michelieae, section *Maingola* of *Magnolia* subgenus *Magnolia, Magnolia* subgenus *Yulania,* section *Alcimandra* of *Magnolia* subgenus *Magnolia,* section *Aromadendron* of *Magnolia* subgenus *Talauma, Pachylarnax,* section *Manglietiastrum* of *Magnolia* subgenus *Talauma,* and section *Gynopodium* of *Magnolia* subgenus *Magnolia* (Fig. 5, Clade I). This clade is divided into three subclades (Fig. 5, Clade Ia–c).

The subclade Ia mainly consists of the species of *Michelia* (Fig. 5). The section *Maingola* of *Magnolia* subgenus *Magnolia* and *Elmerrillia* are also included in the *Michelia* subclade. *Magnolia cathcartii,* the sole member of section *Alcimandra* of *Magnolia* subgenus *Magnolia,* and *M. elegans,* a member of section *Aromadendron* of *Magnolia* subgenus *Ta-*

TABLE 1. Species included in *ndhF* analysis. Taxonomic treatment followed Nooteboom (1985) and species names were referred to Frodin and Govaerts (1996).

Taxa	Voucher, herbarium/source[a]	GenBank accession[b]
Family Magnoliaceae		
Subfamily Magnolioideae		
Tribe Magnolioideae		
Genus *Magnolia*		
Subgenus *Magnolia*		
Section *Magnolia* (1/1)[c]		
virginiana	S. Kim 1027, NPRI/CHOLLIPO 85-304	GBAN-AF107939
Section *Gwillimia* (5/15)		
albosericea	S. Kim 1068, NPRI/SCBG	GBAN-AF107914
championii	S. Kim 1057, NPRI/SCBG	GBAN-AF107915
coco	S. Kim 1005, NPRI/CHOLLIPO 75-61	GBAN-AF107916
delavayi	S. Kim 1009, NPRI/CHOLLIPO 86-152	GBAN-AF107917
henryi	S. Kim 1054, NPRI/SCBG	GBAN-AF107918
Section *Lirianthe* (1/1)		
pterocarpa[d]	M. W. Chase 1304, K/BBG IV.F. 34	GBAN-AF107920
Section *Rytidospermum* (7/9)		
dealbata	S. Kim 1008, NPRI/CHOLLIPO I93-97	GBAN-AF107921
fraseri var. *fraseri*	S. Kim 1111, NPRI/MGA	GBAN-AF216256
fraseri var. *pyramidata*	S. Kim 1011, NPRI/CHOLLIPO 72-124-3	GBAN-AF107922
macrophylla subsp. *macrophylla*	S. Kim 1015, NPRI/CHOLLIPO 74-208	GBAN-AF107923
macrophylla subsp. *ashei*	S. Kim 1016, NPRI/CHOLLIPO 81-854	GBAN-AF107924
obovata	S. Kim 1046, NPRI/CHOLLIPO 72-124-3	GBAN-AF107925
officinalis	S. Kim 1018, NPRI/CHOLLIPO 87-6	GBAN-AF107926
rostrata	S. Kim 1107, NPRI/CHOLLIPO 97-1	GBAN-AF107927
tripetala	S. Kim 1025, NPRI/CHOLLIPO 80-290-2	GBAN-AF107928
Section *Oyama* (3/4)		
globosa	S. Kim 1101, NPRI/WISLEY W927723	GBAN-AF107931
sieboldii var. *sieboldii*	S. Kim 1047, NPRI/Mt. Chiri, Korea	GBAN-AF107933
sieboldii var. *sinensis*	S. Kim 1022, NPRI/CHOLLIPO 82-85	GBAN-AF107932
wilsonii	S. Kim 1028, NPRI/CHOLLIPO 86-7	GBAN-AF107934
Section *Theorhodon* (11/18)		
iltisiana	A. Gentry & E. Jardel 73514, MO/MO	GBAN-AF216258
grandiflora	S. Kim 1012, NPRI/CHOLLIPO 74-202	GBAN-AF107940
guatemalensis	/UCB 72.0658	GBAN-AF107941
pacifica var. *tarahumara*	M. Fishbein 1109, UMBS/UMBS	GBAN-AF216260
panamensis	G. McPherson 15882, MO/MO	GBAN-AF216255
poasana	H. Haber 9830, MO/MO	GBAN-AF216257
portoricensis	C. M. Taylor & E. Jarde 173514, MO/MO	GBAN-AF216254
schediana	S. Kim 1021, NPRI/CHOLLIPO 94-1	GBAN-AF221498
sharpii	/UCB 80.0066	GBAN-AF107942
splendens	S. Kim 1108, NPRI/MGA	GBAN-AF216259
tamaulipana	S. Kim 1026, NPRI/CHOLLIPO 95-36	GBAN-AF107943
Section *Gynopodium* (2/4)		
nitida var. *nitida*	S. Kim 1017, NPRI/CHOLLIPO 95-97	GBAN-AF107935
nitida var. *lotungensis*	S. Kim 1051, NPRI/CHOLLIPO 95-97	GBAN-AF107936
kachirachirai	C. E. Chang 4384, L	GBAN-AF216264
Section *Maingola* (3/7)		
griffithii	S. Kim 1113, NPRI/Assam, India	GBAN-AF216265
gustavii	S. Kim 1114, NPRI/Assam, India	GBAN-AF216266
pealiana	S. Kim 1105, NPRI/Assam, India	GBAN-AF107938
Section *Alcimandra* (1/1)		
cathcartii	S. Kim 1091, NPRI/Xiangpingshan, China	GBAN-AF107945
Subgenus *Yulania*		
Section *Yulania* (7/7)		
amoena	S. Kim 1002, NPRI/CHOLLIPO 79-1256	GBAN-AF107946
campbellii	S. Kim 1004, NPRI/CHOLLIPO 90-134	GBAN-AF107947
dawsoniana	S. Kim 1007, NPRI/CHOLLIPO 74-200-1	GBAN-AF107948
denudata	S. Kim 1010, NPRI/CHOLLIPO B72-123-1	GBAN-AF107949
sargentiana	S. Kim 1102, NPRI/WISLEY W920036	GBAN-AF107950
sprengeri	S. Kim 1023, NPRI/CHOLLIPO 80-39	GBAN-AF107951
zenii	S. Kim 1029, NPRI/CHOLLIPO 84-17	GBAN-AF107952
Section *Buergeria* (5/5)		
biondii	S. Kim 1003, NPRI/CHOLLIPO 74-198	GBAN-AF107953
kobus	S. Kim 1013, NPRI/CHOLLIPO 72-107-2	GBAN-AF107954
salicifolia	S. Kim 1019, NPRI/CHOLLIPO 74-200-1	GBAN-AF107955
stellata	S. Kim 1103, NPRI/WISLEY W922256-A	GBAN-AF107956
cylindrica	S. Kim 1006, NPRI/CHOLLIPO 75-61	GBAN-AF107957
Section *Tulipastrum* (2/2)		
acuminata var. *acuminata*	S. Kim 1001, NPRI/CHOLLIPO 91-85	GBAN-AF107958
acuminata var. *subcordata*	S. Kim 1104, NPRI/WISLEY W891027-A	GBAN-AF107959
liliiflora	S. Kim 1014, NPRI/CHOLLIPO 72-127-2	GBAN-AF107960

TABLE 1. Continued.

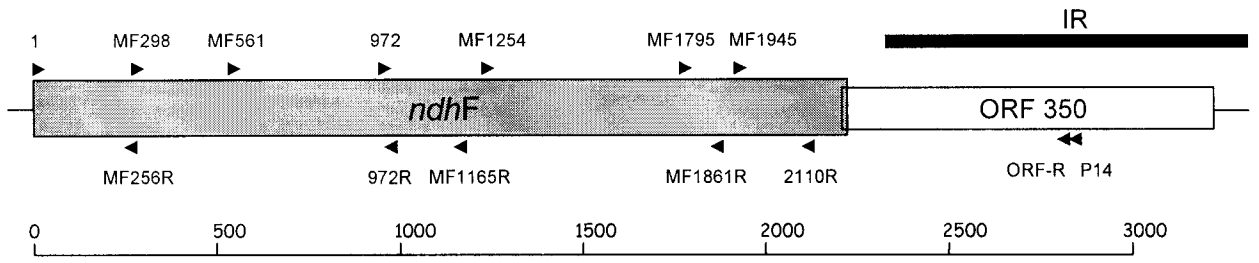
Taxa	Voucher, herbarium/source[a]	GenBank accession[b]
Subgenus *Talauma*		
Section *Talauma* (4/12)		
dodecapetala	S. Kim 1106, NPRI/MGA	GBAN-AF119338
lenticellatum	G. Lozano-Contreras 2272, COL/COL	GBAN-AF216261
mahechae	G. Lozano-Contreras 2161, COL/COL	GBAN-AF216262
mexicana	Thien & Azuma s.n., TI/Coatepec, Mexico	GBAN-AF216263
Section *Blumiana* (2/40)		
liliifera[d]	M. W. Chase 2108, K/BBG XX.D. 154	GBAN-AF107919
gigantifolia[d]	J. Dransfield 7364, K/BBG	GBAN-AF107944
Section *Aromadendron* (1/4)		
elegans	B. A. Krukoff 4213, MO/MO	GBAN-AF262317
Section *Manglietiastrum* (1/1)		
sinica	S. Kim 1098, NPRI/KBG	GBAN-AF107937
Genus *Manglietia* (12/25)		
aromatica	S. Kim 1093, NPRI/KBG	GBAN-AF107983
conifera	S. Kim 1030, NPRI/CHOLLIPO 95-84	GBAN-AF107984
dolichogyna	H. P. Nooteboom 6137, L/Cameron highlands, Malaysia	GBAN-AF107985
duclouxii	S. Kim 1092, NPRI/Xingpingshan, China	GBAN-AF107986
fordiana	S. Kim 1031, NPRI/CHOLLIPO 90-223	GBAN-AF107987
glauca	S. Kim 1073, NPRI/SCBG	GBAN-AF107988
grandis	S. Kim 1084, NPRI/Chuntianping, China	GBAN-AF107989
hebecarpa	S. Kim 1094, NPRI/KBG	GBAN-AF107990
insignis	S. Kim 1032, NPRI/CHOLLIPO 95-48	GBAN-AF107991
megaphylla	S. Kim 1056, NPRI/SCBG	GBAN-AF107992
moto	S. Kim 1033, NPRI/CHOLLIPO 94-46	GBAN-AF107993
szechuanica	S. Kim 1090, NPRI/Xingpingshan, China	GBAN-AF107994
Genus *Kmeria* (2/2)		
duperreana	Smitinand 11692, L/L	GBAN-AF107929
septentrionalis	S. Kim 1053, NPRI/SCBG	GBAN-AF107930
Genus *Pachylarnax* (1/2)		
praecalva	Lehlas 4, L/L	GBAN-AF107995
Tribe Michelieae		
Genus *Elmerrillia* (1/4)		
ovalis[d]	M. W. Chase 1302, K/BBG VIII.G.19	GBAN-AF107982
Genus *Michelia* (20/30)		
baillonii	S. Kim 1064, NPRI/SCBG	GBAN-AF107979
balanse	S. Kim 1060, NPRI/SCBG	GBAN-AF107980
cavaleriei	S. Kim 1034, NPRI/CHOLLIPO 95-87	GBAN-AF107961
champaca	S. Kim 1035, NPRI/CHOLLIPO S89-709	GBAN-AF107962
chapensis	S. Kim 1065, NPRI/SCBG	GBAN-AF107975
doltsopa	S. Kim 1037, NPRI/CHOLLIPO 92-79	GBAN-AF107963
figo	S. Kim 1039, NPRI/CHOLLIPO 83-563-2	GBAN-AF107977
floribunda	S. Kim 1095, NPRI/KBG	GBAN-AF107981
foveolata	S. Kim 1038, NPRI/CHOLLIPO 94-47	GBAN-AF107964
hypolampra	S. Kim 1077, NPRI/SCBG	GBAN-AF107974
lacei	S. Kim 1096, NPRI/KBG	GBAN-AF107966
macclurei	S. Kim 1041, NPRI/CHOLLIPO 95-111	GBAN-AF107967
martinii	S. Kim 1048, NPRI/CHOLLIPO 95-47	GBAN-AF107976
masticata	S. Kim 1097, NPRI/KBG	GBAN-AF107968
maudiae	S. Kim 1042, NPRI/CHOLLIPO 86-48	GBAN-AF107969
montana	H. P. Nooteboom 6138, L/Cameron highlands, Malaysia	GBAN-AF107970
odora	S. Kim 1099, NPRI/SCBG	GBAN-AF107978
shiluensis	S. Kim 1063, NPRI/SCBG	GBAN-AF107971
velutina	S. Kim 1040, NPRI/CHOLLIPO 92-86	GBAN-AF107972
wilsonii	S. Kim 1043, NPRI/CHOLLIPO 95-82	GBAN-AF107973
Subfamily Liriodendroideae		
Genus *Liriodendron* (2/2)		
chinense	S. Kim 1044, NPRI/CHOLLIPO 89-111	GBAN-AF107996
tulipifera	S. Kim 1045, NPRI/CHOLLIPO 74-138	GBAN-AF107997

[a] CHOLLIPO, Chollipo Arboretum, Korea; SCBG, South China Botanical Garden, Guangzhou, China; MGA, Magnolian Grove Arboretum (personal garden of R. B. Figlar), South Carolina; WISLEY, Garden of Royal Horticultural Society, Wisley, U.K.; BBG, Bogor Botanical Garden, Indonesia; KBG, Kunming Botanical Garden, Yunnan, China; UCB, Botanical Garden of the University of California at Berkeley; L, MO, UMBS, COL, leaf material was taken from herbarium specimens.

[b] The prefix GBAN- has been added to all GenBank accession numbers to link the online version of *American Journal of Botany* to GenBank but is not part of the actual accession number.

[c] Number of species included in the molecular analysis/number of species currently recognized in each section or genus.

[d] Total genomic DNA extracts were received from M. W. Chase.



1	5′-ATGGAACAKACATATSAATATGCGTGG-3′	MF256R	5′-TGGGTCGATCAAGTGGCC-3′
MF298	5′-ATCACTACTGTTGGAATCATGG-3′	972R	5′-CATAATATAACCCAATTGAGAC-3′
MF561	5′-TGGTTTATTATTAGGAATCTTAGG-3′	MF1165R	5′-AATTGGCACATATTTGSTTA-3′
972	5′-GTCTCAATTGGGTTATATGATG-3′	MF1861R	5′-TGAAAAGAYGAATAAACAGACCC-3′
MF1254	5′-TGAAATTCTTAATGATAGTTGG-3′	2110R	5′-CCCCCTAYATATTTGATACCTTCTCC-3′
MF1795	5′-GTGACAAATGCAATTTATTCA-3′	ORF-R	5′-TTAGTCAACATTTATCTCTTTCG-3′
MF1945	5′-ATAATAAATGTAATATACAATTGGT-3′	P14	5′-ACCAAGTTCAATGTTAGCGAGATTAGTC-3′

Fig. 2. Map of the chloroplast gene *ndhF* from Magnoliaceae with the relative position of primers. Numbers of primers indicate the 5′ most position of the primer relative to the position in species of Magnoliaceae. Primers 1, 972, and 2110 were described by Olmstead and Sweere (1994) and primer 14 by Jansen (1992). MF stands for Magnoliaceae *ndhF* and R for reverse primers. IR indicates the inverted repeat of chloroplast DNA.

lauma, are placed at the base of the *Michelia–Elmerrillia* subclade, with weak support.

Members of *Magnolia* subgenus *Yulania* form a well-defined group (Fig. 5, Clade Ib). *Magnolia acuminata*, the sole North American species of *Magnolia* subgenus *Yulania* represented here by two varieties, is placed at the base of the *Yulania* clade being separated from all other Asian species of the subgenus *Yulania*. In the *Yulania* clade, four species of section *Yulania* of *Magnolia* subgenus *Yulania*, *M. dawsoniana*, *M. sargentiana*, *M. campbellii*, and *M. sprengeri*, form a very distinctive clade separated by six synapomorphic changes with 100% bootstrap value.

Sections *Gynopodium* and *Manglietiastrum* of the genus *Magnolia* constitute a well-supported clade sharing five synapomorphic changes supported by 99% bootstrap value. The genus *Pachylarnax* is also included in the *Gynopodium–Manglietiastrum* clade (so *Gynopodium* is paraphyletic) and the *ndhF* sequences of *P. praecalva* and *M. sinica* were identical (Fig. 5, Clade Ic).

The genus *Manglietia* is strongly supported as monophyletic (Fig. 5, Clade II). All members of the section *Theorhodon* of *Magnolia* subgenus *Magnolia*, except for *M. portoricensis* and *M. splendens*, which are Caribbean species of section *Splendentes* sensu Vázquez-Garcia (1994), form a clade with *M. virginiana*, which is the sole member of the section *Magnolia* of *Magnolia* subgenus *Magnolia* (Fig. 5, Clade III). The section *Gwillimia* of *Magnolia* subgenus *Magnolia* is closely allied to section *Lirianthe* of *Magnolia* subgenus *Magnolia*, section *Blumiana* of *Magnolia* subgenus *Talauma* (Fig. 5, Clade IV), although they were considered to belong to different subgenera of *Magnolia* (Nooteboom, 1985; Chen and Nooteboom, 1993). The section *Oyama* of *Magnolia* subgenus *Magnolia* constitutes a moderately well-supported clade with Asian spe-

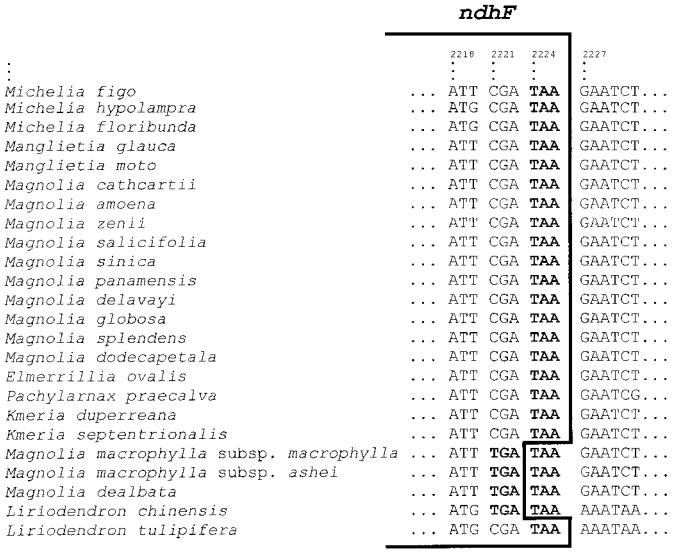


Fig. 3. Sequences at 3′ end of *ndhF* in the Magnoliaceae. Stop codons are indicated in boldface.

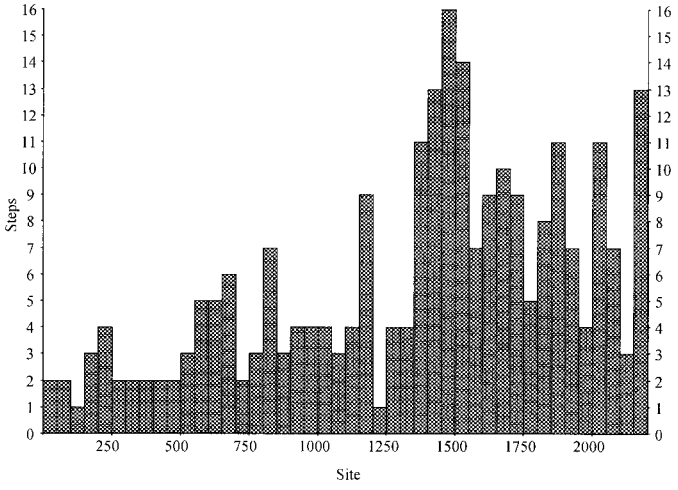


Fig. 4. Distribution of base substitutions across the *ndhF* gene in the Magnoliaceae. Each bar represents the average number of base substitutions per 50 sites. Base substitutions were inferred from the shortest tree.

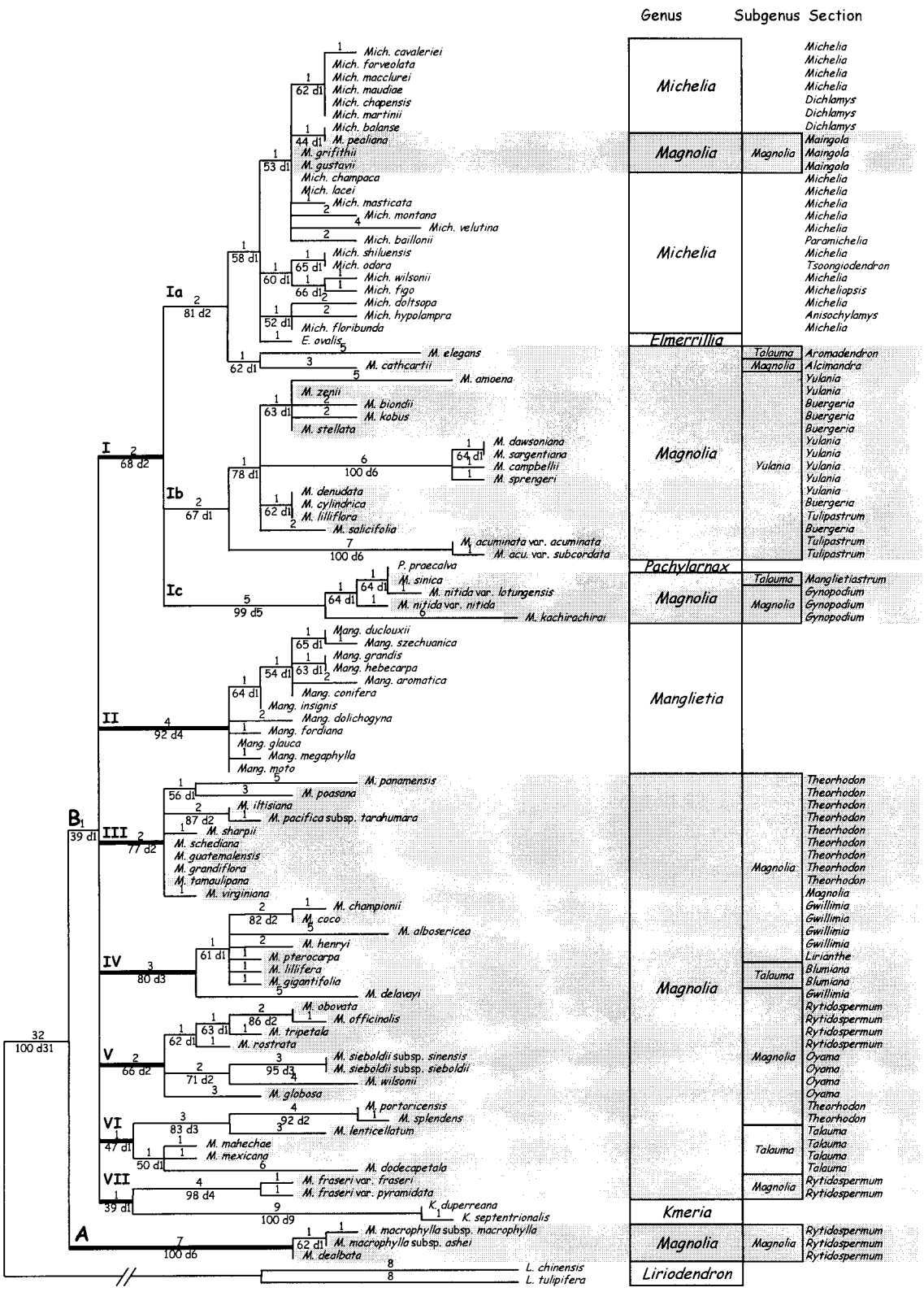


Fig. 5. A single most parsimonious tree based on the *ndhF* sequences (CI = 0.79; RI = 0.93). Gray boxes indicate species of the genus *Magnolia* by Nooteboom (1985). Numbers above the branches are the base changes to support the node. Numbers below the branches are bootstrap values and the decay indices at which the node collapses. *Magnolia portoricensis* and *M. splendens* (Clade VI) were treated as section *Splendentes* by Vázquez-Garcia (1994).

cies of section *Rytidospermum* and *M. tripetala,* a North American species of the section (Fig. 5, Clade V).

New World subgenus *Talauma* constitutes a clade together with *M. splendens* and *M. portoricensis.* The latter two species are separated from the other species of section *Theorhodon* (Fig. 5, Clade VI). They were previously treated as section *Theorhodon* of *Magnolia* subgenus *Magnolia,* but Vázquez-Garcia (1994) described them as a new section *Splendentes. Magnolia fraseri,* another North American species of section *Rytidospermum,* forms a poorly supported clade with *Kmeria* distributed in Southeast Asia (Fig. 5, Clade VII). As a result, the section *Rytidospermum* of *Magnolia* subgenus *Magnolia* is divided into three independent lineages, implying that the traditionally recognized section *Rytidospermum* is polyphyletic (Fig. 5, Clades A, V, and VII).

The NJ tree presents a basically identical topology among major clades with the MP tree in spite of very different assumptions to construct trees (Fig. 6). Both the MP and NJ trees recognized nine major independent lineages including *Liriodendron* in the Magnoliaceae (Figs. 5, 6).

DISCUSSION

The MP analysis produced a single robust tree with a high consistency index (0.79). It discerns several distinctive lineages. The *ndhF* sequence analysis did not provide a complete resolution for all phylogenetic relationships in the family. Despite the poor resolution among major clades, the molecular cladogram clearly challenges the current alignment of species in Magnoliaceae. The high consistency index implies that homoplasy is very low in *ndhF*. Moreover, since both the parsimony and neighbor-joining analysis generated basically identical topologies, the *ndhF* sequence data generate a consistent phylogenetic signal despite the low variability of the gene in the family Magnoliaceae. The sequence divergence in the Magnoliaceae (2.45%) is very low in comparison to other angiosperm families (Olmstead and Palmer, 1994; Clark, Zhang, and Wendel, 1995; Kim and Jansen, 1995; Olmstead and Reeves, 1995; Scotland et al., 1995; Bohs and Olmstead, 1997; Oxelman, Backlund, and Bremer, 1999). The low sequence divergence and the long evolutionary history of the family Magnoliaceae in the fossil record (Dilcher and Crane, 1984) indicate that the *ndhF* gene has evolved slowly in the family. It frequently has been claimed that molecules evolve more slowly in perennial woody plants than in annual herbaceous plants (Wilson, Gaut, and Clegg, 1990; Bousquet et al., 1992; Chase et al., 1993; Suh et al., 1993). The *ndhF* gene in the Magnoliaceae gives an excellent example of the retarded evolutionary rate in woody perennial plants.

Recognition of subfamilies Magnolioideae and Liriodendroideae—It is generally accepted that Magnoliaceae should be divided into two subfamilies, Magnolioideae and Liriodendroideae (Law, 1984; Chen and Nooteboom, 1993; Nooteboom, 1998). The latter, with the sole genus *Liriodendron* of two species, is clearly distinguished from the former by easily recognizable features, such as 2–10 lobed leaves, extrorsely dehiscing anthers, and winged, deciduous and indehiscent samaroid fruits. Molecular phylogenetic analyses based on *rbcL* sequences also strongly support the separation of two subfamilies in the Magnoliaceae (Chase et al., 1993; Qiu et al., 1993). For these reasons, trees were rooted to separate the subfamily Liriodendroideae from the subfamily Magnolioideae in the *ndhF* analyses. Moreover, since the sequence divergence value of the *ndhF* between the subfamily Liriodendroieae and the subfamily Magnolioideae (2.45%) are higher than those within Magnolioideae (1.05%) and Liriodendroideae (0.73%), respectively, the division into two subfamilies seems secure.

Michelieae–Yulania–Gynopodium aggregate—It has been generally agreed that subfamily Magnolioideae should be subdivided into two tribes, Magnolieae and Michelieae (Law, 1984; Nooteboom, 1985). Axillary flowers distinguish tribe Michelieae from tribe Magnolieae, which has terminal flowers. However, the *ndhF* molecular tree does not support the separation based on the flower position (Figs. 5, 6). Although flowers of *Michelia* and *Elmerrillia* (tribe Michelieae) have often been perceived to be axillary, flower buds are actually produced terminally on short shoots (brachyblasts) arising from the leaf axis (Nooteboom, 1985; Figlar, 2000).

In the *ndhF* tree, species in section *Maingola* of *Magnolia* subgenus *Magnolia, M. griffithii, M. pealiana,* and *M. gustavii,* are nested in *Michelia,* and then the clade of the tribe Michelieae is closely related to the clade of *Magnolia* subgenus *Yulania.* The close alliance of *Maingola* with *Michelia* has never been proposed, but species of *Maingola, Michelia,* and *Yulania* have cylindrical fruits in common (Dandy, 1978). One of the main features separating *Magnolia* subgenus *Yulania* from subgenus *Magnolia* relates to the dehiscence of anthers. Pollen is shed introrsely in subgenus *Magnolia,* but laterally in the subgenus *Yulania.* In *Michelia,* pollen is also shed laterally (Dandy, 1978). The close relationship between *Michelia* and *Yulania* is also demonstrated by proleptic growth and the formation of hybrids (Figlar, 2000). *Michelia* and the subgenus *Yulania* have been observed to form branches by prolepsis, in which branches are produced from a dormant axillary bud of the previous year's growth. On the other hand, all species of *Magnolia* subgenus *Magnolia,* except for section *Oyama,* produce branches directly from the current year's growth by syllepsis (Tomlinson, 1983; Figlar, 2000). In addition, the close affinity between *Michelia* and *Yulania* is demonstrated by their cross compatibility. A hybrid was successfully produced between *Michelia figo* and *M. acuminata,* a species of subgenus *Yulania,* but many attempts to produce hybrids between the two subgenera *Magnolia* and *Yulania* have never been successful (Savage, 1989; Figlar, 2000).

Magnolia cathcartii, the sole member of section *Alcimandra* of *Magnolia* subgenus *Magnolia,* was originally described as a species of *Michelia* (Hooker and Thomson, 1855), and Lozano-Contreras (1975) recognized that this species has pseudolateral flowers. Later, Dandy (1927) separated this taxon as the independent genus *Alcimandra* because other species of *Michelia* comprise a well-defined group with consistently axillary flowers. However, since the axillary flowers are interpreted to be actually terminal on brachyblasts, the current taxonomic position of *M. cathcartii* should be reconsidered. In fact, *Alcimandra* has been treated as a distinctive genus from *Magnolia* because it has stipitate gynoecium similar to the condition in all species of *Michelia* (Nooteboom, 1985; Figlar, 2000). The gynophore develops even further in fruit to a short stalk on the fruiting axis between the androecium and the base of gynoecium.

A stipitate gynoecium is also found in *Pachylarnax,* section *Manglietiastrum* of *Magnolia* subgenus *Talauma,* and section *Gynopodium* of *Magnolia* subgenus *Magnolia,* which together

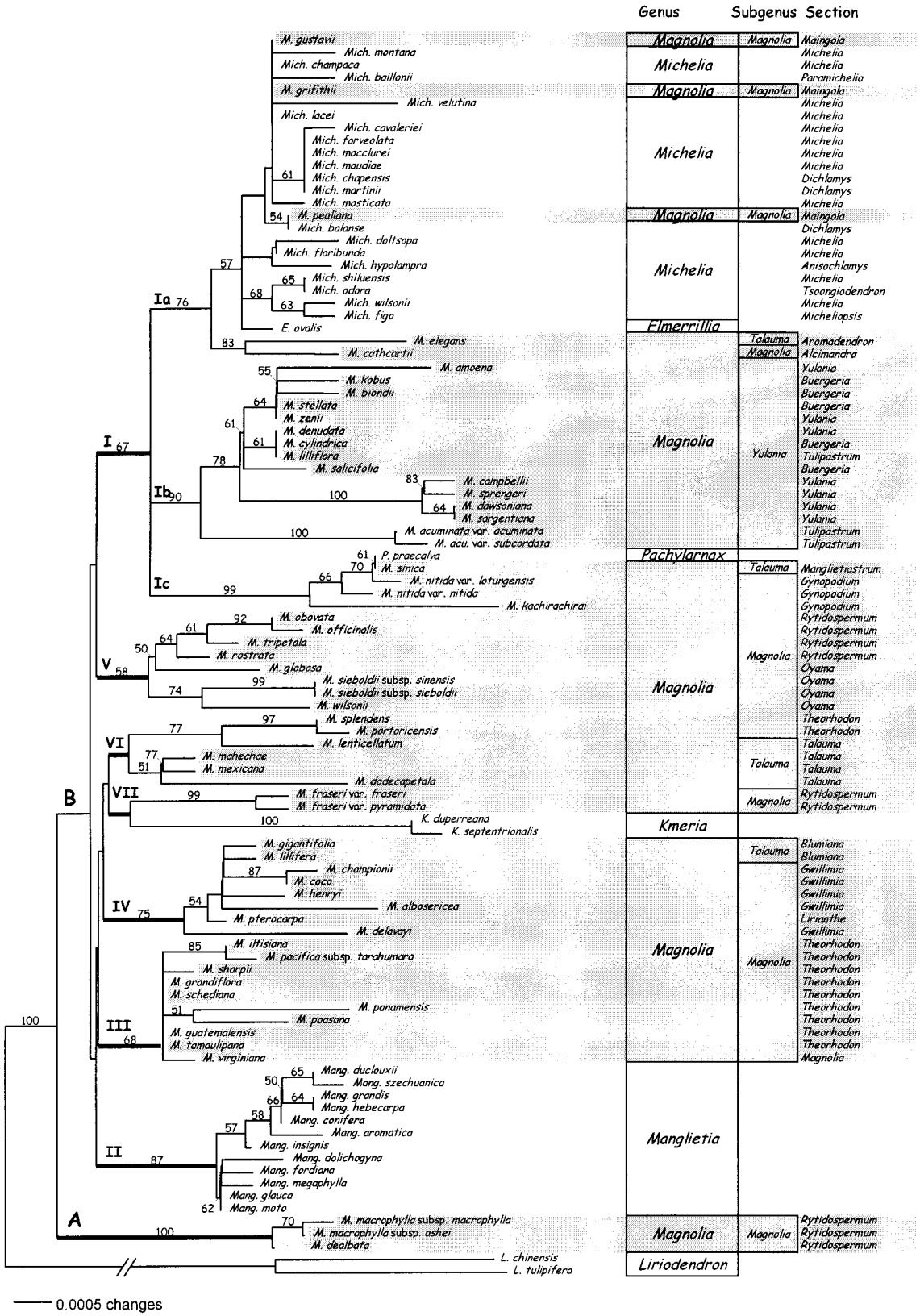


Fig. 6. The neighbor-joining tree. Bootstrap values >50% are shown above the branches.

form Clade Ic. Section *Manglietiastrum* is similar to the genus *Manglietia* but distinguished by petioles without stipular scars and a gynoecium with a short gynophore (Chen and Nooteboom, 1993). In the *ndhF* tree, *Manglietiastrum* is quite distantly related to the clade of *Manglietia. Manglietiastrum* is separated from the well-defined clade of *Manglietia* and allied with *P. praecalva* and species of section *Gynopodium* of *Magnolia* subgenus *Magnolia.* Although *Manglietia* was once considered to belong to the genus *Magnolia* (Baillon, 1866), it has been recognized as a distinctive genus defined by four or more ovules in each carpel (Law, 1984; Nooteboom, 1985). *Manglietiastrum* often has been classified as an independent genus (Law, 1979, 1984, 1996) and sometimes as a section of *Magnolia* subgenus *Talauma* (Nooteboom, 1985) or a section of *Manglietia* (Chen and Nooteboom, 1993). The *ndhF* tree does not support a close association of *Manglietiastrum* with *Manglietia* or with other species of *Talauma.*

Gynopodium has been considered to be a section of *Magnolia* subgenus *Magnolia* (Dandy, 1978; Nooteboom, 1985; Chen and Nooteboom, 1993) or designated as the genus *Parakmeria* (Hu and Cheng, 1951; Law, 1984, 1996). The *ndhF* molecular tree strongly suggests that the clade of *Pachylarnax–Manglietiastrum–Gynopodium* is closely related to the Michelieae and *Yulania* clades. This association is also supported by the character of a stipitate gynoecium.

Pachylarnax has been considered to be a well-distinguished genus based on the unique capsular fruit (Dandy, 1978; Law, 1984, 1996; Nooteboom, 1985). *Pachylarnax praecalva* is placed together with species of sections *Manglietiastrum* and *Gynopodium* in the *ndhF* tree. The *ndhF* sequence of *P. praecalva* is identical with that of *M. sinica,* the only species of section *Manglietiastrum,* implying a strong association.

Michelia odora was once considered a monotypic genus, *Tsoongiodendron,* characterized by crowded, sessile, woody, and large fruits (Chun, 1963). *Michelia baillonii* was also regarded as the distinctive genus *Paramichelia* because it has syncarpous fruits and entirely adnate stipules (Hu, 1940). The separation of *Toongiodendron* and *Paramichelia* as distinctive genera is not supported by the *ndhF* analysis.

Magnolia subgenus Yulania—The subgenus *Yulania* has been divided into three sections, *Tulipastrum, Yulania,* and *Buergeria* (Dandy, 1927, 1950). These sections are recognized by (1) the presence/absence of sepaloid tepals, the tepals of the outermost whorl being smaller than the inner tepals, (2) the time of flowering before or after the production of leaves, and (3) the color of the tepals (Dandy, 1927, 1950). In the *ndhF* tree, *M. acuminata,* the sole North American species of the subgenus *Yulania,* is separated from the Asian species and placed at the base of the *Yulania* clade. Traditionally, *M. liliiflora,* which is an Asian species, and *M. acuminata* constitute section *Tulipastrum* because of the sepaloid tepals and flowers appearing with or after the production of leaves (Dandy, 1927, 1950; Nooteboom, 1985; Chen and Nooteboom, 1993). However, molecular data show that *M. liliiflora* is quite distantly related to *M. acuminata.* The *ndhF* sequence of *M. liliiflora* is identical with those of *M. denudata* and *M. cylindrica* that belong to section *Yulania* and *Buergeria,* respectively. In addition, since other species of section *Yulania* are variously related to species of section *Buergeria,* the *ndhF* data do not support sectional treatments of the subgenus *Yulania* based on sepaloid tepals, time of flowering, and the tepal color (Chen and Nooteboom, 1993). Four species of the section *Yulania, M. dawsoniana, M. sargentiana, M. campbellii,* and *M. sprengeri,* form a strongly supported clade, but there are no discrete characters that define this clade except for the relatively large flowers with pinkish tepals.

Clade of sections Theorhodon–Magnolia of Magnolia subgenus Magnolia—Recently Vázquez-Garcia (1994) separated Caribbean species (*M. portoricensis* and *M. splendens*) from North and Central American taxa in section *Theorhodon* and placed them in section *Splendentes.* Section *Splendentes* is distinguished by stamens with the connective apex extended into a long setiform appendage, while species of *Theorhodon* have stamens with the short connective apex acute to acuminate. The stamen appendages of *Splendentes* become embedded in the gynoecium and support the stamen when it detaches at the base during dehiscence of the anther (Howard, 1948; Vázquez-Garcia, 1994). The *ndhF* data strongly support this segregation of section *Splendentes* by Vázquez-Garcia (1994). *Splendentes* is separated from the clade of *Theorhodon* and associated with South American *Talauma,* being closely allied with *M. lenticellatum. Magnolia lenticellatum,* a Colombian species of *Talauma* that was treated as section *Dugandiodendron* by Lozano-Contreras (1975), also has a long, elongate, hair-like appendage at the tip of stamen. *Magnolia virginiana,* which is a sole member of the section *Magnolia* of *Magnolia* subgenus *Magnolia,* is closely allied with the core members of *Theorhodon* in the *ndhF* tree. The close affinity between *M. virginiana* and section *Theorhodon* has also been demonstrated by chloroplast DNA restriction fragment length polymorphism (RFLP) comparison without the inclusion of *Splendentes* in the analysis (Qiu, Chase, and Parks, 1995).

Clade of sections Gwillimia, Lirianthe, and Blumiana—Sections *Gwillimia* and *Lirianthe* of *Magnolia* subgenus *Magnolia* are placed together, forming a clade. Both sections are distinguished by beaked fruiting carpels, and the beak of the monotypic *Lirianthe* is longer than those found in species of *Gwillimia* and forms a dorsally flattened coriaceous appendage that becomes more or less curved (Nooteboom, 1985). It should be noted that Southeast Asian members of *Talauma,* sections *Blumiana* and *Aromadendron,* are clearly separated from New World *Talauma,* placed in the clade of section *Gwillimia.* The close relationship between *Blumiana* and *Gwillimia* previously has been suggested because they are almost impossible to distinguish without fruits, although have been assigned to different subgenera (Nooteboom, 1985; Chen and Nooteboom 1993). Sections *Aromadendron* and *Blumiana* were assigned to subgenus *Talauma* because of connate carpels in fruits (Dandy, 1978; Nooteboom, 1985). The *ndhF* data suggest that subgenus *Talauma,* as traditionally recognized by connate carpels, is polyphyletic. Since Southeast Asian *Talauma* is distantly related to New World *Talauma,* and *Splendentes* of *Magnolia* subgenus *Magnolia* closely associates with New World *Talauma,* the taxonomic circumscription of subgenus *Talauma* should be adjusted.

Sections Rytidospermum and Oyama of Magnolia subgenus Magnolia—The most striking character that distinguishes section *Rytidospermum* is the whorl-like arrangement of the leaves, as indicated by the name of umbrella tree commonly used for the American species (Dandy, 1978). Dandy (1978) recognized three distinctive lineages in *Rytidospermum*: (1) Asian series comprising *M. hypoleuca, M. officinalis,*

and *M. rostrata,* (2) American series of *M. tripetala, M. fraseri,* and *M. pyramidata* (= *M. fraseri* var. *pyramidata*), and (3) American series of *M. macrophylla, M. ashei* (= *M. macrophylla* subsp. *ashei*), and *M. dealbata.* The *ndhF* analysis clearly distinguishes three lineages, and the only discrepancy is in the placement of *M. tripetala.*

Magnolia tripetala forms a clade together with the Asian series, which has a close affinity with the section *Oyama.* All species of *Oyama* are also Asian. The close affinity between *M. tripetala* and Asian *Rytidospermum* is supported by the extremely similar seed and fruit morphology, identical *rbcL* sequences of *M. tripetala* and *M. obovata* (= *M. hypoleuca*), high genetic identity estimated from allozyme variation, high interspecific cross compatibility between *M. tripetala* and Asian species, and chloroplast DNA RFLP analysis (Parks et al., 1983; Qiu et al., 1993; Qiu and Parks, 1994; Qiu, Chase, and Parks, 1995; Qiu, Parks, and Chase, 1995). Evidence that *Rytidospermum* was polyphyletic resulted from cladistic analysis of chloroplast DNA RFLP. Thus, leaf morphology and wood anatomy shared by Asian and North American *Rytidospermum* were assumed to be convergent (Qiu, Chase, and Parks, 1995). The more extensive sampling of Magnoliaceae presented here supports that conclusion.

Magnolia fraseri constitutes a distinctive lineage, separated from the clade of Asian *Rytidospermum* that contains North American species, *M. tripetala.* Another group of North American *Rytidospermum, M. macrophylla* and *M. dealbata,* is placed at the base of the *ndhF* tree, suggesting they might be the sister group to all other species of the Magnolioideae. Since this position is poorly supported, additional data will be required to identify the earliest split in Magnoliaceae. The basal placement of *M. macrophylla* and *M. dealbata* was also shown in the chloroplast DNA RFLP analysis (Qiu, Chase, and Parks, 1995). In the *ndhF* sequences, *M. macrophylla* subsp. *macrophylla, M. macrophylla* subsp. *ashei, M. dealbata,* and *Liriodendron chinensis* share a three-base deletion at the end of the gene. However, the significance of this deletion is unclear because the other outgroup species, *L. tulipifera,* does not have the deletion. In the phylogenetic analysis of *rbcL* sequences, *M. macrophylla* is also placed at the base among the clade of four species of *Magnolia,* which is closely associated with *Liriodendron* (Qiu et al., 1993).

Phylogenetic position of Kmeria—*Kmeria,* distributed in Southeast Asia, has been treated as a distinctive genus because it has unisexual flowers (Dandy, 1927, 1978; Law, 1984; Nooteboom, 1985; Chen and Nooteboom, 1993) and its phylogenetic associations have never been proposed. The close association of *Kmeria* with *M. fraseri* in the *ndhF* tree remains in question because no other evidence so far supports the relationship. This affinity may rather be explained by long-branch attraction because the *Kmeria–M. fraseri* clade is supported by only one base change at the third position in codon, which is considerably less in comparison to four and nine steps supporting the clades of *M. fraseri* and *Kmeria,* respectively (Felsenstein, 1978; Hendy and Penny, 1989).

Conclusions—This study is the first attempt to elucidate phylogenetic relationships in the family Magnoliaceae from a comprehensive sampling of taxa representing all sections recognized to date. Phylogenetic analysis of Magnoliaceae using *ndhF* sequences not only confirmed many taxonomic relationships based on morphological data, but also provided evidence for phylogenetic relationships previously undetected by systematists. Although *ndhF* sequences do not completely resolve phylogenetic relationships in the family, they clearly delimit major lineages that are supported by other data. This study has also demonstrated that *ndhF* data are phylogenetically informative despite low sequence divergence within Magnoliaceae. Examination of rapidly changing genes and multiple gene analysis, part of an ongoing endeavor, will certainly enhance our understanding of the phylogeny of the family, which is essential to generate a natural classification system.

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