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Phylogenetic analysis of Magnoliales and Myristicaceae based on multiple data sets: implications for character evolution

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Magnoliales, consisting of six families of tropical to warm-temperate woody angiosperms, were long considered the most archaic order of flowering plants, but molecular analyses nest them among other eumagnoliids. Based on separate and combined analyses of a morphological matrix (115 characters) and multiple molecular data sets (seven variable chloroplast loci and five more conserved genes; 14 536 aligned nucleotides), phylogenetic relationships were investigated simultaneously within Magnoliales and Myristicaceae, using Laurales, Winterales, and Piperales as outgroups. Despite apparent conflicts among data sets, parsimony and maximum likelihood analyses of combined data converged towards a fully resolved and well-supported topology, consistent with higher-level molecular analyses except for the position of Magnoliaceae: Myristicaceae + (Magnoliaceae + ((*Degeneria* + *Galbulimima*) + (*Eupomatia* + Annonaceae))). Based on these results, we discuss morphological evolution in Magnoliales and show that several supposedly plesiomorphic traits are synapomorphies of Magnoliineae, the sister group of Myristicaceae (e.g. laminar stamens). Relationships within Annonaceae are also resolved with strong support (*Anaxagorea* basal, then ambavioids). In contrast, resolution of relationships within Myristicaceae is difficult and still incomplete, due to a very low level of molecular divergence within the family and a long stem lineage. However, our data provide good evidence that *Mauloutchia* is nested among other Afro-Malagasy genera, contradicting the view that its androecium and pollen are plesiomorphic *Botanical Journal of the Linnean Society*, 2003, **142**, 125–186.

ADDITIONAL KEYWORDS: Annonaceae – data combination – Magnoliaceae – *matK* – morphological evolution – *ndhF* – *trnK* intron – *trnL* intron – *trnL-trnF* – *trnT-trnL*.

INTRODUCTION

Magnoliales were long thought to be 'the most archaic existing order of flowering plants', as stated by Cronquist (1981, 1988), and because of their presumed primitiveness were listed first in most major pre-cladistic systems of angiosperm classification (including Cronquist, 1968, 1981, 1988; Thorne, 1974, 1992; Takhtajan, 1980, 1997). This interpretation was based on a special concentration of characters assumed to be ancestral in angiosperms, including simple, entire leaves with pinnate venation and monosulcate pollen with granular exine structure. Additional presumed plesiomorphic traits included laminar stamens, cond-

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uplicate carpels, the spiral arrangement of fertile parts in Magnoliaceae, and the absence of vessels in the wood of Winteraceae. Although the concept that Magnoliales are the most primitive angiosperms has been refuted by recent higher-level phylogenetic analyses (as detailed below), they remain one of the most important lineages in the early radiation of angiosperms, with a fossil record extending back to the Cenomanian (Dilcher & Crane, 1984; Frumin & Friis, 1999) and possibly the Albian (Crane, Friis & Pedersen, 1994: stamen type 1) and Aptian (*Lethomasites*: Ward, Doyle & Hotton, 1989; Doyle & Hotton, 1991).

Circumscription of Magnoliales

The taxa assigned to Magnoliales have varied considerably in recent classification systems (Table 1), although the order has always included a central group of three families: Degeneriaceae, Himantandraceae and Magnoliaceae. However, recent progress in angiosperm phylogenetics (Chase *et al*., 1993; Qiu *et al*., 1993, 1999, 2000; Mathews & Donoghue, 1999, 2000; Soltis, Soltis & Chase, 1999; Barkman *et al*., 2000; Doyle & Endress, 2000; Savolainen *et al*., 2000; D. Soltis *et al*., 2000; P. Soltis *et al*., 2000; Zanis *et al*., 2002) has led to a new, stable circumscription of the order as a monophyletic group of six families (Table 2), as formalized by APG (1998), including the three families listed above and Myristicaceae, Annonaceae and Eupomatiaceae. It is this definition that we adopt in the present study.

Annonaceae, Magnoliaceae and Myristicaceae each include many species and have pantropical or Asian-American distributions, whereas Degeneriaceae, Eupomatiaceae and Himantandraceae each consist of a single genus with two species almost entirely restricted to the Australasian area (Table 2). Unlike Annonaceae and Magnoliaceae, none of the genera of Myristicaceae have widely disjunct distributions. All genera of Myristicaceae are endemic to one of the four following areas: South and Central America (six genera), continental Africa (five genera), Madagascar (four genera), and South-east Asia to the western Pacific (six genera). Most Magnoliales are trees, or occasionally shrubs (with some lianas in the Annonaceae), and usually bear conspicuous, bisexual flowers, with the notable exception of Myristicaceae, which are instead characterized by very small, always unisexual flowers, with a single perianth cycle of usually three tepals.

Relationships of Magnoliales

Except for the morphological cladistic analysis of Donoghue & Doyle (1989), which placed Magnoliales as a paraphyletic basal grade or the sister-group of all other angiosperms, all higher-level phylogenetic analyses have contradicted a basal position for Magnoliales. Other analyses of morphological data placed different taxa at the base of the angiosperms: Calycanthaceae (*sensu* APG, 1998; Renner, 1999) (Loconte & Stevenson, 1991), Chloranthaceae (Taylor & Hickey, 1992; Nixon *et al*., 1994), Nymphaeales plus monocots (Doyle, Donoghue & Zimmer, 1994), or Nymphaeales (Doyle, 1996). These variations reflect different outgroup relationships and difficulties in assessment of morphological homologies between angiosperms and other seed plants. However, except for studies of *rbcL* (Chase *et al*., 1993; Qiu *et al*., 1993), which placed *Ceratophyllum* at the base of the angiosperms, analyses of molecular data have converged on trees in which *Amborella*, Nymphaeales, Illiciales, Trimeniaceae and *Austrobaileya* (the ANITA taxa) form a basal, paraphyletic grade (Hamby & Zimmer, 1992; Doyle *et al*., 1994; Goremykin *et al*., 1996; D. Soltis *et al*., 1997; 2000; P. Soltis *et al*., 1999; 2000; Mathews & Donoghue, 1999, 2000; Parkinson, Adams & Palmer, 1999; Qiu *et al*., 1999, 2000, 2001; Barkman *et al*., 2000; Borsch, 2000; Graham & Olmstead, 2000; Graham *et al*., 2000; Savolainen *et al*., 2000; Zanis *et al*., 2002; Borsch *et al*. in press; K. W. Hilu *et al*., unpubl. data). All these analyses place Magnoliales in a more derived (nested) position in the angiosperms, although they are still located in the magnoliid grade that is basal to eudicots and monocots.

Analyses of *rbcL* (Chase *et al*., 1993; Qiu *et al*., 1993), *rbcL* and *atpB* (Savolainen *et al*., 2000), *rbcL*, *atpB* and 18S rDNA (P. Soltis *et al*., 2000), morphology (Doyle & Endress, 2000), and morphology plus *rbcL*, *atpB* and 18S rDNA sequences (Doyle & Endress, 2000) have identified Winteraceae and Canellaceae as the sister-group of Magnoliales. The second outgroup of Magnoliales was Laurales (*sensu* APG, 1998; Renner, 1999) (Doyle & Endress, 2000; P. Soltis *et al*., 2000), Laurales plus Piperales (*sensu* APG, 1998) (Savolainen *et al*., 2000; P. Soltis *et al*., 2000), or Chloranthaceae plus the ANITA taxa (Chase *et al*., 1993; Qiu *et al*., 1993). The clade consisting of Winteraceae and Canellaceae has been supported by all molecular analyses, but it was not formally named by APG (1998). Chase *et al*. (1993) treated it as part of Magnoliales, but because its position is still unresolved (see below), we believe it is better considered a distinct order, for which several recent papers have used the name Winterales (Donoghue, 1994; Qiu *et al*., 1999, 2000; P. Soltis *et al*., 1999, 2000; D. Soltis *et al*., 2000; Barkman *et al*., 2000; Doyle & Endress, 2000; Kuzoff & Gasser, 2000).

A larger number of molecular analyses have identified Laurales as the sister group of Magnoliales and a clade consisting of Winterales and Piperales as their

Table 1. Evolution of the family content of Magnoliales through various classification systems prior to APG (1998). The six families underlined are those included in the order in the APG (1998) phylogenetic system

Hutchinson (1973)	Walker (1976b)	Cronquist (1988)	Dahlgren (1989)	Thorne (1992)	Takhtajan (1997)
MAGNOLIALES	MAGNOLIALES	MAGNOLIALES	Annonales	MAGNOLIALES	MAGNOLIALES
Magnoliaceae	Magnoliineae	Winteraceae	Annonaceae	Illiciineae	Degeneriaceae
Illiciaceae	Magnoliaceae	Degeneriaceae	Myristicaceae	Magnoliineae	Himantandraceae
Winteraceae, incl. Degeneriaceae	Degeneriaceae	Himantandraceae	Eupomatiaceae	Magnoliaceae	Magnoliaceae
Canellaceae	Himantandraceae	Eupomatiaceae	Canellaceae	Degeneriaceae	Eupomatiales
Schisandraceae	Eupomatiaceae	Austrobaileyaceae	Austrobaileyaceae	Himantandraceae	Eupomatiaceae
Himantandraceae	Annonineae	Magnoliaceae	MAGNOLIALES	Eupomatiaceae	Annonales
Lactoridaceae	Annonaceae	Lactoridaceae	Degeneriaceae	Annonaceae	Annonaceae
Trochodendraceae	Canellaceae	Annonaceae	Himantandraceae	Aristolochiaceae	Myristicales
Cercidiphyllaceae	Myristicaceae	Myristicaceae	Magnoliaceae	Myristicaceae	Myristicaceae
Annonales		Canellaceae		Canellaceae	
Annonaceae				Austrobaileyineae	
Eupomatiaceae				Laurineae	
Laurales				Piperineae	
Monimiaceae					
Austrobaileyaceae Trimeniaceae					
Lauraceae					
Gomortegaceae					
Hernandiaceae					
Myristicaceae					

Table 2. Basic information on the six families currently included in Magnoliales (*sensu* APG, 1998)

Family	Genera/species	Distribution	Habit
Magnoliales Bromhead			
Annonaceae Juss.	128–200/2050–2500	Pantropical (except temperate *Asimina*)	Trees, shrubs, or lianas
Degeneriaceae I.W. Bailey & A.C. Sm. (= *Degeneria*)	1/2	Fiji	Trees
Eupomatiaceae Endl. (= *Eupomatia*)	1/2	New Guinea, eastern Australia	Small trees or rhizomatous, wiry shrubs
Himantandraceae Diels (= *Galbulimima*)	1/2	Eastern Malaysia to northern Australia	Trees
Magnoliaceae Juss.	2–13/200–240	Tropical to warm temperate (especially Northern Hemisphere)	Trees or shrubs
Myristicaceae R. Br.	21/440–500	Pantropical	Trees

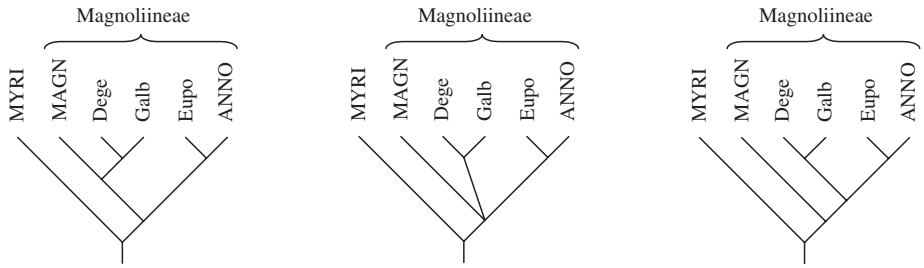
next outgroup (Mathews & Donoghue, 1999, 2000; Qiu *et al*., 1999, 2000; Barkman *et al*., 2000: six-gene analysis; Graham & Olmstead, 2000; Graham *et al*., 2000; Savolainen *et al*., 2000: *atpB* alone; Zanis *et al*., 2002; Borsch *et al*. in press; K. W. Hilu *et al*., unpubl.). However, although the 17-gene analysis of Graham & Olmstead (2000) separated Winterales from Magnoliales, it did reveal one indel shared by the two groups.

Regardless of whether Winterales or Laurales were found to be sister to Magnoliales, most of these studies recovered a four-order clade consisting of Piperales, Winterales, Laurales and Magnoliales, which we will refer to as eumagnoliids (following Qiu *et al*., 2000, but not Savolainen *et al*., 2000, D. Soltis *et al*., 2000, and P. Soltis *et al*., 2000, who used this informal name to refer to a larger clade also including monocots and Chloranthaceae). Two exceptions are the results of Barkman *et al*. (2000: nine-gene analysis) and Doyle & Endress (2000: morphology plus three-gene analysis), which excluded Piperales from the eumagnoliid clade and linked them with either eudicots or monocots, respectively. Surprisingly, the six-gene maximum likelihood analysis of Parkinson *et al*. (1999) differs from all other recent molecular studies in not recovering a eumagnoliid clade at all and linking Magnoliales with Chloranthaceae, although with no statistical support. No studies have identified Piperales as the sister-group of Magnoliales.

Background on the phylogeny of Magnoliales

Phylogenetic analyses of angiosperms based on morphology gave inconsistent relationships within Magnoliales. All trees of Donoghue & Doyle (1989) included a core group of *Degeneria* (= Degeneriaceae), Magnoliaceae, Annonaceae and Myristicaceae, which was associated with *Galbulimima* (= Himantandraceae) and *Eupomatia* (= Eupomatiaceae) in some trees. Loconte & Stevenson (1991) found two sister clades, one consisting of *Galbulimima*, *Degeneria* and Magnoliaceae, the other of *Eupomatia*, Annonaceae and Myristicaceae plus Canellaceae. In studies by Doyle & Le Thomas (1994, 1996), which focused on relationships within Annonaceae, *Eupomatia* and *Galbulimima* were basal to *Degeneria*, Magnoliaceae, Annonaceae and Myristicaceae, but relationships among the last four taxa were unresolved. Finally, in the morphological analysis of Doyle & Endress (2000), Myristicaceae were sister to Magnoliaceae, Annonaceae, *Degeneria*, *Galbulimima* and *Eupomatia*. Several molecular analyses based on *rbcL* data (Chase *et al*., 1993; Qiu *et al*., 1993; Rice, Donoghue & Olmstead, 1997; Doyle, Bygrave & Le Thomas, 2000; Savolainen *et al*., 2000) also gave inconsistent relationships, depending on taxonomic sampling and methods of analysis.

In contrast, all recent higher-level phylogenetic analyses based on combined molecular (Qiu *et al*., 1999, 2000; Savolainen *et al*., 2000; D. Soltis *et al*., 2000; P. Soltis *et al*., 2000; Zanis *et al*., 2002) or morphological and molecular (Doyle & Endress, 2000) data sets have given very similar results (Fig. 1). In all of them, Myristicaceae were basal in the order, sister to the five other families, which we will refer to in this study as Magnoliineae Engl. (although Walker, 1976b, and Loconte & Stevenson, 1991 used this subordinal name with narrower circumscriptions). Within Magnoliineae, two clades were consistently recovered: *Degeneria* plus *Galbulimima*, and *Eupomatia* plus Annonaceae. Relationships of Magnoliaceae remained inconsistent, depending on the position of the root of Magnoliineae.



Qiu *et al.* (1999, 2000: *atpB*+*rbcL*+18S+*atp1*+*matR*)
Savolainen *et al.* (2000: *atpB*+*rbcL*)
D. Soltis *et al.* (2000: B series; *atpB*+*rbcL*+18S)
P. Soltis *et al.* (2000: 110-taxon analysis; *atpB*+*rbcL*+18S)
Zanis *et al.* (2002: 104-taxon analysis)

D. Soltis *et al.* (2000: A series; *atpB*+*rbcL*+18S)
P. Soltis *et al.* (2000: compartmentalization; *atpB*+*rbcL*+18S)

Doyle & Endress (2000: morphology+*atpB*+*rbcL*+18S)

Figure 1. Background on the phylogeny of Magnoliales prior to this study, as indicated by higher-level combined analyses in which all six families were represented and found to form a monophyletic group. Brackets identify Magnoliineae (*sensu* this study). Abbreviations: ANNO = Annonaceae, Dege = *Degeneria*, Eupo = *Eupomatia*, Galb = *Galbulimima*, MAGN = Magnoliaceae, MYRI = Myristicaceae.

Studies in Magnoliaceae

A number of recent studies have investigated the phylogeny of Magnoliaceae using molecular data (Qiu, Chase & Parks, 1995; Azuma, Thien & Kawano, 1999; Jin *et al*., 1999; Pan *et al*., 1999; Shi *et al*., 2000; Ueda, Yamashita & Tamura, 2000; Azuma *et al*., 2001; Kim *et al*., 2001). When outgroups were included to root the trees, these studies supported a basal split between *Liriodendron* and the remaining Magnoliaceae, concurring with the pre-cladistic division of the family into two subfamilies, Liriodendroideae and Magnolioideae (Law, 1984; Nooteboom, 1985). Within Magnolioideae, all studies indicated that *Magnolia* as traditionally defined is paraphyletic or polyphyletic, supporting the inclusion of all other genera of Magnolioideae in an extended, monophyletic circumscription of *Magnolia*.

Studies in Annonaceae

Doyle & Le Thomas (1994, 1996) conducted morphological cladistic analyses of the Annonaceae and recently combined their data set with *rbcL* data (Doyle *et al*., 2000). These studies supported the genus *Anaxagorea* as the basal line in the family, as well as several major clades in the remaining Annonaceae including, in the combined analysis, a malmeoid–piptostigmoid–miliusoid (MPM) clade, an ambavioid plus *Cananga* clade, and a large inaperturate clade. Van Zuilen *et al*. (1996) obtained consistent results in a smaller analysis of *trnL-trnF*. The relationships among the three large clades, however, remained unresolved in the combined analysis of Doyle *et al*. (2000).

Previous views on Myristicaceae

In contrast to the significant recent progress made in phylogenetic reconstruction of Magnoliaceae and Annonaceae, Myristicaceae have so far received very little attention from phylogeneticists. Before the advent of modern phylogenetics, Warburg (1897: 102) proposed a 'hypothetical genealogical tree of the genera of Myristicaceae' in his main monograph of the family. Later botanists who contributed to the systematics of Myristicaceae (including Smith, 1937; Capuron, 1972, 1973; Sinclair, 1958a,b, 1961, 1968, 1974, 1975; Rodrigues 1977, 1980, 1981, 1982, 1989a,b; de Wilde, 1979, 1981, 1984a,b, 1985a,b, 1986, 1987a,b, 1990, 1991a,b, 1994a,b, 1995, 1996, 1998, 2000) hardly ever made suggestions about relationships among the 21 genera now recognized in the family. Two pre-cladistic views on the origin of the family are, however, worth mentioning. Sinclair (1958a: 242–244) thought that Myristicaceae originated in the southeast Asian-Pacific area, based on the highest species diversity in this region, to which a number of other 'primitive' angiosperm families are endemic (including Degeneriaceae, Eupomatiaceae and Himantandraceae). Warburg (1897) suggested that the Malagasy genus *Mauloutchia* was the most basal line in Myristicaceae, based primarily on the fact this genus has filaments in its androecium, unlike all other Myristicaceae, which have strictly sessile anthers.

Walker & Walker (1981) also emphasized the primitiveness of the pollen of *Mauloutchia*, compared to other genera, based especially on its granular exine structure. A basal position for *Mauloutchia* was also found in a morphological analysis by Sauquet (1999), which served as the starting point for the present study.

Goals of this study

This study had two main goals. First, we aimed at reconstructing the relationships among genera of Myristicaceae. Although this part of the study gave few well-supported results, we believe it is important to provide a progress report on this research, because no such attempt has ever been published for the family and some important and novel results have emerged. Second, we aimed at testing and confirming with more taxa and sequence data what recent higher-level analyses had suggested for relationships among families of Magnoliales, and at discussing in detail for the first time what these relationships mean for morphological evolution in this important group of angiosperms. In most cases, we were not expecting topological novelty, but rather corroboration from different genes of previous hypotheses, in order to gain more confidence in using one particular topology as a basis for discussion of biological issues. However, given the low level of support and inconsistency among higher-level studies regarding at least some critical relationships, such as the position of Magnoliaceae and the sister-group of Magnoliales, further testing based on increased character and taxon sampling appeared essential.

Because outgroup relationships can have a great influence on the inference and, especially, rooting of ingroup relationships and vice versa, we decided to address the two phylogenetic issues (Myristicaceae and Magnoliales) in simultaneous analyses. A focus on relationships within Myristicaceae is especially important because of the putative basal position of this family in Magnoliales, which implies that ancestral conditions in Myristicaceae may have a direct influence on reconstruction of character evolution in Magnoliales. Until now, morphological data sets have included Myristicaceae as a single terminal taxon, scored with assumed ancestral states (e.g. Doyle & Endress, 2000), and this may have influenced phylogenetic results and character reconstructions.

In order to reconstruct these relationships, we scored potentially informative morphological variation from all parts of the plant body as cladistic characters and sequenced three plastid DNA regions (*ndhF*, *trnK*/*matK* and *trnT*-*trnF*), selected as among the most variable loci used in lower-level angiosperm phylogenetics. We also added published data from the five more conserved genes used by Qiu *et al*. (1999, 2000) to assemble a sufficient number of characters to reconstruct interfamilial relationships within Magnoliales with confidence. This study therefore provided a good opportunity to compare the performances of individual genes or loci at the level of Magnoliales. Following the current consensus among systematists that individual genes usually lead to incorrect reconstructions, there is a tendency to forgo separate analyses in studies that combine several genes. However, a comparison of separate and combined analyses may help us understand sources of phylogenetic signal and noise and inform us on the confidence we may put in results of studies based on single genes, which are still the only kind of phylogenetic study available for many groups of organisms.

MATERIAL AND METHODS

Taxonomic sampling

Magnoliales were assumed to consist of the six families included in the order by APG (1998), relying on strong evidence from the multiple and at least partially independent analyses summarized above. Annonaceae and Magnoliaceae were also assumed to be monophyletic and were represented as single entries in the morphological data set. They were, however, split up into several lines in the molecular data sets. All four major clades of Annonaceae found in combined analyses of morphology and *rbcL* (Doyle *et al*., 2000) were sampled, using 13 exemplar genera: *Ambavia*, *Anaxagorea*, *Annickia*, *Annona*, *Artabotrys*, *Asimina*, *Cananga*, *Isolona*, *Malmea*, *Mkilua*, *Polyalthia*, *Uvaria* and *Xylopia*. Given current evidence for the polyphyly of *Polyalthia* (Doyle & Le Thomas, 1996; Doyle *et al*., 2000), the same species (*P. suberosa*) was sampled across all data sets. Within Magnoliaceae, evidence for a basal split into Liriodendroideae and Magnolioideae (see above) led us to sample a single exemplar of each subfamily for all molecular data sets. Degeneriaceae, Eupomatiaceae and Himantandraceae were each treated as a single entry in both morphological and molecular data sets.

All 21 genera of Myristicaceae were treated as terminal taxa in the morphological data set, except for two Malagasy genera, *Brochoneura* and *Mauloutchia*, which we split up into all their component species, including four new species of *Mauloutchia*. This approach took into account the high level of morphological variation found in *Mauloutchia* and provided a test for the monophyly of these two genera. At least eight different genera from all four areas of distribution of Myristicaceae were sampled for each new molecular data set, with a maximum of 16 different genera (including all three species of *Brochoneura* and

three species of *Mauloutchia*). Due to the lack of suitable leaf material *Endocomia*, *Osteophloeum*, *Paramyristica* and *Scyphocephalium* could not be sampled for any of the molecular data sets.

In order to provide an optimal rooting for Magnoliales, we have represented Piperales, Winterales and Laurales each by two or more monophyletic groups, based on phylogenetic relationships in these orders (as explained in the Taxa section of Appendix A).

Morphological data set

We attempted to score all potentially informative morphological variations observed throughout Myristicaceae and Magnoliales as cladistic characters. We began with the data set of Doyle & Endress (2000) for basal angiosperms (reduced to the taxa included herein) and an unpublished data set for Myristicaceae (Sauquet, 1999), which we merged and edited with MacClade (version 3; Maddison & Maddison, 1997) and NDE (version 4.0.9; Page, 2001a), combining, redefining and rescoring characters as necessary. Data are derived partly from the literature and partly from our own observations. Specific methods followed for scoring morphological characters, the definitions of taxa included in this data set, an annotated list of all characters, and the morphological matrix itself are all presented in Appendix A.

Molecular data sets

In order to look for substantial variation at the levels of both Myristicaceae and Magnoliales, we sequenced three chloroplast DNA regions (Fig. 2), selected from among the most variable loci used in angiosperm phylogenetics: *ndhF*, a protein-coding gene; the *trnK* region, which consists of two short tRNA-encoding exons and a large intron, inside of which is inserted the *matK* protein-coding gene; and the *trnL* region, which consists of three adjacent noncoding fragments, the *trnT-trnL* intergenic spacer, the *trnL* intron (flanked by two short tRNA-encoding exons), and the

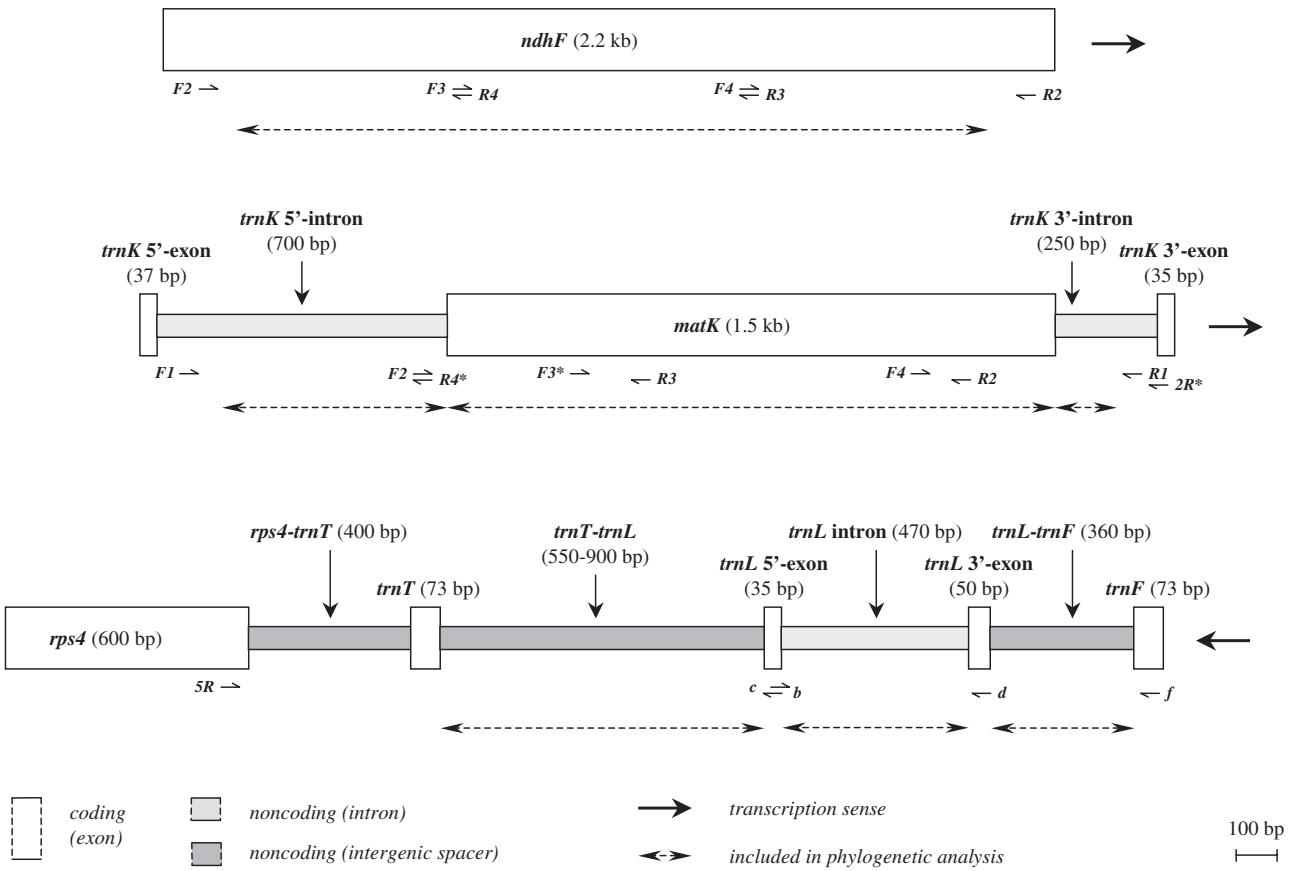


Figure 2. Schematic representation of the three chloroplast regions sequenced for this study. All primers are new (detailed information in Table 3), except for primers b, c, d and f of the *trnL* region, which are described in Taberlet *et al*. (1991). Indicated lengths are average estimates based on the taxa sampled for this study (see Table 4 for details on length variation).

trnL-trnF intergenic spacer. In addition, sequences of the five genes used by Qiu *et al*. (1999, 2000) for reconstructing basal angiosperm relationships were selected from GenBank; *rbcL* data for Annonaceae were supplemented by sequences from Doyle *et al*. (2000) not available on GenBank. Full sampling information for all molecular data sets is provided in Appendix B. An effort was made to represent supraspecific taxa by the same genera and species for all data sets, whenever this was possible.

The sequencing strategy and protocols used by H. Sauquet and T. Scharaschkin were as follows (sequences generated by other authors are indicated in Appendix B). Genomic DNA was extracted from either fresh or silicagel-dried leaf samples, using modified versions of the CTAB protocol (Doyle & Doyle, 1987) or, occasionally, QIAGEN DNeasy Plant Mini Kits. *ndhF* and the *trnK* region were both amplified and sequenced using newly designed primers (Table 3), most of which are specific to eumagnoliids. The *trnL* region was amplified and sequenced using the primers described in Taberlet *et al*. (1991) and a newly designed primer (*rps4*-5R) located near the 3′-end of the *rps4* gene, about 400 bp upstream of the *trnT* exon (Fig. 2). PCR amplifications were typically prepared in 25 μL reactions using 10 μL of 1:100 to 1:1000 diluted DNA solution, 2.5 μL of 10× Taq buffer, 2 μL of dNTPs (2.5 mM each), 1.25 μL of 100% DMSO, 1.25 μL of each primer (at 10 μM), 1 μL of 50 mM $MgCl_2$ solution, and 0.1 μL of 5 U μL^{-1} GibcoBRL Taq polymerase. A typical amplification program began with 3 min at 94°C, then 35 cycles of [1 min at 94°C, 1 min at 52°C, 2 min at 72°C], followed by 10 min at 72°C, and was performed on a Perkin Elmer GeneAmp PCR System 2400 thermocycler. PCR products were purified using the QIAGEN QIAquick PCR Purification Kit and sent to the sequencing facility (using a Perkin Elmer ABI 377 automated sequencer) of the Division of Biological Sciences at the University of California, Davis. Sequence data were edited and assembled using Sequencher (version 4; Gene Codes Corporation, Ann Arbor, Michigan).

Alignments were first generated using ClustalW with the default parameters as implemented in BioEdit (version 5.0.9; Hall, 1999). These automatic alignments were then edited by eye using BioEdit. Alignment of coding genes required very little, if any, editing. In contrast, no satisfactory automatic alignment was obtained with ClustalW for noncoding loci, even after trying different values of gap opening and elongation costs. These alignments thus required much hand-editing and the exclusion of indel (insertion or deletion) regions that could not be aligned unambiguously (i.e. with multiple, overlapping gaps of uncertain placement). However, such regions could occasionally be aligned among groups of taxa, in which case these regions were split into blocks corresponding to these groups of taxa, and unambiguously aligned blocks were included for phylogenetic analysis. In addition, unevenly sequenced ends were trimmed prior to phylogenetic analysis. All gaps (including artificial gaps created by this block-disjunction approach) were treated as missing data, but all parsimony-informative indel patterns were scored as binary or multistate characters in a separate data matrix (following principles outlined in Danforth, Sauquet & Packer, 1999: 608–609). Additional information on the indel data set is provided in Appendix C. All alignments and data sets are available upon request from the first author.

Table 3. New primers used in this study to sequence the three chloroplast regions, as indicated in Figure 2. All primers were designed by the first author, unless otherwise mentioned

Primer name	5′–3′ sequence
ndhF-HSF2	GA CTT CTG CTT GTT CCN AC
ndhF-HSF3	GCA GTT GCK AAA TCY GC
ndhF-HSF4	GGT ATT CCA CCY CTT GCT TG
ndhF-HSR2	C AAA AAA ATG AGT TAS TTB GG
ndhF-HSR3	CA AGC AAG AGG YGG AAT AC
ndhF-HSR4	GC GGA TTT MGC AAC TG
trnK-HSF1	GCAACGGATTCGTCCATACC
trnK-HSF2	CCTTGTTCTGACTGTATCG
trnK-HSF3*	CG CTG CGA TTA GTA TYT TC
trnK-HSF4	CT TCG GKG GTA AGG AKT CA
trnK-2R*[a]	CCCGGAACTAGTCGGATGG
trnK-HSR1	GCACACGGCTTTCCTTATG
trnK-HSR2	GA GTC TGA CGA ATC GGC
trnK-HSR3	GA GGS AGC ATC TTG TAT CCA
trnK-HSR4*	CGATACRGTCAGARCAAGG
rps4–5R[b]	AGG CCC TCG GTA ACG SG

[a]Designed by M.F. Wojciechowski (pers. comm.).
[b]Designed by T. Borsch.

PHYLOGENETIC ANALYSES

Individual and combined data sets

All DNA fragments described above were first treated individually (with the 5′- and the 3′-parts of the *trnK* intron treated as two separate fragments), resulting in 12 molecular data sets (seven from the three chloroplast regions sequenced for this study, five from previous higher-level studies; see Table 4), to which the morphological and indel matrices were added. We emphasize that the distinction between 'slow' and 'fast' loci used throughout this paper is only approximate. It is based on the empirical observation that

Table 4. Sequence length (bp) variation in the 12 molecular data sets included in this study. Sequence lengths are compared only among complete sequences over a defined fragment (as indicated in Fig. 2), before the exclusion of ambiguously aligned internal regions in noncoding data sets. The defined fragments for completely sequenced *matK*, *trnT-trnL*, *trnL* intron and *trnL-trnF* correspond exactly to the named locus. For all remaining data sets, the defined fragment corresponds to a partial segment of the named locus (i.e. with up to 200 bp trimmed on each end). Various 1 bp indels resulting from obvious sequencing mistakes in protein-coding sequences imported from GenBank (e.g. *matK*, *ndhF*) were not excluded for sequence length calculations

Data set	Average ± SD[a]	Range	Scored indels[b]
'Fast':			
ndhF	1884 ± 3	1877–1892	3
trnK 5′-intron	662 ± 14	637–688	14
matK	1531 ± 9	1504–1539	5
trnK 3′-intron	220 ± 9	189–234	8
trnT-trnL	792 ± 115	549–905	12
trnL intron	472 ± 59	323–511	3
trnL-trnF	364 ± 26	259–402	14
'Slow':			
atpB	1457 ± 0	1456–1457	0
rbcL	1398 ± 1	1397–1401	0
18S rDNA	1707 ± 2	1704–1709	0
atp1	1251 ± 1	1248–1251	0
matR	1785 ± 4	1784–1799	0

[a]Standard deviation.
[b]See Appendix C.

atpB, *rbcL*, 18S rDNA, *atp1* and *matR* are each more conserved (i.e. less variable) than *ndhF*, the *trnK* region, or the *trnL* region in the angiosperms, which does not necessarily imply that they had lower evolutionary rates in every taxon. These 14 data sets were analysed separately, then in various combinations following a maximum-taxon approach: when two data sets were combined, any taxon present in one and missing in the other was scored as a line of question marks in the data set for which data were missing. Eleven combined data sets were thus generated (listed and defined in Table 5). In order to avoid confusion, we refer to these combined data sets with capital letters (e.g. NONCOD = combined noncoding DNA loci). In addition, a uniform reduced sample of taxa was prepared for all data sets to limit reconstruction problems associated with taxa with large amounts of missing data (due to poor sampling across data sets) and reduce the effects of sampling heterogeneity on various comparisons among data sets. The arbitrary criterion for this sampling reduction was to discard all taxa having more than 30% missing data (not including alignment-generated gaps) over the seven fast molecular data sets considered together. This procedure led to a reduced set of 30 taxa, including four families of Laurales, five genera of Annonaceae, 12 genera of Myristicaceae, and all the remaining taxa. We will refer to data sets with this reduced taxon sample using the suffix 'red' throughout this paper (e.g. *ndhF*red = *ndhF* data set with reduced taxon sample).

Parsimony analyses

All 25 data sets were analysed with full and reduced taxon sampling with maximum parsimony using PAUP* (version 4.0b10; Swofford, 2002). All characters and substitutions were given equal weights, because we believe there is no rationale for applying any weighting scheme to the data prior to analysis, especially when it would involve the assumption that rarer changes have more phylogenetic value than more frequent changes and should therefore be given greater weights than the latter (e.g. 1st and 2nd vs. 3rd positions, transversions vs. transitions). Unless saturation of particular characters or transformations can be demonstrated, valuable information may be overlooked by downweighting more frequent changes, especially in lower-level studies such as this, in which third positions or transitions may contain most of the useful phylogenetic signal for reconstructing relationships. Furthermore, we prefer to correct for potential saturation by using the more appropriate maximum likelihood approach to this problem (see below).

Heuristic searches were conducted for each data set, using 100 replicates of random taxon addition sequence and TBR (tree bisection and reconnection) branch swapping, with a maximum of 2000 trees per replicate (reaching this limit in the set of trees saved at the end of a replicate resulted in abortion of the search after this replicate). Decay indices (using reverse constraints to search for shortest trees in which particular clades do not appear) and constraint costs were calculated using similar searches of ten replicates, with a maximum of 200 trees per replicate. All constraint trees were drawn using the tree editing tool in TreeView (version 1.6.6; Page, 2001b). Bootstrap support was evaluated using 1000 replicates of similar searches of one replicate each, with a maximum of 100 trees per replicate. Branch support was thus estimated for all data sets with full sampling and for five selected data sets with reduced sampling (listed in Table 6).

Maximum likelihood analyses

In addition, maximum likelihood (ML) analyses were conducted using PAUP* on all individual and combined molecular data sets with reduced taxon

Table 5. Cladistic information on all data sets included in this study. When there were multiple islands of most-parsimonious trees, the size of each island is indicated (e.g. 69+9). Combined data sets as follows: TRNK (*trnK* region) = *trnK* 5′-intron + *matK* + *trnK* 3′-intron; TRNL (*trnL* region) = *trnT-trnL* + *trnL* intron + *trnL-trnF*; FAST = *ndhF* + TRNK + TRNL; SLOW = *atpB* + *rbcL* + 18S rDNA + *atp1* + *matR*; NONCOD (noncoding) = *trnK* 5′-intron + *trnK* 3′-intron + TRNL; COD (coding) = *ndhF* + *matK* + SLOW; CP (chloroplast) = FAST + *atpB* + *rbcL*; MT (mitochondrial) = *atp1* + *matR*; MOLSEQ (molecular without indel matrix) = FAST + SLOW; MOL (molecular) = MOLSEQ + indels; TOT (total) = morphology + MOL. Abbreviations: Ann = Annonaceae, Myr = Myristicaceae, P-inform = parsimony-informative, MP = maximum parsimony, ML = maximum likelihood

	Full taxon sampling										Reduced taxon sampling							
Data set	Total characters	Total taxa	Ann taxa	Myr taxa	Variable characters		P-inform characters		MP trees	MP score	Total taxa	Variable characters		P-inform characters		MP trees	MP score	ML trees	ML score
Individual:																			
morphology	115	48	1	32	115	100%	114	99%	10	409	25	114	99%	103	90%	24	312	–	–
ndhF	1 910	31	6	12	737	39%	454	24%	32	1491	30	710	37%	405	21%	32	1406	1	9 845.95102
trnK 5′-intron	901	27	5	13	300	33%	140	16%	2000	473	26	300	33%	140	16%	2000	473	1	3 649.03726
matK	1 593	39	12	13	756	47%	447	28%	2	1543	30	694	44%	385	24%	3	1319	1	8 903.95128
trnK 3′-intron	260	28	5	13	120	46%	59	23%	2000	199	26	111	43%	56	22%	2000	182	1	1 235.55541
trnT-trnL	1 088	25	1	8	334	31%	141	13%	12+18	579	22	303	28%	120	11%	6	493	1	3 818.03271
trnL intron	556	44	12	20	185	33%	72	13%	2000	281	28	154	28%	52	9%	2000	226	97	1 979.15135
trnL-trnF	537	49	13	20	220	41%	117	22%	2000	445	30	173	32%	85	16%	18	312	1	2 181.33490
atpB	1 457	21	3	3	267	18%	131	9%	16	464	18	240	16%	107	7%	4+6	392	1	4 185.24595
rbcL	1 401	32	13	3	385	27%	207	15%	2	814	21	322	23%	155	11%	4	577	1	5 165.72284
18S rDNA	1 715	23	6	2	252	15%	113	7%	18+1	436	18	240	14%	106	6%	6	396	1	4 683.37071
atp1	1 251	22	4	3	109	9%	46	4%	18	153	19	103	8%	42	3%	36	144	1	2 670.08296
matR	1 808	22	4	3	222	12%	75	4%	18	288	19	201	11%	62	3%	12	252	1	4 216.69450
indels	59	49	13	20	59	100%	59	100%	2000	91	30	58	98%	55	93%	2000	84	–	–
Combined:																			
TRNK	2 754	39	12	13	1176	43%	646	23%	9	2226	30	1105	40%	581	21%	15	1985	1	13 888.34979
TRNL	2 181	49	13	20	739	34%	330	15%	2000	1315	30	630	29%	257	12%	2000	1038	1	8 109.60246
FAST	6 845	49	13	20	2652	39%	1430	21%	2000	5043	30	2445	36%	1243	18%	4	4439	1	32 076.58138
SLOW	7 632	32	13	3	1235	16%	572	7%	3	2184	21	1106	29%	472	6%	1	1781	1	21 406.18366
NONCOD	3 342	49	13	20	1159	35%	529	16%	2000	1994	30	1041	14%	453	14%	72	1698	1	13 085.45928
COD	11 135	42	13	13	2728	24%	1473	13%	24	5236	30	2510	23%	1262	11%	14	4519	1	40 904.55484
CP	9 703	49	13	20	3304	34%	1768	18%	2000	6337	30	3007	31%	1505	16%	4	5414	1	41 853.10740
MT	3 059	22	4	3	331	11%	121	4%	2	447	19	304	10%	104	3%	2	400	1	6 970.39416
MOLSEQ	14 477	49	13	20	3887	27%	2002	14%	2000	7242	30	3551	25%	1715	12%	4	6224	1	54 462.47302
MOL	14 536	49	13	20	3946	27%	2061	14%	2000	7342	30	3609	25%	1770	12%	2	6314	–	–
TOT	14 651	61	13	32	4061	28%	2175	15%	69+9	7788	30	3723	25%	1873	13%	1	6645	–	–

Table 6. Support for selected branches in the parsimony analyses of all individual and combined data sets, as estimated by decay indices (above) and bootstrap values (below). A dash alone indicates that no test was provided for this branch by this data set (insufficient sampling). A dash below a decay index represents a bootstrap value less than 50%. When the branch was not recovered in the strict consensus tree, the number of extra steps required to find this branch is indicated (+0 means this branch was found in some but not all most-parsimonious trees). Occasionally, branches not recovered in the strict consensus tree were supported in the bootstrap analysis; these bootstrap values are indicated as well. Abbreviations: Dege = *Degeneria*, Galb = *Galbulimima*, Eupo = *Eupomatia*, Broc = *Brochoneura*, Maul = *Mauloutchia*, Ceph = *Cephalosphaera*, Doyl = *Doyleanthus*, Coel = *Coelocaryon*, Pycn = *Pycnanthus*

	Unrooted and Magnoliales (Figs 3–5)									Annonaceae (Fig. 6)					Myristicaceae (Figs 7–10)								
	Laurales + Magnoliales	Winterales + Magnoliales	Magnoliales	Magnoliineae	Dege + Galb + Eupo + Anno	*Degeneria* + *Galbulimima*	*Eupomatia* + Annonaceae	Annonaceae	Myristicaceae	*Anaxagorea* basal	ambavioids	MPM + inaperturates	MPM	inaperturates	*Compsoneura* basal	mauloutchioids	Broc + Maul + Ceph + Doyl	Broc + Maul	mauloutchioids + Coel + Pycn	Coel + Pycn	mauloutchioids basal	America	Asia
Data set	A	A′	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
Individual:																							
morphology	D2 72%	+2	D1 –	D6 77%	D1 –	+2	+4	–	D8 98%	–	–	–	–	–	+8	+0	+0	+3	+9	+1	D2 58%	+11	+7
ndhF	D2 73%	+2	D3 80%	+1	+2	D2 57%	D8 98%	D15 100%	D27 100%	D11 100%	D21 100%	+0	–	–	D3 62%	–	D2 87%	D4 98%	D2 76%	D8 100%	+6	+4	+0
trnK 5′-intron[a]	–	–	D5 96%	+0	+1	+1	+0	D12 100%	D20 100%	D5 97%	D6 99%	+1	–	–	D1 65%	–	+0	+0	+0	D2 96%	+2	+4	+0
matK	D5 88%	+5	D5 87%	D8 90%	+4	+3	+2	D25 100%	D68 100%	D11 100%	D21 100%	D2 64%	D2 91%	D2 78%	+4	–	D1 58%	D5 99%	D1 60%	D6 100%	+2	+8	+2
trnK 3′-intron[a]	+2	+0	D2 58%	+0	+1	+0	+1	D4 96%	D4 98%	D2 82%	D3 95%	D2 89%	–	–	+0	–	+0	+0	+0	D2 88%	+0	+1	+0
trnT-trnL	+0	+0	+1	+1	+1	+1	+1	–	D13 100%	–	–	–	–	–	+1	–	D2 82%	D2 82%	D1 64%	–	+2	–	+2
trnL intron[a]	D1 –	+2	D2 –	D2 59%	+0	+0	+0	D5 94%	D5 98%	D1 69%	D1 –	D4 71%	+0	D1 61%	+0	+1	+0	+0	+0	+0	+2	+0	+1
trnL-trnF[a]	D1 –	+3	D1 –	+2	+1	+1	D2 58%	D6 94%	D11 100%	D2 88%	D5 94%	D2 77%	D2 81%	D4 81%	+0	+1	+0	D2 87%	+1	+0	+0	+1	+1
atpB	D3 63%	+3	D4 67%	D3 51%	+0	D2 –	D2 –	D5 97%	D10 100%	–	–	–	–	–	–	–	–	–	–	–	–	–	–
rbcL	+1	D1 –	D2 66%	D1 –	+2	+1	+1	D9 99%	D19 100%	D2 68%	D5 86%	+3	D1 –	D2 72%	–	–	–	–	–	–	–	–	–
18S rDNA	D2 81%	+2	+0	+1	+3	D3 90%	+0	D10 100%	D18 100%	–	–	+4	–	+3	–	–	–	–	–	–	–	–	–
atp1	+1	D1 –	D1 56%	+3	+3	+1	+2	D1 57%	D4 98%	–	–	+1	–	–	–	–	–	–	–	–	–	–	–
matR	+0	+0	D5 97%	+2	+1	+4	D4 98%	D11 100%	D2 93%	–	–	+0	–	–	–	–	–	–	–	–	–	–	–
indels[a]	+0	+0	+0	+0	+0	+0	+1 59%	D2 87%	+0	D2 62%	+0 83%	+2	+1	+0	+2	+0	+0	+0	+1	+0	+1	+1	+3

Table 6. *Continued*

	Unrooted and Magnoliales (Figs 3–5)									Annonaceae (Fig. 6)					Myristicaceae (Figs 7–10)								
	Laurales + Magnoliales	Winterales + Magnoliales	Magnoliales	Magnoliineae	Dege + Galb + Eupo + Anno	*Degeneria* + *Galbulimima*	*Eupomatia* + Annonaceae	Annonaceae	Myristicaceae	*Anaxagorea* basal	ambavioids	MPM + inaperturates	MPM	inaperturates	*Compsoneura* basal	mauloutchioids	Broc + Maul + Ceph + Doyl	Broc + Maul	mauloutchioids + Coel + Pycn	Coel + Pycn	mauloutchioids basal	America	Asia
Data set	A	A′	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
Combined:																							
TRNK	D3	+3	D7	D8	+2	+1	D1	D42	D87	D10	D18	D2	D2	D2	+1	–	D1	D5	D1	D11	+1	+11	+0
	66%		96%	95%			56%	100%	100%	100%	100%	67%	90%	77%			58%	99%	73%	100%			
TRNL[a]	D3	+3	D5	D1	+0	+0	D3	D11	D18	D4	D5	D5	D2	D3	+0	+1	+0	D2	+0	+0	+3	+0	+2
	82%		91%	59%			85%	100%	100%	98%	98%	98%	83%	91%				83%		65%			
FAST[a]	D7	+7	D19	D13	+0	D1	D19	D77	D17	D41	D60	D5	D4	D3	+0	+0	D2	D2	+0	+0	+7	+12	+0
	94%		100%	98%		51%	100%	100%	100%	100%	100%	99%	95%	93%			75%	86%		*3%			
SLOW	D8	+8	D17	D2	+1	D6	D8	D8	D46	D1	D4	+6	+0	D2	–	–	–	–	–	–	–	–	–
	97%		100%	62%		89%	94%	99%	100%	–	79%			71%									
NONCOD[a]	D1	+1	D8	D6	+0	+0	D5	D28	D38	D11	D14	D5	D2	D3	+0	+1	+0	D2	+0	+0	+6	+6	+2
	–		98%	88%			90%	100%	100%	100%	100%	98%	87%	94%	57%			85%		79%			
COD	D13	+13	D27	D15	+1	D8	D21	D45	D42	D17	D5	+3	D2	+0	+0	–	D3	D9	D2	D12	+3	+8	+0
	99%		100%	99%		93%	100%	100%	100%	100%	99%		81%	72%			92%	100%	87%	100%			
CP[a]	D9	+9	D21	D20	+0	D6	D22	D87	D22	D43	D64	D5	D6	D5	+0	+0	D1	D2	+0	+0	+7	+12	+0
	95%		100%	100%		86%	100%	100%	100%	100%	100%	94%	99%	94%			75%	86%		83%			
MT	D4	+4	D9	+2	+2	+5	D4	D14	D9	–	–	+1	–	–	–	–	–	–	–	–	–	–	–
	82%		99%				93%	100%	100%														
MOLSEQ[a]	D14	+14	D28	D20	+0	D9	D28	D87	D21	D43	D14	D6	D6	D5	+0	+0	D1	D2	+0	+0	+7	+12	+0
	98%		100%	100%		92%	100%	100%	100%	100%	100%	87%	99%	96%			78%	88%	50%	83%			
MOL[a]	D14	+14	D31	D20	D1	D10	D30	D91	D40	D46	D67	D8	D6	D5	+0	+0	D2	D2	+0	+0	+7	+13	+0
	99%		100%	100%	66%	94%	100%	100%	100%	100%	100%	91%	98%	96%			90%	89%	54%	83%			
TOT	D17	+17	D17	D21	D5	D12	D17	D26	D10	D25	D19	D8	D6	D5	+0	D2	D1	D1	+2	+0	+2	+12	+4
	99%		100%	100%	90%	93%	100%	100%	100%	100%	100%	91%	99%	95%		67%	64%	62%		69%			
Reduced:																							
morphred	D2	+2	+0	D6	+0	+2	+4	–	D7	–	–	–	–	–	+4	–	+0	+0	+3	+1	D3	+8	+5
	69%			82%	50%				99%												84%		
FASTred	D7	+7	D16	D13	+0	D1	D19	D76	D185	D37	D57	D8	–	–	D1	–	D3	D11	D6	D22	+7	+1	+0
	92%		100%	98%		–	100%	100%	100%	100%	100%	91%			51%		93%	100%	98%	100%			
MOLSEQred	D14	+14	D34	D22	+0	D9	D27	D87	D212	D41	D62	D4	–	–	D1	–	D3	D11	D6	D22	+7	+1	+0
	98%		100%	100%		92%	100%	100%	100%	100%	100%	77%			53%		93%	100%	97%	100%			
MOLred	D14	+14	D36	D24	D1	D10	D29	D91	D226	D44	D65	D6	–	–	D1	–	D4	D11	D6	D22	+7	+1	+0
	98%		100%	100%	66%	94%	100%	100%	100%	100%	100%	79%			–		96%	100%	93%	100%			
TOTred	D16	+16	D35	D37	D6	D12	D33	D94	D258	D44	D65	D6	–	–	+2	–	D8	D14	D2	D20	+2	D2	D1
	99%		100%	100%	92%	94%	100%	100%	100%	100%	100%	79%					100%	100%	81%	100%			

[a]Estimation of branch support may be inaccurate because of tree number limitation (see Table 5).

sampling. The search strategy used (recommended by H. Philippe, pers. comm.; see also Swofford *et al*., 1996: 445) was as follows: (1) estimation of all parameters (using the LScores command in PAUP*) of the GTR + gamma (with 8 rate categories) + I model based on the maximum parsimony topology (first tree in memory if multiple most-parsimonious trees were found); (2) heuristic search (with ten replicates of random taxon addition sequence and TBR branch swapping) using this model (with the previously calculated parameters); (3) reiteration of these two steps starting from the new ML topology until the topology and all scores stabilized. Stability of all scores (likelihood and parameter estimates) was usually reached within two iterations, while the topology itself usually remained unchanged after the first iteration. Although this successive approximation approach may be criticized on the grounds that the final results may depend on the initial (maximum parsimony) topology, it allows implementation of ML searches with a more general and therefore more realistic model than simultaneous estimation of topology and model parameters usually allows within reasonable computational times. Furthermore, we conducted a series of experiments in which we applied this search strategy to the same data set with different initial topologies and observed that alternative arrangements of the families of Magnoliales had no influence on the ML topology obtained. It is therefore unlikely that our ML results were conditioned by incorrect starting trees based on parsimony.

RESULTS

Variation and information in the molecular data sets

Our study provides a good opportunity to compare the evolution and information content of variable chloroplast regions in Magnoliales, together and in comparison with the more slowly evolving genes used in higher-level angiosperm phylogenetics. Although a maximum likelihood approach is required to compare the evolutionary rates of these fragments properly, a rough estimate of relative rates may be provided by comparing proportions of variable characters across data sets (Table 5). Direct comparison of this statistic, however, may be biased by sampling heterogeneity (denser taxonomic sampling will usually increase these proportions) as well as the exclusion of internal ambiguous regions in the noncoding data sets. The latter is particularly true of our *trnT-trnL* data set, since we found in this spacer a large AT-rich hotspot region, starting *c*. 60 bp downstream from the *trnT* exon, that could only be aligned among Myristicaceae. This led us to split this region into an aligned block with Myristicaceae and an unaligned block with all remaining taxa, which we excluded from the data set for all phylogenetic analyses, thus greatly underestimating the variation of this spacer.

In order to decrease the bias introduced by sampling heterogeneity, we have recalculated the proportions of variable and parsimony-informative characters over the set of taxa used in the reduced sampling experiments (Table 5). Most individual data sets do not include all 30 selected taxa (Table 5), but this procedure significantly homogenized the taxonomic sampling among data sets. Surprisingly, the two coding genes we sequenced (*ndhF* and *matK*) are more variable than the noncoding data sets (except for the *trnK* 3′-intron, with about the same level of variation as *matK*). The expected less-constrained evolution of noncoding DNA is instead reflected in length variation (Table 4), due to insertion and deletion events (indels), which are usually selected against in protein- or RNA-coding genes. As a result, many more indels have been scored in the noncoding data sets. *trnT-trnL* has by far the greatest length variation, most of which is concentrated in the large hotspot region.

Although *matK* had the highest proportion of parsimony-informative characters, *ndhF* provided the highest number of them as a result of its greater length. Another advantage of these two protein-coding genes is the fact that heuristic searches based on them quickly yielded small numbers of most-parsimonious trees, whereas most noncoding data sets gave exceedingly high numbers of most-parsimonious trees (Table 5), reducing the chances of finding the shortest trees. This result might seem to be easily attributed to lower ratios of informative characters to taxa in our noncoding data sets, but most of the effect is actually concentrated in the Myristicaceae part of the trees. Indeed, heuristic searches yielded a small number of most-parsimonious trees when there were more parsimony-informative characters than taxa sampled, considering Myristicaceae only (Table 7). This rule does not apply to the combined data sets, because of the great amounts of missing data caused by the addition of unscored taxa in our maximum-sampling approach to data combination. However, once our sampling reduction is applied to these combined data sets (greatly limiting the amount of missing data), the rule seems to apply again (Table 7).

All five slow data sets are less variable than the seven fast data sets (Table 5). Of all the individual data sets, the two mitochondrial genes (*atp1* and *matR*) are the least variable.

Further insight into the phylogenetic potential of all these DNA regions in Magnoliales may be gained by close examination of the results of individual and combined parsimony and maximum likelihood analyses. We did not perform any tests of incongruence among

Table 7. Variation and information content of all data sets within Myristicaceae (all other taxa were excluded for calculation of these statistics). General information on these data sets is provided in Table 5. Abbreviations: Tax = number of (Myristicaceae) taxa, P-inform = parsimony-informative, PI/Tax = ratio of parsimony-informative characters to taxa, – = not applicable (less than four taxa)

	Full taxon sampling						Reduced taxon sampling					
Data set	Tax	Variable		P-inform		PI/Tax	Tax	Variable		P-inform		PI/Tax
Individual:												
morphology	32	47	40.9%	44	38.3%	1.4	12	45	39.1%	32	27.8%	2.7
ndhF	12	83	4.3%	28	1.5%	2.3	12	83	4.3%	28	1.5%	2.3
trnK 5′-intron	13	28	3.1%	9	1.0%	0.7[a]	12	28	3.1%	8	0.9%	0.7[a]
matK	13	68	4.3%	32	2.0%	2.5	12	65	4.1%	25	1.6%	2.1
trnK 3′-intron	13	7	2.7%	3	1.2%	0.2[a]	12	7	2.7%	2	0.8%	0.2[a]
trnT-trnL	8	71	6.5%	14	1.3%	1.8	8	71	6.5%	14	1.3%	1.8
trnL intron	20	17	3.1%	3	0.5%	0.2[a]	12	13	2.3%	3	0.5%	0.3[a]
trnL-trnF	20	18	3.4%	5	0.9%	0.3[a]	12	14	2.6%	5	0.9%	0.4
atpB	3	6	0.4%	–	–	–	3	6	0.4%	–	–	–
rbcL	3	8	0.6%	–	–	–	3	8	0.6%	–	–	–
18S rDNA	2	25	1.5%	–	–	–	2	25	1.5%	–	–	–
atp1	3	7	0.6%	–	–	–	3	7	0.6%	–	–	–
matR	3	4	0.2%	–	–	–	3	4	0.2%	–	–	–
indels	20	9	15.3%	5	8.5%	0.3[a]	12	9	15.3%	4	6.8%	0.3[a]
Combined:												
TRNK	13	103	3.7%	44	1.6%	3.4	12	100	3.6%	35	1.3%	2.9
TRNL	20	106	4.9%	22	1.0%	1.1[a]	12	98	4.5%	22	1.0%	1.8[a]
FAST	20	292	4.3%	94	1.4%	4.7[a]	12	281	4.1%	85	1.2%	7.1
SLOW	3	50	0.7%	–	–	–	3	50	0.7%	–	–	–
NONCOD	20	141	4.2%	34	1.0%	1.7[a]	12	133	4.0%	32	1.0%	2.7
COD	13	201	1.8%	60	0.5%	4.6	12	198	1.8%	53	0.5%	4.4
CP	20	306	3.2%	94	1.0%	4.7[a]	12	295	3.0%	85	0.9%	7.1
MT	3	11	0.4%	–	–	–	3	11	0.4%	–	–	–
MOLSEQ	20	342	2.4%	94	0.6%	4.7[a]	12	331	2.3%	85	0.6%	7.1
MOL	20	351	2.4%	99	0.7%	5.0[a]	12	340	2.3%	89	0.6%	7.4
TOT	32	398	2.7%	143	1.0%	4.5	12	385	2.6%	121	0.8%	10.1

[a]MAXTREES limit (set to 2000 trees) was reached in the parsimony analysis of the corresponding data set (including taxa outside Myristicaceae).

our data sets. Parsimony tests of incongruence have been shown to confuse real incongruence (i.e. different evolutionary histories) with sampling error (cf. Buckley *et al*., 2002), and therefore may lead to mistaken conclusions on whether combination is appropriate. Instead, the phylogenetic signal may occasionally emerge only in the interaction of all the data (Cognato & Vogler, 2001), even (or especially) in cases where incongruence tests yield positive results (e.g. Reeves *et al*., 2001). We thus decided to adopt the alternative approach of making a branch-by-branch comparison of the results of separate and combined analyses (Wiens, 1998). This led us to calculate decay and bootstrap support for selected relationships in Magnoliales, Annonaceae and Myristicaceae over all separate and combined parsimony analyses, and, when a relationship was not found in a particular analysis, to calculate the number of extra steps required to recover it (Table 6).

Unrooted outgroup relationships

Although the exact location of the root in our trees is uncertain, the unrooted tree of Magnoliales and their outgroups provided a minimal test for both the monophyly of Magnoliales and their relationships with the three other eumagnoliid orders. Parsimony analyses of all our combined data sets and most individual data sets support a sister-group relationship of Magnoliales with Laurales rather than Winterales (Figs 3,4), with relatively weak support from individual data sets but good support from most combined data sets

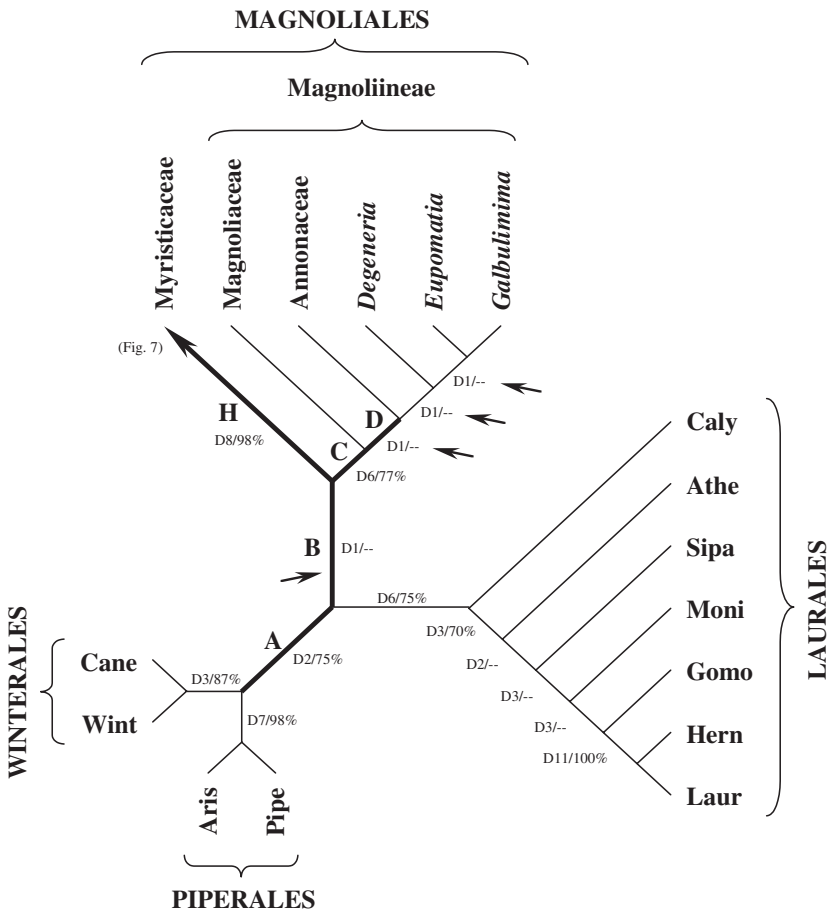


Figure 3. Relationships of Magnoliales and outgroups found in the strict consensus of ten most-parsimonious trees (varying only in relationships within Myristicaceae: Fig. 7) from the morphological analysis (see Table 5). Decay index and bootstrap support are provided for each branch. Thick, lettered branches relate to Table 6. The Myristicaceae part of this tree is developed in Figure 7. The four branches designated by arrows collapse in the strict consensus of 24 most-parsimonious trees from the morphological analysis with reduced taxon sampling (morphred; see Tables 5,6). Abbreviations: Aris = Aristolochiineae (*sensu* Shipunov, 2002: Aristolochiaceae + *Lactoris*), Athe = Atherospermataceae, Caly = Calycanthaceae, Cane = Canellaceae, Gomo = *Gomortega*, Hern = Hernandiaceae, Laur = Lauraceae, Moni = Monimiaceae, Pipe = Piperineae (*sensu* Walker, 1976b: Saururaceae + Piperaceae), Sipa = Siparunaceae, Wint = Winteraceae.

(Table 6A), reaching a decay index of 17 and a bootstrap value of 99% in the total combined (TOT) analysis. Only the individual *rbcL* and *atp1* data sets support the alternative relationship of Winterales and Magnoliales, although with weak support (Table 6A′), and trees in which Laurales and Magnoliales are sister groups are only one step longer.

The monophyly of Laurales (assuming the root is outside the order) is supported by all data sets except the *trnK* 3′-intron and *atp1*. It is rejected only by *atp1* (and by only one step) due to attachment of Calycanthaceae to Saururaceae, but this placement is very weakly supported and is rejected by the maximum likelihood analysis of the same data set, suggesting it is the result of long-branch attraction. Within Laurales, relationships vary considerably across the analyses, but become more stable as the data sets are added together. A topology consistent with the results of Renner (1999), except for the position of Hernandiaceae, is finally obtained in the combined molecular (MOL) and total (TOT) analyses (Fig. 4). Interestingly, whereas the combined molecular (MOL) analysis weakly supports a sister-group relationship between Hernandiaceae and Monimiaceae (decay index 2, bootstrap value 59%), the morphological and total combined (TOT) analyses strongly support an association of Hernandiaceae with Lauraceae (Figs 3,4), as in the morphological and morphology plus three-gene analyses of Doyle & Endress (2000). These results are consistent with the study of Renner & Chanderbali

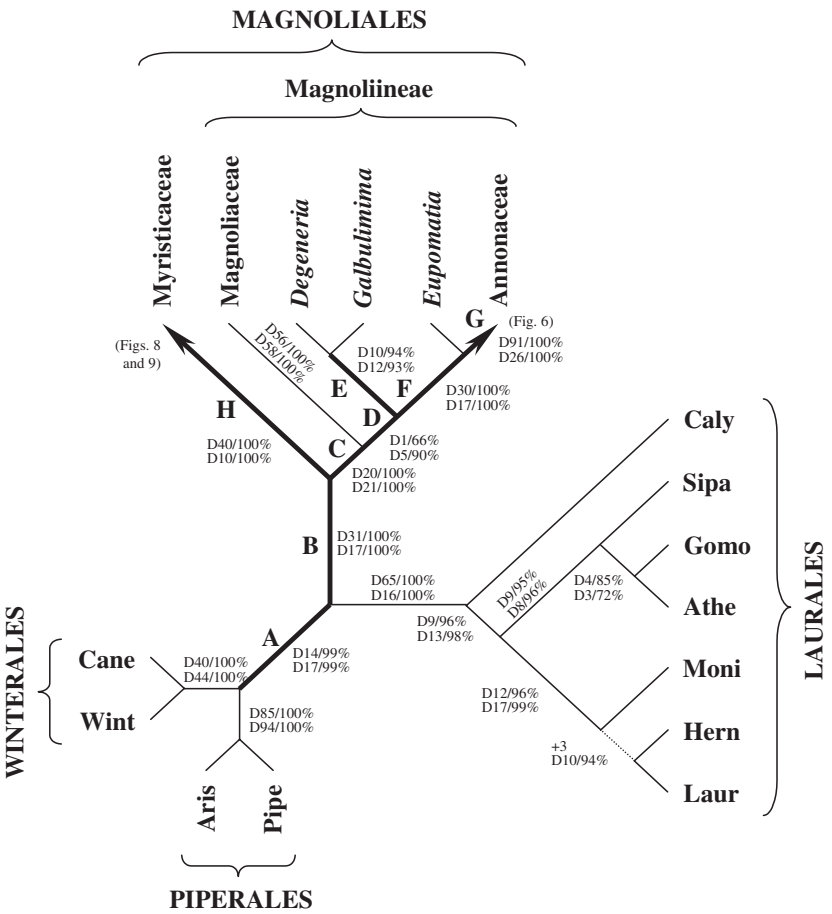


Figure 4. Relationships of Magnoliales and outgroups found in the strict consensuses of 2000 and 78 most-parsimonious trees (varying only in relationships within Myristicaceae: Figs 8,9) from the molecular combined (MOL) and total combined (TOT) analyses, respectively (see Table 5). Decay index and bootstrap support for both analyses are provided for each branch (molecular above, total below). The dotted branch was not recovered in the molecular analysis (where Hernandiaceae are sister to Monimiaceae, with a decay index of 2 and a bootstrap support of 59%). Thick, lettered branches relate to Table 6. The Annonaceae and Myristicaceae parts of this tree are developed in Figures 6, 8 and 9, respectively, as indicated by the arrows. Abbreviations as in Figure 3.

(2000), which suggested that molecular data are insufficient for resolving the relationship among these three families.

The monophyly of Magnoliales (assuming the root is outside the order) is supported by all data sets except *trnT-trnL*, 18S rDNA and morphology with reduced sampling (Table 6B). It is rejected only by *trnT-trnL* (by only one step) because of separation of *Ambavia* (the only member of Annonaceae sampled in this data set) from the rest of Magnoliales. However, the remaining Magnoliales are only very weakly supported as a clade (decay index 1, bootstrap value 50%), and the ML analysis of the same data set links *Ambavia* to *Eupomatia* and recovers the monophyly of Magnoliales (Fig. 5), suggesting the parsimony result was due to long-branch attraction with Winterales.

PHYLOGENY OF MAGNOLIALES

In almost all individual and combined parsimony analyses, Annonaceae (tested by molecular data only), Magnoliaceae (tested only in the molecular analyses by the association of *Liriodendron* with *Magnolia*), and Myristicaceae were all monophyletic, almost always with very strong support (Table 6G,H; results not shown for Magnoliaceae). The only minor exceptions concern the *trnL* intron, *atpB* and *atp1* data sets for Magnoliaceae but in no case was the monophyly of the family completely rejected (i.e. it was observed in some but not all most-parsimonious trees).

The analysis of our morphological data set (Fig. 3) led to exactly the same asymmetric topology as the morphological analysis of Doyle & Endress (2000). An

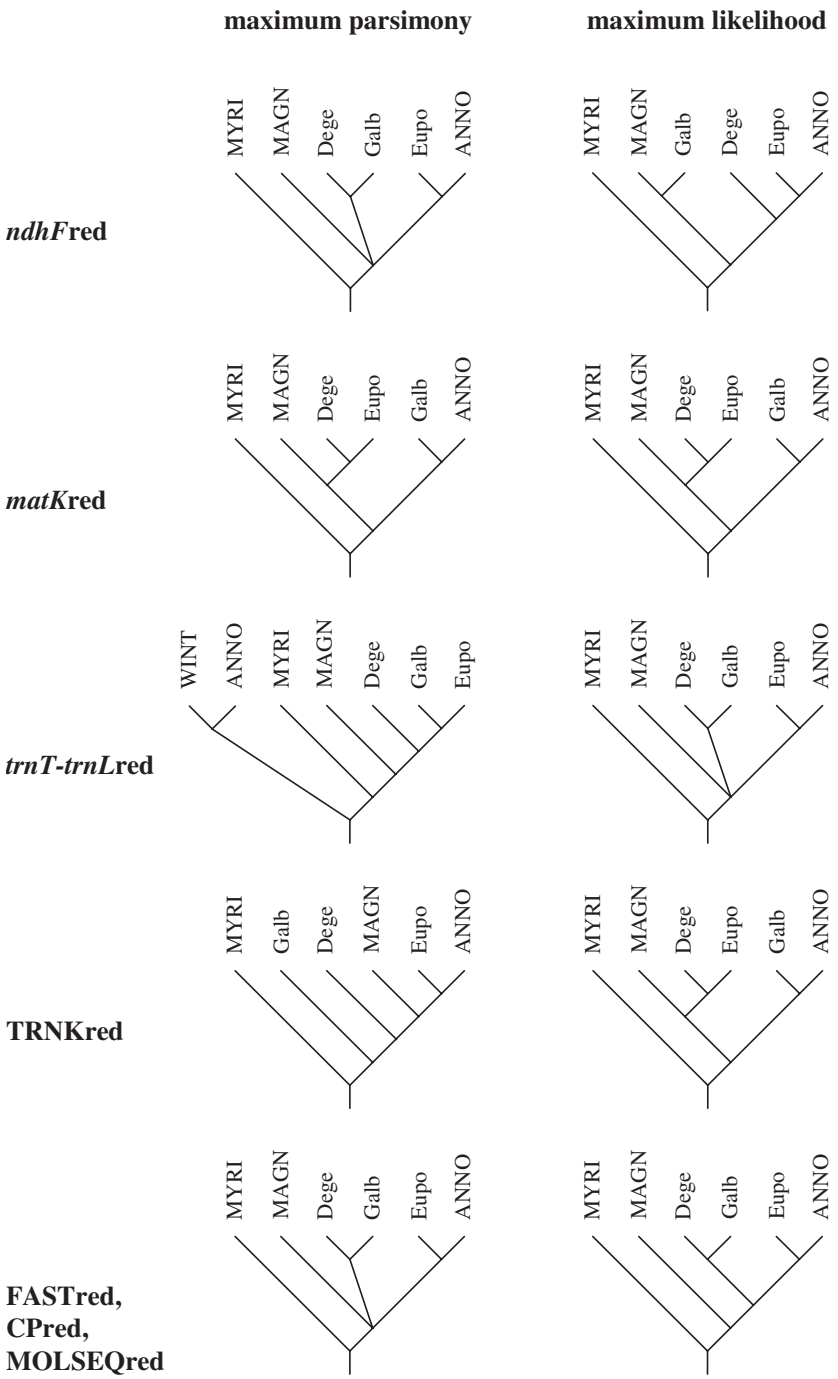


Figure 5. Summary of relationships among the six families of Magnoliales found in parsimony and maximum likelihood analyses of selected individual and combined data sets with reduced taxon sampling (see Table 5). Abbreviations: ANNO = Annonaceae, Dege = *Degeneria*, Eupo = *Eupomatia*, Galb = *Galbulimima*, MAGN = Magnoliaceae, MYRI = Myristicaceae, WINT = Winterales.

important difference, however, lies in our stronger support (decay index 6 and bootstrap value 77% vs. 1 and 60%) for a Magnoliineae clade (and thus the basal position of Myristicaceae). All remaining branches within Magnoliineae are very weakly supported in both analyses, indicating that this asymmetric tree may not be correct.

The relationships among the six magnolialian families fluctuate across the molecular data sets, but become more stable as data sets are added together

(Table 6C–F). The topology obtained in the combined molecular (MOL) and total (TOT) analyses (Fig. 4) had been previously found only in the combined morphology and three-gene analysis of Doyle & Endress (2000; Fig. 1). Interestingly, none of the other combined data sets nor any of the individual data sets unambiguously supported this topology, although a few did not reject it (i.e. it was found in some but not all most-parsimonious trees). The *Degeneria* plus *Galbulimima*, *Eupomatia* plus Annonaceae, and Magnolineae clades were each recovered in many analyses, but the clade consisting of *Degeneria*, *Galbulimima*, *Eupomatia* and Annonaceae together only appeared in the two final combined analyses (MOL and TOT; Fig. 4) and the morphological analysis (with a different arrangement of the four taxa; Fig. 3).

Finally, our reduced taxon sampling (involving a limited number of taxa in Annonaceae and Myristicaceae) had little or no influence on the relationships among the six families and support for the branches involved (Table 6C–F). Our reduced sample of taxa was therefore suitable for investigations of the phylogeny of Magnoliales with maximum likelihood methods (see below).

Phylogeny of Annonaceae

The topology we obtained for Annonaceae based on molecular data (Fig. 6) is consistent with the *trnL-trnF* analysis of van Zuilen *et al*. (1996) and the *rbcL* and *rbcL* plus morphology analyses of Doyle *et al*. (2000), and was very well supported in our final combined analyses (MOL and TOT; Fig. 6). The basal position of *Anaxagorea* in the family was found in all analyses where the genus was represented (Table 6I). In most analyses, the rest of the family was consistently distributed into an ambavioid clade (*Ambavia* + *Cananga*), a MPM clade (here represented by *Annickia*, *Malmea* and *Polyalthia*), and an inaperturate clade (all remaining genera; Table 6J,L,M). The particularly strong support we found for the association of *Cananga* with *Ambavia* (ambavioids; Table 6J) definitely rules out the placement of *Cananga* in the inaperturate clade (with *Xylopia*) found in the morphological analysis of Doyle & Le Thomas (1996), confirming the hypothesis based on *rbcL* data in Doyle *et al*. (2000). But, conversely, our combined molecular data indicate that the ambavioids are the next most-basal line after *Anaxagorea* (i.e. the MPM and inaper-

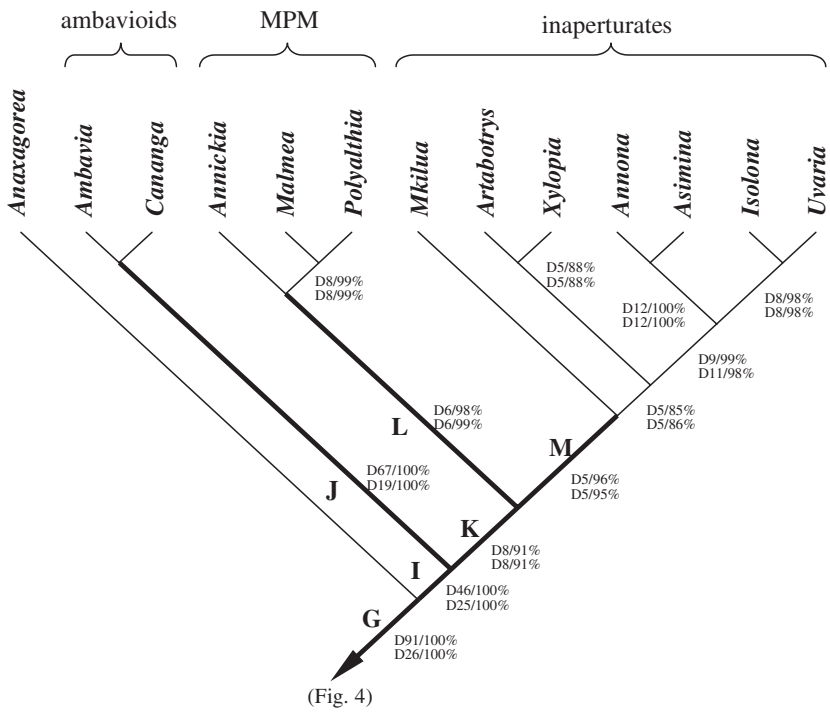


Figure 6. Annonaceae relationships found in the strict consensuses of 2000 and 78 most-parsimonious trees (varying only in relationships within Myristicaceae: Figs 8,9) from the molecular combined (MOL) and total combined (TOT) analyses, respectively (see Table 5). Decay index and bootstrap support for both analyses are provided for each branch (molecular above, total below). Thick, lettered branches relate to Table 6. The relationships of Magnoliales and outgroups are developed in Figure 4, as indicated by the arrow. MPM = malmeoid-piptostigmoid-miliusoid clade.

turate clades are sister groups; Fig. 6), as in the morphological analysis of Doyle & Le Thomas (1996), whereas the *rbcL* analysis of Doyle *et al*. (2000) supported a sister-group relationship of the ambavioids and the inaperturates. Our own analyses of available data from *rbcL* and the other slow genes, whether taken individually or in combination, also support the placement of the ambavioids as the sister-group of the inaperturates (Table 6K). Our final result on the position of the ambavioids is therefore due to our fast data sets, which overcome the conflicting signal of the slow genes. However, decay support for the near-basal position of ambavioids increased by one step when the SLOW data set was added to the FAST data set (Table 6K), suggesting there is also some evidence for this relationship in the slow genes.

Phylogeny of Myristicaceae

In contrast with the results found in Magnoliales and Annonaceae, our parsimony analyses have not revealed any clear, well-supported phylogenetic structure for Myristicaceae, even when all data sets are added together. Indeed most analyses, including the morphological (Fig. 7), combined molecular (MOL; Fig. 8), and total combined (TOT; Fig. 9) analyses, yielded strict consensus trees with a polytomy at the base of the family, and whenever resolution is found it is always weakly supported.

Mauloutchioids

One weakly supported clade, however, was recovered in most individual and all combined analyses and was

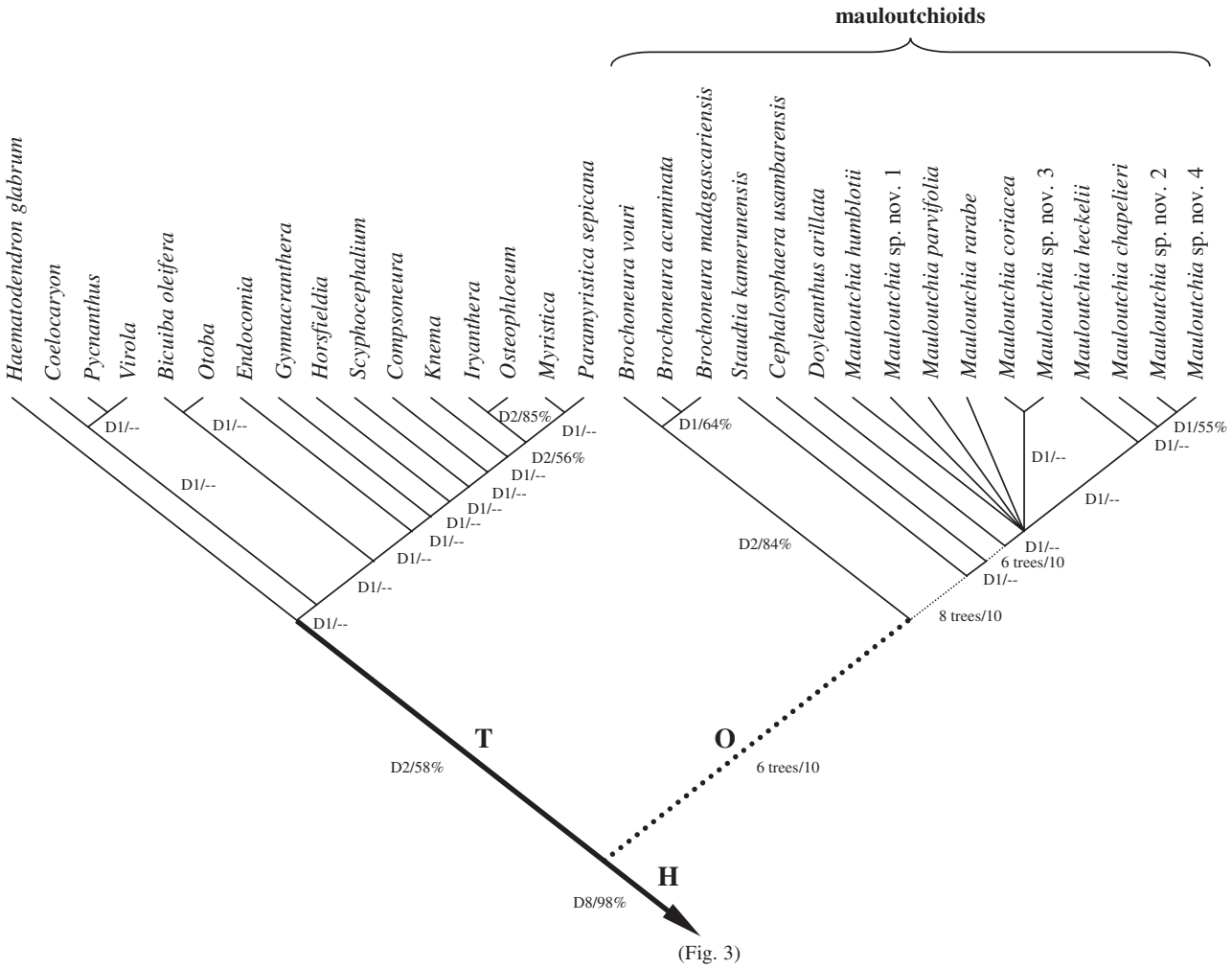


Figure 7. Myristicaceae relationships found in the majority-rule consensus of ten most-parsimonious trees from the morphological analysis (see Table 5). Decay index and bootstrap support are provided for each branch recovered in the strict consensus tree. Dotted branches collapse in the strict consensus tree (numbers of most-parsimonious trees in which these branches were recovered are indicated). Thick, lettered branches relate to Table 6. The relationships of Magnoliales and outgroups are developed in Figure 3, as indicated by the arrow.

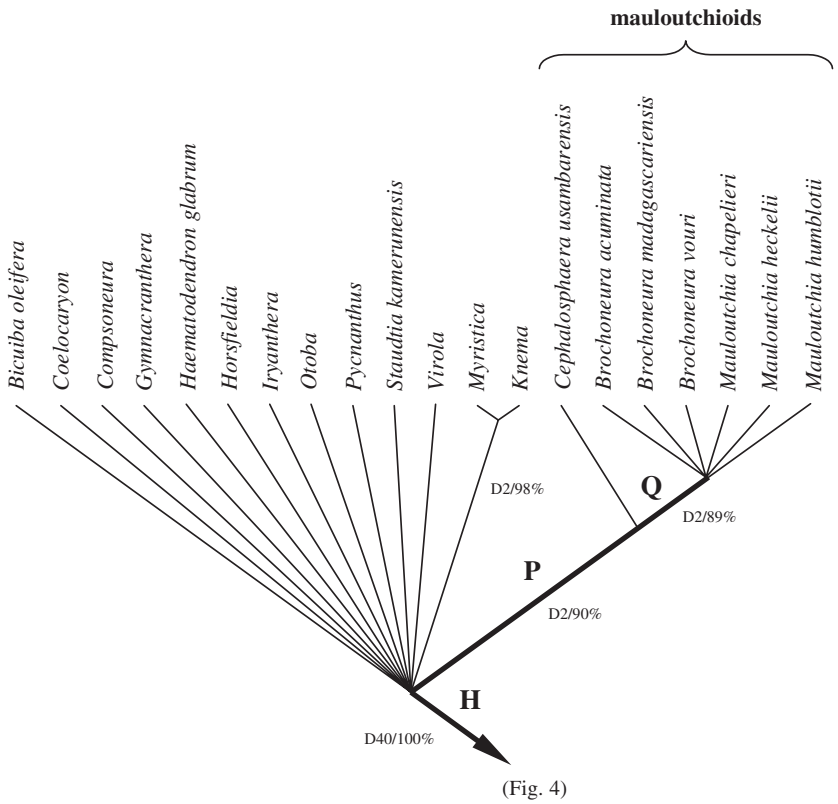


Figure 8. Myristicaceae relationships found in the strict consensus of 2000 most-parsimonious trees from the molecular combined (MOL) analysis (see Table 5), with decay and bootstrap measures of support. Thick, lettered branches relate to Table 6. The relationships of Magnoliales and outgroups are developed in Figure 4, as indicated by the arrow.

never unambiguously rejected. This consists of three Malagasy genera (*Brochoneura*, *Doyleanthus* and *Mauloutchia*) and a Tanzanian monotypic genus (*Cephalosphaera*) (Table 6P and Figs 8–10). Interestingly, all species of this clade sampled in the *trnL-trnF* data set share a 43 bp repeat insertion near the 3′-end of this spacer (character 54 of our indel matrix; Appendix C), which is absent in all other Myristicaceae, Magnoliales and eumagnoliids sampled. In addition, the monotypic genus *Staudtia*, which is widely distributed in central and western tropical Africa, is recurrently associated with this clade (Figs 7,9), although this result is not found in the strict consensus tree of any analysis (Table 6O), except for the total combined analysis (TOT; Fig. 9). Although *Staudtia* is nested among the other lines of this clade in the morphological analysis (Fig. 7), it does not have the insertion shared by *Brochoneura*, *Mauloutchia* and *Cephalosphaera*, supporting its position as the sister-group of these three genera plus *Doyleanthus* (not sampled in any molecular data set), as found in the total combined analysis (Fig. 9). From now on, we will refer to this clade of five genera as the mauloutchioids (an informal name used for the sake of convenience only).

Interestingly, whereas the morphological analysis weakly supports a sister-group relationship of *Cephalosphaera*, *Doyleanthus* and *Mauloutchia* (Fig. 7), molecular data unambiguously relate *Brochoneura* and *Mauloutchia* (Table 6Q; Figs 8–10), between which virtually no molecular variation is observed. Out of 14 536 total molecular characters (including indels), only 21 vary among the six species sampled from these two genera, and none of them are parsimony-informative (although only the *trnL* intron and *trnL-trnF* data sets included more than one species of each genus); hence the polytomy of these six species in the combined molecular analysis (MOL; Fig. 8). Thus, the monophyly and internal topology of *Brochoneura* and *Mauloutchia* in the total combined analysis (TOT; Fig. 9) are entirely the result of morphological information.

Remaining Myristicaceae

Apart from the mauloutchioid clade, relationships among the remaining genera fluctuate greatly across the various analyses and remain poorly resolved. However, a few associations do emerge from the molecular analyses. First, a sister-group relationship

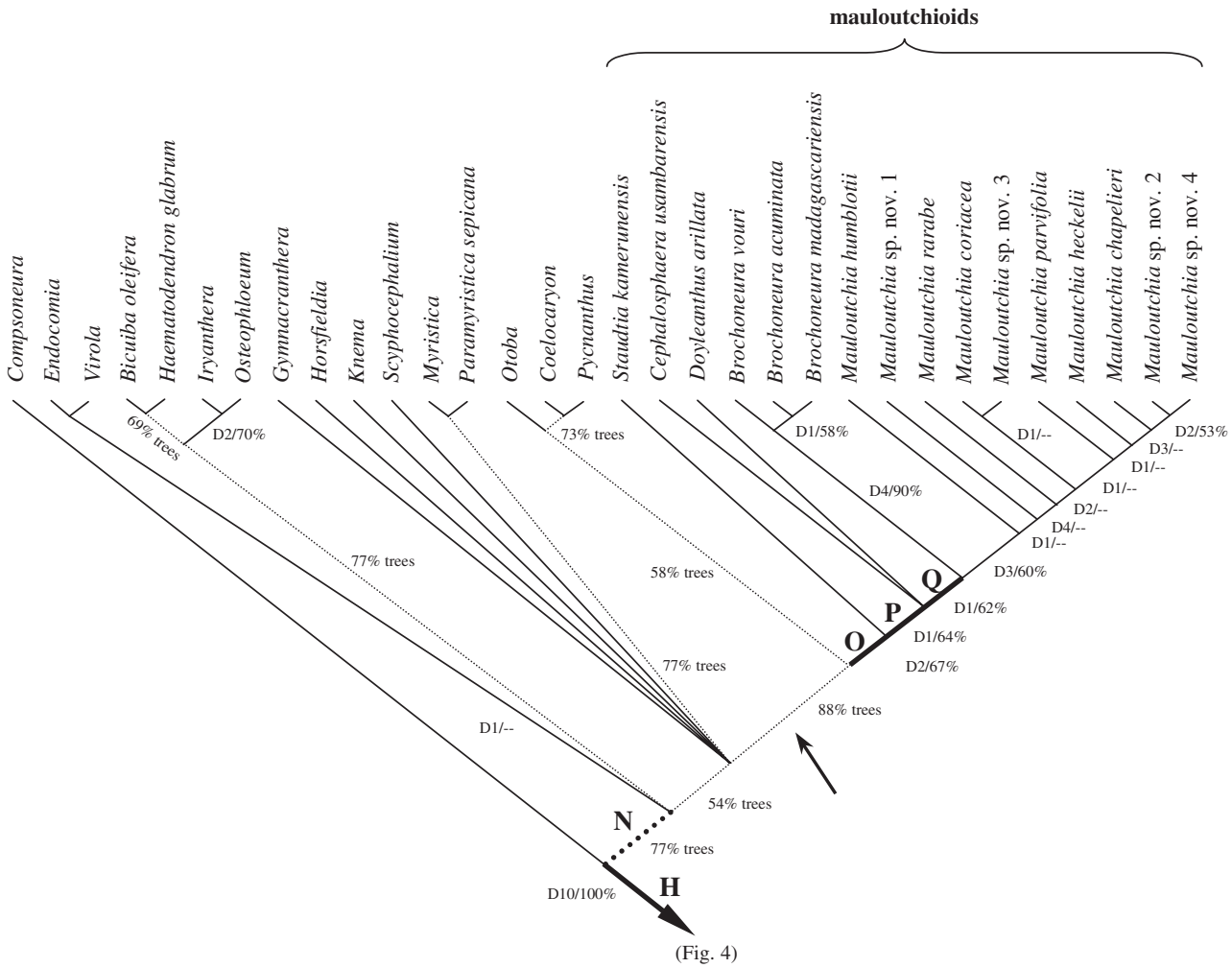


Figure 9. Myristicaceae relationships found in the majority-rule consensus of 78 most-parsimonious trees from the total combined (TOT) analysis (see Table 5). Decay index and bootstrap support are provided for each branch recovered in the strict consensus tree. Dotted branches collapse in the strict consensus tree (percentages of most-parsimonious trees in which these branches were recovered are indicated). Thick, lettered branches relate to Table 6. The relationships of Magnoliales and outgroups are developed in Figure 4, as indicated by the arrow. The arrow indicates where this tree was rerooted for discussion of morphological evolution (e.g. Fig. 14).

between *Coelocaryon* and *Pycnanthus* (both from central and western tropical Africa) is recovered in a number of molecular analyses and never unambiguously rejected (Table 6S). In addition, these two genera form the sister-group of mauloutchioids in a number of molecular analyses (Table 6R). This relationship is recovered in 33 out of 78 trees in the total combined (TOT) analysis, but in the remaining 45 trees the American genus *Otoba* is associated with the two genera (Fig. 9). Interestingly, when *Otoba* is not represented, the sister-group relationship between *Coelocaryon* and *Pycnanthus* is strongly supported and their association with mauloutchioids is recovered (e.g. *ndhF*, *matK*, TRNK, FASTred, MOLred and TOTred; Table 6R,S). Among the remaining genera, a sister-group relationship between the Asian genera *Knema* and *Myristica* is supported by the molecular data (Fig. 8), but not by morphology or total combined data, because of the interference of additional genera not sampled in the molecular data sets. When such genera are deleted, the relationship of *Knema* and *Myristica* is recovered with good support (TOTred analysis: Fig. 10).

In all our analyses, neither the Malagasy genera nor the African genera nor the Afro-Malagasy genera together formed monophyletic groups, mainly because

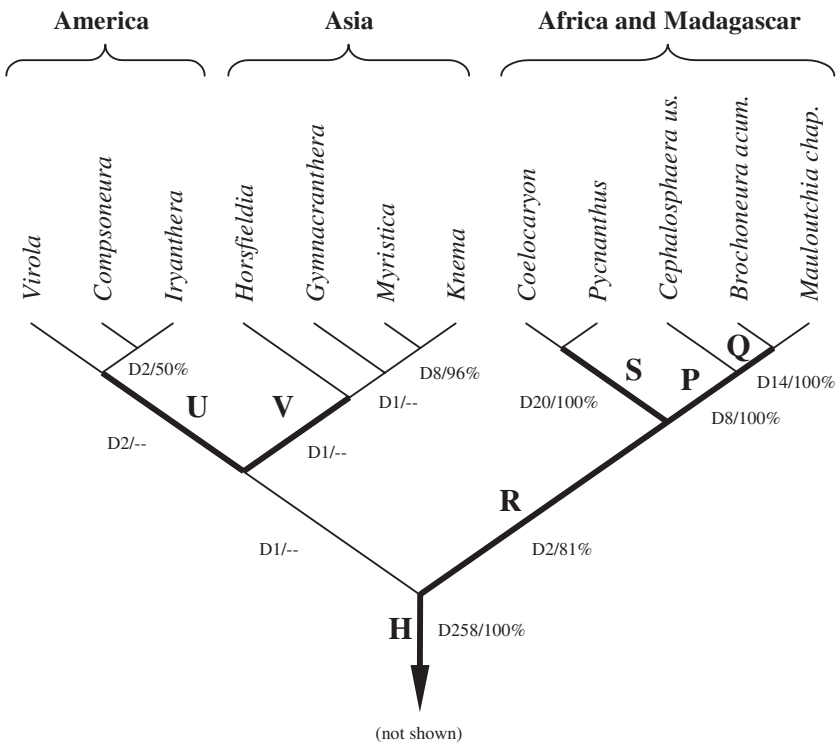


Figure 10. Myristicaceae relationships found in the single most-parsimonious tree from the total combined analysis with reduced taxon sampling (TOTred; see Table 5), with decay and bootstrap measures of support. Thick, lettered branches relate to Table 6. The rest of the tree is not developed in any figure, but its topology is entirely consistent with Figures 4 (Magnoliales and outgroups) and 6 (Annonaceae).

Haematodendron (Madagascar) and *Scyphocephalium* (Africa) were intermixed with American and Asian lines (e.g. Fig. 9). In addition, the monophyly of the American genera is strongly rejected by most data sets (Table 6U), whereas the monophyly of the Asian genera is only weakly rejected overall and is actually compatible with a number of data sets (Table 6V). Interestingly, both the American and the Asian groups are monophyletic (although with low support) in the reduced-sampling analysis when all data sets are combined (TOTred; Fig. 10), but not when morphology and molecules are dissociated (morphred and MOLred analyses; Table 6U,V).

Rooting of Myristicaceae

Our various parsimony analyses have suggested three alternative rootings for the family.

The first, found only in the morphological analysis, involves a basal split between the mauloutchioids and the rest of Myristicaceae (Fig. 7). Even though the mauloutchioids are monophyletic in only six out of ten most-parsimonious trees (because *Brochoneura* is basal or linked with the rest of the family in four trees), the clade comprising all remaining Myristicaceae is recovered in all ten trees. However, its monophyly is never unambiguously supported and almost always rejected by the molecular and combined data sets (Table 6T).

The second rooting, seen in the majority-rule consensus tree from the total combined analysis (Fig. 9), places the American genus *Compsoneura* as the sister group of all remaining Myristicaceae. Even though this rooting was seen many times in our molecular analyses, it is actually supported by only two individual data sets (*ndhF* and the *trnK* 5′-intron), but it was never strongly rejected, except by the morphological analysis (in which it is eight steps worse than the most-parsimonious solution; Table 6N). Interestingly, our sampling-reduction experiment resulted in some weak support for this rooting in combined molecular data sets (FAST, MOLSEQ and MOL) that did not support it when all taxa were included.

A third rooting is found when molecular and morphological data are combined and taxon sampling is reduced (TOTred; Fig. 10). In this case, the *Compsoneura* rooting is rejected by two steps and replaced with a rooting rather similar to the morphological rooting, but with *Coelocaryon* and *Pycnanthus* placed

on the line leading to mauloutchioids rather than among the remaining Myristicaceae. This third rooting appears to be a compromise between the conflicting influences of morphology (supporting a basal position for the mauloutchioids) and molecules (favouring a basal position for *Compsoneura* and an association of *Coelocaryon* and *Pycnanthus* with the mauloutchioids).

These three alternative rootings involve very similar unrooted trees of Myristicaceae, suggesting that the inconsistencies among our various analyses are largely the result of a rooting problem, rather than strongly conflicting signals within the family. For instance, in the morphological analysis, *Coelocaryon* and *Pycnanthus* are more closely related to other Myristicaceae than they are to mauloutchioids, but they still diverge close to the base of the sister clade of mauloutchioids (Fig. 7).

Reasons for this uncertainty

The almost complete absence of strong support for any relationship in Myristicaceae seems to be explained by an unexpected lack of molecular variation in the family (Table 7). For instance, only five parsimony-informative characters were found in the *trnL-trnF* data set, despite the sampling of 16 genera and 20 species across the family. The amount of molecular information was thus far from sufficient to resolve relationships within Myristicaceae, resulting in exceedingly high numbers of most-parsimonious trees and poorly resolved consensus trees (e.g. Fig. 8).

In addition, the conflicting results on the rooting of Myristicaceae may be partly explained by the combination of very limited variation within the family and the particularly long stem branch leading to it (Fig. 11). This was true of all our molecular data sets, individual and combined, but also to a lesser extent of morphology. Such a situation favours long-branch attraction between the longest branch in the unrooted tree of Myristicaceae and their long stem branch (similar cases of long-branch attraction have been documented in various studies, e.g. Donoghue, 1994; Philippe & Laurent, 1998; Philippe, 2000). This factor may be responsible for trees rooted on *Compsoneura*, which often had a somewhat longer terminal branch than any other taxon sampled in the molecular analyses. For instance, in the fast combined analysis with reduced sampling (FASTred; Fig. 11), the longest terminal branch in the unrooted tree of Myristicaceae is *Compsoneura* (49–81 changes, as calculated by PAUP*), followed by *Horsfieldia* (36–42) and *Gymnacranthera* (28–32). Indeed, *Compsoneura* is basal in all four trees found in this analysis, and *Horsfieldia* and *Gymnacranthera* were frequently found at the base of Myristicaceae in analyses in which *Compsoneura* was more nested within the family.

Maximum likelihood analyses

The purpose of our analyses using a maximum likelihood approach was to address two specific issues on which the parsimony analyses gave inconsistent and weakly supported results, even when all data sets were combined: the position of Magnoliaceae within Magnoliineae, and the rooting of Myristicaceae. We did not expect to obtain better resolution of relationships within Myristicaceae, given their very low level of molecular variation. Besides, the reduced taxon sample used in these analyses may reduce the significance of their results compared to parsimony analyses that included more taxa. We will therefore focus on the rooting of the family, for which the greatest shifts between parsimony and ML analyses were observed.

Rooting of Magnoliineae

Interestingly, the FASTred, CPred, and MOLSEQred ML analyses converged towards a basal position of Magnoliaceae in Magnoliineae, whereas parsimony analyses of these data sets left this question unresolved (Fig. 5). A signal supporting this relationship is therefore indeed present in the molecular data. This is also suggested by two other lines of evidence from the parsimony analyses. First, although neither the MOLSEQ (all molecular sequence data combined) nor the indel data set supported this relationship, their combination (MOL data set) did (Table 6D). Since there is only one indel that unambiguously supports this relationship (a 2 bp deletion in the *trnK* 5′-intron of Annonaceae, *Degeneria*, *Eupomatia* and *Galbulimima*: character 13 in Appendix C), this result comes mostly from signal in the sequence data (MOLSEQ data set). Second, although support for this relationship remained weak in both the MOL and morphological data sets (decay index 1, bootstrap value 66%; decay index 1, bootstrap value <50%, respectively), it became much stronger when these two data sets were combined (decay index 5, bootstrap value 90%).

The difficulty in reconstructing this relationship even with large amounts of data (over 3500 variable sites in the MOLSEQ data set) may be simply explained by an extremely short branch (Fig. 11). If this actually reflects a very short period of divergence before the split of the sister lineage of Magnoliaceae into (*Degeneria* + *Galbulimima*) and (*Eupomatia* + Annonaceae), it is not surprising that no previously published higher-level molecular studies recovered this relationship, because of insufficient numbers of characters that are variable at this level.

Rooting of Myristicaceae

A more spectacular result of the ML analyses was a complete shift in the rooting of Myristicaceae in four data sets: *ndhF*red, FASTred, CPred and MOLSEQred.

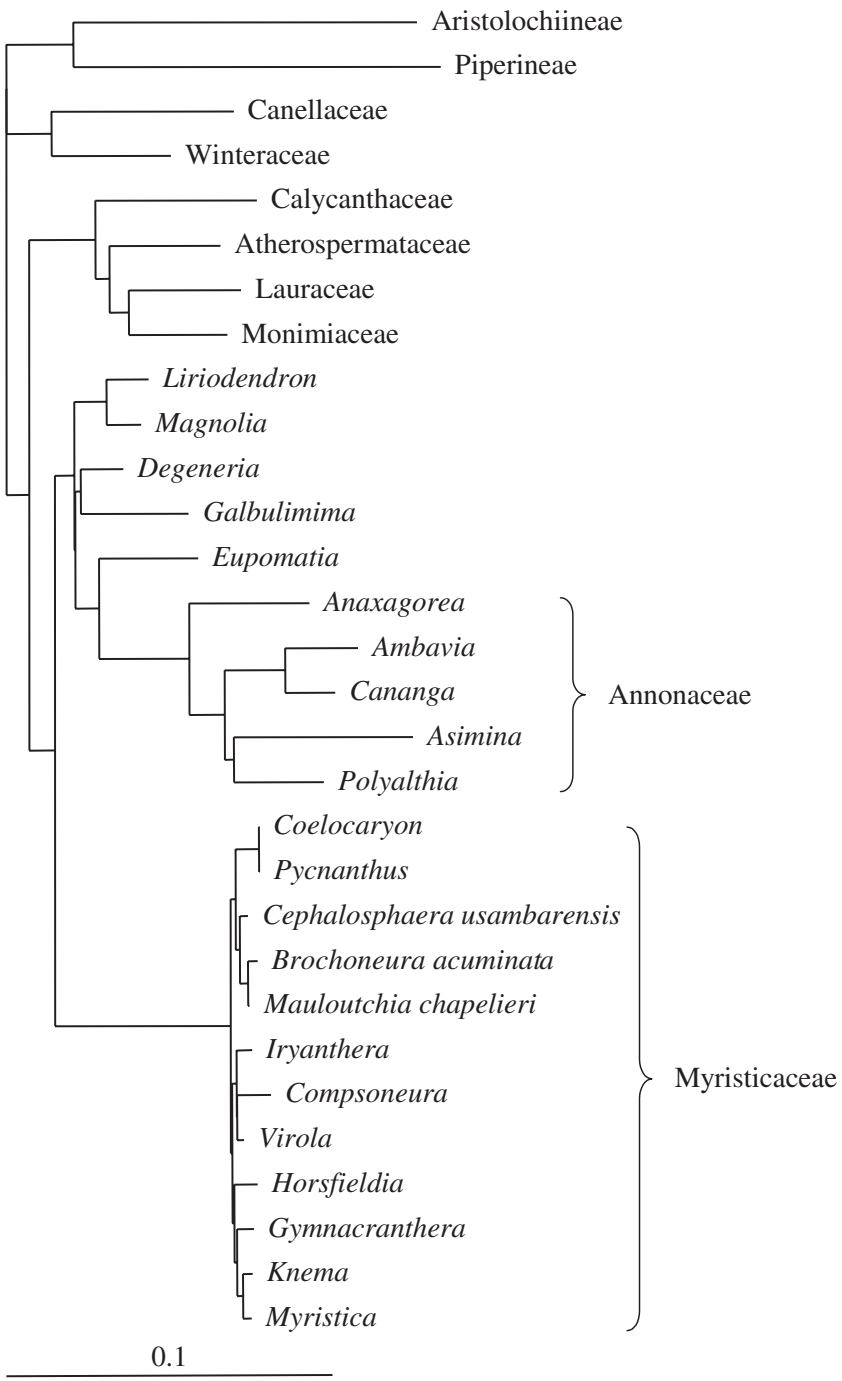


Figure 11. Phylogram from the maximum likelihood analysis of the combined fast molecular data sets with reduced taxon sampling (FASTred; no more than 30% total missing data per taxon). Three other analyses yielded very similar topologies: maximum likelihood analyses of the combined chloroplast data sets (CPred; identical topology) and the combined molecular data sets (MOLSEQred; differing only in relationships among Annonaceae), and total combined parsimony analysis with reduced taxon sampling (TOTred; differing only in relationships among neotropical genera of Myristicaceae: see Fig. 10).

While parsimony analyses of these data sets yielded trees rooted on *Compsoneura*, ML analyses (e.g. Fig. 11) instead supported the rooting found in the TOTred (parsimony) analysis (Fig. 10). This is consistent with the weak support for the rooting on *Compsoneura* in the few parsimony analyses in which it was found (Table 6N) and definitely favours the interpretation of this rooting as the result of long-branch attraction.

DISCUSSION

Contribution and interaction of data sets

A striking conclusion of this study is that several results that were well supported in the total combined analysis did not appear until the individual data sets were combined, whether parsimony or ML was used as the optimality criterion. The relationships among the six families of Magnoliales are particularly illustrative of this point. For instance, the sister-group relationship of *Eupomatia* and Annonaceae was recovered in the parsimony analyses of only four individual molecular data sets (*ndhF*, *trnL-trnF*, *atpB* and *matR*), only two of which (*ndhF* and *matR*) supported it well. Similarly, the ML analyses of only three data sets (*ndhF*red, *trnT-trnL*red and *matR*red) supported this relationship. Yet it was found in all parsimony and most ML analyses of combined data sets and received very good support (decay index 30, bootstrap value 100%) in the parsimony analysis that combined all molecular data sets (MOL; Fig. 4). Interestingly, none of the three fragments of the *trnK* region recovered this relationship when analysed separately, but they did when analysed in combination (TRNK), although not with maximum likelihood (Fig. 5). These results reinforce the growing view that individual genes may often fail to reconstruct a phylogeny, not because the underlying signal is misleading, but because they do not provide enough characters to overcome effects of homoplasy. It seems as if when data sets are added together, individual conflicting sources of noise cancel each other out, whereas the signal resulting from a common phylogenetic history is additive. Depending on levels of variation and homoplasy in individual data sets, some actual relationships may not appear until a large number of characters are analysed simultaneously (cf. Soltis *et al*., 1998; Gatesy, O'Grady & Baker, 1999; Cognato & Vogler, 2001; Reeves *et al*., 2001). In our study, this was best illustrated by the new rooting of Magnoliineae on Magnoliaceae, which was only observed in our two last combined parsimony analyses (MOL and TOT; Fig. 4) and four combined ML analyses (FASTred, CODred, CPred and MOLSEQred; Fig. 5), presumably because of the very short branch (and therefore very few characters) supporting this relationship (Fig. 11).

Despite our use of one of the most general models of molecular evolution available (GTR + gamma + I), maximum likelihood analyses did not always perform better than parsimony analyses. First, consistency among separate analyses was not improved by the use of this more elaborate method (e.g. Fig. 5). Second, assuming our total combined analysis did reveal the true phylogeny of Magnoliales, ML did not always recover more branches of this tree than parsimony did. In some cases, it actually widened the gap with the presumed correct tree (e.g. *ndhF*red and TRNKred; Fig. 5). In others, it had no effect on the incorrect topology (e.g. *matK*red; Fig. 5). In fact, only when parsimony already yielded almost correct trees did ML lead to even more accurate results (e.g. FASTred, CPred and MOLSEQred; Fig. 5). Significant changes were only observed when long-branch attraction seems to have affected relationships in the parsimony analysis, as illustrated by *trnT-trnL*red in Magnoliales (Fig. 5) and by drastic shifts in the rooting of Myristicaceae.

Phylogenetic structure of Annonaceae

Our molecular analyses confirm most aspects of the global phylogenetic structure for Annonaceae found in the molecular analyses of van Zuilen *et al*. (1996) and Doyle *et al*. (2000), while supporting the near-basal position of ambavioids found in the morphological analysis of Doyle & Le Thomas (1996). Our new molecular data also provided increased support for the basal position of *Anaxagorea* and the monophyly of the ambavioid, MPM and inaperturate clades. As discussed further below, the variation in these genes reflects higher rates of molecular evolution in Annonaceae than in other Magnoliales.

Phylogenetic problems in Myristicaceae

Reconstruction of the phylogeny of Myristicaceae turned out to be a particularly difficult problem, which our current data were insufficient to resolve. The unexpected lack of molecular variation in this family contrasts with the recently proven usefulness of molecular data for resolving the phylogenies of Annonaceae (van Zuilen *et al*., 1996; Doyle *et al*., 2000) and Magnoliaceae (e.g. Azuma *et al*., 1999, 2001; Ueda *et al*., 2000; Kim *et al*., 2001). In addition, the rooting problem (presumably an effect of this lack of variation and the very long stem branch of the family) and the negative influence of taxa with abundant missing data on the accuracy of combined analyses contributed to the current uncertainty on relationships among the genera of Myristicaceae.

A striking illustration of these factors is the large basal polytomy in the strict consensus tree from the

total combined analysis (not shown in Fig. 9, but note dotted branches that collapse in the strict consensus). In 60 out of 78 most-parsimonious trees, *Compsoneura* is basal (Fig. 9) and *Scyphocephalium* is usually found in a more nested position, intermixed with Asian genera, but in the remaining 18 trees *Scyphocephalium* branches first, followed by *Compsoneura*. The resulting polytomy may thus be largely explained by uncertainty on the position of *Scyphocephalium*, for which we were unable to obtain molecular data.

Even though discarding such annoying taxa with large amounts of missing data did allow us to avoid these problems (experiments with the reduced taxon sample; Fig. 10) and clarify the rooting of the family (cf. also maximum likelihood analyses; Fig. 11), we are convinced that full representation of the family is needed for interpretation of our results (see also Wiens & Reeder, 1995). In particular, ignoring the nine poorly sampled genera may lead to incorrect conclusions regarding morphological evolution in Myristicaceae and Magnoliales. In this regard, we prefer to consider a poorly resolved consensus tree based on maximum taxon and character sampling over a well-resolved but simplistic phylogenetic hypothesis that ignores problematic data. Moreover, as emphasized by Kearney (2002), taxa with abundant missing data do not always have a 'wildcard' behaviour in phylogenetic analyses, and when they do so it is not always the result of missing data alone (homoplasy, unevenly distributed among taxa, may also interact). Indeed, although *Scyphocephalium* seems to behave like a 'wildcard', other taxa with no molecular data, such as *Doyleanthus* or various species of *Mauloutchia*, definitely do not. Furthermore, the introduction of huge amounts of missing data in Myristicaceae not sampled in the SLOW data set (i.e. all genera except *Knema*, *Mauloutchia* and *Myristica*) did not seem to increase uncertainty when the SLOW data were combined with the more densely sampled FAST data set (MOLSEQ analysis).

Despite all the ambiguity on our results in Myristicaceae, the clear emergence of a mauloutchioid clade represents an encouraging result of our investigations and has important implications for character evolution in the family.

Choice of trees for reconstruction of character evolution

For discussion of implications for morphological evolution in Magnoliales and Myristicaceae in the following sections, we have considered the majority-rule consensus tree of the total combined analysis (see Fig. 9 for Myristicaceae; identical outside the family to the strict consensus tree: Fig. 4). Because we believe the rooting of Myristicaceae in this tree is an artifact of long-branch attraction in the molecular data and may greatly distort character reconstruction both within and outside the family, we have rerooted Myristicaceae to take into consideration the alternative rooting indicated by the parsimony (Fig. 10) and ML (Fig. 11) analyses with reduced taxon sampling. This was accomplished by rerooting the tree at the position indicated in Figure 9 and moving the outgroups of Myristicaceae to their original arrangement at the base.

Although the resulting tree is less parsimonious in terms of the total combined data set than the tree rooted on *Compsoneura*, we believe it represents a good trade-off between the more consistent rooting found in the reduced-sampling analyses and the full taxonomic representation of the TOT analysis. Unfortunately, an alternative solution, namely analysing the TOT data set with the reduced-sampling rooting enforced as a backbone constraint, yielded even lower levels of resolution. Furthermore, although it might be more rigorously correct to reroot the strict consensus tree of Myristicaceae as a basis for character discussion, the low level of resolution would have allowed us to draw very few conclusions. On the belief that it is more constructive to discuss ambiguous relationships than it is to discuss nothing at all, we thus selected the majority-rule consensus tree as a compromise between the poorly resolved strict consensus tree and one arbitrarily selected, fully resolved most-parsimonious tree.

Outgroup relationships

The unrooted trees of the four eumagnoliid orders obtained from our combined analyses are all consistent with a sister-group relationship between Laurales and Magnoliales (Fig. 4), as found in many, although not all, recent higher-level phylogenetic studies of angiosperms (Mathews & Donoghue, 1999, 2000; Qiu *et al*., 1999, 2000; Barkman *et al*., 2000; Graham & Olmstead, 2000; Graham *et al*., 2000; Zanis *et al*., 2002; Borsch *et al*., in press; K. W. Hilu *et al*., unpubl.). One of the surprising results of this study is that our morphological matrix supports this relationship (Fig. 3), whereas the larger data set from which it was derived (Doyle & Endress, 2000) recovered the alternative relationship of Winterales and Magnoliales, although with low support (decay index 1, bootstrap value <50%). It might be suspected that this shift was due to the removal of all non-eumagnoliid taxa, especially the monocots, with which Piperales were linked in Doyle & Endress (2000). However, we refuted this explanation by reanalysing the Doyle & Endress data set with taxon sampling reduced to the eumagnoliids: in the resulting unrooted trees, Winterales remain with Magnoliales. This suggests that the change in

topology is due to increased taxon sampling and new inferred ancestral states in Myristicaceae, which, given the basal position of this family in Magnoliales, had an effect on relationships of the order. It should be noted that this topology does not necessarily imply that Winterales and Piperales form a clade: it is equally consistent with a tree in which Winterales are the sister-group of Laurales plus Magnoliales.

Assuming that the root is outside the order, the monophyly of Magnoliales is supported by only two unambiguous morphological synapomorphies (Fig. 12), stratified phloem and adaxial plate of vascular tissue in the midrib. However, both of these characters show very low homoplasy (Doyle & Endress, 2000).

Rooting of Magnoliales

Although the basal position of Myristicaceae in Magnoliales had already been suggested by higher-level studies (Fig. 1), our analyses have provided much additional support for it, from both morphological and molecular data (Figs 3–5). For instance, it was only supported with a decay index of 1 and a bootstrap value of 60% in the morphological analysis of Doyle & Endress (2000), whereas our morphological matrix supports it with a decay index of 6 and a bootstrap value of 77%, possibly as a result of a better representation of Myristicaceae and therefore a better estimation of their ancestral states. With molecular data, Qiu *et al*. (2000) found this relationship supported with a

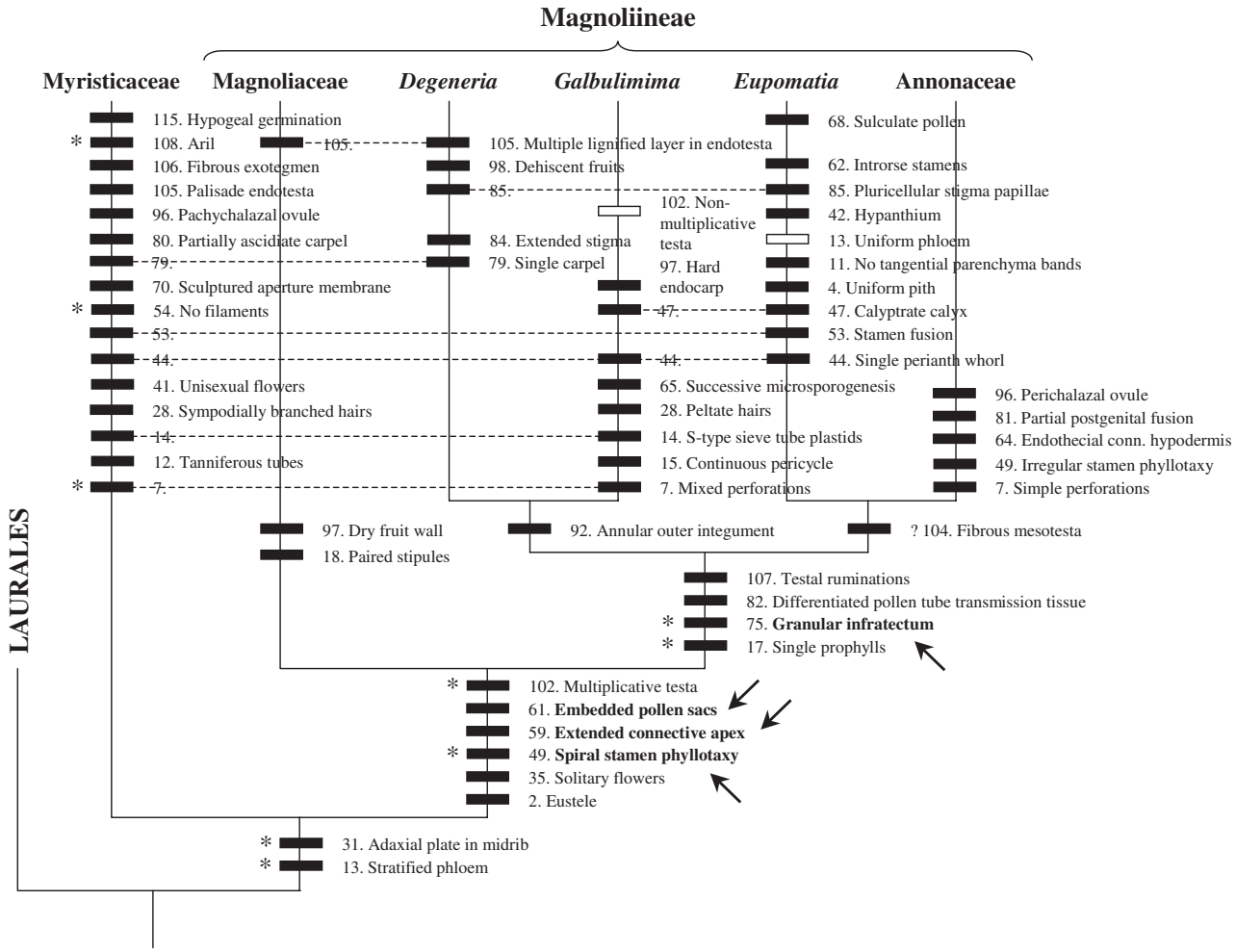


Figure 12. Unambiguous morphological transformations in Magnoliales, based on the total combined analysis (Fig. 4). Character numbers refer to Appendix A. White boxes represent reversals. Boxes marked with an asterisk represent characters that subsequently reversed or changed to a different state within the lineage on which they are placed. Dashed lines represent parallelisms or convergences. The arrows indicate important transformations that contradict previous assumptions on character polarity (as discussed in the text).

bootstrap value of 62%, and so did we in our SLOW combined analysis (based on the same data, with almost the same sampling for Magnoliales), whereas our FAST combined analysis supported it with a decay index of 13 and a bootstrap value of 98%. This result probably reflects the greater number of informative characters in the FAST data set (about three times more than in SLOW: Table 5), but may also be partly explained by lower levels of homoplasy in the FAST data sets. However, the significant increase in support for this relationship when both sources of data were combined (MOLSEQ analysis: decay index 20, bootstrap value 100%) suggests there is also considerable underlying support for it in the SLOW data set.

The monophyly of Magnoliineae, and therefore the basal position of Myristicaceae in Magnoliales, is supported by six unambiguous morphological synapomorphies (Fig. 12). In particular, the extended connective apex and the embedded pollen sacs represent two main aspects of the laminar stamens characteristic of the five families of Magnoliineae. Their unambiguously derived status in this study contradicts the long-held assumption that laminar stamens were plesiomorphic for the angiosperms as a whole (e.g. Canright, 1952; Cronquist, 1988: 207). The same is true of the spiral stamen phyllotaxy of Magnoliineae (secondarily irregular in Annonaceae).

All these derived states correspond to as many symplesiomorphies of Laurales and Myristicaceae, and their preponderance over synapomorphies of Magnoliales may have contributed to the placement of Myristicaceae in Laurales rather than Magnoliales in some early classifications (such as Hutchinson, 1973; Table 1). This may also explain the weak support for the monophyly of Magnoliales in our morphological analysis (Fig. 3) and the fact that this order breaks up in the reduced-sampling analysis of the same data set (morphred; see branch that collapses in Fig. 3). The low support for Magnoliales may also reflect the great amount of evolution on the long stem lineage leading to Myristicaceae, which may have erased some original conditions of the order. The family is, indeed, supported by 16 unambiguous synapomorphies (Fig. 12).

Previous systematists, including Cronquist (1981) and Takhtajan (1997), linked Myristicaceae to Canellaceae and Annonaceae, based on their ruminate seeds and stamen fusion in Canellaceae, but our results clearly indicate that both features evolved independently in these families. Indeed, the ruminations in Canellaceae are restricted to one group (*Cinnamosma*: Parameswaran, 1961), which is nested within the family (Karol *et al*., 2000). The testal ruminations of Annonaceae, which are in fact shared with *Degeneria*, *Eupomatia* and in reduced form *Galbulimima* (Doweld & Shevyryova, 1998), are inferred to be a synapomorphy of these four taxa (Fig. 12), whereas the structurally different, tegminal and/or chalazal ruminations of Myristicaceae were independently derived. As for stamen fusion, our results unambiguously indicate that this is a parallelism in Canellaceae and Myristicaceae (with a third partial convergence in the basally fused stamens of *Eupomatia*).

Relationships and character evolution within Magnoliineae

Despite apparent conflicts among data sets, our parsimony and maximum likelihood analyses of combined data converged towards a single, well-supported hypothesis of relationships within Magnoliineae (Figs 4, 5). Most previous molecular studies, based on the slow genes only, linked Magnoliaceae with *Degeneria* plus *Galbulimima* (Chase *et al*., 1993; Qiu *et al*., 1993, 1999, 2000; Rice *et al*., 1997; Savolainen *et al*., 2000; D. Soltis *et al*., 2000; P. Soltis *et al*., 2000; Zanis *et al*., 2002; Fig. 1; surprisingly, a relationship that we never observed in any of our analyses) or did not resolve the placement of this family within Magnoliineae (D. Soltis *et al*., 2000; P. Soltis *et al*., 2000; Fig. 1), as in several of our analyses (Fig. 5). Our study instead places Magnoliaceae unambiguously as the sister-group of the four remaining families of Magnoliineae, which only the combined morphological plus three-gene analysis of Doyle & Endress (2000) had suggested so far (Fig. 1), with lower support (decay 3 and bootstrap 73% vs. decay 5 and bootstrap 90% in this study).

The clade consisting of *Degeneria*, *Galbulimima*, *Eupomatia* and Annonaceae is supported by four unambiguous morphological synapomorphies (Fig. 12): single prophylls, granular infratectum, differentiated pollen tube transmission tissue and testal ruminations. In addition, the inner staminodes of *Degeneria*, *Galbulimima*, *Eupomatia* and some Annonaceae (*Anaxagorea* and *Xylopia*) may represent another synapomorphy of this clade, unless they are a synapomorphy of Laurales and Magnoliales that was lost in Hernandiaceae, Magnoliaceae and Myristicaceae (scored '?' because of unisexuality and stamen fusion).

In turn, *Degeneria* and *Galbulimima* only share one unambiguous morphological synapomorphy (annular outer integument; Fig. 12). On strict parsimony grounds *Eupomatia* and Annonaceae do not share any, although a fibrous mesotesta could be a synapomorphy (reconstruction of changes in this character is equivocal because it has two states in the outgroups: sarcotesta in *Degeneria* and *Galbulimima*, undifferentiated or sarcotesta in Magnoliaceae, and undifferentiated elsewhere). It is therefore not surprising that neither of these clades was found in the morphological analysis, but they are unambiguously supported by

the present and previous combined analyses of molecular data. However, these associations had been suggested to some extent by earlier systematists: Dahlgren (1989) and Takhtajan (1997) (Table 1) proposed a restricted circumscription of Magnoliales to include only *Degeneria*, *Galbulimima* and Magnoliaceae; Hutchinson (1973; Table 1) split out *Eupomatia* and Annonaceae as a separate order (Annonales); and a relationship of the last two taxa based on fibrous mesotesta was proposed by Corner (1976).

An interesting result of this study is that a number of important traits shared by individual families of Magnoliales are inferred to be convergences within the order (Fig. 12), for instance the single carpel of Myristicaceae and *Degeneria*, the mixed perforations of Myristicaceae and *Galbulimima*, the single perianth whorl of Myristicaceae, *Galbulimima* and *Eupomatia*, and the calyptrate calyx of *Galbulimima* and *Eupomatia*. This homoplasy may partly explain why this and previous attempts to reconstruct relationships in Magnoliales based solely on morphology (Donoghue & Doyle, 1989; Loconte & Stevenson, 1991; Doyle & Le Thomas, 1996; Doyle & Endress, 2000) recovered a few or none of the well-supported clades found in our combined analyses.

Character evolution in Myristicaceae

Despite the ambiguity on most internal relationships in Myristicaceae, some conclusions regarding the evolution of several important morphological characters in the family can still be drawn from our analyses, relying on the weak but recurrent support for a mauloutchioid clade. For practical purposes, we introduce here two additional informal names to refer to the two main clades comprising the rest of the family in the rerooted tree used for this discussion: pycnanthoids are defined as consisting of *Coelocaryon*, *Pycnanthus* and *Otoba*, and myristicoids as comprising the 13 remaining genera (see Fig. 14).

Under the rooting found in the parsimony and ML analyses with reduced taxon sampling, the only unambiguous synapomorphy of mauloutchioids is a shift in pollen exine structure from columellar to mixed (i.e. with both granular and columellar elements; Fig. 13). Other characteristic features of this clade are 'first rank' leaf venation (i.e. with festooned brochidodromous to cladodromous secondary veins and reticulate tertiary veins; Hickey & Wolfe, 1975), pollen with a continuous tectum, and lack of ruminations in the seed. Often considered primitive in angiosperms, these traits are also ancestral character states of Magnoliales. However, their optimization in Myristicaceae is equivocal: they may be either plesiomorphies or reversals, and thus additional synapomorphies of the mauloutchioids, because more derived states are present in the pycnanthoids. This situation may partly explain why the mauloutchioids were found at the base of Myristicaceae in our morphological analysis (Fig. 7). In turn, the mauloutchioids other than *Staudtia* are supported by two unambiguous synapomorphies (Fig. 13), the ulcerate/ulceroid pollen aperture and the 43 bp repeat insertion near the 3′-end of the *trnL-trnF* spacer (scored in the indel matrix).

Androecium characters

Many androecial features of the mauloutchioids that were previously considered ancestral in Myristicaceae now appear to have originated later within the mauloutchioid clade (Fig. 13). In particular, spiral/irregular stamen phyllotaxy, great number of anthers (up to 60), and partially free filaments (plus free connectives), which characterize some or all species of *Mauloutchia*, are all inferred to be derived. The most common and best known species of this genus, *M. chapelieri*, represents the extreme end of this unexpected trend in androecium evolution, whereas *M. humblotii*, which has hardly any filament and a small number of anthers (5–10), is much closer to the ancestral condition of the genus. As a consequence, our results imply that the ancestral Myristicaceae had a single whorl of a small number (two to 12) of entirely fused anthers (i.e. borne as a crown at the apex of a sterile column, with no free filaments and with fused connectives). This indicates a remarkable reversal within *Mauloutchia* towards the androecial morphology of other eumagnoliids. To claim that these states are plesiomorphic would require assuming that the species showing them are basal in Myristicaceae, which is contradicted by the very low molecular divergence in *Mauloutchia* and *Brochoneura*. Androecial evolution in Myristicaceae is discussed in greater detail in Sauquet (in press).

Pollen characters

Similarly, the ulcerate to ulceroid aperture, granulate tectal microsculpture, and mixed infratectum that characterize most mauloutchioids are derived features in this clade, whereas the ancestral pollen of Myristicaceae instead had a sulcate to sulcoid aperture, a smooth tectal surface, and a columellar infratectum. Conversely, mauloutchioids seem to have inherited a globose pollen shape from the common ancestor of the family, whereas most myristicoids have boat-shaped pollen. Finally, it remains uncertain whether the ancestral tectum of Myristicaceae was continuous (as observed in mauloutchioids) or reticulate to rugulate. In any case, the granular infratectum of a few species of *Mauloutchia* was derived from a mixed infratectum (at least twice independently: Fig. 13), and the ancestral infratectum of the family was at least partly columellar. Our results thus contradict the view of

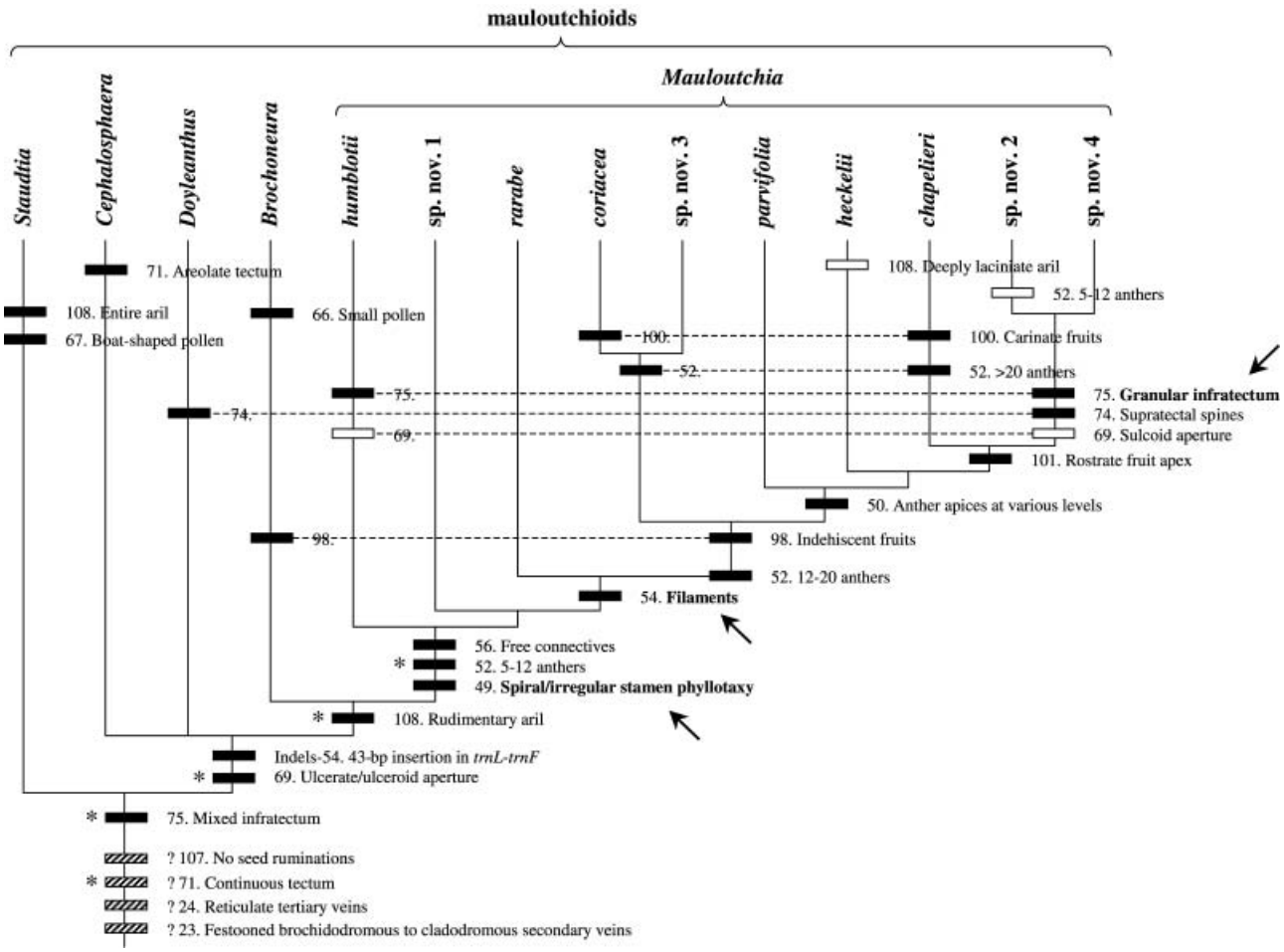


Figure 13. Unambiguous morphological transformations in the mauloutchioid clade of Myristicaceae, based on the total combined analysis (Figure 9). The internal resolution of *Brochoneura* is not developed here. Character numbers refer to Appendix A. White boxes represent reversals. Boxes marked with an asterisk represent characters that subsequently reversed or changed to a different state within the lineage on which they are placed. Dashed lines represent parallelisms or convergences. The arrows indicate important transformations that contradict previous assumptions on character polarity (as discussed in the text).

Walker & Walker (1981) that granular structure is primitive and are inconsistent with the phylogenetic relationships of five species of *Mauloutchia* that they hypothesized (p.16) based primarily on pollen characters. Pollen evolution in Myristicaceae is discussed in more detail in Sauquet & Le Thomas (in press).

Aril

The aril around the seed (Fig. 14) represents an unambiguous synapomorphy of Myristicaceae, which originated independently from the arils observed in some Annonaceae and Canellaceae. It was originally deeply laciniate and later evolved towards an entire aril (occasionally with the apex still laciniate) in several lineages (Fig. 14). In addition, it became rudimentary in the common ancestor of *Brochoneura* and *Mauloutchia* (with reduced laciniations visible in some species: our observations on fresh specimens) and was totally lost independently in *Haematodendron* (Fig. 14). It would be tempting to interpret the reduction or disappearance of the aril as a functional consequence of a shift to an indehiscent fruit (once the aril is no longer visible, it can no longer attract animals for dispersal), but these two features are not always correlated (see notes under character 108 in Appendix A).

In conclusion, as illustrated in Figure 13, many important morphological transformations have occurred within *Mauloutchia*. Ignoring the internal evolution of this genus by treating it as a single taxon (albeit with polymorphic states) probably contributed to its incorrect placement at the base of Myristicaceae in the pre-

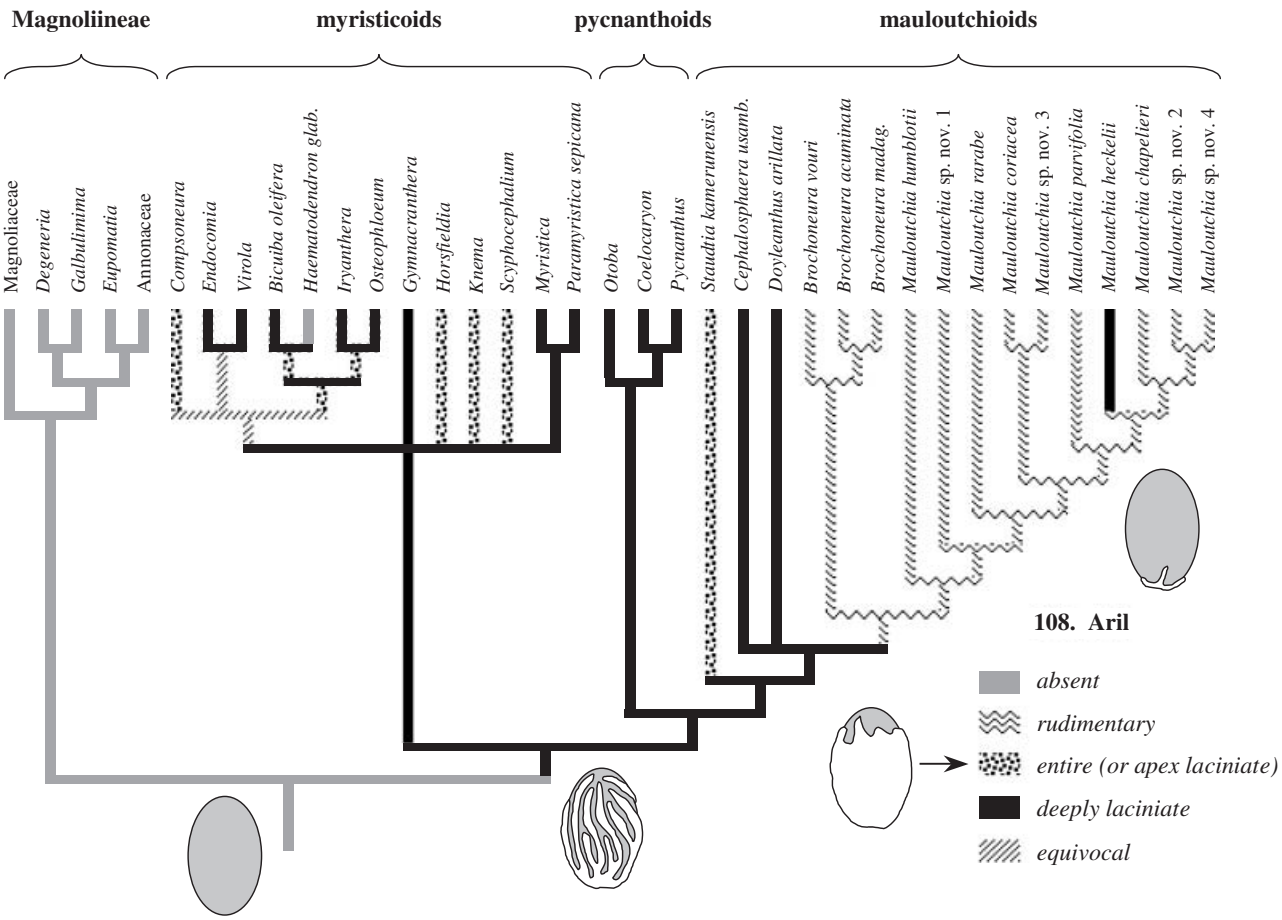


Figure 14. Parsimony optimization of the aril character in Magnoliales and Myristicaceae, based on the total combined tree of Myristicaceae rerooted according to the parsimony and maximum likelihood analyses with reduced taxon sampling. *Mauloutchia* spp. nov. 1 and 4 were scored as unknown (?) for this character, and *Endocomia* was scored as entire/deeply laciniate.

liminary morphological analysis of Sauquet (1999) and the hand-constructed tree of Warburg (1897). The careful examination and scoring of all component species of *Mauloutchia* in our new morphological data set was thus crucial for rejecting this basal position, resulting in a shift towards the more derived position supported by molecular data.

Fossils, molecules, and the geographical history of Myristicaceae

The wide pantropical distribution of Myristicaceae and their limited dispersal capabilities (by large animals only: Sinclair, 1958a: 213; Alexandre, 1978; Howe & Vande Kerckhove, 1980; van der Pijl, 1982; Steentoft, 1988; Forget & Milleron, 1991; Beehler & Dumbacher, 1996; Julliot, 1997; Sabatier, 1997; Galetti, Laps & Pizo, 2001) together suggest that the first steps of their diversification predated separation of the Gondwana continents by wide oceanic barriers. This would extend the age of crown-group Myristicaceae back to the Cretaceous, as inferred for Annonaceae from biogeographical and fossil data (Doyle & Le Thomas, 1997).

In contrast, the remarkably low molecular divergence within Myristicaceae, as compared to the length of their stem lineage and divergences in other Magnoliales (Fig. 11), might suggest that the family is much younger than expected. This possibility is hard to exclude based on palaeobotanical data: except for some convincing Tertiary fossil seeds (Berry, 1924; Gregor, 1977) and flowers (Poinar & Poinar, 1999), most supposed myristicaceous fossils are questionably diagnostic woods (e.g. Boureau, 1950), leaves (Wolfe, 1977), and pollen (e.g. Frederiksen, 1973; Jan du Chêne, Onyike & Sowunmi, 1978; Salard-Cheboldaeff, 1978). Their distinctive seeds are conspicuously absent in the Eocene London Clay, which does contain seeds of Annonaceae (Reid & Chandler, 1933; Collinson, 1983).

Opposing this reasoning, however, is the fact that our molecular data appear to deviate strongly from a molecular clock in Magnoliales (Fig. 11). Annonaceae clearly had higher rates of molecular evolution than other Magnoliales, whether due to an acceleration in Annonaceae or a deceleration in the other families. The observed branch lengths might be reconciled with a Cretaceous origin of Myristicaceae by hypothesizing that the rate of molecular evolution was high during the phase when their major innovations originated, but then decelerated markedly during their diversification.

The great difficulty we faced in resolving the phylogeny of Myristicaceae, itself an effect of the lack of molecular variation, also makes it difficult to reconstruct the geographical history of the family. Whereas the *Compsoneura* rooting might favour a neotropical origin, our preferred rooting of the ingroup topology (Figs 10,11) is consistent with an origin in either South America or the Afro-Malagasy region. In any case, an Asian origin is rejected and, because it is possible that all genera in this area form a clade, they may represent a single dispersal from Gondwana into Asia. This would contradict the views of Sinclair (1958a: 242–244) but would agree closely with Walker (1971: 48–49). These issues are to be discussed in detail in J.A. Doyle, H. Sauquet, T. Scharaschkin & A. LeThomas (unpubl. data) .

CONCLUSIONS AND FUTURE PROSPECTS

Our combination of morphological and molecular data proved effective in reconstructing a well-supported phylogeny of Magnoliales and demonstrated the value of studies that focus on particular lower-level taxa with denser taxonomic sampling, increased amounts of data, and more thorough exploration of relationships to confirm and increase support for phylogenetic hypotheses emerging from higher-level molecular studies (e.g. Qiu *et al*., 2000; D. Soltis *et al*., 2000). Furthermore, our results on the position of Magnoliaceae illustrate the need for caution when relying solely on higher-level studies to understand relationships among terminal taxa.

In addition, the incorporation of morphological data in our study allowed us to discuss the biological implications of such emerging hypotheses, which are barely if ever addressed at this level in large-scale studies based solely on molecular data. Thus, we showed that a number of morphological traits that were once assumed plesiomorphic and taken as evidence for the basal status of Magnoliales in angiosperms are synapomorphies of particular clades within the order and were independently derived in other magnoliid angiosperms (e.g. laminar stamens and granular exine structure: cf. Doyle & Endress, 2000).

In contrast, we were unable to reconstruct relationships within Myristicaceae with confidence, because of an unusually low level of molecular divergence within the family, despite our effort to sequence and combine multiple molecular loci selected from among the most variable regions used in lower-level angiosperm phylogenetics. Searching for even more variable loci or combining many more loci with this level of variation may ultimately provide enough characters for a better understanding of relationships among the genera. However, we believe it is important at this point to make available our preliminary results on this family, which has never been investigated from a phylogenetic perspective, and to discuss their most secure morphological implications, some of which contradict long-standing assumptions on character polarity (such as the derived status of androecial, pollen and fruit characters in mauloutchioids). We also hope that these preliminary results will help orientate future research on the phylogeny of Myristicaceae and taxa within this family.

In addition, our comparison of individual and combined analyses illustrates the need for caution concerning results from studies based on one or a few genes, even when analysed with more elaborate methods such as maximum likelihood.

Finally, our results suggest it will be difficult to rely on molecular data to date the main cladogenetic events in the history of Magnoliales, giving prime importance to palaeobotanical research in this order. Because of their special position at the base of Magnoliales, additional investigations on the phylogeny of Myristicaceae will be essential for further clarification of the early morphological and geographical history of Magnoliales.

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APPENDIX A
MORPHOLOGICAL DATA SET

Approach

Whenever prior phylogenetic results were available, supraspecific taxa included in this study were scored with reconstructed ancestral states, as inferred from the ingroup topology (independent of outgroup relationships). This applied for all supraspecific taxa outside Myristicaceae (see Taxa section). Since no phylogenetic data were available at any level within Myristicaceae, variable non-monospecific genera were scored conservatively as polymorphic (e.g. 0/1), except when features restricted to individual species were assumed to be autapomorphies and thus ignored (all such assumptions are mentioned in the Characters section). Polymorphic taxa with species in which a given character was not applicable were scored for this character with the state(s) observed in the remaining species where it is applicable (e.g. –/0 scored 0). Autapomorphies of terminal taxa in the present data set (including states found in more than one taxon in Doyle & Endress, 2000) were kept in the matrix, since they may be synapomorphies of supraspecific terminal taxa and thus represent essential information for future development of the data set at lower taxonomic levels. This procedure resulted in one parsimony-uninformative character (number 18).

Taxa

All taxa listed below, when comprising more than one species, are assumed to be monophyletic. Taxa are designated by the lowest-rank name that includes all their component organisms. Numbers of genera and species are based mostly on Kubitzki, Rohwer & Bittrich (1993) and Takhtajan (1997) for taxa outside Myristicaceae, and on our personal compilation of taxonomic literature for Myristicaceae.

Piperales (*sensu* APG, 1998)

Aristolochiineae (*sensu* Shipunov, 2002; 8–13 gen./411–601 spp.): Aristolochioideae (10 gen./404 spp.) + Asaroideae (2 gen./71 spp.) + *Lactoris fernandeziana* (= Lactoridaceae) merged. Since relationships of these three lines remain uncertain, states have been optimized assuming a trichotomy.

Piperineae (*sensu* Walker, 1976b; Shipunov, 2002; 16–19 gen./1497–2107 spp.): Piperaceae (11–14 gen./1490–2100 spp.) + Saururaceae (5 gen./7 spp.) merged.

Winterales

Canellaceae (5–6 gen./16–20 spp.)

Winteraceae (5–9 gen./60–90 spp.): *Takhtajania perrieri* assumed to be basal, *Tasmannia* basal in the remaining taxa (Karol *et al*., 2000).

Laurales (*sensu* APG, 1998; Renner, 1999)

Atherospermataceae (7 gen./16 spp.)

Calycanthaceae (= Calycanthales *sensu* Loconte & Stevenson, 1991; 4 gen./10 spp.): Calycanthaceae *s.s.* (3 gen./9 spp.) + *Idiospermum australiense* (= Idiospermaceae)

Gomortega nitida Ruiz & Pav. (= Gomortegaceae)

Hernandiaceae (4–5 gen./58–68 spp.): Hernandioideae (2–3 gen./43 spp.) + Gyrocarpoideae (2 gen./16 spp.) merged.

Lauraceae (45–54 gen./2500–3500 spp.): *Hypodaphnis zenkeri* assumed to be basal (Renner & Chanderbali, 2000; Rohwer, 2000).

Monimiaceae (25 gen./266 spp.): Monimioideae (3 gen./18 spp.) + Mollinedioideae (21 gen./245 sp.) + *Hortonia* (= Hortonioideae; 2–3 spp.) merged, assuming that Monimioideae are basal to Mollinioideae and *Hortonia*, as suggested by molecular data (Renner, 1998, 1999), despite the great number of plesiomorphies in *Hortonia*.

Siparunaceae *(*2 gen./154 spp.): *Siparuna (*150 spp.) + *Glossocalyx* (3–4 spp.).

Magnoliineae (*sensu* this study)

Annonaceae (128–200 gen./2050–2500 spp.): *Anaxagorea* assumed to be basal (Doyle & Le Thomas, 1996; Doyle *et al*., 2000).

Degeneria (= Degeneriaceae; 2 spp.)

Eupomatia (= Eupomatiaceae; 2 spp.)

Galbulimima (= Himantandraceae; 2 spp.)

Magnoliaceae (7–13 gen./200–240 spp.): *Liriodendron* (= Liriodendroideae; 2 spp.) + Magnolioideae.

Myristicaceae

Bicuiba oleifera (Schott) W.J. de Wilde (= *Bicuiba*; Brazil): described in de Wilde (1991b).

Brochoneura acuminata (Lam.) Warb. (Madagascar): following Capuron (1973).

Brochoneura madagascariensis (Lam.) Warb. (Madagascar): following Capuron (1973).

Brochoneura vouri (Baill.) Warb. (Madagascar): following Capuron (1973).

Cephalosphaera usambarensis (Warb.) Warb. (= *Cephalosphaera*; Tanzania)

Coelocaryon Warb. (4 spp.; central and western tropical Africa): following Fouilloy (1974).

Compsoneura Warb. (12 spp.; southern Mexico to tropical South America): following Smith (1937, 1950, 1956) and Rodrigues (1989b). According to J. Janovec (pers. comm.), possibly a polyphyletic genus (taking into account many new species), which would be best split up into two or three putatively monophyletic genera. This information, however, was not taken into account in this version of our data set, and characters for this genus are based only on described species.

Doyleanthus arillata Capuron ex Sauquet (Madagascar): new genus described in Sauquet (in press).

Endocomia W.J. de Wilde (4 spp.; south-east Asia, from southern China to New Guinea): described in de Wilde (1984b).

Gymnacranthera (A. DC.) Warb. (7 spp.; southern India and south-east Asia, from southern Thailand to New Guinea and the Bismarck Archipelago): following Sinclair (1958a,b) and Schouten (1986).

Haematodendron glabrum Capuron (= *Haematodendron*; Madagascar): described in Capuron (1972).

Horsfieldia Willd. (104 spp.; India, Sri Lanka, Malay Peninsula, Indonesia, Philippines, New Guinea and Micronesia): following de Wilde (1984a,b, 1985a,b, 1986, 1987a, 1996, 2000).

Iryanthera Warb. (25 spp.; Panama and northern South America): following Smith (1937, 1950, 1956), Ducke (1945, 1947), Gentry (1975, 1981), Rodrigues (1981, 1982).

Knema Lour. (95 spp.; India, Nepal and Sri Lanka to south-east Asia): following de Wilde (1979, 1981, 1987b, 1996, 1998, 2000).

Mauloutchia chapelieri (Baill.) Warb. (Madagascar): includes both varieties *chapelieri* and *media*, which seem to differ only in leaf size, and forma *ecristata* of var. *chapelieri* (with less pronounced but still carinate fruits, and similar leaves), as described in Capuron (1973), but not forma *sambiranensis* of var. *media* (with not at all carinate fruits and quite different leaves), herein considered instead a new species (sp. nov. 3).

Mauloutchia coriacea Capuron (Madagascar): following Capuron (1973).

Mauloutchia heckelii Capuron (Madagascar): known only from Capuron 8952 SF (with female organs only; type specimen), and our collection H. Sauquet 6 (with both male and female flowers), on the belief (supported by pollen, inflorescence, and leaf characters) that the assignment of specimen 10574 SF to this species in its original description (Capuron, 1973) was a mistake (misled by leaf shape); instead, we consider this specimen an entirely new species (sp. nov. 4).

Mauloutchia humblotii (H. Perrier) Capuron (Madagascar): following Capuron (1973).

Mauloutchia parvifolia Capuron (Madagascar): following Capuron (1973).

Mauloutchia rarabe (H. Perrier) Capuron (Madagascar): following Capuron (1973).

Mauloutchia sp. nov. 1 (Madagascar): specimen 23961 SF (with monoecious flowers and fruits), and possibly doubtful specimens 12395 SF and 2867 SF. Distribution: Farafangana, and possibly Ambila-Lemaitso.

Mauloutchia sp. nov. 2 (named *'echinocarpa'* on herbarium specimens by R. Capuron; Madagascar): specimens 3795 SF (with fruits), 11800 SF (with fruits), and Capuron 28666 SF (with male and possibly female flowers). Very distinctive in the unique, echinate ornamentation of its fruits. Distribution: Mahatalaky and Vohibe-Manantenina.

Mauloutchia sp. nov. 3 (syn. *Mauloutchia chapelieri* var. *media* forma *sambiranensis* Capuron; Madagascar): specimens 7503 SF (with flowers), 7696 SF (with male and perhaps female flowers), 11379 SF (with fruits; not in description of forma), 11468 SF (with fruits; type of forma), and 13110 SF (with fruit). Exclusion from *Mauloutchia chapelieri* is justified by the completely non-carinate fruit (unlike *Mauloutchia chapelieri* var. *chapelieri* forma *ecristata*, found to be softly carinate), different leaf shape (narrower and glabrous, much closer to *Mauloutchia rarabe*), and distinctive, separate distribution: Ambanja/Sambirano.

Mauloutchia sp. nov. 4 (Madagascar): specimen 10574 SF (with male flowers; fruits unknown), mistakenly assigned to *Mauloutchia heckelii* in Capuron (1973), from which it differs in distinct inflorescence and pollen characters (and also leaves that are not so hairy, though similarly shaped, and distribution). First thought to belong to *Mauloutchia* sp. nov. 2 because of its very similar pollen, but now considered a different species because it has very different inflorescences and significantly longer, almost ovate leaves. Distribution: Mahatalaky.

Myristica Gronov. (144 spp.; India and south-east Asia

to northern Australia and the western Pacific): following de Wilde (1990, 1991a, 1994b, 1995, 1998, 2000).

Osteophloeum Warb. (2 spp.; Amazon basin): following Little (1969).

Otoba DC. ex H. Karst. (syn. *Dialyanthera* Warb.; 7 spp.; Costa Rica to tropical South America): following Gentry (1979).

Paramyristica sepicana (Foreman) W.J. de Wilde (= *Paramyristica*; Papua New Guinea): described in de Wilde (1994a).

Pycnanthus Warb. (3 spp.; central and western tropical Africa): following Fouilloy (1974).

Scyphocephalium Warb. (2 spp.; Nigeria, Cameroon, and Gabon): following Fouilloy (1974).

Staudtia kamerunensis Warb. (= *Staudtia*; central and western tropical Africa): includes both varieties *kamerunensis* and *gabonensis*, which may differ in fruit size only (Fouilloy, 1974).

Virola Aubl. (54 spp.; Guatemala and Guadeloupe to tropical South America): following Smith (1937, 1950, 1953, 1956), Ducke (1945, 1947), Duke (1962), Williams (1964), Little (1970), Dwyer (1972), Gentry (1975), Rodrigues (1977, 1980, 1989a), de Paula & Heringer (1979), Sabatier (1997).

Characters

DE-X = character number X in Doyle & Endress (2000), with states not found in this data set eliminated; for references on scoring of these characters in taxa outside Myristicaceae, see Doyle & Endress (2000). Data collected and compiled by J. A. Doyle and H. Sauquet, with contributions by A. Le Thomas for pollen characters. All characters are unordered except for seven of them: characters 20, 39, 52, 66, 75, 78 and 94. Character 18 is parsimony-uninformative. 'Harvard' and 'Kew' refer to anatomical collections at Harvard University and Royal Botanic Gardens, Kew.

Vegetative morphology (1–34)

1. Habit: (0) tree or shrub; (1) rhizomatous, scandent, or acaulescent. DE-1. Lianas occur in Annonaceae (inaperturates) and Lauraceae (*Cassytha*), but based on internal phylogenies (Doyle *et al.*, 2000; Renner & Chanderbali, 2000; Rohwer, 2000) these are assumed to be derived. Within Myristicaceae, *Pycnanthus dinklagei* is reported to be a liana (de Wilde, 1991b; F. Hallé, pers. comm.), but other species are trees.

Stem anatomy (2–15)

2. Stele: (0) eustele; (1) (pseudo)siphonostele. DE-2. We have rescored Winteraceae as (1) rather than (0/1) based on presence of a pseudosiphonostele in *Takhtajania* (Keating, 2000), which is sister to the rest of the family. In Myristicaceae, *Myristica* has been described as siphonostelic (Benzing, 1967; Metcalfe, 1987), *Knema* as pseudosiphonostelic (Siddiqi & Wilson, 1975b); our observations on slides at Kew indicate that *Knema* has more distinct primary vascular bundles than *Myristica*, but both fall in state (1) as here defined.

3. Nodal anatomy: (0) multilacunar; (1) unilacunar one-trace; (2) unilacunar two-trace (leaf traces derived from two adjacent stem bundles or protoxylem areas, may split or fuse in petiole); (3) trilacunar. DE-21. Genera of Myristicaceae characterized by Benzing (1967), Siddiqi & Wilson (1975b), Sugiyama (1979), and Metcalfe (1987).

4. Pith: (0) uniform; (1) septate (with plates of sclerenchyma). DE-14. Hernandiaceae: our observations (Kew). Although Vander Wyk & Canright (1956) described septations as characteristic of Myristicaceae and Siddiqi & Wilson (1975b) reported them in *Knema*, Doyle & Endress (2000) scored Myristicaceae as non-septate, based on Metcalfe's (1987) description of *Myristica* as having only axial rows and local groups of stone cells and observations on the lack of septations in *Mauloutchia* (then assumed to be basal in the family) and *Iryanthera*. However, our observations on *Brochoneura*, *Compsoneura*, *Haematodendron*, *Iryanthera*, *Mauloutchia*, *Myristica*, *Pycnanthus* and *Virola* indicate that septations are present in some taxa and absent in others.

5. Storied structure (in tracheids and axial parenchyma, phloem): (0) absent; (1) present. DE-6. We assume that storied structure is absent in all genera of Myristicaceae studied by Garratt (1933).

6. Vessel grouping: (0) predominantly solitary; (1) mostly pairs or multiples. DE-8. Scoring of genera of Myristicaceae based on figures and descriptions in Garratt (1933), Siddiqi & Wilson (1974), Armstrong & Wilson (1980), and Metcalfe (1987).

7. Vessel perforations (end-wall pits in vesselless taxa): (0) scalariform; (1) mixed (scalariform and simple in the same wood); (2) simple only. DE-9. The descriptions of Garratt (1933), Siddiqi & Wilson (1974), and Metcalfe (1987) indicate that most genera of Myristicaceae have both scalariform and simple perforations, with simple predominating in some taxa, but Siddiqi & Wilson and Metcalfe reported that simple perforations are very rare or absent in *Gymnacranthera* and *Knema*. Siddiqi & Wilson (1974) cited Garratt (1933) for absence of scalariform perforations in *Horsfieldia*, *Osteophloeum*, *Brochoneura*, *Cephalosphaera* and *Pycnanthus*, but this appears to be based on a misreading.

8. Fibre pitting (lateral pitting of tracheids in vesselless taxa): (0) distinctly bordered; (1) reduced (minutely bordered or simple). DE-10. Scoring of genera of Myristicaceae based on Garratt (1933), Siddiqi & Wilson (1974), and Metcalfe (1987).

9. Ray width: (0) narrow (generally not more than four cells wide); (1) wide. DE-11. Scoring of genera of Myristicaceae based on Garratt (1933), Siddiqi & Wilson (1974), Armstrong & Wilson (1980), and Metcalfe (1987). Garratt (1933) described rays in Myristicaceae as predominantly uni- and biseriate but 3–6 cells wide in *Pycnanthus*, *Staudtia*, *Compsoneura*, and occasional specimens of *Horsfieldia*, *Knema* and *Virola*. However, figures of *Pycnanthus* and *Virola* in Metcalfe (1987) show only narrow rays.

10. Paratracheal parenchyma: (0) absent or scanty; (1) well developed. DE-12. Scoring of genera of Myristicaceae based on Garratt (1933), Siddiqi & Wilson (1974), Armstrong & Wilson (1980), and Metcalfe (1987).

11. Tangential apotracheal parenchyma bands: (0) absent; (1) present. DE-13. Scoring of genera of Myristicaceae based on Garratt (1933), Siddiqi & Wilson (1974), Armstrong & Wilson (1980), and Metcalfe (1987). Garratt (1933) listed *Brochoneura* sp. as lacking

parenchyma, but because of uncertainty on assignment of the species studied, we have scored this genus as unknown.
12. Tanniferous tubes: (0) absent; (1) present. Assumed to be present in all Myristicaceae, because they produce the red sap that is the best diagnostic character for assigning a tree to the family in the field.
13. Secondary phloem: (0) uniform; (1) stratified. DE-15. Highly stratified phloem was described by Roth (1969, 1981) in *Iryanthera* and *Virola*.
14. Sieve tube plastids: (0) S-type (starch); (1) PI-type; (2) PII-type. DE-16. Behnke (1981, 1988, 1991).
15. Pericycle (including modified protophloem) with: (0) separate fibre bundles; (1) more or less continuous ring of fibres (or fibres and non-U-shaped sclereids); (2) fibres alternating with U-shaped sclereids. DE-17. Separate fibre bundles have been described in *Knema* (Siddiqi & Wilson, 1975b) and *Myristica* (Metcalfe, 1987), but Vander Wyk & Canright (1956) described lateral extension of the bundles by lignification, forming an almost continuous ring, in *Osteophloeum* and *Pycnanthus*, and contrasted this with the condition in Annonaceae. Our observations at Harvard showed separate bundles in *Cephalosphaera*, *Coelocaryon*, *Compsoneura*, *Gymnacranthera*, *Horsfieldia*, *Iryanthera*, *Myristica*, *Pycnanthus* and *Staudtia*, but an almost continuous ring in *Osteophloeum*, *Otoba* and *Virola*. Given the observed variation within genera, we have scored taxa described as almost continuous as (0/1), the rest as (0).

Leaf attachment (16–18)
16. Phyllotaxy: (0) spiral; (1) distichous (at least on lateral branches); (2) opposite. DE-20. Within Myristicaceae, the spiral phyllotaxy of the orthotropic main stem extends to the lateral branches only in a few species of *Horsfieldia* (de Wilde, 1991b, 2000).
17. Prophylls (first appendages on vegetative branch): (0) paired lateral; (1) single (variously orientated). DE-22 modified, with numbers for states reversed to correct an editing error in that paper. Siparunaceae: our observations. *Degeneria*, scored (?) in Doyle & Endress (2000), is reported by P. F. Stevens (pers. comm.) to have a single abaxial prophyll, so we have rescored it (1) for consistency with the present state definition. Myristicaceae: P. F. Stevens (pers. comm.) for *Brochoneura*, *Cephalosphaera*, *Gymnacranthera*, *Horsfieldia* and *Mauloutchia*; our observations for *Brochoneura acuminata*, *Horsfieldia*, *Iryanthera*, *Mauloutchia coriacea*, *M. humblotii*, *M. parvifolia* and *M.* sp. nov. 1.
18. Stipules: (0) absent; (1) adaxial/axillary; (2) paired. DE-23 modified, with addition of a new state (2) for Magnoliaceae.

Leaf architecture (19–25)
Data on new characters (not used by Doyle & Endress, 2000) and on taxa within Myristicaceae are based on our observations on herbarium material and figures in de Wilde (1991b, 2000), with exceptions noted. Terminology follows Hickey (1973, 1979).
19. Leaf shape: (0) elliptical to oblong to obovate (including oblanceolate); (1) ovate (including lanceolate) to cordate. DE-26 modified. In scoring this character, we ignored the long acuminate apex of *Mauloutchia* sp. nov. 1. Because we frequently observed variation from elliptical to weakly ovate and obovate in a given specimen, we scored taxa that show this type of variation as (0) and restricted state (1) to taxa that are consistently ovate. We scored taxa as (0/1) if they vary intraspecifically from elliptical to more strongly ovate (e.g. *Bicuiba*, described as oblong-lanceolate to lanceolate: de Wilde, 1991b) or vary between species (e.g. Calycanthaceae, with ovate leaves in *Calycanthus occidentalis* and elliptical leaves in *C. floridus*: our observations).
20. Leaf base: (0) rounded (angle >90°) to cordate; (1) moderately acute (<90°); (2) decurrent (<< 90°). Ordered. We have not scored this and the following character (21) in taxa outside Myristicaceae. Although they seem potentially useful at the species level, they vary so much within genera in both Myristicaceae and other taxa that their value at higher levels seems questionable. With many larger taxa scored as polymorphic, taxa outside Myristicaceae with one or two species might also have an excessive influence on polarization of these characters within Myristicaceae.
21. Leaf apex: (0) obtuse to emarginate; (1) acute to acuminate (= with 'drip tip'). See remarks on character 20.
22. Major venation: (0) pinnate with secondaries at more or less constant angle; (1) palmate (actinodromous or acrodromous) or crowded (pinnate with crowded basal secondaries, upward decreasing angle). DE-27.
23. Outer secondary venation: (0) festooned brochidodromous to cladodromous; (1) brochidodromous; (2) eucamptodromous. This and character 24 based on Carlquist (1964), Lorence (1985), and our observations. Intergradation between festooned brochidodromous and cladodromous is seen in *Brochoneura*, *Cephalosphaera* and *Mauloutchia*.
24. Tertiary venation: (0) reticulate (with frequent intersecondary veins); (1) weakly percurrent (often with intersecondary veins near the midrib and percurrent tertiaries towards the margin); (2) strongly percurrent (without intersecondary veins). Comparable to character 8 of Doyle & Le Thomas (1996).
25. Marginal teeth: (0) absent; (1) monimioid. DE-30.

Leaf anatomy (26–34)
26. Stomata (predominant type on leaf): (0) paracytic; (1) anomocytic or tetracytic. DE-31 modified, assuming that the tetracytic condition in Piperineae is more closely related to anomocytic than to paracytic. Solereder (1908) for *Virola*; Koster & Baas (1981) for *Endocomia*, *Gymnacranthera*, *Horsfieldia*, *Knema* and *Myristica*.
27. Stomata level: (0) level with surface, or somewhat sunken but not overarched by surrounding cells; (1) sunken and overarched by surrounding cells. Aristolochiineae: Metcalfe (1987) for *Lactoris*, Dickison (1996) for *Saruma*. Piperineae: Baranova (1987, 1992). Winteraceae: sometimes concealed by papillae in *Drimys*, but flush with surface in *Takhtajania* (Metcalfe, 1987; Feild, Zwieniecki & Holbrook, 2000). Canellaceae, Atherospermataceae, Calycanthaceae, *Gomortega*, Hernandiaceae, Lauraceae (mostly level but sunken and overarched by lobed cells in the near-basal genera *Caryodaphnopsis* and *Beilschmiedia*), Monimiaceae, *Siparuna*, *Degeneria*, *Eupomatia*, *Galbulimima*

and Magnoliaceae based on Metcalfe (1987). Annonaceae: level with leaf surface except when overlapped by papillae, level in *Anaxagorea* (Metcalfe, 1987; Maas & Westra, 1984). According to Koster & Baas (1981), stomata are sunken and overarched by papillae in *Knema* and most (though not all) *Myristica* species, but not in *Gymnacranthera* and *Horsfieldia*. Stomata are somewhat sunken but lack papillae and are not overarched in *H. crassifolia* figured in Metcalfe (1987); Koster & Baas (1981) describe them as sunken in *H. iryaghedhi* and slightly so in *H. crassifolia* and *H. sylvestris*. Hence we define state (1) as overarched by surrounding cells but not necessarily papillate. *Endocomia* based on Koster & Baas (1981) (as *H. macrocoma*).

28. Hairs on leaf: (0) unbranched or absent; (1) stellate or peltate, not sympodially branched; (2) sympodially branched, hair cells one-armed; (3) sympodially branched, hair cells two-armed. Data on taxa outside Myristicaceae from Metcalfe (1987), plus Metcalfe & Chalk (1950) for Piperineae and Kubitzki (1993) for *Degeneria*. Some Annonaceae have stellate and peltate hairs, but phylogenetic analyses indicate that these are derived in the family (Doyle & Le Thomas, 1996; Chatrou, Koek-Norman & Maas, 2000; Doyle *et al*., 2000). Data on Myristicaceae from Koster & Baas (1981), Bernardi & Spichiger (1980), and our observations. Scoring of *Brochoneura* and *Mauloutchia* spp. is based on our SEM observations on hairs on the perianth and pedicel.

29. Alveolar material: (0) absent; (1) present. Aristolochiineae based on *Lactoris* (Metcalfe, 1987) and *Saruma* (Dickison, 1996); Piperineae on Baranova (1987, 1992). Distribution in Myristicaceae based on Koster & Baas (1981) (*Endocomia* = *H. macrocoma*). Alveolar material is not mentioned or figured by Metcalfe (1987) in any taxa included here except Myristicaceae and Winteraceae; in Winteraceae it is absent in the basal genus *Takhtajania* (Feild *et al*., 2000).

30. Large druses in spongy tissue: (0) absent; (1) present. Koster & Baas (1981). Aristolochiineae based on *Lactoris* (Metcalfe, 1987) and *Saruma* (Dickison, 1996), Piperineae on Metcalfe & Chalk (1950). Outside Myristicaceae, Metcalfe (1987) cited a few cases of cluster crystals in the epidermis or near veins (e.g. Canellaceae), but not druses in the mesophyll, except in Winteraceae (*Zygogynum*, nested well within the family: Karol *et al*., 2000) and Annonaceae (including the basal genus *Anaxagorea*: Koek-Noorman in Maas & Westra, 1984; van Setten & Koek-Noorman, 1986). In Annonaceae, the druses are smaller and do not occur in special idioblasts like those of Myristicaceae (J. Koek-Noorman, pers. comm.). None of these are considered comparable to druses in Myristicaceae. Koster & Baas (1981) reported druses as present in all species of *Horsfieldia*, so we have scored them as also present in *Endocomia* (= *H. macrocoma*).

31. Midrib vasculature: (0) simple arc; (1) arc with adaxial plate; (2) ring. DE-32. Myristicaceae based on Koster & Baas (1981) for *Endocomia* (as *Horsfieldia macrocoma*), *Gymnacranthera*, *Horsfieldia*, *Knema* and *Myristica*; Sugiyama (1979) for *Knema* and *Myristica*; and our observations of rehydrated herbarium material and Harvard anatomy slides for *Brochoneura*, *Cephalosphaera*, *Coelocaryon*, *Compsoneura*, *Haematodendron*, *Iryanthera*, *Knema*, *Mauloutchia* and *Virola*. Since an adaxial plate may be present in both the midrib and the petiole or in the midrib and distal petiole only, we scored taxa represented by petiole sections only (at Harvard) as (1) when a plate was present but as (?) when it was not (*Cephalosphaera*, *Osteophloeum*, *Otoba*).

32. Palisade parenchyma: (0) absent (mesophyll homogeneous); (1) present (mesophyll dorsiventral). DE-33. Myristicaceae based on Solereder (1908) for *Virola*, Koster & Baas (1981) for the Asian genera (found isobilateral in *Horsfieldia sabulosa* only, genus scored as (0/1)).

33. Astrosclereids or rarely branched, filiform sclereids in mesophyll: (0) absent; (1) present. DE-34, modified to include rarely branched, filiform sclereids in state (1), since the two types sometimes intergrade. Solereder (1908) and Rao (1991) for *Iryanthera*; Rao & Wee (1966) and Koster & Baas (1981) for *Gymnacranthera*, *Horsfieldia* and *Knema*; Koster & Baas (1981) for *Myristica* and *Endocomia* (= *H. macrocoma*).

34. Mucilage cells in mesophyll: (0) absent; (1) present. DE-36. In Asian Myristicaceae, Koster & Baas (1981) reported that oil cells sometimes appear to have tannin-like or (in *Horsfieldia iryaghedhi*) even mucilaginous contents, but since mucilage is not clearly present in any case, we have scored all genera studied as (0). *Virola* based on Solereder (1908).

Reproductive morphology (35–115)

Inflorescence morphology (35–40)

35. Inflorescence type: (0) solitary or occasionally with 1–2 additional lateral flowers; (1) consistently multiflowered. DE-37, modified by combination of (1) spike, raceme, or botryoid and (2) richly branched states. Without this simplification, the character of Doyle & Endress (2000) overlaps in part with the following characters, which appear to be more informative within Myristicaceae. The distinction between simple and richly branched can also be questioned on the grounds that it may be less important than the distinction between determinate and indeterminate inflorescence units and frequently shows variation within taxa (Winteraceae, Canellaceae, Siparunaceae, Atherospermataceae).

36. Inflorescence grouping: (0) single-type (one basic unit only); (1) plural-type (compound raceme of multiple basic units). De Wilde (1991b). Applies only in Myristicaceae. This character is mainly diagnosed by the structure of the very base of the main axis. Note that the single-type architecture may be repeated indefinitely in every main branch of the basic unit (as illustrated in fig. 2A of de Wilde, 1991b). *Paramyristica*, a few New Guinean species of *Myristica*, and a few *Virola* species (de Wilde, 1991b, 2000) provide mixed conditions and/or insights into how these two types may have evolved from one another, in both directions. According to our observations, *Haematodendron* may very well be of the single-type, contrary to what was assumed (though with a question mark) by de Wilde (1991b), who did not have access to material.

37. Axillary branches: (0) absent (a single inflorescence originating in the leaf axil = 'distally branched plural-type'); (1) present (three inflorescences originating in

the leaf axil, two of which result from development of the axillary buds of the two lateral prophylls of the main axis = 'proximally branched plural-type'). De Wilde (1991b). Applies only in Myristicaceae with inflorescences of the plural type. The main axis may or may not develop, and the two axillary basal branches may or may not be of the single-type.

38. Flower clustering: (0) not or loosely clustered; (1) condensed raceme-like short shoots (brachyblasts); (2) umbel-shaped bundles or fascicles (subumbels); (3) globose or hemispherical heads (capitula). De Wilde (1991b). Applies only in Myristicaceae. States correspond to de Wilde's (1991b) four types of clustering. However, we prefer to include saucer-shaped receptacles in umbels rather than in heads. Loosely clustered flowers usually appear as few-flowered fascicles, intergrading with a few solitary flowers, whereas in umbels or sub-umbels (whether few-flowered or not) all flowers in the inflorescence appear to be clustered. In *Iryanthera* and *Otoba*, flowers are always clustered into few-flowered fascicles, interpreted by de Wilde (1991b) as reduced single-type units but scored here as (2). These sessile fascicles may indeed be historically different from stalked (pedunculate) clusters found elsewhere in the family. Hemispherical heads of *Brochoneura* are interpreted as sessile heads fused to the main axes of the inflorescence. Flowers may appear clustered as an artifact of pedicel absence, as in *Mauloutchia* sp. nov. 4, here scored (0). Interpretation of the almost spike-like branches in inflorescences of *Mauloutchia heckelii* is ambiguous. *Haematodendron* was mistakenly assumed to correspond to state (0) by de Wilde (1991b), who did not have access to material, whereas our observations instead indicate that all flowers are clustered into fascicles, as is also the case in *Mauloutchia coriacea*.

39. Pedicel: (0) distinct (long or short); (1) short and widening continuously into the perianth (subsessile flowers); (2) absent (strictly sessile flowers). Ordered. Data from de Wilde (1991b) and our observations. Applies to male flowers only, since female flowers are often subsessile when male flowers are distinctly pedicellate, at least in *Mauloutchia*. Pedicellate flowers may appear subsessile in immature regions of the inflorescence. *Pycnanthus* was drawn as pedicellate by de Wilde (1991b) but drawn as and stated to be sessile by Fouilloy (1974); we score it (2). *Staudtia* was described as shortly pedicellate by de Wilde (1991b) and subsessile by Fouilloy (1974); so far we have observed it to be strictly sessile, but because Fouilloy observed more specimens, we have scored it (1/2).

40. Bracteoles: (0) absent or rudimentary; (1) present. Not applicable when flowers are strictly sessile. Scored only in Myristicaceae, with data from Sinclair (1958a) and de Wilde (1991b), supplemented by our own observations.

General floral morphology (41–48)

Data on conventional floral characters in Myristicaceae based on Sinclair (1958a), de Wilde (1991b, 2000), and our own observations, particularly on the Afro-Malagasy genera.

41. Sex distribution: (0) bisexual; (1) unisexual monoecious; (2) unisexual dioecious. DE-38, modified to distinguish monoecious and dioecious. Data on taxa outside Myristicaceae primarily from Kubitzki *et al*. (1993). In Siparunaceae, based on the phylogeny of Renner & Won (2001), it is equally parsimonious to reconstruct the ancestral state as monoecious or dioecious. Unisexual taxa in Annonaceae (e.g. *Uvariopsis*) and Magnoliaceae (*Kmeria*) are assumed to be autapomorphic. According to de Wilde (2000: 359), *Myristica fragrans* may occasionally be monoecious, and *Myristica crassa* is paroecious; these exceptions are considered autapomorphic and the genus is scored as dioecious, but they provide additional illustrations of the flexibility of monoecy and dioecy in Myristicaceae.

42. Ovary position: (0) superior; (1) surrounded by hypanthium; (2) inferior. DE-39.

43. Perianth phyllotaxy: (0) spiral; (1) whorled. DE-40.

44. Perianth whorls (series when phyllotaxy is spiral): (0) more than two; (1) two; (2) one; (3) absent. DE-41. As in Doyle & Endress (2000), we have scored the calyptrate calyx of *Eupomatia* and *Galbulimima* as a single perianth whorl, although it has also been interpreted as composed of one or two bracts, and we have assumed that the petaloid organs in *Galbulimima* are staminodes (cf. Endress, 1977).

45. Perianth merosity: (0) irregular; (1) in threes; (2) in twos. DE-42. Based on outer rather than inner cycle(s) when these differ. In Myristicaceae, deviations from three (from two to five) hardly ever seem to be fixed within species, and when they are, this is assumed to be autapomorphic. A major exception, however, is the genus *Horsfieldia*, in which the number two has been fixed in many species, most of which belong to section *Irya* (de Wilde, 2000).

46. Outer perianth cycle (series): (0) not clearly differentiated (or continuum of forms); (1) sepaloid. DE-43. Taxa with one cycle, such as Myristicaceae, are scored (?).

47. Calyx (outermost perianth whorl or series): (0) separate or basally fused; (1) fused most of length (usually calyptrate). DE-44. Provisionally scored (0) in all Myristicaceae; it is possible that some members might actually be scored (1), but this would require proper examination and treatment of the sexual dimorphism that exists in the family (fusion is usually more pronounced in female flowers).

48. Inner staminodes: (0) absent; (1) present. DE-70. All Myristicaceae are scored (?) because of unisexuality and stamen fusion.

Androecium morphology (49–65)

For Myristicaceae, literature data (Sinclair, 1958a; Hutchinson, 1964; Capuron, 1972, 1973; de Wilde, 1991b, 2000) were supplemented by many of our own observations, including SEM photographs of pickled or herbarium specimens of *Brochoneura*, *Cephalosphaera*, *Knema*, *Mauloutchia* and *Staudtia*.

49. Androecium phyllotaxy: (0) spiral; (1) whorled; (2) irregular. DE-46. It is still unclear whether stamen phyllotaxy is spiral or irregular in *Mauloutchia* (for further detail, see Sauquet in press).

50. Anther apices: (0) all at (almost) same level; (1) at various levels. This character accounts for potentially informative differences within *Mauloutchia*. Taxa outside Myristicaceae are scored (?) because it is unclear

whether variations in the level of anthers can be compared in taxa with free and highly fused stamens.
51. Stamen merosity: (0) irregular; (1) in threes. DE-47. In Myristicaceae, only taxa in which the number of anthers seems to be fixed at three are scored (1), or (0/1) when there is recurrent variation (sometimes within the same inflorescence) around three. All others, including genera that may have individual species with anther number fixed at multiples of three, are scored (0).
52. Number of anthers: (0) 2–4–(5); (1) 5–≈12; (2) ≈12–≈20; (3) over 20. Ordered. This character is not redundant with character 51, because the number of anthers in trimerous taxa may vary from three to many. The states recognized are based on apparent natural breaks within Myristicaceae. We have scored taxa outside Myristicaceae (?), since it is unclear whether variations in anther number in the fused androecium of Myristicaceae can be compared with those in taxa with free stamens, in which numbers appear to vary continuously or in multiples of three.
53. Stamen fusion: (0) free to base; (1) partially or completely fused. DE-48. Even in *Mauloutchia chapelieri*, where stamens are most nearly free, it is unambiguous that they are partially fused, whether as a result of overgrowth of the receptacle or a more complex evolutionary history.
54. Filament (free sterile portion below anther): (0) present; (1) absent (whether by reduction or fusion among filaments).
55. Stamen base: (0) short (<length of anther) and wide (typical laminar); (1) long (>length of anther) and wide (>1/2 width of anther); (2) narrow (<1/2 width of anther) and either long or short (typical filament). DE-49. Following Doyle & Endress (2000), we have scored Canellaceae and all Myristicaceae as (?). Although some *Mauloutchia* species have a short, narrow filament (character 54), the androecium is so fused that it seems questionable to compare the form of the free portion of the stamen base with the narrow stamen base in taxa with free stamens, especially if the partially free condition is secondarily derived within *Mauloutchia*.
56. Connectives: (0) free (basifixed anthers); (1) fused for part or all of the length of the anthers (to a central mass), or centrifixed sessile anthers. Condition varies within *Compsoneura* (basifixed in section Compsoneura, fused for the whole length in section Coniostele), *Knema* (with basifixed, fused, and ambiguous species), and possibly *Iryanthera,* and it is ambiguous in *Mauloutchia* sp. nov. 2; all of these are scored (0/1).
57. Upper part of column (sterile tissue bearing anthers, = sterile part of synandrium *sensu* de Wilde, 2000): (0) truncate to deeply hollowed; (1) shortly and narrowly elongated (i.e. with a sterile apex); (2) greatly widened (including peltate disc). Applies only to Myristicaceae with nonbasifixed anthers. Range of conditions in state (0) based on high polymorphism and existence of all intermediate forms in *Horsfieldia* (de Wilde, 2000).
58. Paired basal stamen glands: (0) absent; (1) present. DE-50.
59. Connective apex: (0) extended; (1) truncate or smoothly rounded. DE-51. The short sterile apex of the synandrium of *Osteophloeum* and most species of *Myristica* (a peculiarity described by state (1) of character 57) may or may not be derived from fusion of extended connective apices, so we have scored both genera (?).
60. Number of microsporangia: (0) four; (1) two. DE-52.
61. Pollen sacs: (0) protruding; (1) embedded. DE-53.
62. Orientation of stamen dehiscence: (0) introrse; (1) latrorse; (2) extrorse. DE-54.
63. Mode of stamen dehiscence: (0) longitudinal slit; (1) H-valvate; (2) valvate with upward-opening flaps. DE-55.
64. Connective hypodermis: (0) unspecialized; (1) endothecial or sclerenchymatous. DE-56.
65. Microsporogenesis: (0) simultaneous; (1) successive. DE-58. In Myristicaceae, this character appears to have been studied only in *Myristica* (Bhandari, 1971; Yakovlev, 1981).

Pollen morphology (66–79)

Data on characters not used by Doyle & Endress (2000) in groups outside Myristicaceae (67, 69, 72, 77, 78) from Praglowski (1974, 1979), Walker (1976a,b), Sampson (1993, 1995, 1996, 2000), Le Thomas (1980–81), Hesse & Kubitzki (1983), Jérémie, Lugardon & Le Thomas (1984), Prakash, Foreman & Griffith (1984), Takahashi (1986), Zavada & Taylor (1986), Foreman & Sampson (1987), Sampson & Foreman (1988, 1990), Doyle & Hotton (1991), Dickison (1992), Pignal *et al*. (1999), J.-M. Groult (pers. comm., for Canellaceae). Primary source of data for Myristicaceae is the comprehensive SEM and TEM survey by Walker & Walker of the American (1979, 1983), African (1980), and Malagasy (1981) genera, complemented by our own SEM survey of *Staudtia*, the Asian genera, and the Malagasy genera (including species not examined by Walker & Walker, 1981). Additional, complementary data found in Wodehouse (in Smith, 1937: 397–402), Canright (1963), Walker (1974), Siddiqi & Wilson (1975a), Straka & Friedrich (1988), Tissot, Chikhi & Nayar (1994), Sampson (2000) and J-M. Groult (unpublished SEM data on *Brochoneura*, *Compsoneura*, *Gymnacranthera*, *Horsfieldia*, *Mauloutchia* and *Myristica*).
66. Pollen size (average): (0) large (>50 μm); (1) medium; (2) small (<20 μm). Ordered. DE-62. Measures for Myristicaceae from Wodehouse (in Smith, 1937: 397–402), Walker & Walker (1979, 1980, 1981, 1983), Straka & Friedrich (1988) and Tissot *et al*. (1994).
67. Pollen shape: (0) boat-shaped; (1) intermediate; (2) globose. DE-60 modified, with the new intermediate state including various ambiguous shapes not readily assignable to either boat-shaped or globose. *Cephalosphaera* is the best illustration of the globose condition in Myristicaceae.
68. Aperture type: (0) one distal (monosulcate, including monoulcerate and disulcate, with distal and proximal sulcus); (1) none (inaperturate); (2) equatorial (sulculate). DE-61.
69. Shape of aperture: (0) sulcate (= long and narrow) to sulcoid (= long but wide); (1) ulcerate to ulceroid. Applicable only in taxa with state (0) of character 68. Sulcoids differ from sulcates in their wider aperture, and sometimes in a reduction in length apparently correlated with globose or intermediate shape, although some quite globose pollen grains are very nicely sulcate (*Otoba*). No clearly boat-shaped pollen with an ulceroid

or ulcerate aperture has been found so far. Again, the perfectly rounded aperture in *Cephalosphaera* is the best illustration of the ulcerate condition in Myristicaceae. Based on currently available data (including unpublished photos of J-M. Groult), *Brochoneura acuminata* seems to vary between sulcoid and ulceroid. Shape of the aperture in *Mauloutchia coriacea* is confused by the strongly sculptured membrane (see TEM section of Walker & Walker, 1981): the aperture is difficult to circumscribe, but very likely ulceroid according to our SEM data.

70. Aperture membrane: (0) smooth; (1) sculptured. DE-68. Sculptured in all surveyed Myristicaceae, where exine sculpture always changes gradually at the aperture margin.

71. Tectum: (0) continuous (including microperforate); (1) reticulate to rugulate; (2) areolate to verrucate; (3) reduced (not distinguishable from underlying granules). DE-64, modified by addition of state (2). Reticulate and rugulate were united in one state because they intergrade completely in *Knema* (our observations). The verrucate condition differs from areolate (in which elements are also wider than tall) only in the wider spacing of the elements. We distinguish verrucate from compound granulate microsculpture (as in *Mauloutchia heckelii*), scored as continuous (and possibly microperforate). Walker & Walker's (1981) figures of *Mauloutchia chapelieri* show an areolate tectum with a slight rugulate tendency, whereas ours show a continuous tectum, so we scored this species (0/2). Apart from this ambiguous case, an areolate tectum was found only in *Cephalosphaera* and *Mauloutchia coriacea*. A trend towards gemmate sculpture in *Knema* (especially *K. laurina*) is assumed to be autapomorphic and derived from the rugulo-reticulate condition of most *Knema* species and hence ignored in scoring the genus. Similarly, the unusual intectate pollen of *Horsfieldia sinclairii* figured by Walker (1974; as *H. erubescens*) is assumed to be autapomorphic (though it may be compared to *Knema laurina*). The continuous face in *Otoba* (Walker & Walker, 1983) is the only example so far in Myristicaceae of a smooth, continuous tectum without microperforations (unlike *Brochoneura* and *Staudtia*); because of the very different sculpture of the proximal and distal hemispheres, we have scored *Otoba* as (0/1). The continuous microperforate tectum in *Staudtia* tends somewhat towards a very densely rugulate condition, from which it may have originated. Conversely, the very densely rugulate tectum of some 'type I' *Virola* species (Walker & Walker, 1979; especially *V. calophylla*) approaches the microperforate condition, from which it can still be distinguished in whole-grain views, where the tectum appears more lumpy than in continuous microperforates like *Staudtia* and *Brochoneura*. However, the limit between states (0) and (1) in *Staudtia* and these peculiar *Virola* species remains ambiguous. Although *M. coriacea* clearly has an areolate tectum, it also exhibits microperforations.

72. Free bacula: (0) absent; (1) present. Applicable only when pollen is reticulate with large perforations. Aristolochiaceae based on *Saruma*, the only genus with large perforations (Dickison, 1992). Characteristic of *Myristica*, *Paramyristica* and *Osteophloeum*, but also found in some species of *Iryanthera* and *Virola*. Taxa with both inapplicable (rugulate or microreticulate) and reticulate species are scored based on the condition observed in the latter (e.g. absent in *Knema*). Assumed to be absent in the columellar region of *Otoba*, though overgrowth of columellae might hide small, free bacula. Scored (?) in *Haematodendron* and *Pycnanthus*, in which the rugulo-reticulum is very fine and any free bacula would be difficult to see, as well as in *Scyphocephalium*, in which the lumina are obstructed by the heavy crotonoid microsculpturing.

73. Tectal microsculpture: (0) smooth (with or without microperforations); (1) granulate (supratectal elements smaller than the width of tectal muri, when the latter are present); (2) annulate to crotonoid (with large, triangular-shaped elements on the muri). DE-66, modified by addition of state (2). State (2) applies only when the tectum is reticulate or rugulate. Annulate and crotonoid conditions are lumped, because crotonoid muri in *Haematodendron* and *Scyphocephalium* intergrade with muri with a few rings and are probably derived from annulate. Granulate, spinulate and echinulate conditions are combined; they differ only in having rounded (granulate) or acute (spinulate, echinulate) apices. Such elements are also occasionally referred to as (micro)verrucate, but our definition also includes elements that are taller than wide. A slight tendency to spinulate in some *Virola* species (Walker & Walker, 1979) is assumed to be derived from annulate and thus ignored. SEM close-ups of the surface of *Mauloutchia humblotii* and *Mauloutchia rarabe* by Walker & Walker (1981; B and C, respectively, in fig. 1) may have been reversed, but this does not affect their scoring.

74. Prominent spines (larger than spinules, easily visible with light microscopy): (0) absent; (1) present. DE-67. When tectal microsculpture is granulate, prominent spines are additional larger conical/pointed projections, differentiated from the finer granules. Thus, *Doyleanthus* is scored as having prominent spines, although they are lower and less developed than those of *Mauloutchia* sp. nov. 2 and *Pycnanthus*.

75. Infratectum: (0) columellar; (1) mixed (with both granular and columellar elements); (2) granular. DE-63, with state numbers reversed and (1) slightly modified. Ordered. In Myristicaceae, strictly granular structure has so far been found only in *Mauloutchia humblotii* and *Mauloutchia* sp. nov. 2, based on unambiguous TEM sections of Walker & Walker (1981; the second species as *M. heckelii*). *Brochoneura acuminata* is assumed to be mixed by comparison of our SEM section with the TEM sections of the two other species in Walker & Walker (1981). Data on *Staudtia* are only available from our unsatisfactory SEM sections, where granules could be identified but columellae only assumed because of the thin infratectum. *Cephalosphaera* looks columellar but is assumed to be mixed because it has a very thin infratectum like that of *Brochoneura*, suggesting that it also has granules. However, a thin infratectum may not always indicate mixed structure: *Pycnanthus* has a thin infratectum (as in *Cephalosphaera*), but also a very thin (reticulate) tectum. If granules were present in *Pycnanthus*, they could only be located at the outside of its unusually thick nexine, but in the absence of any evidence for this, we scored it as strictly columellar. The TEM section of *Mauloutchia parvifolia* (Walker &

Walker, 1981) may not be reliable. Though granular at first sight, it does not look like other granular forms and could very well be mixed, with the infratectum crushed as an artifact of preparation (plus it is otherwise very similar to the TEM section of *Mauloutchia* sp., d'Alleizette s.n., which is clearly mixed); thus the scoring of this species is based on our SEM section (7713 RN), which clearly appears mixed. Our SEM sections of *Mauloutchia rarabe* and *Mauloutchia* sp. nov. 1 are difficult to interpret but very similar to each other; they are both scored (1/2). The same difficulty was also encountered in *Mauloutchia heckelii* and *Doyleanthus*. *Otoba* is scored (0/1) because of the very different structure of the proximal and distal hemispheres.

76. Nexine stratification (in extra-apertural regions): (0) footlayer only; (1) footlayer and endexine; (2) reduced (absent or discontinuous). DE-69.

77. Nexine thickness: (0) thin (less than a third of total exine); (1) thick (more than a third of total exine). In Myristicaceae, the clearest break occurs at 1/3, although this leaves *Compsoneura* ambiguous. When thickness varies over a range that reaches but does not overlap this limit, the taxon is scored based on its average thickness. Thickness ratio estimated without prominent spines when these occur. The estimates of Walker & Walker (1979, 1980, 1981, 1983; expressed as a percentage of total nexine thickness) are always concordant with our rough estimates, except for *Mauloutchia parvifolia*, where we concluded that the TEM section of Walker & Walker (1981) is not reliable (see notes on infratectum).

78. Foliations: (0) absent; (1) 1–2 discontinuous; (2) multiple. Character 51 of Doyle & Le Thomas (1996), modified. Ordered. Myristicaceae based on Walker & Walker (1979, 1980, 1981, 1983) and Sampson (2000) for *Myristica fragrans*.

Gynoecium morphology: carpel characters (79–88)

Scoring of groups outside Myristicaceae for carpel and ovule characters (79–96) is based on Doyle & Endress (2000) and references cited therein. For Myristicaceae, characters not covered in the general references are scored in *Horsfieldia*, *Myristica* and *Virola* based on observations by Igersheim & Endress (1997; P.K. Endress, pers. comm.).

79. Number of carpels: (0) more than one; (1) one. DE-71. Unicarpellate taxa in Annonaceae are restricted to a few genera in the inaperturate clade (*Cyathocalyx* p.p., *Dasoclema*, *Dendrokingstonia*, *Dielsiothamnus* and *Tridimeris*; van Heusden, 1992) and are thus assumed to be apomorphic within the family.

80. Form of carpel: (0) plicate; (1) partially ascidiate (both plicate and ascidiate zones present below the stigma) with ovule(s) on the ascidiate zone. DE-72, with elimination of completely ascidiate. The condition in Myristicaceae is problematic, since an ascidiate zone is not developed, but the ovule is in a median/basal position. Doyle & Endress (2000) scored the family as partially ascidiate, and this is followed here for the three genera studied by Igersheim & Endress (1997).

81. Sealing of carpel: (0) complete postgenital fusion with no secretion; (1) postgenital fusion to apex with partial canal containing secretion (= Type 3 of Endress & Igersheim, 2000); (2) partial postgenital fusion with continuous unfused canal containing secretion (= Type 2 of Endress & Igersheim, 2000). DE-73, with elimination of sealing by secretion only.

82. Pollen tube transmitting tissue: (0) not prominently differentiated; (1) one layer prominently differentiated; (2) multilayered (more than one layer differentiated). DE-74.

83. Style: (0) absent (stigma sessile or capitate); (1) present (elongated apical portion of carpel distinctly constricted relative to the ovary). DE-75. In Annonaceae, presence of a style is a derived condition (Doyle & Le Thomas, 1996; Chatrou *et al*., 2000). Most Myristicaceae have a sessile stigma, but species of *Mauloutchia* (including *M. coriacea*) have more or less elongate stigmatic lobes and are therefore scored as (?).

84. Stigma: (0) extended (all around ventral slit or extending half or more of the way down the style-stigma zone); (1) restricted (above slit or around its upper part). DE-76.

85. Stigma papillae: (0) unicellular only; (1) some or all uniseriate pluricellular. DE-77, with elimination of pluriseriate pluricellular (in this data set found only in *Idiospermum*, included in Calycanthaceae). In Myristicaceae, the condition in *Horsfieldia* is uncertain (P.K. Endress, pers. comm.).

86. Compitum: (0) absent; (1) extragynoecial. DE-78. Unicarpellate taxa such as Myristicaceae scored (?). Doyle & Endress (2000) scored Annonaceae as (0/1), since both conditions occur in the taxa sampled by Igersheim & Endress (1997). However, an extragynoecial compitum occurs in the basal genus *Anaxagorea* (our observations), so we have rescored the family as (1).

87. Carpel fusion: (0) apocarpous (including pseudosyncarpous); (1) parasyncarpous. DE-79, with elimination of eusyncarpous (in this data set found only in Aristolochiaceae). Unicarpellate taxa scored as apocarpous (0).

88. Oil cells in carpels: (0) not visible (absent or internal); (1) superficial (intrusive). DE-80.

Gynoecium morphology: ovule characters (89–96)

89. Ovule number: (0) one; (1) mostly two (occasionally one or a few more than two); (2) more than two. DE-82.

90. Ovule direction: (0) pendent; (1) horizontal; (2) ascendent. DE-84.

91. Ovule curvature: (0) anatropous (or nearly so); (1) orthotropous (including hemitropous). DE-85. All Myristicaceae studied by Corner (1976) and Igersheim & Endress (1997) are anatropous except *Horsfieldia sylvestris*, described by Corner (1976) as suborthotropous.

92. Shape of outer integument: (0) semiannular; (1) annular. DE-87. *Mauloutchia chapelieri* scored based on SEM photos by H. Sauquet and P. K. Endress.

93. Lobation of outer integument: (0) unlobed; (1) lobed. DE-88. *Mauloutchia chapelieri* scored based on SEM photos by H. Sauquet and P. K. Endress.

94. Thickness of outer integument (at middle of integument length): (0) two cells; (1) two and three to four; (2) four and five, or more. Ordered. DE-89. Myristicaceae based on Corner (1976) and Igersheim & Endress (1997).

95. Thickness of inner integument: (0) two and three cells, or three; (1) three and more cells. DE-90. Myristi-

caceae based on Corner (1976) and Igersheim & Endress (1997).

96. Chalaza: (0) unextended; (1) pachychalazal; (2) perichalazal. DE-91. Myristicaceae based on Corner (1976) and Igersheim & Endress (1997).

Fruit morphology (97–101)

Corner (1976), Takhtajan (1988), and Kubitzki *et al*. (1993) for taxa outside Myristicaceae; Perrier de la Bâthie (1952), Capuron (1972, 1973), de Wilde (2000) and our observations for Myristicaceae.

97. Fruit wall: (0) fleshy; (1) fleshy with hard endocarp (= drupe); (2) dry (= achenes, follicles). DE-93. Scored as fleshy in all Myristicaceae for which fruits are known, but often very leathery.

98. Fruit dehiscence: (0) indehiscent; (1) dehiscent. DE-94.

99. Fruit symmetry: (0) more or less radial (circular in transverse section); (1) elongated along the axis perpendicular to the suture plane (referred to as transversely ellipsoid in the literature).

100. Fruit ornamentation: (0) smooth or simply verrucate; (1) carinate; (2) echinate. Fruits that are smooth when fresh may become verrucate when dried (e.g. *Mauloutchia rarabe*), so these two conditions are lumped (ornamentation may also change during maturation). Defined this way, these three states can be determined in young fruits as well. Carinate fruits (which may also be somewhat verrucate) are a potential synapomorphy of *Mauloutchia chapelieri* (though var. *chapelieri* forma *ecristata* is only slightly carinate) and *M. coriacea* (with less pronounced carinations than *M. chapelieri*). Echinate fruits are most likely an autapomorphy of *Mauloutchia* sp. nov. 2.

101. Fruit apex: (0) obtuse to slightly acute; (1) elongate, acuminate, or rostrate. Applies to mature fruits only (young fruits, at least in *Mauloutchia*, may appear rostrate but later become obtuse to slightly acute, as in *Mauloutchia humblotii* and *M. rarabe*). Young fruit of *Mauloutchia* sp. nov. 1 is rostrate, but scored (?) because no mature fruit is known so far. Occasionally rostrate in *Myristica* (de Wilde, 2000).

Seed morphology (102–115)

Taxa outside Myristicaceae based primarily on Corner (1976) and Takhtajan (1988). Myristicaceae: literature data supplemented by our observations on fresh, pickled, and herbarium material of *Brochoneura*, *Cephalosphaera*, *Haematodendron*, *Mauloutchia* and *Staudtia*.

102. Testa: (0) slightly or non-multiplicative; (1) multiplicative. DE-95. This character concerns multiplication of cell layers in development of the testa from the outer integument. Corner (1976) described Myristicaceae as having 7–10 layers in the outer integument (6–8 in *Myristica*), Igersheim & Endress (1997) as having 5–7 layers. Corner (1976) described *Gymnacranthera* (with 6–7 layers in the testa) as '? not multiplicative', *Myristica* (10–23 layers) as multiplicative. By these criteria, *Horsfieldia* (testa 9 cells thick: Corner, 1976) and some but not all *Knema* species (testa 14–17, 6–8 cells thick) are multiplicative, but *Pycnanthus* and *Virola* (both 6–7 cells thick) are not.

103. Exotesta: (0) unspecialized, (1), palisade or shorter, sclerotic cells. DE-96. Corner (1976) described Myristicaceae as having a slightly lignified (*Gymnacranthera*), thickened (*Pycnanthus*, *Virola*), or slightly thickened (*Horsfieldia*, *Myristica*) exotesta, but based on his figures this is less specialized than the palisade layer in Winteraceae.

104. Mesotesta: (0) unspecialized; (1) fibrous; (2) sarcotesta; (3) spongy. DE-97. Taxa with two cell layers in the outer integument scored (?). Corner (1976) described Myristicaceae as aerenchymatous in *Knema* or aerenchymatous and pulpy in *Myristica*, *Pycnanthus* and *Virola*, but did not mention this in *Gymnacranthera*; given the unclear comparisons within the family and with the spongy mesotesta of Hernandiaceae, we have scored these as unspecialized.

105. Endotesta: (0) unspecialized; (1) multiple lignified layer (with fibrous endoreticulum); (2) tracheidal (or similar tangentially elongate but non-lignified cells); (3) palisade of thick-walled prismatic or shorter sclerotic cells. DE-107.

106. Tegmen: (0) unspecialized; (1) sclerotic (both ecto- and endotegmen thick-walled); (2) exotegmen fibrous to sclerotic. DE-99. Fibrous and sclerotic intergrade in Myristicaceae. According to Corner (1976), *Myristica fragrans* differs from *M. lowiana* in having an unspecialized exotegmen.

107. Ruminations: (0) absent; (1) tegminal (lateral) and/or chalazal; (2) testal. DE-100, modified by addition of state (1) and specification of testal origin for state (2). Doweld & Shevyryova (1998) reported ruminations in *Galbulimima*, but these are poorly developed; thus we have scored this genus (?) rather than (0) in Doyle & Endress (2000). Myristicaceae based on Sinclair (1958a), Corner (1976), Takhtajan (1988), and our observations.

108. Aril: (0) absent; (1) rudimentary (shortly laciniate or not, usually whitish); (2) entire or apex laciniate; (3) deeply laciniate. DE-102, modified by splitting of aril types. Myristicaceae: Sinclair (1958a), Capuron (1973), de Wilde (2000) and our observations on fresh and/or dried material of *Brochoneura*, *Haematodendron*, *Mauloutchia* and *Staudtia*. Observations on dry specimens, especially when the fruit is indehiscent, may be tricky, since the testa may separate from inside of the seed (mostly endosperm) during drying, as the inside shrinks and the lignified testa remains appressed to the fruit wall. The aril must be sought between the testa and the fruit wall, which may be difficult to separate (e.g. in *Brochoneura* and *Mauloutchia*), and impressions of the fibrous exotegmen on the inside of the testa and outside of the tegmen may be very conspicuous and confused with an aril (e.g. Capuron, 1973; figs 6,7, mistakenly labelled as seed). An aril is usually absent or rudimentary in indehiscent fruits, but not always: *Doyleanthus*, *Scyphocephalium*, and possibly *Mauloutchia heckelii* and *Staudtia* have indehiscent fruits, yet they have fully developed arils, whereas *Mauloutchia humblotii* and *M. rarabe* both have dehiscent fruits but almost invisible, rudimentary arils. Rudimentary arils may not be visible in dry specimens. *Haematodendron* may be the only genus in the family with no aril at all. The condition in *Endocomia* is intermediate between states (2) and (3), since the aril is incised about halfway (de Wilde, 2000).

109. Endosperm development: (0) cellular; (1) nuclear. DE-103. In Myristicaceae, this character appears to have been studied only in *Myristica* (Bhandari, 1971); Yakovlev, 1981).
110. Endosperm in mature seed: (0) present; (1) absent. DE-104.
111. Starch in seed: (0) absent; (1) present. Groups outside Myristicaceae based on Corner (1976), Takhtajan (1988), and Kubitzki (1993). Myristicaceae: Sinclair (1958a).
112. Embryo: (0) minute (less than 1/2 length of seed interior); (1) large. DE-106. Corner (1976), Yakovlev (1981), Takhtajan (1988). Assumed to be minute in all Myristicaceae.
113. Cotyledon habit: (0) (sub)erect; (1) divaricate or horizontal. Taxa outside Myristicaceae based on Takhtajan (1988). Taxa with undifferentiated embryo (*Galbulimima*) or massive cotyledons (Lauraceae, Hernandiaceae) are scored (?). Myristicaceae: Sinclair (1958a).
114. Cotyledon fusion: (0) free; (1) fused (at the base). Myristicaceae: Sinclair (1958a). Taxa outside Myristicaceae scored (?) because of uncertainty of comparison with conditions in Myristicaceae.
115. Germination: (0) epigeal; (1) hypogeal *s.l.* DE-108, modified. Duke (1969), de Vogel (1980), Takhtajan (1988). In Myristicaceae, de Vogel (1980) placed most genera studied in his *Horsfieldia* type/*Horsfieldia* subtype, which is typically hypogeal. His *Horsfieldia* type/*Pseuduvariopsis* subtype, found in *Virola*, is technically epigeal, since the seed is lifted above the soil surface by hypocotyl elongation, but it is most closely related to the *Horsfieldia* subtype in being cryptocotylar and in other respects (as implied by de Vogel's classification), so we have retained it in the same state.

DATA MATRIX

Symbols: ? = missing data; – = not applicable; a = 0/1; b = 0/2; c = 0/3; d = 1/2; e = 1/3; f = 2/3; g = 0/1/2; h = 1/2/3; i = 0/1/2/3

	1 5 10 15 20 25 30 35 40 45 50 55
	. . . .
Aristolochiineae	10f0112a10000ga11aa??10001000001000---??0b1d11001?1?a02
Piperineae	100011g010000011111??10001000001001---??00?3???01?1?002
Canellaceae	013000000a0001a0000??0000a00000a001---??001011001?a?11?
Winteraceae	01300?001?000a00000??0000000a000001---??001021a01?0?001
Atherospermataceae	012000000000122000??0001101000100a---??01a00001a?0?00b
Calycanthaceae	0120012100a0012200a??00100000000000---??010000010?0?000
Gomortega nitida	002000000000122?00??0??00000001001---??020000010?0?002
Hernandiaceae	00100021010001b0001??1??00000021011---??021d00001?0?002
Lauraceae	002000d10100a12b00a??a??00a00001011---??01111001?1?00b
Monimiaceae	002001a1100001220002??000aa010001001---??21100001a?0?002
Siparunaceae	01d001a1000001020002??0dd1001002a001---??d1120?0?1?0?00a
Annonaceae	0a31012a1a101101100??0a0000000a1100---??0010110a2?0?000
Degeneria	000101011010111011 00??00000000010000---??000011010?0?000
Eupomatia	000001011000010110 0??00000000011000---??01?2??110?0?100
Galbulimima	0031011a00101011100??0000001001?000---??00?2??110?0?000
Magnoliaceae	000101aa00101a0a020??0aa00000011100---??0010aa000?0?000
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Brochoneura acuminata	0??0???????1???100a010000??2??????11032-10121?0?101011?
Brochoneura madagascariensis	0??a???????1???1?00010000??2??1???11032-10121?0?101011?
Brochoneura vouri	0??1???????1???1?00210000?????1???11032-10121?0?101011?
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Gymnacranthera	0?3?01010011??0100aa101200031101101100002 0121?0?100111?
Haematodendron glabrum	0??0???????1???1?00100000?????0???1a0b0020121?0?10a011?
Horsfieldia	0?3?011100a1?a0a0001101d0002a11a10110c002012d?0?100i11?
Iryanthera	0?3101110011001000a10110??3??1?1?111201d0121?0?10aa11?
Knema	013101010011?a01?0aaa012001210111010-10120121?0?100i11?
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Mauloutchia coriacea	0??????????1???1000000000??2??????110bg010121?0?b00310?
Mauloutchia heckelii	0??????????1???1?0a010aa0??2??????110c2-10121?0?b1021??
Mauloutchia humblotii	0??1???????1???1000110000??3??0???1100001012 1?0?b00111?
Mauloutchia parvifolia	0??1???????1???1000100000?????1???1100001012 1?0?b10d10?
Mauloutchia rarabe	0??????????1???1?00010000??2??????1100001012 1?0?b00d10?
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Mauloutchia sp. nov. 2	0??????????1???1?00010000?????????110000d0121?0?b1011??
Mauloutchia sp. nov. 3	0??????????1???1?000100?0?????????110000d0121?0?b?031??
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Osteophloeum	0?3?01110011??a1?00d00110?????????111201201 21?0?100211?
Otoba	0?3?01110001??a1?00??0120??3??????1112a020121?0?101011?
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Scyphocephalium	0???01110011???1?00a10120??2??????10-3a020121?0?100111?
Staudtia kamerunensis	0?3?01110001??01?00110000??3??1???1103d010121?0?10a011?
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APPENDIX B
MOLECULAR SEQUENCE DATA SETS

Table B1. Sampling information for the three chloroplast regions (split into seven data sets in our analyses) sequenced for this study. Voucher information only applies to unpublished or unreleased sequences. Taxa as defined in Appendix A. In a few cases, different genera had to be sequenced to represent a particular taxon in the different data sets; in these cases, genera are identified by a capital letter. Likewise, different species of the same genus are identified by a lowercase letter and different vouchers of the same species by a number. Initials between parentheses refer to the authors of sequences generated for this study. * = Sequence not yet submitted to GenBank at the time this paper went to press.

		trnK region			*trnL* region		
Species and voucher	*ndhF*	*trnK* 5′-intron	*matK*	*trnK* 3′-intron	*trnT-trnL*	*trnL* intron	*trnL-trnF*
PIPERALES							
Aristolochiineae							
(A) *Aristolochia pistolochia* L.: T. Borsch 3257 (FR); (B) *Lactoris fernandeziana* Phil.	(B) AF123809 (Graham & Olmstead, 2000)	(A) PROV01* (T.B.)	(A) AF543724 (T.B.)		(A) AY145341 (Borsch *et al.*, in press)	(A) AY145341 (Borsch *et al.*, in press)	(A) AY145341 (Borsch *et al.*, in press)
Piperineae							
(A) *Houttuynia cordata* Thunb.: T. Borsch 3481 (FR); (B) *Saururus cernuus* L.: T. Borsch & V. Wilde 3108 (BONN)	(B) AF123811 (Graham & Olmstead, 2000)	(A) PROV05* (T.B.)	(A) AF543737 (T.B.)	(A) PROV06* (T.B.)	(B) AY145343 (Borsch *et al.*, in press)	(B) AY145343 (Borsch *et al.*, in press)	(B) AY145343 (Borsch *et al.*, in press)
WINTERALES							
Canellaceae							
Canella winterana (L.) Gaertn.: (1) T. Borsch 3466 (BONN); (2) Y-L. Qiu 90017 (NCU)	(2) AY218191 (H.S.)	(1) PROV10* (T.B.)	(1) AF543731 (T.B.)		(1) AY145348 (Borsch *et al.*, in press)	AY004152 (Karol *et al.*, 2000)	AY004152 (Karol *et al.*, 2000)
Winteraceae							
(A) *Drimys* (a) *lanceolata* (Poir.) Baill.: T. Borsch 3484 (BONN); (b) *winteri* J.R. Forster & G. Forster: T. Borsch 3479 (BONN); (B) *Zygogynum pauciflorum* Vink: Y-L. Qiu 90025 (NCU)	(B) AY218192 (H.S.)	(Aa) PROV12* (T.B.)	(Aa) AF543735 (T.B.)	(Aa) PROV13* (T.B.)	(Ab) AY145347 (Borsch *et al.*, in press)	(Ab) AY004143 (Karol *et al.*, 2000)	(Ab) AY004143 (Karol *et al.*, 2000)

LAURALES						
Atherospermataceae						
(A) *Doryphora sassafras* Endl.: Y-L. Qiu 98109 (Z); (B) *Laurelia* (a) *novae-zelandiae* A. Cunn.; (b) *sempervirens* (Ruiz & Pav.) Tul. R. Greissl 643–99 (MJG)	(A) AY218165 (T.S.)	(Bb) PROV15* (K. W. Hilu *et al.*, unpubl.)		(Ba) AF129032 (Renner, 1999)		(Ba) AF040674 (Renner, 1998)
Calycanthaceae						
Calycanthus floridus L.: T. Borsch 3455 (BONN)	AF123802 (Graham & Olmstead, 2000)	AF543730 (K.W.H.)		AY145349 (Borsch *et al.*, in press)	AY145349 (Borsch *et al.*, in press)	AY145349 (Borsch *et al.*, in press)
Gomortegaceae						
Gomortega nitida Ruiz & Pav.				AF264020 (Renner & Chanderbali, 2000)	AF264020 (Renner & Chanderbali, 2000)	AF012404 (Renner *et al.*, 1997)
Hernandiaceae						
(A) *Gyrocarpus americanus* Jacq.; (B) *Hernandia nymphaeifolia* (C. Presl) Kubitzki: J.G. Rohwer 166 (MJG)		(B) PROV19* (K. W. Hilu *et al.*, unpubl.)	(B) AJ247165 (Rohwer, 2000)	(A) AF129025 (Renner, 1999)		(A) AF012398 (Renner *et al.*, 1997)
Lauraceae						
(A) *Persea americana* Mill.: Botanical Conservatory of the University of California, Davis; (B) *Umbellularia californica* (Hook. & Arn.) Nutt.: T. Borsch 3471 (BONN)	(A) AY218166 (T.S.)	(A) AJ247179 (Rohwer, 2000)	(A) AJ247179 (Rohwer, 2000)	(B) AY145350 (Borsch *et al.*, in press)	(B) AY145350 (Borsch *et al.*, in press)	(B) AY145350 (Borsch *et al.*, in press)
Monimiaceae						
(A) *Hedycarya angustifolia* A. Cunn.: Arboretum of the University of California, Santa Cruz 75.176; (B) *Peumus boldus* Molina	(A) AY218167 (T.S.)	(B) AJ247183 (Rohwer, 2000)	(B) AJ247183 (Rohwer, 2000)	(B) AF129041 (Renner, 1999)		(B) AF012403 (Renner *et al.*, 1997)

Table B1. *Continued*

Species and voucher	*ndhF*	*trnK* region			*trnL* region		
		trnK 5′-intron	*matK*	*trnK* 3′-intron	*trnT-trnL*	*trnL* intron	*trnL-trnF*
Siparunaceae							
Siparuna aspera (Ruiz & Pav.) A. DC.					AF129042 (Renner, 1999)		AF040695 (Renner, 1998)
MAGNOLIALES							
Annonaceae							
Ambavia gerrardii (Baill.) Le Thomas: H. Sauquet 23 (P)	AY218168 (T.S.)	AY218193 (H.S.)	AY220435 (H.S.)	AY220381 (H.S.)	AY220401 (T.S. & H.S.)	AY220411 (T.S. & H.S.)	AY220358 (T.S. & H.S.)
Anaxagorea (a) *acuminata* (Dunal) A. DC.: M.J. Jansen-Jacobs *et al*. 5774 (U); (b) *phaeocarpa* Mart.: L.W. Chatrou & G.B. Ozinga 263 (U)	(a) AY218169 (T.S.)	(a) AY218194 (H.S.)	(a) AY220436 (H.S.)	(a) AY220382 (H.S.)		(b) AY231284 (L.W.C.)	(b) AY238944 (L.W.C.)
Annickia kummeriae (Engl. & Diels) Setten: D.M. Johnson 1942 (OWU)			AY23896 (L.W.C.)			AY231285 (L.W.C.)	AY238945 (L.W.C.)
Annona muricata L.: (1) T. Borsch 3460 (BONN); (2) Y-L. Qiu 90031 (NCU)	(2) AY218170 (T.S.)		(1) AF543722 (K.W.H.)			(1) AY145352 (Borsch *et al.*, in press)	(1) AY145352 (Borsch *et al.*, in press)
Artabotrys hexapetalus (L. f.) Bhandari: Utrecht University Botanic Gardens 94GR01614			AY238962 (L.W.C.)			AY231286 (L.W.C.)	AY238946 (L.W.C.)
Asimina triloba (L.) Dunal: Y-L. Qiu 15 (NCU)	AY218171 (H.S.)	AY218195 (H.S.)	AY220437 (H.S.)	AY220383 (H.S.)		AY220412 (H.S.)	AY220359 (H.S.)
Cananga odorata (Lam.) Hook. f. & Thomson: M.W. Chase 219 (NCU)	AY218172 (H.S.)	AY218196 (H.S.)	AY220438 (H.S.)	AY220384 (H.S.)		AY220413 (H.S.)	AY220360 (H.S.)
Isolona campanulata Engl. & Diels: Utrecht University Botanic Gardens 86GR00241			AY238963 (L.W.C.)			AY231287 (L.W.C.)	AY238947 (L.W.C.)

Malmea dielsiana R.E. Fr.: L.W. Chatrou *et al.* 122 (U) *Mkilua fragrans* Verdc.			AY238964 (L.W.C.)			AY231288 (L.W.C.)	AY238948 (L.W.C.) van Zuilen *et al.* (1996)
Polyalthia suberosa (Roxb.) Thwaites: (1) Utrecht University Botanic Gardens 83GR00317; (2) Y-L. Qiu 94008 (IND)	(2) AY218173 (T.S.)	(2) AY218197 (H.S.)	(2) AY220439 (H.S.)	(2) AY220385 (H.S.)		(1) AY231289 (L.W.C.)	(1) AY238949 (L.W.C.)
Uvaria lucida Benth.: Utrecht University Botanic Gardens 84GR00334			AY238966 (L.W.C.)			AY231290 (L.W.C.)	AY238950 (L.W.C.)
Xylopia peruviana R.E. Fr.: Utrecht University Botanic Gardens 84GR00271			AY238967 (L.W.C.)			AY231291 (L.W.C.)	AY238951 (L.W.C.)
Degeneriaceae							
Degeneria roseiflora J.M. Miller: J.M. Miller 1189–63 (SUVA)	AY218174 (H.S.)	AY218198 (H.S.)	AY220440 (H.S.)	AY220386 (H.S.)	AY220402 (T.S. & H.S.)	AY220414 (T.S. & H.S)	AY220361 (T.S. & H.S.)
Eupomatiaceae							
Eupomatia bennettii F. Muell.: (1) P.K. Endress 5197 (BONN); (2) Y-L. Qiu 90022 (NCU)	(2) AY218175 (H.S.)	(2) AY218199 (H.S.)	1+2 PROV47* (H.S. & T.B.)	1+2 PROV48* (H.S. & T.B.)	(1) PROV49* (T.B.)	(1) PROV50* (T.B.)	(1) PROV51* (T.B.)
Himantandraceae							
Galbulimima belgraveana (F. Muell.) Sprague: Y-L. Qiu 90034 (NCU)	AY218176 (H.S.)	AY218200 (H.S.)	AY220441 (H.S.)	AY220387 (H.S.)	AY220403 (T.S. & H.S.)	AY220415 (T.S. & H.S.)	AY220362 (T.S. & H.S.)
Magnoliaceae							
Liriodendron chinense (Hemsl.) Sarg.: (1) T. Borsch 3483 (BONN); (2) Y-L. Qiu 28 (NCU)	(2) AY218177 (H.S.)	AB021016 (Azuma *et al.*, 1999)	AB021016 (Azuma *et al.*, 1999)	AB021016 (Azuma *et al.*, 1999)	(1) PROV52* (T.B.)	(1) PROV53* (T.B.)	(1) PROV54* (T.B.)
Magnolia (a) *tripetala* L.: Y-L. Qiu 3 (NCU); (b) *virginiana* L.: T. Borsch & C. Neinhuis 3280 (VPI)	(a) AY218178 (H.S.)	(a) AB021001 (Azuma *et al.*, 1999)	(a) AB021001 (Azuma *et al.*, 1999)	(a) AB021001 (Azuma *et al.*, 1999)	(b) AY145354 (Borsch *et al.*, in press)	(b) AY145354 (Borsch *et al.*, in press)	(b) AY145354 (Borsch *et al.*, in press)

Table B1. *Continued*

Species and voucher	*ndhF*	*trnK* region			*trnL* region		
		trnK 5′-intron	*matK*	*trnK* 3′-intron	*trnT-trnL*	*trnL* intron	*trnL-trnF*
Myristicaceae							
Bicuiba oleifera (Schott) W.J. de Wilde: H. Sauquet 1 (P)						AY220416 (H.S.)	AY220363 (H.S.)
Brochoneura acuminata (Lam.) Warb.: H. Sauquet 19 (P)	AY218179 (T.S. & H.S.)	AY218201 (H.S.)	AY220442 (H.S.)	AY220388 (H.S.)	AY220404 (H.S.)	AY220417 (H.S.)	AY220364 (H.S.)
Brochoneura madagascariensis (Lam.) Warb.: H. Sauquet 18 (P)						AY220418 (H.S.)	AY220365 (H.S.)
Brochoneura vouri (Baill.) Warb.: H. Sauquet 15 (P)						AY220419 (H.S.)	AY220366 (H.S.)
Cephalosphaera usambarensis (Warb.) Warb.: J. Lovett s.n. (coll. Tanzania)	AY218180 (T.S. & H.S.)	AY218202 (H.S.)	AY220443 (H.S.)	AY220389 (H.S.)		AY220420 (H.S.)	AY220367 (H.S.)
Coelocaryon oxycarpum Stapf: Chèvreloup Arboretum 8488	AY218181 (T.S. & H.S.)	AY218203 (H.S.)	AY220444 (H.S.)	AY220390 (H.S.)	AY220405 (H.S.)	AY220421 (H.S.)	AY220368 (H.S.)
Compsoneura sprucei (A. DC.) Warb.: S.W. Brewer 297 (CAS)	AY218182 (H.S.)	AY218204 (H.S.)	AY220445 (H.S.)	AY220391 (H.S.)	AY220406 (H.S.)	AY220422 (H.S.)	AY220369 (H.S.)
Gymnacranthera farquhariana (Hook. f. & Thomson) Warb.: A.R. Khalit 63586 (Z)	AY218183 (H.S.)	AY218205 (H.S.)	AY220446 (H.S.)	AY220392 (H.S.)	AY220407 (H.S.)	AY220423 (H.S.)	AY220370 (H.S.)
Haematodendron glabrum Capuron: H. Sauquet 3 (P)		AY218206 (H.S.)	AY220447 (H.S.)	AY220393 (H.S.)		AY220424 (H.S.)	AY220371 (H.S.)
Horsfieldia punctatifolia J. Sinclair: A.R. Khalit 64832 (Z)	AY218184 (H.S.)	AY218207 (H.S.)	AY220448 (H.S.)	AY220394 (H.S.)	AY220408 (H.S.)	AY220425 (H.S.)	AY220372 (H.S.)

Iryanthera (a) *hostmanni* (Benth.) Warb.: S. Jésel s.n. (French Guiana); (b) sp. Vincentini 655 (K)	(b) AY218185 (H.S.)	(a) AY218208 (H.S.)	(a) AY220449 (H.S.)	(a) AY220395 (H.S.)		(a) AY220426 (H.S.)	(a) AY220373 (H.S.)
Knema (a) *latericia* Elmer; (b) *laurina* (Blume) Warb.: A.R. Khalit 6197 (Z)	(b) AY218186 (H.S.)	(b) AY218209 (H.S.)	(b) AY220450 (H.S.)	(b) AY220396 (H.S.)	(b) AY220409 (H.S.)	(b) AY220427 (H.S.)	(a) AF040694 (Renner, 1999)
Mauloutchia chapelieri (Baill.) Warb.: (1) G.E. Schatz 3847 A (MO); (2) H. Sauquet 30 (P); (3) H. Sauquet 37 (P)	(1) AY218187 (H.S.)	(2) AY218210 (H.S.)	(2) AY220451 (H.S.)	(2) AY220397 (H.S.)	(2) AY220410 (H.S.)	(2) AY220428 (H.S.)	(3) AY220374 (H.S.)
Mauloutchia heckelii Capuron: H. Sauquet 6b (P)						AY220429 (H.S.)	AY220375 (H.S.)
Mauloutchia humblotii (H. Perrier) Capuron: H. Sauquet 5 (P)						AY220430 (H.S.)	AY220376 (H.S.)
Myristica (a) *fragrans* Houtt.: (1) T. Borsch 3473 (BONN); (2) Y-L. Qiu 92014 (NCU); (b) *maingayi* Hook. f.: A.R. Khalit 15762 (Z)	(a2) AY218188 (H.S.)	(b) AY218211 (H.S.)	(b) AY220452 (H.S.)	(b) AY220398 (H.S.)	(a1) AY145351 (Borsch *et al.*, in press)	(a1) AY145351 (Borsch *et al.*, in press)	(a1) AY145351 (Borsch *et al.*, in press)
Otoba parvifolia (Markgr.) A.H. Gentry: J. Janovec 1999 (NY)						AY220431 (H.S.)	AY220377 (H.S.)
Pycnanthus angolensis (Welw.) Warb.: Abidjan Botanical Garden	AY218189 (T.S. & H.S.)	AY218212 (H.S.)	AY220453 (H.S.)	AY220399 (H.S.)		AY220432 (H.S.)	AY220378 (H.S.)
Staudtia kamerunensis Warb.: Tadjouteu & Achoundong 433 (YA)						AY220433 (H.S)	AY220379 (H.S)
Virola (a) *michelii* Heckel: S. Jésel s.n. (French Guiana); (b) *sebifera* Aubl. Litt 6 (NY); (c) *surinamensis* (Rol.) Warb.: S. Jésel s.n. (French Guiana)	(b) AY218190 (H.S.)	(a) AY218213 (H.S.)	(a) AY220454 (H.S.)	(a) AY220400 (H.S.)		(c) AY220434 (H.S.)	(c) AY220380 (H.S.)

Table B2. Sampling information for the five additional data sets included in the analyses of this study. See Table B1 for comments and notes.

	Chloroplast		Nucleus	Mitochondrion	
Species and voucher	*atpB*	*rbcL*	18S rDNA	*atp1*	*matR*
PIPERALES					
Aristolochiineae					
Aristolochia macrophylla Lam.	AJ235399 (Savolainen *et al*., 2000)	L12630 (Qiu *et al*., 1993)	AF206855 (D. Soltis *et al*., 2000)	AF197669 (Qiu *et al*., 2000)	AF197732 (Qiu *et al*., 2000)
Piperineae					
Saururus cernuus L.	AF187061 (Graham & Olmstead, 2000)	L14294 (Olmstead *et al*., 1993)	U42805 (Soltis *et al*., 1997)	AF197633 (Qiu *et al*., 2000)	AF197748 (Qiu *et al*., 2000)
WINTERALES					
Canellaceae					
Canella winterana (L.) Gaert.	AJ235424 (Savolainen *et al*., 2000)	AJ131928 (Qiu *et al*., 1993)	AF206879 (D. Soltis *et al*., 2000)	AF197676 (Qiu *et al*., 2000)	AF197757 (Qiu *et al*., 2000)
Winteraceae					
Drimys winteri J.R. Forster & G. Forster	AF093425 (Hoot *et al.*, 1999)	AF093734 (Hoot *et al*., 1999)	U42823 (Soltis *et al*., 1997)	AF197673 (Qiu *et al*., 2000)	AF197781 (Qiu *et al*., 2000)
LAURALES					
Atherospermataceae					
Atherosperma moschatum Labill.	AF197604 (Qiu *et al.*, 2000)	AF121362 (Renner, 1999)	AF197578 (Qiu *et al.*, 2000)	AF197683 (Qiu *et al*., 2000)	AF197799 (Qiu *et al*., 2000)
Calycanthaceae					
Calycanthus floridus L.	AJ235422 (Savolainen *et al*., 2000)	L14291 (Chase *et al*., 1993)	U38318 (Soltis *et al*., 1997)	AF197678 (Qiu *et al*., 2000)	AF197777 (Qiu *et al*., 2000)
Gomortegaceae					
Gomortega nitida Ruiz & Pav.	AF209593 (D. Soltis *et al*., 2000)	Ueda *et al*. (1997)	AF206918 (D. Soltis *et al*. 2000)		
Hernandiaceae					
Gyrocarpus americanus Jacq.	AJ235487 (Savolainen *et al*., 2000)	L12647 (Qiu *et al*., 1993)	AF206923 (D. Soltis *et al*. 2000)	AF197701 (Qiu *et al*., 2000)	AF197805 (Qiu *et al*., 2000)

Lauraceae					
(A) *Laurus nobilis* L.; (B) *Persea americana* Mill.	(A) AJ235518 (Savolainen *et al*., 2000)	(B) L14620 (Golenberg *et al*., 1990)	(A) AF197580 (Qiu *et al*., 2000)	(A) AF197682 (Qiu *et al*., 2000)	(A) AF197798 (Qiu *et al*., 2000)
Monimiaceae					
Peumus boldus Molina	AF209650 (D. Soltis *et al*., 2000)	AF040664 (Renner, 1998)	AF206988 (D. Soltis *et al*., 2000)	AF197686 (Qiu *et al*., 2000)	AF197803 (Qiu *et al*., 2000)
Siparunaceae					
Siparuna (a) *decipiens* A. DC.; (b) *lepidota* (Humb., Bonpl. & Kunth) A. DC.		(b) AF040667 (Renner, 1998)	(a) AF197687	(a) AF197809 (Qiu *et al*., 2000)	(Qiu *et al*., 2000)
MAGNOLIALES					
Annonaceae					
Ambavia gerrardii (Baill.) Le Thomas		Doyle *et al*. (2000)			
Anaxagorea dolichocarpa Sprague & Sandwith		Doyle *et al*. (2000)			
Annickia kummeriae (Engl. & Diels) Setten		Doyle *et al*. (2000)			
Annona muricata L.	AJ235393 (Savolainen *et al*., 2000)	L12629 (Qiu *et al*., 1993) Doyle *et al*. (2000)	AF206850 (D. Soltis *et al*., 2000)	AF197695 (Qiu *et al*., 2000)	AF197766 (Qiu *et al*., 2000)
Artabotrys hexapetalus (L. f.) Bhandari	AF209532 (D. Soltis *et al*., 2000)	L12631 (Qiu *et al*., 1993)	AF206856 (D. Soltis *et al*., 2000)	AF197696 (Qiu *et al*., 2000)	AF197765 (Qiu *et al*., 2000)
Asimina triloba (L.) Dunal		L12636 (Qiu *et al*., 1993)	AF469770 (D. Soltis *et al*., 2000)	AF197700 (Qiu *et al*., 2000)	AF197763 (Qiu *et al*., 2000)
Cananga odourata (Lam.) Hook. f. & Thomson: M.W. Chase 219 (NCU)		Doyle *et al*. (2000) AY238955 (L.W.C)	L54061 (Soltis *et al*., 1997)		
Isolona sp.		Doyle *et al*. (2000)	L54060 (Soltis *et al*., 1997)		
Malmea dielsiana R.E. Fr.: L.W. Chatrou *et al*. 122 (U)					
Mkilua fragrans Verdc.					

Table B2. *Continued*

Species and voucher	Chloroplast		Nucleus	Mitochondrion	
	atpB	*rbcL*	18S rDNA	*atp1*	*matR*
Polyalthia suberosa (Roxb.) Thwaites	AF293856 (Qiu *et al.*, 2000)	AF193971 (Parkinson *et al.*, 1999)	AF193938 (Parkinson *et al.*, 1999)	AF197694 (Qiu *et al.*, 2000)	AF197764 (Qiu *et al.*, 2000)
Uvaria lucida Benth.		Doyle *et al.* (2000)			
Xylopia nitida Dunal		Doyle *et al.* (2000)			
Degeneriaceae					
Degeneria roseiflora J.M. Miller	AJ235451 (Savolainen *et al.*, 2000)	L12643 (Qiu *et al.*, 1993)	AF206898 (D. Soltis *et al.*, 2000)	AF293752 (Qiu *et al.*, 2000)	AF197771 (Qiu *et al.*, 2000)
Eupomatiaceae					
Eupomatia bennettii F. Muell.	AJ235473 (Savolainen *et al.*, 2000)	L12644 (Qiu *et al.*, 1993)	AF469771 (D. Soltis *et al.*, 2000)	AF197692 (Qiu *et al.*, 2000)	AF197772 (Qiu *et al.*, 2000)
Himantandraceae					
Galbulimima belgraveana (F. Muell.) Sprague	AJ235478 (Savolainen *et al.*, 2000)	(Qiu *et al.*, 1993)	L12646 (D. Soltis *et al.*, 2000)	AF206916 (Qiu *et al.*, 2000)	AF197693 (Qiu *et al.*, 2000) AF197773
Magnoliaceae					
Liriodendron (a) *chinense* (Hemsl.) Sarg.; (b) *tulipifera* L.	(b) AJ235522 (Savolainen *et al.*, 2000)	(a) L12654 (Qiu *et al.*, 1993)	(a) AF206954 (D. Soltis *et al.*, 2000)	(a) AF197690 (Qiu *et al.*, 2000)	(a) AF197774 (Qiu *et al.*, 2000)
Magnolia tripetala L.	AJ235526 (Savolainen *et al.*, 2000)	AJ131927 (Qiu *et al.*, 1995b)	AF206956 (D. Soltis *et al.*, 2000)	AF197691 (Qiu *et al.*, 2000)	AF197770 (Qiu *et al.*, 2000)
Myristicaceae					
Knema latericia Elmer	AF209611 (D. Soltis *et al.*, 2000)	L12653 (Qiu *et al.*, 1993)	AF206946 (D. Soltis *et al.*, 2000)	AF197697 (Qiu *et al.*, 2000)	AF197767 (Qiu *et al.*, 2000)
Mauloutchia chapelieri (Baill.) Warb.	AF197606 (Qiu *et al.*, 2000)	AF197594 (Qiu *et al.*, 2000)		AF197699 (Qiu *et al.*, 2000)	AF197769 (Qiu *et al.*, 2000)
Myristica fragrans Houtt.	AJ235539 (Savolainen *et al.*, 2000)	AF206798 (D. Soltis *et al.*, 2000)	AF206968 (D. Soltis *et al.*, 2000)	AF197698 (Qiu *et al.*, 2000)	AF197768 (Qiu *et al.*, 2000)

APPENDIX C
INDEL DATA SET

APPROACH

Only parsimony-informative indel patterns were scored. Overlapping (or nested) gaps were treated as a single multistate character, with no prior historical assumption: overlapping gaps of different lengths were always scored as different character states. Autapomorphic patterns were either scored as a separate state (when included in the limits of the defined indel region) or considered ambiguous and scored as missing data (when extending beyond the limits of the indel region).

CHARACTERS

All characters are unordered. Following each character number, the length of the indel region covered is given. Unless otherwise indicated, characters are binary, with the following state definition: (0) gap; (1) no gap. For indel regions with overlapping gaps (scored as multistate characters), the length of each observed gap is given with its corresponding state.

ndhF (1–3): **1.** 3 bp **2.** 6 bp **3.** 3 bp.
trnK 5′-intron (4–17): **4.** 1 bp **5.** 3 bp **6.** 1 bp **7.** 1 bp **8.** 1 bp **9.** 15 bp: (0) gap15; (1) gap13; (2) gap7; (3) gap6. **10.** 5 bp **11.** 1 bp **12.** 10 bp: (0) gap10; (1) gap5; (2) no gap. **13.** 2 bp **14.** 5 bp **15.** 1 bp **16.** 1 bp **17.** 9 bp: (0) gap9; (1) gap8; (2) no gap.
matK (18–22): **18.** 6 bp **19.** 6 bp **20.** 3 bp **21.** 3 bp **22.** 6 bp.
trnK 3′-intron (23–30): **23.** 9 bp **24.** 3 bp **25.** 2 bp **26.** 2 bp: (0) gap2; (1) gap1; (2) no gap. **27.** 8 bp: (0) gap8; (1) gap6; (2) gap5; (3) no gap. **28.** 5 bp **29.** 5 bp **30.** 5 bp.
trnT-trnL (31–42): **31.** 1 bp **32.** 5 bp: (0) gap5; (1) gap4; (2) no gap. **33.** 11 bp: (0) gap11; (1) gap1+5; (2) gap1. **34.** 10 bp **35.** 12 bp: (0) gap12; (1) gap7; (2) gap6; (3) no gap. **36.** 5 bp **37.** 1 bp **38.** 4 bp **39.** 1 bp **40.** 1 bp **41.** 10 bp: (0) gap10; (1) gap4; (2) no gap. **42.** 4 bp.
trnL intron (43–45): **43.** 1 bp **44.** 5 bp **45.** 5 bp.
trnL-trnF (46–59): **46.** 5 bp **47.** 13 bp: (0) gap13; (1) gap1+4; (2) gap1; (3) no gap. **48.** 8 bp **49.** 1 bp **50.** 4 bp **51.** 10 bp: (0) gap10; (1) gap5; (2) no gap. **52.** 1 bp **53.** 4 bp **54.** 43 bp **55.** 1 bp **56.** 6 bp: (0) gap6; (1) gap1; (2) no gap. **57.** 1 bp **58.** 6 bp: (0) gap6; (1) gap1; (2) no gap. **59.** 5 bp.

DATA MATRIX

Symbols: ? = ambiguous or missing fragment of sequence; – = not sampled.

	1 5 10 15 20 25 30 35 40 45 50 55 59
	. . . .
Aristolochiineae	???10001?100???0000010--------01??0001101?10?010??111?10010
Piperineae	???10001110010000000010010221??01??000110111O?110??111?100?0
Canellaceae	000110011000100010000--------02??10001011000??00?111010000
Winteraceae	0001100111001000100000010221100 2??00001???1001100?111010000
Atherospermataceae	001----------00010--------01??00000021---01000211010000
Calycanthaceae	???----------00010--------01??000010211??000000??010000
Gomortega	----------01??000000211??01000211010000
Hernandiaceae	----------000100102111011??000010?1---030000????0001
Lauraceae	001----------????00102211011??00001121100 01?00111010000
Monimiaceae	001----------????00102311011??20000121---01000?11010000
Siparunaceae	----------01??00001?1?---01000111010001
Ambavia	1101011131000111201010110030 10????000010??01101011100010000
Anaxagorea	00010011?1000000200010010030 10----------11?00011100010000
Annickia	----------?????----------11101111100010000
Annona	000----------00010----------01?01111100011020
Artabotrys	----------?????----------01101111100010000
Asimina	00010111?1000100200010010030 10----------??101111100011020
Cananga	11?1011131000111200010110030 10----------?1101011100010000
Isolona	----------?????----------0110111110?011000
Malmea	----------?????----------01101011100010000
Mkilua	----------?1111100012000
Polyalthia	1001011131000110200010010030 10----------01101111100010000
Uvaria	----------?????----------01?01111100011000
Xylopia	----------?????----------0??01111100010000
Degeneria	00010?1111000000000010010130100 1??000010101000101011101000?
Eupomatia	000100111100000 0????00100301 0?????0001010?0001010110010000
Galbulimima	00010011110000 0??0101001301 0????0000101?10001?10111010000
Liriodendron	000100101100100??000100101301001??0000100?11001010111010000
Magnolia	00010?101100100??000100101301001??000010001100101011101000 0

Continued

	1 5 10 15 20 25 30 35 40 45 50 55 59
	. . . .
Bicuiba oleifera	----------??0010101111000100
Brochoneura madagascariensis	----------10001010111100100
Brochoneura vouri	----------??0010101111100100
Brochoneura acuminata	0000001100121 00??10111001201 01?00131101010??0010101111100100
Cephalosphaera usambarensis	0000001120111 00??10111001201 01----------??0010101111100100
Coelocaryon	0000001120111 00??10111001201 01?0113110101?10001010111000100
Compsoneura	0000001120111 00??10111001201 0100003110101010001010111000100
Gymnacranthera	0000001120111 00??10111001201 01?0103010101010001010111000100
Haematodendron glabrum	---0001120121 00??10111001201 01----------??0010101111000100
Horsfieldia	0000001120111 00??10111001201 01?0?131101010100010101111000100
Iryanthera	0000001120121 00??10111001201 01----------10001010111000100
Knema	0000001120111 00??10111001201 0100213110101010001000111010100
Mauloutchia chapelieri	0000001100121 00??10111001201 0100003110101010001010111100100
Mauloutchia heckelii	----------??0010101111100100
Mauloutchia humblotii	----------10001010111100100
Myristica	0000001120121 00??10111001201 0100113110101010002010111 0?????
Otoba	----------?0001010?????????
Pycnanthus	0000001120111 00??10111001201 01----------?0001010111000100
Staudtia kamerunensis	----------?000101011 10?????
Virola	0000001120111 00??10111001201 01----------?0001010?11000100