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CIRCUMSCRIPTION AND PHYLOGENY OF THE LAURALES: EVIDENCE FROM MOLECULAR AND MORPHOLOGICAL DATA[1]

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The order Laurales comprises a few indisputed core constituents, namely Gomortegaceae, Hernandiaceae, Lauraceae, and Monimiaceae sensu lato, and an equal number of families that have recently been included in, or excluded from, the order, namely Amborellaceae, Calycanthaceae, Chloranthaceae, Idiospermaceae, and Trimeniaceae. In addition, the circumscription of the second largest family in the order, the Monimiaceae, has been problematic. I conducted two analyses, one on 82 *rbcL* sequences representing all putative Laurales and major lineages of basal angiosperms to clarify the composition of the order and to determine the relationships of the controversal families, and the other on a concatenated matrix of sequences from 28 taxa and six plastid genome regions (*rbcL, rpl16, trnT-trnL, trnL-trnF, atpB-rbcL,* and *psbA-trnH*) that together yielded 898 parsimony-informative characters. Fifteen morphological characters that play a key role in the evolution and classification of Laurales were analyzed on the most parsimonious molecular trees as well as being included directly in the analysis in a total evidence approach. The resulting trees strongly support the monophyly of the core Laurales (as listed above) plus Calycanthaceae and Idiospermaceae. Trimeniaceae form a clade with Illiciaceae, Schisandraceae, and Austrobaileyaceae, whereas Amborellaceae and Chloranthaceae represent isolated clades that cannot be placed securely based on *rbcL* alone. Within Laurales, the deepest split is between Calycanthaceae (including Idiospermaceae) and the remaining six families, which in turn form two clades, the Siparunaceae (Atherospermataceae-Gomortegaceae) and the Hernandiaceae (Monimiaceae s.str. [sensu stricto]-Lauraceae). Monimiaceae clearly are polyphyletic as long as they include Atherospermataceae and Siparunaceae. Several morphological character state changes are congruent with the molecular tree: (1) Calycanthaceae have disulculate tectate-columellate pollen, while their sister clade has inaperturate thin-exined pollen, with the exception of Atherospermataceae, which have columellate but meridionosulcate or disulcate pollen. (2) Calycanthaceae have two ventral ovules while their sister clade has solitary ovules. Within this sister clade, the Hernandiaceae (Lauraceae-Monimiaceae) have apical ovules, while the Siparunaceae (Atherospermataceae-Gomortegaceae) are inferred to ancestrally have basal ovules, a condition lost in *Gomortega,* the only lauralean genus with a syncarpous ovary. (3) Calycanthaceae lack floral nectaries (except for isolated nectarogeneous fields on the inner tepals), while their sister clade ancestrally has paired nectar glands on the filaments. Filament glands were independently lost in higher Monimiaceae and in Siparunaceae concomitant with pollinator changes away from nectar-foraging flies and bees to non-nectar feeding beetles and gall midges. (4) Disporangiate stamens with anthers dehiscing by two apically hinged valves are ancestral in Siparunaceae-(Atherospermataceae-Gomortegaceae) and evolved independently within Hernandiaceae and Lauraceae. Depending on the correct placement of Calycanthaceae-like fossil flowers, tetrasporangiate anthers with valvate dehiscence (with the valves laterally hinged) may be ancestral in Laurales and lost in modern Calycanthaceae and Monimiaceae.

Key words: Atherospermataceae; Calycanthaceae; Gomortegaceae; Hernandiaceae; Lauraceae; Monimiaceae; Siparunaceae; Trimeniaceae.

The Laurales are a small order of flowering plants that comprises ~2400 species. As well as being economically important as a source of hardwoods, the group is of great phylogenetic interest because Laurales are among the oldest known flowering plants. Paleobotanical interest has focused especially on Chloranthaceae, Calycanthaceae, and Lauraceae, families with fossil records that go back to the Early and mid-Cretaceous (Drinnan et al., 1990; Pedersen et al., 1991; Herendeen, Crepet, and Nixon, 1993; Crane, Friis, and Pedersen, 1994; Crepet and Nixon, 1994; Friis et al., 1994; Friis, Crane, and Pedersen, 1997; Eklund and Kvacek, 1998). With the advent of molecular systematic data, however, it has become clear that Chloranthaceae do not belong in Laurales (contra Takthajan, 1973, 1997, and Thorne, 1974, in press). More recently, attention has turned to another putative lauralean taxon, the monotypic Amborellaceae. *Amborella trichopoda* (Baillon, 1869) was placed as sister to all other angiosperms or in a grade with *Illicium, Austrobaileya, Schisandra,* the Piperales, and Nymphaeaceae in the 18S rDNA trees of Soltis et al. (1997a), and in a clade with *Austrobaileya, Illicium,* and Nymphaeaceae in a combined analysis of *rbcL* and 18S sequences (Soltis et al., 1997b; compare also Fig. 4B in Chase et al., 1993).

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TABLE 1. Putative members of Laurales according to recent authors.

Putative Laurales	Cronquist (1988)	Kubitzki (1993a)	Takhtajan (1997)	Thorne (in press)
Amborellaceae	+	+	+	+
Atherospermataceae	incl. in Monimiaceae	incl. in Monimiaceae	incl. in Monimiaceae	+
Calycanthaceae	+	+	−	+
Chloranthaceae	−	+	−	+
Gomortegaceae	+	+	+	+
Hernandiaceae	+	+	+	+
Idiospermaceae	+	incl. in Calycanthaceae	−	incl. in Calycanthaceae
Lauraceae	+	+	+	+
Monimiaceae	+	+	+	+
Siparunaceae	incl. in Monimiaceae	incl. in Monimiaceae	incl. in Monimiaceae	+
Trimeniaceae	+	+	+	+

An analysis of combined *atpB, rbcL,* and 18S sequences again places *Amborella* as sister to all other angiosperms (M. Chase, D. Soltis, and P. Soltis, personal communication, 1998).

Prior to molecular studies, Amborellaceae and Chloranthaceae had been interpreted as close to Trimeniaceae (Endress and Sampson, 1983; Philipson, 1993a). Trimeniaceae are a family with four or five species in one or two genera, for which the first discovered species, *Trimenia weinmanniifolia* (Seemann, 1865) and *Piptocalyx moorei* (Oliver in Bentham, 1870; = *Trimenia moorei* (Oliver) Philipson), were regarded as probably belonging in Monimiaceae by Bentham and Hooker (1880) because their flowers shared some traits with those of *Xymalos. Xymalos* is one of two Monimiaceae with numerous stamens but a single carpel. Monimiaceae at the time also encompassed *Amborella.* Similar to Chloranthaceae, *Amborella* and *Trimenia* have flower and inflorescence modifications typical of wind-pollination, such as inconspicuous flowers, single ovules, stigmas with large receptive surfaces, reduced or lacking tepals or petals, and separation of sexual function, making them similar to each other and difficult to homologize with animal-pollinated potential relatives. As will be shown here, Trimeniaceae do not belong in Laurales. Instead, *Trimenia* is closest to *Illicium, Schisandra,* and *Austrobaileya.*

Partly because the circumscription of Laurales (summarized in Table 1) is unclear, partly because their second-largest family, Monimiaceae, as traditionally conceived (e.g., Money, Bailey, and Swamy, 1950; Philipson, 1993b; Takhtajan, 1997) is blatantly polyphyletic (Renner, 1998), relationships of and within Laurales have remained obscure. Hallier (1905), who in his *Natürliche* (*phylogenetische*) *System der Blütenpflanzen* first interpreted the Laurales as a natural group, thought them characterized by perigynous flowers, that is, flowers with a well-developed fleshy receptacle enveloping the carpels. As circumscribed by Hallier, Laurales comprised three families, the Calycanthaceae, the Monimiaceae s.l. (sensu lato), into which Hallier had sunk another highly aberrant group, the monospecific Gomortegaceae (Reiche, 1896), and the Lauraceae, which in Hallier's conception included the Hernandiaceae. Note that the most divergent member of the Calycanthaceae, *Calycanthus* (*Idiospermum*) *australiensis,* was first described in 1912, after Hallier's paper. Calycanthaceae and Monimiaceae were seen as related to Lauraceae, and the entire order was seen as derived from (''abstammend von'') Magnoliales (Hallier, 1905). The Magnoliales were characterized by hypogynous flowers, i.e., with the carpels exposed on the receptacle rather than embedded in it. Together, Laurales and Magnoliales formed the Polycarpicae (both had ancestrally numerous carpels per flower). Chloranthaceae do not have carpels embedded in the receptacle nor do they have numerous carpels, and Hallier (1905) accordingly did not include them in the Laurales; instead he placed them with Piperaceae and Saururaceae in Piperales. He either had not seen material of *Amborella, Piptocalyx,* and *Trimenia,* all of which lack cup-shaped receptacles and thus might have been deemed misfits in Laurales, or else he was not concerned about intrafamilial variation in receptacle development. As it was, Trimeniaceae and Amborellaceae remained buried in Monimiaceae until 1917 and 1948 when they were raised to family rank by Gibbs and Pichon, respectively (which became widely accepted after the landmark Monimiaceae study by Money, Bailey and Swamy, 1950). No morphologist excluded them from Laurales, however.

To securely circumscribe a monophyletic Laurales and to resolve intralauralean relationships, both large amounts of sequencing data and dense taxon sampling are needed (cf. Chase and Cox, 1998; Graybeal, 1998). Taxon addition minimizes the effects of spurious groupings among clades rich in autapomorphies, i.e., relatively long branches, which are liable to produce long-branch-attraction artifacts, while large amounts of sequence data increase phylogenetic signal. Previous large-scale molecular studies of basal angiosperms have included a small number of core lauralean taxa. Thus, Qiu et al. (1993) and the angiosperm-wide *rbcL* analysis of Chase et al. (1993) both included *Calycanthus, Chimonanthus,* and *Idiospermum* of Calycanthaceae, *Hernandia* and *Gyrocarpus* of Hernandiaceae, *Cinnamomum* and *Persea* of Lauraceae, and *Hedycarya* of Monimiaceae s.str. (sensu stricto). The 18S analysis of Soltis et al. (1997a) included *Calycanthus* and *Sassafras.* The combined *rbcL* + *atpB* analysis of Savolainen et al. (in press) includes the same taxa as Chase et al. (1993) plus an additional member of Monimiaceae (*Kibara*) and minus one genus each of Calycanthaceae and Hernandiaceae. Other studies that combined morphological characters and rDNA (Doyle, Donoghue, and Zimmer, 1994) or *rbcL* (Nandi, Chase, and Endress, 1998) have included, respectively, *Calycanthus, Persea,* and *Hedycarya,* and *Chimonanthus.* Lacking from all these analyses are Atherospermataceae, Gomortegaceae, Siparunaceae, and Trimeniaceae, as well as

first-branching members of Lauraceae and Monimiaceae s.str.

The present study addresses the circumscription and internal relationships of Laurales using a two-pronged approach. Broad *rbcL* sampling of all relevant clades is used to establish whether the perigynous-flowered basal angiosperm families form a monophyletic group (thus evaluating Hallier's concept of Laurales). Then, dense taxon sampling and data from five additional genome regions (the *rpl16* intron and the *trnT-trnL*, *trnL-trnF*, *atpB-rbcL*, and *psbA-trnH* intergenic spacers) were used to resolve relationships within Laurales. Fifteen morphological characters that have traditionally been of key importance in the classification of Laurales were studied on the molecular trees a posteriori as well as being included directly in the analysis in a total evidence approach.

MATERIALS AND METHODS

Taxon sampling—Eighty-nine *rbcL* sequences were used to evaluate the monophyly of Laurales (see Table 2 for a list of *rbcL* sequences and their provenance). All taxa that have been suggested as belonging in Laurales were sampled, usually with all or most of their genera, the exception being the Lauraceae. This family of ~50 genera in three tribes is represented by four genera (following the classification of van der Werff and Richter, 1996). The Cryptocaryeae are represented by *Cryptocarya*, the Perseeae by *Persea* and *Cinnamomum*, and the Laureae by *Litsea* and *Sassafras*. The monophyly of Lauraceae is unquestioned (J. Rohwer, personal communication 1998, University of Mainz; A. Chanderbali, personal communication, 1998, University of Missouri-St. Louis), and ongoing molecular systematic work on the family by J. Rohwer and A. Chanderbali guided the choice of taxa to be included. In addition to 40 *rbcL* sequences from putative Laurales (see Table 2), 42 sequences were included to represent all lineages that have been suggested by earlier authors (Hallier, 1905; Endress, 1972; Thorne, 1974, in press; Takhtajan, 1973, 1997; Cronquist, 1981, 1988; Kubitzki, 1993a) or in published or ongoing molecular work (cf. Introduction) as potential lauralean outgroups: Magnoliales s.l. (sensu lato) (Annonaceae, Degeneriaceae, Eupomatiaceae, Himantandraceae, Magnoliaceae, Myristicaceae), Winterales (Canellaceae, Winteraceae), and monocots (Araceae). Because molecular systematic analyses have shown that *Amborella* groups with the ''expanded Nymphaeales'' (cf. Fig. 1 for members of this informal grouping), *rbcL* sequences were included of Austrobaileyaceae, Cabombaceae, Nymphaeaceae, Illiciaceae, and Schisandraceae. Note that Austrobaileyaceae have sometimes also been included in Laurales, e.g., by Melchior (1964), Smith (1972), Takhtajan (e.g., 1973), Thorne (1974), and Walker (1976). Because of the unclear position of Chloranthaceae among basal angiosperms, representatives of Aristolochiaceae, Lactoridaceae, Piperaceae, and Saururaceae were also included to cover all lineages with which Chloranthaceae might group. The tree was rooted with seven *rbcL* sequences from *Ephedra*, *Gnetum*, and *Welwitschia*.

For the study of intralauralean relationships (see Table 3 for taxa, sequences, and GenBank numbers), Atherospermataceae are represented by eight species from all seven of their genera; Calycanthaceae by one species from each of their three genera; Gomortegaceae by their sole species; and Hernandiaceae by one species from each of their four genera (Kubitzki, 1969, 1993b; the latter work accords the former *Hernandia* subgenus *Hazomalania* generic rank). Monimiaceae are represented by four genera from their two main lineages (Renner, 1998, and unpublished data), and Siparunaceae are represented by one species each of their two genera (Renner, 1998, and unpublished data). Lauraceae are represented by species from the same three tribes as in the broad-scale *rbcL* study. Magnoliaceae and Myristicaceae are used as outgroups based on Hallier's morphological hypothesis outlined above, the results of Nandi, Chase, and Endress (1998, fig. 4), and unpublished analyses of combined *rbcL*, *atpB*, and 18S sequences (M. Chase, personal communication, 1998, Royal Botanic Gardens Kew; M. Chase, D. Soltis, P. Soltis, unpublished data).

Genome regions and laboratory methods—Total DNA was extracted from silica gel-dried, herbarium, or (rarely) fresh leaves using DNeasy extraction kits (QIAGEN Inc., Valencia, California) and following the protocol provided by QIAGEN (1995, 1997). The plant material was either ground in Eppendorf tubes with sterilized sea-sand or in porcelain mortars under liquid nitrogen. The *rbcL* gene was then amplified using a 26-nucleotide forward primer (1F, 5′-ATG TCA CCA CAA ACA GAA ACT AAA GC-3′) and a 24-nucleotide reverse primer (1460R, CTT TTA GTA AAA GAT TGG GCC GAG). Primer sequences are those of Fay, Swensen, and Chase (1997). To amplify the entire gene, two internal primers were used in addition to the two mentioned above, namely 724R (3′-CAT GTA CCT GCA GTA GC-5′) and 636F (5′-GCG TTG GAG AGA TCG TTT CT-3′); where the 636F primer did not work, a complement of the 724R primer was used instead. To detect sequencing errors, the *rbcL* sequences were translated into amino acids using the program SeqPup (which was also used to align sequences; below) and then compared with Fig. 4 in Kellogg and Juliano (1997), which lists variable and invariable sites in 499 seed plant *rbcL* sequences. Alternative amino acids found in Laurales all occurred at sites already known to be variable within angiosperms, and no stop codons were found in any of the sequences. A total of 1434 nucleotides, from positions 30–1395 of the *rbcL* exon, plus 39 base pairs (bp) following the exon were sequenced, but only the first 1331 nucleotides were used in the analyses because several of the sequences downloaded from GenBank ended at around position 1331.

Five plastid chloroplast intron and spacer regions were used, in addition to *rbcL* gene sequences, to resolve intralauralean relationships. The first of these was the large intron that interrupts the *rpl16* gene in all angiosperms thus far investigated (cf. Kelchner and Clark, 1997; Baum, Small, and Wendel, 1998). Primers 1067F (5′-CTT CCT CTA TGT TGT TTA CG-3′) and 18R (3′-GCT ATG CTT AGT GTG TGA CTC-5′) designed by C. B. Asmussen (personal communication, 1997, University of Copenhagen) were used to amplify an ~800-bp long region including the entire intron. The completed alignment (with gaps) comprised 1064 positions.

To amplify the *trnT-trnL* and *trnL-trnF* intergenic spacer regions, I used the universal primers *a*, *b*, *e*, and *f* of Taberlet et al. (1991). The *trnT-trnL* sequences ranged in length from 569 nucleotides (*Illigera*) to 895 nucleotides (*Knema*), with the Monimiaceae, Hernandiaceae, and Lauraceae sequences being similar to each other but differing strikingly from the remaining sequences near the 5′ end of the locus. The first 212 nucleotides were therefore excluded from the analysis so that the completed alignment (472 positions with gaps) comprised only the second half of the spacer. The *trnL-trnF* data set included ~365 nucleotides and the completed alignment comprised 451 positions.

The *atpB-rbcL* spacer (Golenberg et al., 1993) was amplified using the forward primer 'oligo 2' of Manen, Natali, and Ehrendorfer (1994); a primer complementary to the *rbcL* forward primer 1F (sequence given above) was used for the reverse reaction. A total of ~740 nucleotides were used in the analysis, and the completed *atpB-rbcL* alignment (with gaps) comprised 898 positions.

The ~390-bp long *psbA-trnH* intergenic spacer was amplified using the *psbA*-F forward and *trnH*-R reverse primers designed by Sang, Crawford, and Stuessy (1997). Their forward primer amplifies the last 41 base pairs of the *psbA* gene, a region that varies little among the 25 members of Laurales. However, alignment difficulties occurred toward the 3′ end of this rapidly evolving spacer, and therefore only the first ~170 nucleotides of each sequence were used in the analysis. The completed *psbA-trnH* alignment with gaps was 186 positions long.

PCR amplification followed standard protocols. PCR products were cleaned either by running the entire product on a low-melting point

TABLE 2. Species included in the study of the circumscription of the Laurales, listed alphabetically by family except for seven gymnosperms included for rooting purposes and listed together at the end. Also given are ordinal memberships of families in the classification of the Angiosperm Phylogeny Group (APG, 1998) as well as GenBank accession numbers, literature citations for published sequences, and source and voucher information for previously unpublished sequences.

Family/ordinal placement species	GenBank accession no.[a]	Citation or source and voucher
Amborellaceae[b]		
Amborella trichopoda Baill.	GBAN-L12628	Qiu et al., 1993
Annonaceae/Magnoliales		
Annona muricata L.	GBAN-L12629	Qiu et al., 1993
Asimina triloba (L.) Dunal	GBAN-L12631	Qiu et al., 1993
Cananga odorata (Lam.) Hook. f. & Thomson	GBAN-L12636	Qiu et al., 1993
Acoraceae/Acorales		
Acorus calamus L.	GBAN-M91625	Duvall et al., 1993
Araceae/Alismatales		
Anchomanes difformis (Blume) Engl.	GBAN-L10254	Duvall et al., 1993
Gymnostachys anceps R. Br.	GBAN-M91629	Duvall et al., 1993
Aristolochiaceae/Piperales		
Aristolochia macrophylla Lam.	GBAN-L12630	Qiu et al., 1993
Asarum canadense L.	GBAN-L14290	Olmstead et al., 1993
Saruma henryi Oliv.	GBAN-L12664	Qiu et al., 1993
Atherospermataceae/Laurales		
Atherosperma moschatum Labill.	GBAN-AF121362	Y.-L. Qiu, aliquot
Daphnandra repandula (F. Muell.) F. Muell.	GBAN-AF052195	Renner, 1998
Doryphora aromatica (F.M. Bailey) L.S. Smith	GBAN-L77211	Ablett, Playford, and Mills, 1997
Dryadodaphne novoguineensis (Perk.) A.C. Smith	GBAN-AF121363	Takeuchi 7095 (MO)
Laurelia novae-zelandiae Cunn.	GBAN-AF052196	Renner, 1998
Laurelia sempervirens (R. & P.) Tul.	GBAN-AF052612	Renner, 1998
Laureliopsis philippiana (Looser) Schodde	GBAN-AF040662	Renner, 1998
Nemuaron vieillardii (Baill.) Baill.	GBAN-AF121366	McKee 12800 (K)
Austrobaileyaceae[b]		
Austrobaileya scandens C. T. White	GBAN-L12632	Qiu et al., 1993
Cabombaceae (incl. in Nymphaeaceae)[b]		
Brasenia schreberi J. F. Gmelin	GBAN-M77031	Les, Garvin, and Wimpee, 1991
Cabomba caroliniana A. Gray	GBAN-M77027	Les, Garvin, and Wimpee, 1991
Calycanthaceae/Laurales		
Calycanthus chinensis Cheng & S. Y. Chang	GBAN-L12635	Qiu et al., 1993
Calycanthus floridus L.	GBAN-L14291	Chase et al., 1993
Calycanthus occidentalis Hook. & Arn.	GBAN-AF022951	Renner, 1998
Chimonanthus praecox (L.) Link	GBAN-L12639	Qiu et al., 1993
Idiospermum australiense (Diels) S. T. Blake	GRAN-L12651	Qiu et al., 1993
Chloranthaceae[b]		
Chloranthus japonicus Siebold	GBAN-L12640	Qiu et al., 1993
Hedyosmum arborescens Sw.	GBAN-L12649	Qiu et al., 1993
Hedyosmum bonplandianum Kunth[c]	GBAN-121364	Madríñan et al. 1501, COL
Sarcandra grandifolia (Miq.) Subr. & Henry	GBAN-L12663	Qiu et al., 1993
Degeneriaceae/Magnoliales		
Degeneria sp.	GBAN-L12643	Qiu et al., 1993
Eupomatiaceae/Magnoliales		
Eupomatia bennetii F. Muell.	GBAN-L12644	Qiu et al., 1993
Gomortegaceae/Laurales		
Gomortega nitida R. & P. (= *G. keule* (Molina) I. M. Johnson)	GBAN-D89561	Ueda et al., 1997
Hernandiaceae/Laurales		
Gyrocarpus americanus Jacq.	GBAN-L12647	Qiu et al., 1993
Hernandia albiflora (C. T. White) Kubitzki	GBAN-L77210	Ablett et al. 1997
Hernandia moerenhoutiana Guillem.	GBAN-AF052617	Renner, 1998
Hernandia ovigera L.	GBAN-L12650	Qiu et al., 1993
Illigera luzonensis (Presl) Merr.	GBAN-AF050222	Renner, 1998
Sparattanthelium wonotoboense Kosterm.	GBAN-AF052197	Renner, 1998
Himatandraceae/Magnoliales		
Galbulimima belgraveana (F. Muell.) Sprague	GBAN-L12646	Qiu et al., 1993
Illiciaceae[b]		
Illicium parviflorum Michx. ex Vent.	GBAN-L12652	Qiu et al., 1993
Lactoridaceae/Piperales		
Lactoris fernandeziana Phil.	GBAN-L08763	Chase et al., 1993
Lauraceae/Laurales		
Cinnamomum camphora (L.) T. Nees & Eberm.	GBAN-L12641	Qiu et al., 1993
Cryptocarya obovata R. Br.	GBAN-L28950	Martin and Dowd, unpubl.
Litsea japonica (Thun.) Juss.	GBAN-U06843	Martin and Dowd, unpubl.
Persea americana Mill.	GBAN-X54347	Golenberg et al., 1990
Magnoliaceae/Magnoliales		
Liriodendron chinense (Hemsl.) Sarg.	GBAN-L12654	Qiu et al., 1993
Magnolia hypoleuca Siebold & Zucc.	GBAN-L12655	Qiu et al., 1993
Magnolia macrophylla L.	GBAN-X54345	Golenberg et al., 1990

TABLE 2. Continued.

Family/Ordinal placement species	GenBank accession no.[a]	Citation or source and voucher
Magnolia salicifolia (Siebold & Zucc.) Maxim.	GRAN-L12656	Qiu et al., 1993
Michelia figo (Lour.) Spreng.	GBAN-L12659	Qiu et al., 1993
Talauma ovata A. St.-Hil.	GBAN-L12666	Qiu et al., 1993
Talauma singaporensis Ridl.	GBAN-L12667	Qiu et al., 1993
Monimiaceae/Laurales		
Hedycarya arborea J. & G. Forst.	GBAN-L12648	Qiu et al., 1993
Hennecartia omphalandra Poisson	GBAN-AF022950	Renner et al., 1997
Hortonia floribunda Wight ex Arn.	GBAN-AF040663	Renner, 1998
Kibara rigidifolia A. C. Smith	GBAN-AF050221	Renner, 1998
Mollinedia ovata R. P.	GBAN-AF050218	Renner, 1998
Monimia ovalifolia Thouars	GBAN-AF121365	Strasberg s.n., REU
Palmeria scandens F. Muell.	GBAN-AF052613	Renner, 1998
Peumus boldus Molina	GBAN-AF040664	Renner, 1998
Steganthera hirsuta (Warb.) Perkins	GBAN-AF121368	Kiapranis et al. 69621 (LAE)
Tambourissa tau Lorence	GBAN-AF050219	Renner, 1998
Wilkiea huegeliana A. DC.	GBAN-AF040665	Renner, 1998
Xymalos monospora (Harvey) Baill.	GBAN-AF050220	Renner, 1998
Myristicaceae/Magnoliales		
Knema latericia Elmer	GBAN-L12653	Qiu et al., 1993
Nymphaeaceae[b]		
Euryale ferox Salisb.	GBAN-M77035	Les, Garvin, and Wimpee, 1991
Nuphar variegata Durand	GBAN-M77029	Les, Garvin, and Wimpee, 1991
Nymphaea odorata Aiton	GBAN-M77034	Les, Garvin, and Wimpee, 1991
Victoria cruziana Orb.	GBAN-M77036	Les, Garvin, and Wimpee, 1991
Saururaceae/Piperales		
Houttuynia cordata Thunb.	GBAN-L08762	Chase et al., 1993
Saururus cernuus L.	GBAN-L14294	Chase et al., 1993
Schisandraceae[b]		
Schisandra sphenanthera Rehder & Wilson	GBAN-L12665	Qiu et al., 1993
Siparunaceae/Laurales		
Bracteanthus glycycarpus Ducke	GBAN-AF129016	Ribeiro 1802 (MO)
Glossocalyx longicuspis Benth.	GBAN-AF070666	Renner, 1998
Siparuna brasiliensis (Spreng.) A. DC.	GBAN-AF013246	Renner, 1998
Siparuna lepidota (H.B.K.) A. DC.	GBAN-AF040667	Renner et al., 1997
Trimeniaceae[b]		
Trimenia moorei (Oliv.) Philipson (= *Piptocalyx moorei* Oliv.)	GBAN-AF121367	ANBG 701680
Winteraceae[b]		
Belliolum sp.	GBAN-L12633	Qiu et al., 1993
Drimys winteri J. R. & G. Forster	GBAN-L01905	Albert et al., 1992
Tasmannia insipida DC.	GBAN-L01957	Albert et al., 1992
Gnetaceae (gymnosperms)		
Gnetum gnemon L.	GBAN-L12680	Chase et al., 1993
Gnetum leyboldii Tul.	GBAN-U72820	Price et al., 1992
Gnetum parvifolium (Warb.) W. C. Cheng	GBAN-D10734	Hasebe et al., 1992
Ephedra distachya L.	GBAN-U72821	Price, 1996
Ephedra sinica Stapf	GBAN-D10732	Hasebe et al., 1992
Ephedra tweediana C. A. Mey.	GBAN-U72822	Price, 1996
Welwitschia mirabilis Hook. f.	GBAN-AJ235814	Chase et al., 1993

[a] The prefix GBAN has been added for linking the online version of *American Journal of Botany* to GenBank and is not part of the actual GenBank accession number.

[b] Family of uncertain position (APG, 1998).

[c] This sequence is suspected to represent a duplicated copy of the *rbcL* gene (Renner and Qiu, unpublished data).

agarose gel and then recovering the amplified DNA with the help of QIAquick gel extraction kits (QIAGEN, 1997) or by using the QIAquick PCR purification columns directly without a prior gel purification step. Double-stranded PCR products were used as sequencing templates, and sequencing was done on an ABI 377 automated sequencer (University of Missouri—DNA Core Sequencing Facility). Usually (but not for all *psbA-trnH* spacer sequences; above), both strands of DNA were sequenced and used to generate a consensus sequence using Sequencher version 3.1 (GeneCodes Corporation, 1998). All alignment was done manually in Sequencher and/or in SeqPup version 0.6 (D. Gilbert, Indiana University, Bloomington, 1996). None of the insertions or deletions in the five intron and spacer sequence data sets were potentially informative at the between-family level, and gaps were therefore not used as characters. A total of 170 sequences, including a new *Hedyosmum* sequence, were generated for this study and its precursor (Renner, 1998) and have been deposited in GenBank (see Tables 2 and 3 for accession numbers).

Morphology—The morphological, palynological, and karyological characters scored for Laurales (Table 4) were the same as in a previously published matrix (Renner, Schwarzbach, and Lohmann, 1997), which includes descriptions of characters and their states, and lists literature and herbarium sources used. Of the taxa used in that study, two of the outgroups (Austrobaileyaceae and Winteraceae) and six of the nine genera of Monimiaceae s.str. were dropped because molecular data indicated they were irrrelevant to an analysis of intralauralean family re-

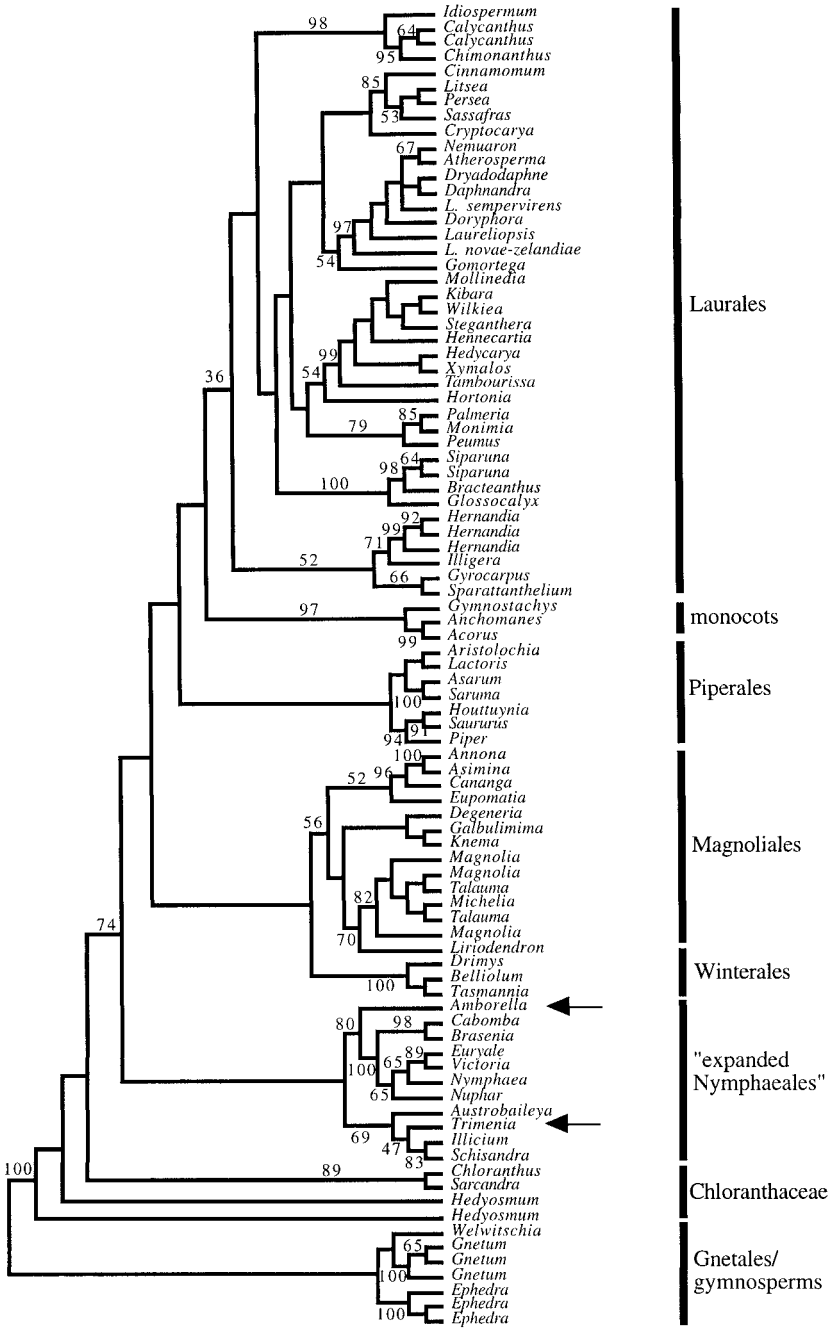

Fig. 1. One of 17 500 equally parsimonious trees resulting from an analysis of 89 *rbcL* sequences representing all putative Laurales, major lineages of basal angiosperms, and Gnetales. At this level, *rbcL* provides 403 parsimony-informative characters that identify four suprafamily-level clades and most families except Chloranthaceae, likely because one of the *Hedyosmum* sequences represents a duplicated copy of the *rbcL* gene. Numbers below branches are parsimony jackknife values (10 000 replicates). See Table 2 for family assignments of genera.

lationships; on the other hand, *Palmeria* was added because it is among the first-branching Monimiaceae (Renner, 1998). For the new matrix, the terminal taxa Atherospermataceae, Calycanthaceae, Hernandiaceae, and Lauraceae were replaced by their genera sampled in the molecular study, and *Knema* (Myristicaceae) was added as another outgroup. For character 1, vegetative phyllotaxy, I followed Nandi, Chase, and Endress (1998) in combining the states ''alternate'' and ''spiral-alternate'' into one state, while for character 5, anther dehiscence, I added the third state, ''opening by two laterally hinged valves,'' to account for variation within Monimiaceae and Hernandiaceae. Character states for *Knema* are taken from Kühn and Kubitzki (1993).

Phylogenetic analyses—Phylogenetic analyses of the aligned sequences were conducted using test version 4.0d64 of PAUP* written by D. L. Swofford. A broadscale *rbcL* heuristic search, in which the seven gymnosperms were designated as the outgroup, was begun using the random taxon addition sequence (RAS), tree bisection and reconnection branch swapping (TBR), and multiple parsimony (MULPARS

TABLE 3. Species and plastid genome regions included in the study of within-Laurales relationships in addition to the *rbcL* sources listed in Table 2. Author names are provided only for species not listed in Table 2. Missing sequences marked by an asterisk were replaced with sequences from a close relative as discussed in the text.

Species	*rp116* (1064, 250)[a]	*trnT-trnL* (472, 176)	*trnL-trnF* (451, 122)	*atpB-rbcL* (898, 172)	*psbA-trnH* (186, 43)	Source or voucher
Atherospermataceae						
Atherosperma moschatum	GBAN-AF127249	GBAN-AF129013	—	GBAN-AF127603	GBAN-AF129044	Y.-L. Qiu, aliquot
Daphnandra repandula	GBAN-AF127250	GBAN-AF129022	GBAN-AF040670	GBAN-AF127608	GBAN-AF129049	leg. B. Hyland Sep 97
Doryphora sassafras (Endl.) Endl.	GBAN-AF127252	GBAN-AF129023	GBAN-AF040672	GBAN-AF127609	GBAN-AF129050	Sydney BG, bed 130
Dryadodaphne novoguineensis	GBAN-AF127253	GBAN-AF129024	GBAN-AF040673	GBAN-AF127610	GBAN-AF129051	Takeuchi 7095 (MO)
Laurelia novae-zelandiae	GBAN-AF127254	GBAN-AF129032	GBAN-AF040674	GBAN-AF127618	GBAN-AF129059	leg. B. Sampson Oct 97
Laurelia sempervirens	GBAN-AF127255	—*	GBAN-AF012402	GBAN-AF127619	GBAN-AF129060	Edinburgh BG 19931681
Laureliopsis philippiana	GBAN-AF127256	GBAN-AF129033	GBAN-AF040675	GBAN-AF127620	GBAN-AF129061	Landrum & Landrum 8160 (MO)
Nemuaron vieillardii	GBAN-AF127257	GBAN-AF129039	GBAN-AF040676	GBAN-AF127625	GBAN-AF129066	McKee 12800 (K)
Calycanthaceae						
Calycanthus occidentalis	GBAN-AF127250	GBAN-AF129017	GBAN-AF012396	GBAN-AF127605	GBAN-AF129046	Missouri BG 897432
Chimonanthus praecox	GBAN-AF127258	GBAN-AF129018	GBAN-AF040677	GBAN-AF127606	GBAN-AF129047	Missouri BG 896912
Idiospermum australiense	GBAN-AF127259	GBAN-AF129029	GBAN-AF040678	GBAN-AF127615	GBAN-AF129056	Qiu et al., 1993, aliquot
Gomortegaceae						
Gomortega nitida	GBAN-AF127260	—	GBAN-AF012404	GBAN-AF127612	GBAN-AF129053	Rodriguez 3070 (CONC)
Hernandiaceae						
Gyrocarpus americanus	GBAN-AF127261	GBAN-AF129025	GBAN-AF012398	GBAN-AF127613	GBAN-AF129054	Qiu et al., 1993, aliquot
Hernandia moerenhoutiana	GBAN-AF130310	GBAN-AF129026	GBAN-AF052198	GBAN-AF127614	GBAN-AF129055	Brisbane BG s.n.
Illigera luzonensis	GBAN-AF127264	GBAN-AF129030	GBAN-AF052199	GBAN-AF127616	GBAN-AF129057	Munich BG s.n.
Sparattanthelium wonotoboense	GBAN-AF127262	GBAN-AF129043	GBAN-AF053342	GBAN-AF127629	GBAN-AF129070	Munich BG 97/134
Lauraceae						
Beilschmiedia obovata Kosterm.	GBAN-AF127265	GBAN-AF129015	GBAN-AF129014	GBAN-AF127604	GBAN-AF129045	Yasuda 1313(MO)
Cinnamomum camphora	GBAN-AF129019	GBAN-AF129021	GBAN-AF129020[1]	GBAN-AF127607[2]	GBAN-AF129048	Missouri BG 897519 [1]K. Ueda, Univ. of Osaka [2]Mainz BG (J. Rohwer)
Litsea glaucescens Kunth	GBAN-AF127266	GBAN-AF129036	GBAN-AF129035	GBAN-AF127622	GBAN-AF129063	Lorea 5496 (MO)
Magnoliaceae						
Liriodendron chinense	GBAN-AF127267	GBAN-AF129034	GBAN-AF040679	GBAN-AF127621	GBAN-AF129062	Missouri BG 890888
Magnolia hypoleuca	GBAN-AF127268	GBAN-AF129037	GBAN-AF012395	GBAN-AF127623	GBAN-AF129064	Missouri BG 732556
Monimiaceae						
Hortonia floribunda	GBAN-AF129027	GBAN-AF129028	GBAN-AF040683	GBAN-AF129029	GBAN-AF129071	Colombo BG s.n.
Monimia ovalifolia	GBAN-AF127269	GBAN-AF129038	GBAN-AF054896	GBAN-AF127624	GBAN-AF129065	Strasberg s.n. (REU)
Plameria scandens	GBAN-AF127270	GBAN-AF129040	GBAN-AF052200	GBAN-AF127626	GBAN-AF129067	Bradford 878 (MO)
Peumus boldus	GBAN-AF127454	GBAN-AF129041	GBAN-AF012403	GBAN-AF127627	GBAN-AF129068	Edinburgh BG 19870707
Myristicaceae						
Knema latericia	GBAN-AF127453	GBAN-AF129031	GBAN-AF040694	GBAN-AF127617	GBAN-AF129058	Qiu et al., 1993, aliquot
Siparunaceae						
Glossocalyx longicuspis	GBAN-AF127452	—*	GBAN-AF012405	GBAN-AF127611	GBAN-AF129052	Bos 4659 (MO)
Siparuna aspera (R. & P.) A. DC.	—*	GBAN-AF129042	GBAN-AF040695	GBAN-AF127628	GBAN-AF129069	Mardiñán et al. 1502 (COL)
Siparuna guianensis Aubl.	GBAN-AF127455					Chanderbali 247 (MO)

[a] Numbers in parentheses refer to number of nucleotide positions and number of informative sites, respectively.

[b] The prefix GBAN has been added for linking the online version of *American Journal of Botany* to GenBank and is not part of the actual GenBank accession number.

TABLE 4. (A) Morphological matrix and (B) Coding of morphological characters used in the cladistic analysis. See Renner, Schwarzbach, and Lohmann (1997) for a discussion of character states, and for sources. *L. = Laurelia.*

A)

Idiospermum	1	1	0	0	0	0	2	1	0	2	3	0	1	1	1
Calycanthus	1	1	0	0	0	0	2	0	0	2	3	0	1	1	1
Chimonanthus	1	1	0	0	0	0	2	0	?	2	3	0	1	1	1
Cinnamomum	0/1	1	0	1	1	2	3	1	0	2	1	0	0	1	1
Litsea	0/1	1	0	1	1	2	3	1	0	2	1	0	0	1	1
Beilschmiedia	0/1	1	0	1	1	2	3	1	0	2	1	0	0	1	1
Hortonia	1	1	0	0	1	0	3	0	0	0	1	0	0	0	1
Palmeria	1	1	0	0	0	0	3	0	0	0	1	0	3	0	1
Monimia	1	1	0	0	1	0	3	0	0	4	1	0	3	0	1
Peumus	1	1	0	0	1	0	3	0	0	3	1	0	0	0	1
Hernandia	0	1	0	1	1	1/3	3	1	1	?	1	0	0	1	1
Illigera	0	1	0	1	1	3	3	1	1	?	1	0	0	1	1
Gyrocarpus	0	1	0	1	1	1	3	1	1	?	1	0	0	1	1
Sparattanthelium	0	1	0	1	1	1	3	1	1	?	1	0	0	1	1
L. novae-zelandiae	1	1	0	0	1	1	1	0	0	1	2	0	1	0	1
L. sempervirens	1	1	0	0	1	1	1	0	0	1	2	0	1	0	1
Laureliopsis	1	1	0	0	1	1	1	0	0	1	2	0	1	0	1
Atherosperma	1	1	0	0	1	1	1	0	0	1	2	0	1	0	1
Nemuaron	1	1	0	0	1	1	1	0	0	1	2	0	1	0	1
Dryadodaphne	1	1	0	0	1	1	1	0	0	1	2	0	1	0	1
Daphnandra	1	1	0	0	1	1	1	0	0	1	2	0	1	0	1
Doryphora	1	1	0	0	1	1	1	0	0	1	2	0	1	0	1
Gomortega	1	1	0	0	1	1	3	?	1	1	1	0	0	0	1
Siparuna	1	1	1	0	0	1	3	0	0	1	2	1	3	0	1
Glossocalyx	1	1	1	0	0	1	3	0	0	?	2	1	3	0	1
Knema	0	0	0	0	0	0	0	1	0	5	2	0	2	0	0
Liriodendron	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Magnolia	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0

B)

1 Phyllotaxy 0, alternate or spiral-alternate; 1, decussate
2 Cup-shaped receptacle 0, absent; 1, present
3 Innermost tepals 0, free; 1, connate
4 Fixed stamen numbers 0, absent; 1, present
5 Paired glands on filament bases 0, absent; 1, present
6 Anther dehiscence 0, longicidal; 1, by 2 apically-hinged valves; 2, by 4 apically-hinged values; 3, by 2 laterally-hinged values
7 Apertures 0, monosulcate; 1, meridionosulcate or disulcate; 2, disulculate; 3, inaperturate
8 Carpels 0, several; 1, one (the syncarpy in *Gomortega* is autapomorphic)
9 Epigyny 0, absent; 1, present
10 Chromosome number 0, n = 19; 1, n = 21–22; 2, n = 11–12; 3, n = 39; 4, n = 44; 5, n = 8
11 Ovule number and position 0, several ventral; 1, one apical; 2, one basal; 3, two ventral
12 Number of integuments 0, two; 1, one
13 Fruit type 0, drupes; 1, achenes; 2, capsules; 3, drupelets (enclosed in receptacle until full maturity); 4, berries
14 Endosperm 0, present; 1, absent
15 Nodes 0, tri- or multilacunar; 1, unilacunar

in which all equally parsimonious trees are saved and swapped on). This search was aborted after 90 h, and the majority rule consensus tree of all 17 500 equally parsimonious trees found at the time saved. Searches using the closest taxon addition and nearest neighbor interchange options in PAUP likewise resulted in memory overflow. The within-Laurales analyses were performed using heuristic searches, 1000 random taxon addition replicates, TBR swapping, and MULPARS. The COLLAPSE, but not the STEEPEST DECENT, options of PAUP were in effect during all searches. Characters were equally weighted and unordered, and gaps were treated as missing data (cf. section on Genome Regions). Tree length, branch lengths (using the ACCTRAN character-state optimization), consistency index (CI), and retention index (RI) were taken from PAUP.

Bootstrap support (Felsenstein, 1985) for each clade in the intralauralean analyses was estimated based on 100 or 1000 replications, using the closest taxon addition and TBR branch swapping options. For the broadscale *rbcL* analysis, relative clade support was assessed via two parsimony jackknife analyses, each with 10 000 replicates (Farris et al., 1996 [1997]). Bremer support (Bremer, 1988, 1994), also called the decay index, was calculated as outlined in Bremer (1994).

Morphological characters were investigated, using MacClade 3.0, by optimizing most parsimonious state assignments onto the best molecular trees of Laurales found (Maddison and Maddison, 1992). In a second step, they were appended to the combined molecular matrix and included in a heuristic analysis to investigate their effect on clade support.

Data concatenation and ambiguity coding—Parsimony analyses of the six data sets followed by bootstrapping showed that there were no statistically well-supported (''hard'') incongruencies among the topologies based on these data, and they were therefore combined. (See the Results section: Effects of amount of data and missing data, for further exploration of individual data sets.) For the combined 6-genome regions-28-taxon analysis, sequences from the same species (usually from a single total DNA extract) were spliced together with the following exceptions (Tables 2, 3): a *Doryphora aromatica rbcL* sequence was combined with *rpl16, trnT-trnL, trnL-trnF, atpB-rbcL,* and *psbA-trnH* sequences from *Doryphora sassafras*. *Doryphora* comprises just these two species. In the Lauraceae, spacer and intron sequences from *Beilschmiedia obovata* and *Litsea glaucescens* were supplemented by *rbcL* sequences from *Cryptocarya obovata* and *Litsea japonica,* respectively. *Cryptocarya and Beilschmiedia* are both members of the Cryptocaryeae. For *Siparuna,* four sequences from *S. aspera* were combined with one from *S. lepidota* and one from *S. guianensis.* For *Laurelia sempervirens* and *Glossocalyx longicuspis,* the sequence to be spliced came from their respective closest relatives, which were also in the data set (compare Table 3). Specifically, the genus *Laurelia* (Atherospermataceae) comprises two species that grouped with each other in the *rpl16* data set (63% bootstrap support), while the other four data sets contained too few informative nucleotide changes to yield statistically well-supported groupings within atherosperms with the exception of an *Atherosperma-Nemuaron* clade that appeared in two of the data sets. Basing myself mainly on the *rpl16* data, I decided to use the *L. novae-zelandiae trnT-trnL* sequence to complement the five other sequences from *L. sempervirens* rather than dropping *L. sempervirens* from the analysis (see below for an alternative approach). *Glossocalyx* is a monotypic genus that based on morphological, *rbcL,* and *trnL-trnF* data is sister to *Siparuna* (Renner, Schwarzbach, and Lohmann, 1997; Renner, 1998); a *Siparuna trnT-trnL* sequence was used to complement the *Glossocalyx* set of sequences.

In two cases, I decided against matching and fusion of terminals. Gomortegaceae comprise a single species, *Gomortega nitida,* for which no *trnT-trnL* sequence could be generated. Likewise, *Atherosperma* comprises a single species from which I was unable to amplify *trnL-trnF*. Both sequences were coded as missing (''nnnn'') and then spliced in with the remaining five *Gomortega* and *Atherosperma* sequences, which added 472 and 451 ambiguous sites, respectively (out of 4402 total nucleotide sites used for each taxon). These 923 ambiguous sites represent 0.74% of all nucleotide positions (923 of 28 × 4402 = 123 256 positions).

I explored the following alternative approaches to the problem of missing sequences. First, the two sequences that contained missing characters, *Gomortega* and *Atherosperma,* were excluded from the analysis. Second, the two data sets containing ''holes'' that could not be filled by splicing (*trnT-trnL* for *Gomortega*; *trnL-trnF* for *Atherosperma*) were sequentially and simultaneously excluded.

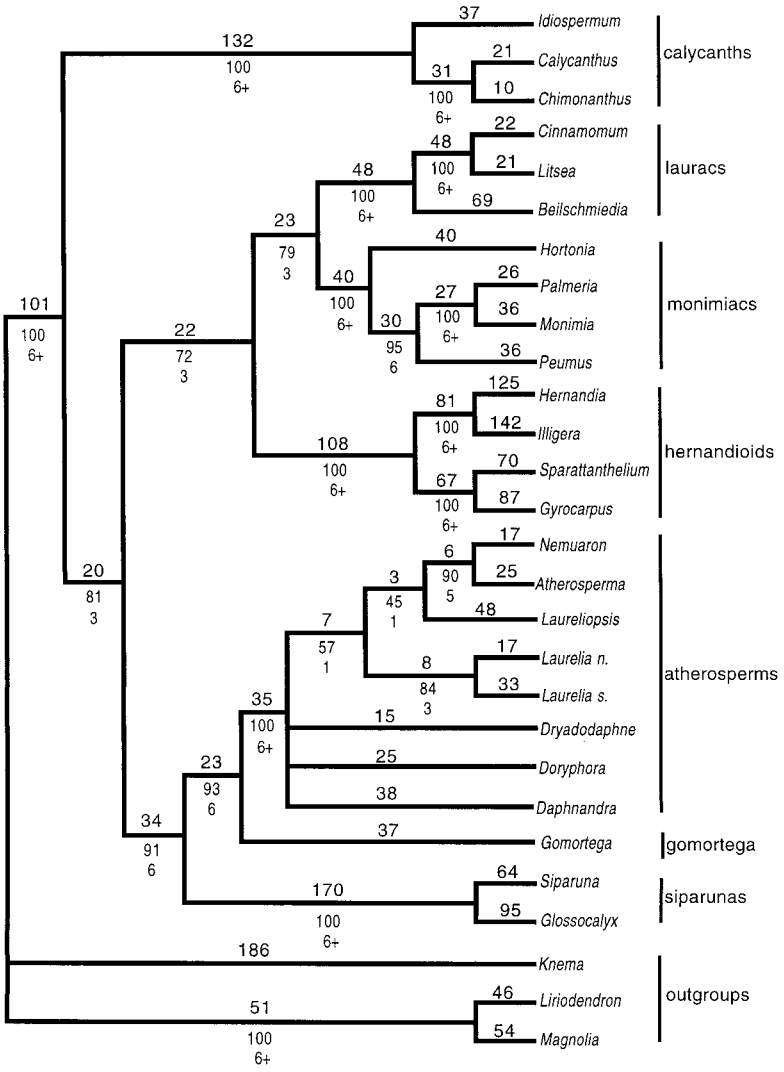

Fig. 2. Internal structure of Laurales. The strict consensus of the four shortest trees found in the 28-taxa-6-plastid-region analysis after 1000 unconstrained TBR/RAS searches with MULPARS is shown. Numbers above branches are branch lengths (in number of nucleotide changes); numbers below branches are bootstrap (in % of 1000 replications) and decay values. The tree was rooted at the branch between the outgroup (Magnoliales) and the remaining taxa as suggested by other molecular analyses (see text). *Laurelia s.* = *L. sempervirens*, *Laurelia n.* = *L. novae-zelandiae*.

RESULTS

Identification of the lauralean clade—The 89-taxa *rbcL* data set included 403 potentially informative characters. By the time the PAUP search was aborted it had found 17 500 equally parsimonious trees (L = 1895, CI = 0.399, RI = 0.708). Figure 1 shows one of these trees with jackknife values (based on 10 000 replicates) on the branches. The tree exhibits robust phylogenetic structure in the form of four suprafamily-level clades supported by jackknife values of >50% (Fig. 1): (1) Illiciaceae, Schisandraceae, Trimeniaceae, and Austrobaileyaceae; (2) Amborellaceae, Cabombaceae, and Nymphaeaceae; (3) a magnolialean clade (Annonaceae, Eupomatiaceae, Degeneriaceae, Himantandraceae, Magnoliaceae, and Myristicaceae); and (4) Piperaceae and Saururaceae. Within core Laurales, Gomortegaceae and Atherospermataceae appear as sisters (but only at 49% jackknife support). The *rbcL* sequences contain enough variation to group genera into families with the striking exception of the four representatives of Chloranthaceae (Fig. 1). As found in the molecular analyses cited in the Introduction, *Amborella*, formerly included in Laurales, groups with Nymphaeaceae/Cabombaceae (80% jackknife support) and Chloranthaceae (also sometimes included in Laurales; Table 1) represent a highly diverse and isolated group. This is the first study, however, to indicate that *Trimenia* belongs with *Illicium*, *Schisandra*, and *Austrobaileya* (69% jackknife support).

Structure of the Laurales—The matrix for the 25 core lauralean taxa (plus three outgroups) included 4402 characters of which 898 were informative. An unconstrained search yielded four equally parsimonious trees of length 2569 (including all characters), with a CI of 0.743 and a

RI of 0.741, located in a single island found in all 1000 random taxon addition replicates. The four trees, which differed from each other only in the placement of the three first-branching genera of Atherospermataceae (Fig. 2), were rooted at the branch between Magnoliales and the remaining taxa based on published and unpublished analyses that show Magnoliales and Laurales as sister groups (Nandi, Chase, and Endress, 1998, fig. 4; Magnoliales here are seen as including Annonales and Myristicaceae; M. Chase, personal communication, 1998, Royal Botanic Gardens Kew; M. Chase, D. Soltis, and P. Soltis, unpublished data).

The addition of 15 morphological characters to the matrix resulted in the same four trees (L = 2611, CI = 0.742, RI = 0.746). Bootstrap support for most clades remained unchanged or changed minimally. Support for the Monimiaceae-Lauraceae sister-group relationship, however, dropped to 43%, which was not unexpected because several morphological characters conflict with such a relationship, supporting instead a Lauraceae-Hernandiaceae relationship (see Discussion).

Effects of amount of data and missing data—Of the six molecular data sets, four by themselves yield statistically supported resolution at the family level (>50% bootstrap support): *rpl16* and *atpB-rbcL* support the Siparunaceae (atherosperm-*Gomortega*) clade (at 82 and 71%, respectively) as well as the atherosperm-*Gomortega* clade (at 74 and 60%, respectively), while *rbcL* supports only the atherosperm-*Gomortega* sister-group relationship (at 54%). *Rpl16* and *atpB-rbcL* also both support the node making Calycanthaceae sister to all remaining families (at 85 and 58%, respectively), and *atpB-rbcL* and *trnT-trnL* both support the Hernandiaceae-Monimiaceae-Lauraceae clade (at 59 and 77%, respectively). No ''hard'' topological conflicts among trees based on individual or variously combined data sets were found.

To explore the effects of absolute numbers of informative characters included and of particular sets of data, such as the *trnT-trnL* or *trnL-trnF* matrices that each included ~450 ambiguous sites due to lacking sequences (Materials and Methods), I sequentially added or excluded data sets. These searches and bootstrap analyses were run using the closest taxon addition option rather than random taxon addition. The exclusion of the *trnT-trnL* data (472 characters of which 176 were parsimony informative) yielded six equally parsimonious trees (L = 2112, CI = 0.746, RI = 0.738), the strict consensus of which was identical to the tree resulting from the full analysis, except that the Hernandiaceae-(Lauraceae-Monimiaceae) clade collapsed. When bootstrapped, support for the remaining clades remained virtually the same as in the full analysis. The exclusion of the *trnL-trnF* data (451 characters of which 122 were parsimony informative) yielded four equally parsimonious trees (L = 2208, CI = 0.750, RI = 0.746). Their strict consensus was identical to the topology found in the full analysis except for intra-atherosperm relationships, which were less resolved; clade support was almost unaffected. The exclusion of the *atpB-rbcL* data (898 characters of which 172 were parsimony informative) resulted in three equally parsimonious trees (L = 2015, CI = 0.745, RI = 0.744) with the same general topology as before, but support values for the Hernandiaceae-(Lauraceae-Monimiaceae) and Lauraceae-Monimiaceae clades dropped to 55%. The exclusion of the *rbcL* data (1331 characters of which 135 were informative) resulted in four shortest trees (L = 2114, CI = 0.766, RI = 0.758) with the same topology as found in the full analysis except that the Lauraceae-Monimiaceae node now had only 49% bootstrap support; support for the remaining clades remained essentially unchanged. The exclusion of the *rpl16* data (1064 characters of which 250 were parsimony informative) resulted in lower support for the node making Calycanthaceae sister to all other Laurales (49%) and for the Monimiaceae-Lauraceae node (48%). Finally, exclusion of the *psbA-trnH* data (186 characters of which 43 were parsimony informative) had almost no effect on topology or bootstrap values.

To explore the effects of the 472 and 451 ambiguous sites contained in the concatenated sequences of *Gomortega* and *Atherosperma*, an analysis was run with both these taxa excluded. This yielded two shortest trees of the same topologies as found in the full analysis (L = 2499, CI = 0.749, RI = 0.742). Bootstrap percentages remained virtually unchanged. Next, I re-included *Gomortega* and *Atherosperma* and instead excluded *Illigera* and *Hernandia* because these and other Hernandiaceae are among the longest branches in the analysis (Fig. 2). This resulted in two shortest trees of the same general topology as found before except that the Monimiaceae no longer grouped with the Lauraceae but instead with the remaining two Hernandiaceae (43% bootstrap support). The exclusion of any two (of four) Monimiaceae or two (of three) Calycanthaceae, by contrast, did not significantly affect topology or clade support.

DISCUSSION

Effects of taxon vs. character sampling density—The effects on phylogenetic accuracy, resolution, and clade support of adding taxa and/or characters have been explored by Graybeal (1998), who found that when the total number of characters is held constant, accuracy is much higher if the characters are distributed across a larger number of taxa. Similar conclusions, namely that denser species sampling greatly improves the ability of an analysis to reconstruct phylogeny, have been reached by others (Lecointre et al., 1993, 1994; Hillis, 1996; Purvis and Quickie, 1997). Although I did not address the relationship between adding taxa vs. adding characters systematically, the experimental exclusion or inclusion of taxa vs. characters showed the sometimes striking impact of lowering taxon density. Thus, the exclusion of two Hernandiaceae, a group particularly rich in nucleotide changes and thus easily forming long branches, had the same impact as an ''across the board'' exclusion of up to a quarter of all informative characters (cf. Results). These results underline the importance of sampling taxa specifically so as to enhance signal by breaking up long branches, which in the Laurales involves sampling several Hernandiaceae.

Circumscription of the Laurales—The broadscale *rbcL* analysis shows that a monophyletic order Laurales includes the following seven lineages (using available

family names): Atherospermataceae, Calycanthaceae (incl. Idiospermaceae), Gomortegaceae, Hernandiaceae, Lauraceae, Monimiaceae, and Siparunaceae. The best contender for a morphological synapomorphy for these seven families is their perigynous flowers, in which the carpel(s) is/are often deeply embedded in a fleshy receptacle that may or may not become woody in fruit (Hallier, 1905; Endress and Igersheim, 1997). Other characters often listed as typically lauralean, such as uniovulate carpels, unilacunar nodes, opposite leaves, and inaperturate pollen, while being useful for distinguishing Laurales from their presumed closest relatives (Magnoliales?), vary within Laurales and/or are also found in a number of other basal angiosperms.

In the single most-parsimonious tree resulting from the combined *rbcL* + morphology analysis of Nandi, Chase, and Endress (1998), Laurales appear as sister to a clade comprising Myristicaceae, Magnoliales, and Annonales (= Magnoliales s.l.), albeit with less than 50% bootstrap support. A problem in this study that conceivably contributed to this low support is that the Laurales were circumscribed as including Calycanthaceae, Monimiaceae s.l., Lauraceae, Hernandiaceae s.str., and Gyrocarpaceae (mislabelled as ''Gyrostemonaceae'' on p. 199; P. Endress, personal communication, September 1998). Gomortegaceae were not included in the study. For the molecular matrix the highly autapomorphic Calycanthaceae (compare the length of their subtending branch in Fig. 2) were chosen as a place holder, while for the morphological matrix presumed apomorphic tendencies were scored across the entire order. Thus, Laurales were scored as having valvate anther opening (character 230, absent/present), inaperturate or monosulcate pollen (character 129 with four states), a reticulate sexine (character 135 with four states), marginal placentation (character 241 with four states), and oil or proteins stored in the endosperm (character 161 with four states). These characters are variable within Laurales (as are many other characters scored as monomorphic) and do not occur together in any of the families.

Families excluded from Laurales—Of the excluded families (see Results) none was morphologically strongly linked with the core Laurales. Generally, the inclusion of Amborellaceae, Chloranthaceae, Trimeniaceae, and sometimes Austrobaileyaceae in Laurales was based on such widespread lower angiosperm traits as unilacunar nodes, opposite leaves, or a spiral floral phyllotaxy, and in the case of the first three also the single apical ovule. Typically, Chloranthaceae were thought to be closest to Trimeniaceae and Amborellaceae (Endress and Sampson, 1983; Sampson and Endress, 1984; Endress, 1987), which in turn remained in Laurales mostly because of tradition (they had originally been described as Monimiaceae; see Introduction). In a strikingly inconsistent decision, Cronquist (1981) excluded Chloranthaceae from Laurales but left *Amborella* and *Trimenia* in the order, stating ''[*Amborella*] is clearly a member of the Laurales, in which its primitively vesselless wood, alternate leaves, essentially hypogynous flowers, several carpels, abundant endosperm, and stamens dehiscent by slits mark it as an archaic type. The virtual absence of ethereal oil cells is anomalous in the group. . . '' Takhtajan (1997) adopts the same line, adding ''Trimeniaceae also approach Chloranthaceae, which I prefer to put in a separate order.'' In keeping with a phenetic approach to classification, he excluded Calycanthaceae from Laurales, placing them in a monotypic order next to Laurales.

Although earlier work had stressed the overall similarity between Chloranthaceae, Trimeniaceae, and Amborellaceae (see especially Endress and Sampson, 1983), these families do not group together based on *rbcL*. Rather, *Amborella* groups with Nymphaeaceae/Cabombaceae, and *Trimenia* with *Schisandra*/*Illicum*/*Austrobaileya*, while Chloranthaceae occupy an isolated position (Fig. 1). Sequences of 18S and *atpB* are currently being produced for *Trimenia* to add this taxon to combined *rbcL* + *atpB* + 18S analyses.

Relationships within Laurales—Within Laurales, the oldest split is between Calycanthaceae and the remaining six families, which in turn form two clades, the Siparunaceae (Atherospermataceae-Gomortegaceae) and the Hernandiaceae (Monimiaceae-Lauraceae). Calycanthaceae, Lauraceae, and Hernandiaceae are clearly monophyletic. Whether or not the single species of *Idiospermum* is recognized as a family (Idiospermaceae) depends on taste (Stevens, 1997; Backlund and Bremer, 1998) as does the recognition of Gyrocarpaceae. As shown by Shutts (1960), Kubitzki (1969), and others, the two subgroups of Hernandiaceae, *Gyrocarpus* and *Sparattanthelium* on the one hand and *Hernandia* and *Illigera* on the other, are divided by a major morphological gap, which historically led to their repeated assignment to different higher taxa.

Several morphological characters are consistent with the molecular results (as is also apparent from the morphological analysis of Donoghue and Doyle [1989] once Amborellaceae, Trimeniaceae, Chloranthaceae, and Austrobaileyaceae are removed from the Laurales). The deep split between Calycanthaceae and the remaining families is paralleled by at least three characters. (1) Calycanthaceae have two lateral ovules per carpel (of which only the lower one forms a mature embryo sac; Schaeppi, 1953; Nicely, 1965), while all other families have a single ovule per carpel. (2) Calycanthaceae have disulculate columellate pollen, while the remaining families with one exception have inaperturate pollen with rather thin, often spinulose exines. The disulculate state in Calycanthaceae may have been acquired relatively recently in their evolution, since a fossil flower attributed to that family (Friis et al., 1994) has monosulcate pollen. Another difference between extant Calycanthaceae and the fossil is that modern calycanth pollen has a nearly closed tectum, while the fossil has a reticulate tectum. If the ancestral lauralean pollen is assumed to have been monosulcate with a thick exine, the Calycanthaceae would have retained the thick exine but modified the single sulcus into two sulculi, while their sister lineage would have lost both the thick exine and the sulcus. Inaperturate pollen with thin, granular-spinulose exines as found in the sister clade to calycanths is rare in basal angiosperms and therefore represents a clear synapomorphy. (3) A third character, paralleling the ovule and pollen characters, is the presence or absence of floral nectary glands. Calycanthaceae are beetle-pollinated and devoid of nectaries (except for iso-

lated nectarogeneous fields on the inner tepals of *Chimonanthus*; Vogel, 1998), whereas large nectary glands on the filament bases characterize Lauraceae, Hernandiaceae, first-branching Monimiaceae, Atherospermataceae, and Gomortegaceae. The only lineages without filament glands are *Palmeria* and other higher Monimiaceae and Siparunaceae (Renner, Schwarzbach, and Lohmann, 1997), both apparently cases of secondary loss. Glands were likely lost in conjunction with increasing closure of flowers, which as far as known are pollinated by small non-nectar feeding beetles in Monimiaceae (Lorence, 1985) and by ovipositing (again, non-nectar foraging) gall midges in Siparunaceae (Feil and Renner, 1991; Feil, 1992; see Renner, Schwarzbach, and Lohmann [1997] for details on pollen tube growth in *Siparuna*). Lorence (1985, p. 66) observed flies as pollinators in some species of *Tambourissa* that offer mucilaginous stigma exudates as a reward (in addition to pollen) and hypothesized a secondary switch from beetle to fly pollination.

In summary, the molecular data support Kubitzki's (1993c) view that ''the Calycanthaceae are certainly the most aberrant element, yet not as primitive as claimed by Loconte and Stevenson (1991), but rather autapomorphic.''

The Monimiaceae-Lauraceae-Hernandiaceae clade—These three families formed a moderately well-supported clade (Fig. 2) that morphologically appears supported mainly by the apical position of their ovules (the ovules are inserted at or near the locule apex). Their sister clade settled on basal ovules (ovules inserted at or near the base of the locule) except for *Gomortega* (below), while Calycanthaceae have two lateral ovules. Some earlier workers have stressed the close relationship of Monimiaceae, Lauraceae, and Hernandiaceae (Shutts, 1960; Takhtajan, 1973; Rohwer, 1993), but assessments of relationships were handicapped by the prevailing broad concept of Monimiaceae, which greatly confused the picture of character variability in this family. As long as Siparunaceae and Atherospermataceae are included in Monimiaceae, the last combine basal and apical ovules, tetrasporangiate and disporangiate stamens with anthers opening by slits or apically hinged valves, wood with narrow rays and wood with very broad rays, and inaperturate thin-exined pollen as well as meridionosulcate columellate pollen.

The sister-group relationship between Lauraceae and Monimiaceae (Fig. 2) found here implies three parallelisms in Lauraceae and Hernandiaceae or reversals in Monimiaceae. First, the fixed unicarpellate condition found in both families must have evolved independently. Elsewhere within Laurales, solitary carpels evolved in Calycanthaceae (*Idiospermum*) and within higher Monimiaceae (*Xymalos, Hennecartia*), but all remaining groups, including Monimiaceae, have numerous carpels per flower. Second, the fixation of stamen and tepal numbers in flowers of Lauraceae (which are 3-merous) and Hernandiaceae [which are 3–4(–6) or 4–8-merous] likely evolved independently, and future morphological analyses should no longer treat the ''fixed number of floral parts'' as a single character as done here. Third, the absence of endosperm from the mature seeds of Lauraceae and Hernandiaceae must have evolved independently. Endosperm is also lost in Calycanthaceae.

The molecular phylogeny implies that disporangiate two-valvate anthers in Lauraceae and Hernandiaceae evolved independently, confirming the traditional view that tetrasporangiate stamens are basal in Lauraceae (Rohwer, 1993; Crane, Friis, and Pedersen, 1994). Tetrasporangiate stamens are plesiomorphic in the order, and they open by slits in two of the seven families of Laurales, Calycanthaceae and Monimiaceae. Notably, Monimiaceae anthers may dehisce longicidally or rarely transversally and may have short lateral incisions at the top and bottom of the slits, which results in a saloon door-like opening of the thecae (Baillon, 1869, fig. 339; Endress and Hufford, 1989, figs. 83–84). This can be interpreted as either a tendency towards valvate anther opening or a remnant of a former valvate dehiscence. The disporangiate anthers of Hernandiaceae dehisce in various ways; *Gyrocarpus* and *Sparattanthelium* have apically hinged valves, while *Illigera* and *Hernandia* (except the single species of *H.* subgenus *Hazomalania*) have laterally hinged valves. In *Hazomalania,* according to Kubitzki (1993b) ''the most primitive element of the family,'' valves are hinged apically (work on a molecular phylogeny of Hernandiaceae is in progress; Renner, unpublished data). Friis et al. (1994) have described a calycanthaceous fossil flower, *Virginianthus,* that had tetrasporangiate anthers opening by laterally hinged valves. It is thus possible that valvate dehiscence (with the valves laterally hinged) is ancestral in Laurales and was lost in Calycanthaceae and Monimiaceae, however, this interpretation hinges on the correct placement of *Virginianthus.*

The Atherospermataceae-Gomortegaceae-Siparunaceae clade—This clade receives 91% bootstrap support (Fig. 2) and agrees with several nonmolecular characters. Thus, all three families have disporangiate two-valvate stamens. Atherospermataceae and Siparunaceae have a chromosome number of $n = 22$ (Philipson, 1993b), while *Gomortega nitida* has $n = 21$ (Goldblatt, 1976; under the synonymous name *G. keule*). The ancestral condition in the clade is basal ovules (in contrast to the condition in its sister clade). The gynoecium of *Gomortega* is unique in Laurales in being syncarpous (two to three merous) and inferior (Leinfellner, 1968; Endress and Igersheim, 1997), and it may be surmised that the evolution of apical ovules in *Gomortega* occurred with the fusion and embedding of the ovary. Gomortegaceae have traditionally been placed close to, or even in, Monimiaceae. For example, Philipson (1987) stated ''The coherence of the Monimiaceae and the gap which separates them from other families of Laurales justify its continued recognition as a single family. Gomortegaceae, with a single species, represents the sole possible reservation. If its syncarpous inferior gynoecium is not considered sufficient to separate it from the Monimiaceae, then its union with that family would be a solution preferable to the fragmentation of the Monimiaceae.'' By contrast Rohwer (1993) thought ''*Gomortega* looks definitely lauraceous, and at least a cross-section of the ovary is needed to reveal features incompatible with Lauraceae.'' That the true relations of *Gomortega* lie with Atherospermataceae was first recognized by Schodde (1969, 1970) based on a phenetic analysis of some 40 morphological characters. A cladistic analysis of morphological characters (Renner, Schwarz-

bach, and Lohmann, 1997) also found Atherospermataceae and Gomortegaceae as sister groups, but support for this was weak. Among the few morphological or anatomical features suggesting a sister-group relationship between Atherospermataceae and *Gomortega* is the peculiar structure of their sieve tube plastids (Behnke, 1981, 1988). Most Monimiaceae s.str. and all Lauraceae, Hernandiaceae, and Siparunaceae have protein-containing plastids of the same kind as found in most Magnoliaceae and many Myristicaceae (Psc-type plastids in the notation of Behnke, 1988). By contrast, Calycanthaceae, Atherospermataceae, and *Gomortega* have plastids of the rare Pcsf-type (Behnke, 1988).

The DNA topology implies a return from inaperturate pollen with granulate exines, as found in most Laurales including Siparunaceae and *Gomortega*, to tectate-columellate exines and aperturate pollen along the stem lineage of Atherospermataceae. Transitions between granular and columellate tectal and/or supratectal sculptures have also occurred in the presumed closest relative of Laurales, the Magnoliales, in Annonaceae, Magnoliaceae, and Myristicaceae (Walker, 1976; Doyle and Le Thomas, 1994, 1996). Interestingly, Hesse and Kubitzki (1983) described *Gomortega* pollen as having short columellae, which might indicate a tendency towards thickening of the exine already along the stem lineage of atherosperms and *Gomortega*. The apertures in atherosperms are of the meridionosulcate or disulcate kind, which is very rare (Sampson, 1996, 1997) and possibly a hint of their de novo evolution.

Siparunaceae have many autapomorphic characters, such as disporangiate anthers that open by a single flap (a unique state in the Magnoliidae; Endress and Hufford, 1989, p. 77), unitegmic ovules (Renner, Schwarzbach, and Lohmann, 1997), and flowers closed by a roof.

Taxonomic conclusions—Laurales comprise seven major lineages for which family names are available. Trimeniaceae (like Amborellaceae, Austrobaileyaceae, and Chloranthaceae) are not part of Laurales. The recognition of Gyrocarpaceae and Idiospermaceae as families separate from Hernandiaceae and Calycanthaceae, respectively, is purely a matter of ranking, whereas the Monimiaceae s.l. are polyphyletic and need to be dismantled, as already argued by Schodde (1970). The information provided by this study should allow a clearer interpretation of the morphological and chemical characters found in Laurales (along the lines of Nandi, Chase, and Endress, 1998) and help to represent the group appropriately in the broader scale studies needed to link it to putative sister groups, such as the Magnoliales.

LITERATURE CITED

ABLETT, E. M., J. PLAYFORD, AND S. MILLS. 1997. The use of rubisco DNA sequences to examine the systematic position of *Hernandia albiflora* (C. T. White) Kubitzki (Hernandiaceae), and relationships among the Laurales. *Austrobaileya* 4: 601–607.

ANGIOSPERM PHYLOGENY GROUP. 1998. An ordinal classification for the families of flowering plants. *Annals of the Missorui Botanical Garden* 85: 531–553.

BAILLON, H. E. 1869. Histoire des Plantes, vol. 1. L. Hachette et Cie, Paris.

BACKLUND, A., AND K. BREMER. 1998. To be or not to be—principles of classification and monotypic plant families. *Taxon* 47: 391–400.

BAUM, D. A., R. L. SMALL, AND J. F. WENDEL. 1998. Biogeography and floral evolution of baobabs (*Adansonia*, Bombacaceae) as inferred from multiple dat sets. *Systematic Biology* 47: 181–207.

BEHNKE, H.-D. 1981. Sieve-element characters. *Nordic Journal of Botany* 1: 381–400.

———. 1988. Sieve-element plastids, phloem protein, and evolution of flowering plants: III. Magnoliidae. *Taxon* 37: 699–732.

BENTHAM, G. 1870. Flora Australiensis, vol. V. Lovell Reeve & Co., London.

———, AND J. D. HOOKER F. 1880. Genera Plantarum, vol. 3. Lovell Reeve & Co., London.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795–803.

———. 1994. Branch support and tree stability. *Cladistics* 10: 295–304.

CHASE, M. W., ET AL. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. *Annals of the Missouri Botanical Garden* 80: 528–580.

———, AND A. V. COX. 1998. Gene sequences, collaboration, and analysis of large data sets. *Australian Journal of Systematic Botany* 11: 215–229.

CRANE, P. R., E. M. FRIIS, AND K. R. PEDERSEN. 1994. Palaeobotanical evidence on the early radiation of magnoliid angiosperms. *Plant Systematics and Evolution*, Supplement 8: 51–72.

CREPET, W. L., AND K. C. NIXON. 1994. Flowers of Turonian Magnoliidae and their implications. *Plant Systematics and Evolution*, Supplement 8: 73–91.

CRONQUIST, A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York, NY.

———. 1988. The evolution and classification of flowering plants. New York Botanical Garden, Bronx, NY.

DONOGHUE, M. J., AND J. A. DOYLE. 1989. Phylogenetic analysis of angiosperms and the relationships of Hamamelidae. *In* P. R. Crane and S. Blackmore [eds.], Evolution, systematics, and fossil history of the Hamamelidae, vol. 1, 17–45. Clarendon Press, Oxford.

DOYLE, J. A., M. J. DONOGHUE, AND E. A. ZIMMER. 1994. Integration of morphological and ribosomal RNA data on the origin of angiosperms. *Annals of the Missouri Botanical Garden* 81: 419–450.

———, AND A. LE THOMAS. 1994. Cladistic analysis and pollen evolution in Annonaceae. *Acta Botanica Gallica* 141: 149–170.

———, AND ———. 1996. Phylogenetic analysis and character evolution in Annonaceae. *Bulletin du Muséum national d'Histoire naturelle, section B, Adansonia* 18: 279–334.

DRINNAN, A. N., P. R. CRANE, E. M. FRIIS, AND K. R. PEDERSEN. 1990. Lauraceous flowers from the Potomac Group (mid-Cretaceous) of eastern North America. *Botanical Gazette* 151: 370–384.

DUVALL, M. R., M. T. CLEGG, M. W. CHASE, W. D. CLARK, W. J. KRESS, H. G. HILLS, L. E. EGUIARTE, J. F. SMITH, B. S. GAUT, E. A. ZIMMER, AND G. H. LEARN, JR. 1993. Phylogenetic hypotheses for the monocotyledons constructed from *rbc*L sequence data. *Annals of the Missouri Botanical Garden* 80: 607–619.

EKLUND, H., AND J. KVACEK. 1998. Lauraceous inflorescences and flowers from the Cenomanian of Bohemia (Czech Republic, Central Europe). *International Journal of Plant Sciences* 159: 668–686.

ENDRESS, P. K. 1972. Zur vergleichenden Entwicklungsmorphologie, Embryologie und Systematik bei Laurales. *Botanische Jahrbücher für Systematik* 92: 331–428.

———. 1987. The Chloranthaceae: reproductive structures and phylogenetic position. *Botanische Jahrbücher für Systematik* 109: 153–226.

———, AND L. D. HUFFORD. 1989. The diversity of stamen structures and dehiscence patterns among Magnoliidae. *Botanical Journal of the Linnean Society* 100: 45–85.

———, AND T. IGERSHEIM. 1997. Gynoecium diversity and systematics of the Laurales. *Botanical Journal of the Linnean Society* 125: 93–168.

———, AND F. B. SAMPSON. 1983. Floral structure and relationships of the Trimeniaceae (Laurales). *Journal of the Arnold Arboretum* 64: 447–473.

FARRIS, J. S., V. A. ALBERT, M. KÄLLERSJÖ, D. LIPSCOMB, AND A. G. KLUGE. 1996 [1997]. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12: 99–124.

FAY, M. F., S. M. SWENSEN, AND M. W. CHASE. 1997. Taxonomic af-

finities of *Medusagyne oppositifolia* (Medusagynaceae). *Kew Bulletin* 52: 111–120.

FEIL, J. P. 1992. Reproductive ecology of dioecious *Siparuna* (Monimiaceae) in Ecuador—a case of gall midge pollination. *Botanical Journal of the Linnean Society* 110: 171–203.

———, AND S. S. RENNER. 1991. The pollination of *Siparuna* (Monimiaceae) by gall-midges (Cecidomyiidae): another likely ancient association. *American Journal of Botany* 78: 186.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

FRIIS, E. M., P. C. CRANE, AND K. R. PEDERSEN. 1997. Fossil history of magnoliid angiosperms. *In* K. Iwatsuki and P. H. Raven [eds.], Evolution and diversification of land plants, 121–156. Springer-Verlag, Tokyo.

———, H. EKLUND, K. R. PEDERSEN, AND P. R. CRANE. 1994. *Virginianthus calycanthoides* gen. et sp. nov.: a calycanthaceous flower from the Potomac Group (Early Cretaceous) of eastern North America. *International Journal of Plant Sciences* 155: 772–785.

GIBBS, L.S. 1917. A contribution to the phytogeography and flora of the Arfak mountains. Taylor and Francis, London.

GOLDBLATT, P. 1976. Chromosome number in *Gomortega keule*. *Annals of the Missouri Botanical Garden* 63: 207–208.

GOLENBERG, E. M., M. T. CLEGG, M. L. DURBIN, J. DOEBLEY, AND D. P. MA. 1993. Evolution of a noncoding region of the chloroplast genome. *Molecular Phylogenetics and Evolution* 2: 52–64.

———, D. E. GIANNASI, M. T. CLEGG, C. J. SMILEY, M. DURBIN, D. HENDERSON AND G. ZURAWSKI. 1990. Chloroplast DNA sequences from a Miocene *Magnolia* species. *Nature* 344: 656–658.

GRAYBEAL, A. 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? *Systematic Biology* 74: 9–17.

HALLIER, H. 1905. Ein zweiter Entwurf des natürlichen (phylogenetischen) Systems der Blütenpflanzen. *Berichte der Deutschen Botanischen Gesellschaft* 23: 85–91.

HASEBE, M., R. KOFUJI, M. ITO, M. KATO, K. IWATSUKI, AND K. UEDA. 1992. Phylogeny of gymnosperms inferred from *rbcL* gene sequences. *Botanical Magazine, Tokyo* 105: 673–679.

HERENDEEN, P. S., W. L. CREPET, AND K. C. NIXON. 1993. *Chloranthus*-like stamens from the Upper Cretaceous of New Jersey. *American Journal of Botany* 80: 865–871.

HESSE, M., AND K. KUBITZKI. 1983. The sporoderm ultrastructure in *Persea, Nectandra, Hernandia, Gomortega* and some other lauralean genera. *Plant Systematics and Evolution* 141: 299–311.

HILLIS, D. M. 1996. Inferring complex phylogenies. *Nature* 383: 130–131.

KELCHNER, S. A., AND L. G. CLARK. 1997. Molecular evolution and phylogenetic utility of the chloroplast *rpl16* intron in *Chusquea* and the Bambusoideae (Poaceae). *Molecular Phylogenetics and Evolution* 8: 385–397.

KELLOGG, E. A., AND N. D. JULIANO. 1997. The structure and function of RuBisCo and their implications for systematic studies. *American Journal of Botany* 84: 413–428.

KUBITZKI, K. 1969. Monographie der Hernandiaceen. *Botanische Jahrbücher für Systematik* 89: 78–148.

———. 1993a. Introduction. *In* K. Kubitzki, J. G. Rohwer, and V. Bittrich [eds.], The families and genera of vascular plants, vol. 2, 1–12. Springer Verlag, Berlin.

———. 1993b. Hernandiaceae. *In* K. Kubitzki, J. G. Rohwer, and V. Bittrich [eds.], The families and genera of vascular plants, vol. 2, 334–338. Springer Verlag, Berlin.

———. 1993c. Calycanthaceae. *In* K. Kubitzki, J. G. Rohwer, and V. Bittrich [eds.], The families and genera of vascular plants, vol. 2, 197–200. Springer Verlag, Berlin.

KÜHN, U., AND K. KUBITZKI. 1993. Myristicaceae. *In* K. Kubitzki, J. G. Rohwer, and V. Bittrich [eds.], The families and genera of vascular plants, vol. 2, 457–467. Springer Verlag, Berlin.

LECOINTRE, G., H. PHILIPPE, H. L. VAN LE, AND H. LE GUYADER. 1993. Species sampling has a major impact on phylogenetic inference. *Molecular Phylogenetics and Evolution* 2: 205–224.

———, ———, ———, AND ———. 1994. How many nucleotides are required to resolve a phylogenetic problem? The use of a new statistical method applicable to available sequences. *Molecular Phylogenetics and Evolution* 3: 292–309.

LEINFELLNER, W. 1968. Über die Karpelle verschiedener Magnoliales. VI. *Gomortega keule* (Gomortegaceae). *Österreichische Botanische Zeitschrift* 115: 113–119.

LES, H. D., D. K. GARVIN, AND C. F. WIMPEE. 1991. Molecular evolutionary history of ancient aquatic angiosperms. *Proceedings of the National Academy of Sciences, USA* 88: 10 119–10 123.

LOCONTE, H., AND D. W. STEVENSON. 1991. Cladistics of the Magnoliidae. *Cladistics* 7: 267–296.

LORENCE, D. H. 1985. A monograph of the Monimiaceae (Laurales) of the Malagasy region (Southwest Indian Ocean). *Annals of the Missouri Botanical Garden* 72: 1–165.

MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade: analysis of phylogeny and character evolution (version 3). Sinauer, Sunderland, MA.

MANEN, J.-F., A. NATALI, AND F. EHRENDORFER. 1994. Phylogeny of Rubiaceae-Rubieae inferred from the sequence of a cpDNA intergenic region. *Plant Systematics and Evolution* 190: 195–211.

MELCHIOR, H. [ED.] 1964. A. Engler's Syllabus der Pflanzenfamilien. II. Bornträger, Berlin.

MONEY, L. L., I. W. BAILEY, AND B. G. L. SWAMY. 1950. The morphology and relationships of the Monimiaceae. *Journal of the Arnold Arboretum* 31: 372–404.

NANDI, O. I., M. W. CHASE, AND P. K. ENDRESS. 1998. A combined cladistic analysis of angiosperms using *rbcL* and non-molecular data sets. *Annals of the Missouri Botanical Garden* 85: 137–212.

NICELY, K. A. 1965. A monographic study of the Calycanthaceae. *Castanea* 30: 38–81.

OLMSTEAD, R. G., B. BREMER, K. M. SCOTT, AND J. D. PALMER. 1993. A parsimony analysis of the Asteridae sensu lato based on *rbcL* sequences. *Annals of the Missouri Botanical Garden* 80: 700–722.

PEDERSEN, K. P., P. R. CRANE, A. N. DRINNAN, AND E. M. FRIIS. 1991. Fruits from the mid-Cretaceous of North America with pollen grains of the *Clavatipollenites* type. *Grana* 30: 577–590.

PHILIPSON, W. R. 1987. A classification of the Monimiaceae. *Nordic Journal of Botany* 7: 25–29.

———. 1993a. Trimeniaceae. *In* K. Kubitzki, J. G. Rohwer, and V. Bittrich [eds.], The families and genera of vascular plants, vol. 2, 596–599. Springer Verlag, Berlin.

———. 1993b. Monimiaceae. *In* K. Kubitzki, J. G. Rohwer, and V. Bittrich [eds.], The families and genera of vascular plants, vol. 2, 426–437. Springer Verlag, Berlin.

PICHON, M. 1948. Les Monimiacées, famille hétérogène. *Bulletin du Muséum Nationale d'Histoire Naturelle, Sér.* 2, 20, 383–384.

PRICE, R. A. 1996. Systematics of the Gnetales: a review of morphological and molecular evidence. *International Journal of Plant Science* 157, Supplement: S40–S49.

PURVIS, A., AND D. L. J. QUICKIE. 1997. Building phylogenies: are the big easy? *Trends in Ecology and Evolution* 12: 49–50.

QIAGEN. 1995. QIAGEN genomic DNA handbook. QIAGEN Inc., Santa Clarita, CA.

———. 1997. QIAquick spin handbook. QIAGEN Inc., Santa Clarita, CA.

QIU, Y.-L., M. W. CHASE, D. H. LES, AND C. R. PARKS. 1993. Molecular phylogenies of the Magnoliidae: cladistic analysis of nucleotide sequences of the plastid gene *rbcL*. *Annals of the Missouri Botanical Garden* 80: 587–606.

REICHE, K. 1896. Zur Kenntnis von *Gomortega nitida* R. et Pav. *Berichte der Deutschen Botanischen Gesellschaft* 14: 225–233, Tabula XVI.

RENNER, S. S. 1998. Phylogenetic affinities of Monimiaceae based on cpDNA gene and spacer sequences. *Perspectives in Plant Ecology, Evolution and Systematics* 1: 61–77.

———, A. E. SCHWARZBACH, AND L. LOHMANN. 1997. Phylogenetic position and floral function of *Siparuna* (Siparunaceae: Laurales). *International Journal of Plant Sciences* 158, Supplement: S89–S98.

ROHWER, J. 1993. Lauraceae. *In* K. Kubitzki, J. G. Rohwer, and V. Bittrich [eds.], The families and genera of vascular plants, vol. 2, 366–391. Springer Verlag, Berlin.

SAMPSON, F. B. 1993. Pollen morphology of the Amborellaceae and Hortoniaceae (Hortonioideae: Monimiaceae). *Grana* 32: 154–162.

———. 1996. Pollen morphology and ultrastructure of *Laurelia, Laureliopsis* and *Dryadodaphne* (Atherospermataceae [Monimiaceae]). *Grana* 35: 257–265.

———. 1997. Pollen morphology and ultrastructure of Australian Monimiaceae—*Austromatthaea, Hedycarya, Kibara, Leviera, Steganthera* and *Tetrasynandra*. *Grana* 36: 135–145.

———, AND P. K. ENDRESS. 1984. Pollen morphology in the Trimeniaceae. *Grana* 23: 129–137.

SANG, T., D. J. CRAWFORD, AND T. F. STUESSY. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* 84: 1120–1136.

SAVOLAINEN, V., M. W. CHASE, S. B. HOOT, C. M. MORTON, D. E. SOLTIS, C. BAYER, M. F. FAY, A. Y. DE BRUIN, S. SULLIVAN, AND Y.-L. QIU. In press. Phylogenetics of flowering plants based upon a combined analysis of plastid *atpB* and *rbcL* gene sequences. *Systematic Biology*.

SCHAEPPI, H. 1953. Morphologische Untersuchungen an den Karpellen der Calycanthaceen. *Phytomorphology* 3: 112–117.

SCHODDE, R. 1969. A monograph of the family Atherospermataceae R. Br. Ph.D. dissertation, University of Adelaide, Australia.

———. 1970. Two new suprageneric taxa in the Monimiaceae alliance (Laurales). *Taxon* 19: 324–328.

SEEMANN, B. C. 1865. Flora Vitiensis, vol. I. Lovell Reeve & Co., London.

SHUTTS, C. F. 1960. Wood anatomy of Hernandiaceae and Gyrocarpaceae. *Tropical Woods* 113: 85–123.

SMITH, A. C. 1972. An appraisal of the orders and families of primitive extant angiosperms. *Journal of the Indian Botanical Society* 50A: 215–226.

SOLTIS, D. E., ET AL. 1997a. Angiosperm phylogeny inferred from 18S ribosomal DNA sequences. *Annals of the Missouri Botanical Garden* 84: 1–49.

———, C. HIBSCH-JETTER, P. S. SOLTIS, M. W. CHASE, AND J. S. FARRIS. 1997b. Molecular phylogenetic relationships among angiosperms: an overview based on *rbc*L and 18S rDNA sequences. *In* K. Iwatsuki and P. H. Raven [eds.], Evolution and diversification of land plants, 157–178. Springer Verlag, Tokyo.

STEVENS, P. 1997. What kind of classification should the practising taxonomist use to be saved? *In* J. Dransfield, M. J. E. Coode, and D. A. Simpson [eds.], Plant diversity in Malesia III, 295–319. Royal Botanical Gardens, Kew.

TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

TAKHTAJAN, A. 1973. Evolution und Ausbreitung der Blütenpflanzen. Gustav Fischer Verlag, Jena.

———. 1997. Diversity and classification of flowering plants. Columbia University Press, New York, NY.

THORNE, R. F. 1974. A phylogenetic classification of the Annoniflorae. *Aliso* 8: 147–209.

———. In press. An updated phylogenetic classification of the flowering plants. *Aliso*.

UEDA, K., A. NAKANO, R. RODRIGUEZ, C. RAMÍREZ, AND H. NISHIDA. 1997. Molecular phylogeny of the Gomortegaceae, a Chilean endemic monotypic, and endangered family. *Noticiero de Biologia* 5: 124.

VAN DER WERFF, H., AND H. G. RICHTER. 1996. Toward an improved classification of Lauraceae. *Annals of the Missouri Botanical Garden* 83: 409–418.

VOGEL, S. 1998. Remarkable nectaries: structure, ecology, organophyletic perspectives. II. Nectarioles. *Flora* 193: 1–29.

WALKER, J. W. 1976. Comparative pollen morphology and phylogeny of the ranalean complex. *In* C. B. Beck [ed.], Origin and early evolution of angiosperms, 241–299. Columbia University Press, New York, NY.