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PHYLOGENETIC POSITION AND FLORAL FUNCTION OF *SIPARUNA* (SIPARUNACEAE: LAURALES)

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Nucleotide sequences for two chloroplast genome regions, the *rbc*L gene and the *trn*L-*trn*F spacer region, were obtained for 22 genera and 30 species representing all major lineages of Laurales, with a special sampling effort being made in the phylogenetically problematic Monimiaceae *sensu lato.* Magnoliaceae, Winteraceae, Austrobaileyaceae, and Saururaceae were used as outgroups. A morphological character matrix for the same taxa was compiled and includes new floral-anatomical data for Siparunaceae, a lineage traditionally placed in Monimiaceae. Phylogenetic analyses of the molecular and morphological data based on maximum parsimony reveal that *Siparuna* and its sister taxon, the monotypic West African genus *Glossocalyx,* are not closely related to the remaining Monimiaceae, supporting the view that the Monimiaceae in the wide sense are polyphyletic. Based on morphology and chromosome numbers, *Siparuna* forms a clade with Atherospermataceae and Gomortegaceae, while based on *rcbL* sequences, it is in a clade with Hernandiaceae, Atherospermataceae, and Gomortegaceae. The *trn*L-*trn*F sequences provide no resolution for basal nodes in Laurales but agree with the *rbc*L and morphological analyses in strongly supporting a placement of Siparunaceae away from Monimiaceae s.str. Based on the phylogenetic hypotheses, we analyze some of the reported floral-morphological trends noted in Monimiaceae and Laurales, such as miniaturization of ovules, increasingly complete enclosure of reproductive organs, and aquisition of functional syncarpy. Both *Siparuna* and *Glossocalyx* have polycarpellate gynoecia embedded in massive receptacles covered by a membrane and uniovulate carpels with unitegmic ovules. The membrane, or floral roof, has a small, central pore for the styles or anthers to emerge at anthesis. It is more developed and thicker in female flowers than in male ones. Consequently, the styles in particular are forced into close physical contact. Flowers are pollinated by gall midges that oviposit into them through the pore in the floral roof, whereby they contact stamens or styles. Anatomical studies of flowers representing eight species show that styles fuse postgenitally at the height where they emerge through the pore, resulting in a joint transmission track for pollen tubes that originally landed on different stigmas. Lateral growth of pollen tubes, which results in switching between carpels, was observed in an experimentally pollinated species that had received large pollen loads.

Introduction

Comparative studies of floral evolution in Monimiaceae have emphasized morphological trends that seem to characterize basal angiosperm lineages (Endress 1980*a,* 1980*b,* 1986, 1990, and 1994, pp. 230–232). One such trend discerned by Endress (see esp. Endress 1990) is toward miniaturization of the ovules, resulting in unitegmic and/or tenuinucellar conditions. While most basal angiosperms have bitegmic ovules with abundant endo- or perisperm, minute ovules occur in both Laurales and Piperales: Calycanthaceae, Lauraceae, and Hernandiaceae have endospermless seeds; *Peperomia* has ovules with a single integument (Tucker 1980; Tucker et al. 1993). Within Laurales, only *Siparuna* was known until recently to have a single integument (Heilborn 1931; Endress 1972; S. Renner, personal observation), but a screening of potential relatives of *Siparuna* revealed the presence of unitegmic ovules in the monotypic West African genus *Glossocalyx,* thus providing a potential synapomorphy for a clade consisting of these two genera. *Siparuna* comprises ca. 72 species of shrubs, straggling shrubs, and trees, occurring from tropical Mexico throughout the West Indies and northern South America to Bolivia and Paraguay (Renner, ongoing revision). The flowers are strictly unisexual and measure a few millimeters in diameter (figs. 1, 2).

A second floral evolutionary trend emphasized by Endress (1979, 1980*b,* 1982; Endress and Lorence 1983) is from completely free carpels, without any intercellular communication between their inner spaces, to "functionally syncarpous" gynoecia. Functional syncarpy can be achieved in different ways, with morphologically free carpels sharing either a joint pollen-receiving region formed by massive secretions or a pollen-exchange zone at their bases as in *Illicium,* where tubes can enter adjacent carpels via their unfused margins (Williams et al. 1993). Ecologically, these mechanisms could be increasing reproductive efficiency. This may be particularly important in groups in which flowers have numerous carpels but solitary ovules: if insect-deposited pollen loads can be distributed to more than one carpel, this must increase the number of ovules that can be fertilized. In Laurales, functional syncarpy via a common pool of mucilage is known from the monimiaceous genera *Faika, Hedycarya, Hennecartia, Tambourissa,* and *Wilkiea* (Endress, 1979, 1980*b,* 1982, and 1994, p. 232). Below we report a new type of functional syncarpy by postgenital fusion of styles in *Siparuna.*

A third trend noticed by Endress in the Monimiaceae is toward an increasingly complete enclosure of the reproductive organs in massive cup-like receptacles that raise the floral periphery around or above the gynoecium. While fleshy cupules were present in now extinct higher seed plants (K. Nixon, personal communication), within angiosperms they characterize a group of families first united under the name Laurineae by Hallier (1905). The Laurineae (or Laurales) include the Calycanthaceae (three genera; including *Idiosper-*

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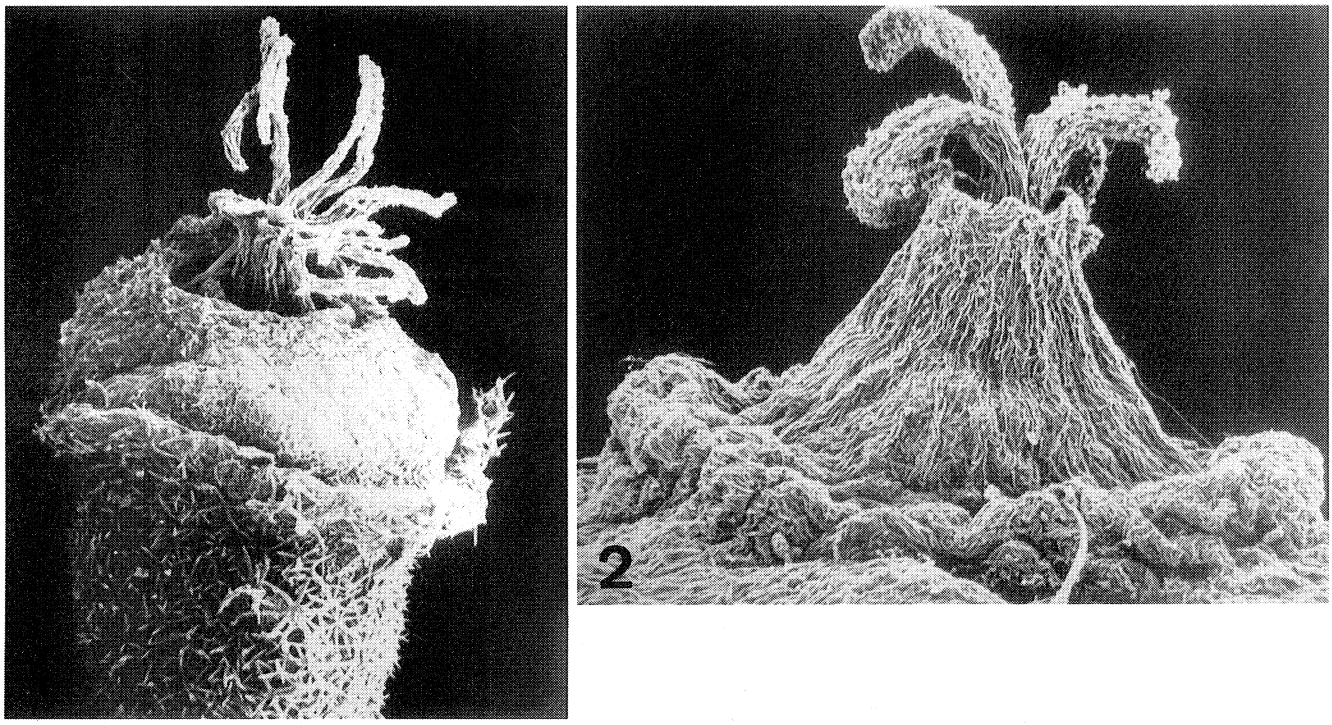

Figs. 1, 2 Female flowers of *Siparuna*. Fig. 1, *S. thecaphora*. The floral roof in this species is glabrous and consists of an outer bulge and an inner, narrower collar just visible to the right of the stylar complex. (Real height of flower ca. 1.6 mm) Fig. 2, *S. muricata*. The floral roof consists of a wide outer area (not shown) and an innermost collar that tightly surrounds the styles. Pollen grains visible on stigmatic papillae.

mum), Gomortegaceae (monotypic), Hernandiaceae (five genera; including *Gyrocarpus*), Lauraceae (50 genera), and Monimiaceae s.l. (i.e., Atherospermataceae with seven genera, Siparunaceae with two or three genera, and Monimiaceae with ca. 18). These taxa share several characters besides the cup-like receptacles, including inaperturate pollen (except Calycanthaceae and Atherospermataceae) usually with spinulose exines, unilacunar two-trace nodes (also present in some potential outgroups and perhaps plesiomorphic), decussate leaves (perhaps plesiomorphic and lost in Lauraceae and Hernandiaceae), and carpels with a single ovule (except Calycanthaceae, which have two ovules of which only one forms a mature embryo sac [Nicely 1965], and Gomortegaceae, which have a 2- or 3-merous syncarpous gynoecium). Four of seven lauralean families have stamens dehiscing by apically-hinged valves and with large, paired nectar glands at the filament bases. Both characters seem to have arisen early in the evolution of the order and to have become coupled or uncoupled several times. Thus, the Siparunaceae have valvate anther dehiscence but no glands, while three genera of Monimiaceae s.str. (*Hortonia, Peumus,* and *Monimia* with together five or six species) have longicidal or also lateral (in *Monimia*) dehiscence and glands on the filament bases.

This article places the three floral-morphological trends, miniaturization of ovules, increasingly complete enclosure of reproductive organs, and aquisition of functional syncarpy, in a phylogenetic context. It also examines the hypothesis that Monimiaceae in the wide sense are polyphyletic as suggested by R. Brown (1814), Schodde (1969, 1970), Endress (1972), and A. C. Smith (1972). Our understanding of (floral) evolutionary trends in Monimiaceae, Siparunaceae, and Atherospermataceae stands to be improved by knowledge of their closest relatives in Laurales. However, trends also need to be related to the function of flowers in pollination or seed dispersal. To do this we report new results of floral-anatomical studies on *Glossocalyx* and *Siparuna* and observations on pollen tube growth in *Siparuna* and relate them to the pollination of these plants by ovipositing gall midges.

The molecular data presented are part of ongoing work on the phylogenetic systematics of Laurales.

Material and methods

Nucleotide sequences of *rbc*L and/or the *trn*L_{UAA} 3′-*trn*F_{GAA} 5′ intergenic spacer region of the chloroplast genome were obtained from 30 species (table 1, which also lists voucher information for each taxon), representing 22 genera and all major lauralean lineages identified by previous morphological and molecular studies (Qiu et al. 1993, personal communication; J. A. Doyle, personal communication; K. Ueda, personal communication). Thirteen *rbc*L sequences were obtained from GenBank (table 1); two *rbc*L and 19 *trn*L-*trn*F sequences were produced for this study; and an *rbc*L sequence of *Gomortega* (Ueda et al. 1997) was contributed by K. Ueda (personal communication). As outgroups we used *Magnolia macrophylla* and *M. hypoleuca* (Magnoliaceae), *Drimys winteri* (Winteraceae), *Austrobail-*

Table 1

SOURCES OF *rbc*L AND *trn*L-*trn*F SEQUENCES USED IN THIS STUDY

Taxon	Voucher or citation	GenBank accession numbers	
		*rbc*L	*trn*L-*trn*F
Atherospermataceae:			
Doryphora aromatica (F.M. Bail.) L.S. Smith	Ablett et al. 1997	L77211	···
Laurelia sempervirens (R. & P.) Tul.	Edinburgh Bot. Gard. 19931681	···	AF012402
Calycanthaceae:			
Calycanthus occidentalis Hook. & Arn.	Missouri Bot. Gard. 897423	···	AF012396
Calycanthus floridus L.	Chase et al. 1993	L14291	···
Chimonanthus praecox (L.) Link	Qiu et al. 1993	L12639	···
Idiospermum australiense (Diels) S.T. Blake	Qiu et al. 1993	L12651	···
Gomortegaceae:			
Gomortega keule (Molina) I.M. Johnson	Ueda et al. 1997	D89561	···
Gomortega keule (Molina) I.M. Johnson	*Rodriquez 3070* (CONC)	···	AF012404
Hernandiaceae:			
Gyrocarpus americanus Jacq.	Qiu et al. 1993, DNA aliquot	L12647	AF012398
Hernandia albiflora (C.T. White) Kubitzki	Ablett et al. 1997	L77210	···
Hernandia ovigera L.	Qiu et al. 1993, DNA aliquot	L12650	AF012397
Lauraceae:			
Cinnamomum camphora (L.) T. Nees & Eberm.	Qiu et al. 1993	L12641	···
Litsea japonica (Thun.) Juss.	P.G. Martin and J. Dowd, unpubl.	U06843	···
Nectandra turbacensis (Kunth) Nees	*Taylor 11746* (MO)	···	AF012400
Ocotea leucoxylon (Sw.) Lanessan	*Taylor 11733* (MO)	···	AF012399
Persea americana Mill.	Golenberg et al. 1990	X54347	···
Persea americana Mill.	Missouri Bot. Gard. 897543	···	AF012401
Monimiaceae:			
Hedycarya arborea J.R. & G. Forst.	Qiu et al. 1993, DNA aliquot	L12648	AF012412
Hennecartia omphalandra Poisson	*Peña s.n.* (MO)	AF022950	···
Kibara coriacea (Bl.) Tul.	*Coode 7879* (K), DNA aliquot	···	AF012413
Peumus boldus Molina	Edinburgh Bot. Gard. 19870707	···	AF012403
Tambourissa ficus (Tul.) A. DC.	National Trop. Bot. Gard. 940043	···	AF012410
Tambourissa tau Lorence	National Trop. Bot. Gard. 940044	···	AF012411
Siparunaceae:			
Glossocalyx longicuspis Benth.	*Sothers 2109-74* (INPA)	···	AF012407
Siparuna brasiliensis (Spreng.) A. DC.	*Ståhl 2254* (QCA)	···	AF012406
Siparuna depressa Jangoux	*Madriñán et al. 1504* (COL)	···	AF012409
Siparuna lepidota (H.B.K.) A. DC.	*Pignal 309* (P)	AF013246	AF012409
Siparuna sessiliflora (H.B.K.) A. DC.	*Bos 4659* (MO)	···	AF012405
Magnoliaceae:			
Magnolia hypoleuca Siebold & Zucc.	Qiu et al. 1993	L12655	···
Magnolia hypoleuca Siebold & Zucc.	Missouri Bot. Gard. 732556	···	AF012395
Winteraceae:			
Drimys winteri J.R. & G. Forster	Albert et al. 1992	L01905	···
Austrobaileyaceae:			
Austrobaileya scandens C.T. White	Qiu et al. 1993	L12632	···
Saururaceae:			
Saururus cernuus L.	Chase et al. 1993	L14294	···

eya scandens (Austrobaileyaceae), and *Saururus cernuus* (Saururaceae), a choice reflecting the current uncertainty about relationships in the Magnoliidae.

Total DNA was extracted from fresh or silica gel–dried leaves, using the CTAB method (Doyle and Doyle 1987). Following DNA purification with QIAGEN Genomic-tip columns (G-20; QIAGEN 1995), target loci were amplified by PCR with a Hybaid Thermal Cycler. A 5′ primer that anneals to the first 16 base pairs of the *rbc*L gene (*1F*) and a 3′ primer (*1460R*) that anneals to a flanking region downstream from the 3′ end was used to amplify the entire chloroplast-encoded *rbc*L gene, while the *e* and *f* primers (Taberlet et al. 1991) were used for amplification of the intergenic spacer. Single-stranded DNA products were purified with the QIAquick PCR Purification kit (QIAGEN 1997) and automatically sequenced.

Sequences were aligned visually (the *rbc*L sequences had no gaps, the *trn*L-*trn*F sequences, very few gaps). The molecular data were analyzed with PAUP 3.1.1 (Swofford 1993), using Multiple Parsimony (MULPARS) and the branch-and-bound search option. Number of potentially informative characters, number and length of most-parsimonious trees, consistency index (uninformative characters excluded), and retention index were obtained from PAUP. The robustness of trees was evaluated by bootstrapping (Felsenstein 1985), using 100 replicates. The present article focuses on the position of *Siparuna* and its immediate relatives in Laurales, and therefore only a small number of representatives of other lauralean lineages were included in the analyses as place holders once the monophyly of these lineages was clear (cf. table 1). This is the case for Atherospermataceae (Schodde 1969; Renner et al. 1997, and ongoing

work), Lauraceae (Rohwer 1993; H. van der Werff, personal communication; ongoing work in our lab), and Calycanthaceae (including *Idiospermum;* Qiu et al. 1993). The monophyly of Hernandiaceae (including *Gyrocarpus*) needs further testing (see also Kubitzki 1993*c*); currently, sequences are available for three species representing two of five genera, *Hernandia* and *Gyrocarpus,* and these do not always group together.

For the morphological cladistic analysis, data were derived from S. S. Renner's observations of herbarium and living material as well as from the literature, as noted below in the discussion of individual characters. Particularly useful were the family summaries found in Kubitzki et al. (1993) and the character matrix compiled by Donoghue and Doyle (1989) for their phylogenetic analysis of basal angiosperms. Donoghue and Doyle (1989) found that Monimiaceae s.l., Gomortegaceae, Hernandiaceae, and Lauraceae (termed "core Laurales" and abbreviated "MON" in their trees) were united by stamen filaments with basal nectaries and inaperturate pollen. In a preliminary analysis, not published in Donoghue and Doyle (1989), they had broken up the Monimiaceae into Atherospermataceae, Siparunaceae, Monimiaceae s.str., and *Hortonia.* The resulting tree, but not the data matrix, was published in Doyle et al. (1994) as figure 8*b,* and an unpublished electronic version of the matrix was kindly sent to us by Jim Doyle. It contains 28 characters of which 10 appear in our matrix, albeit sometimes with different codings. To achieve greater resolution within Laurales, we added five characters to yield a total of 15 (appendix); all were unordered and polarized using Magnoliaceae, Winteraceae, and Austrobaileyaceae as outgroups.

1. Vegetative phyllotaxy: 0 = alternate, 1 = decussate, 2 = spiral-alternate.
2. Floral cup, cupular receptacle, or cupule: 0 = absent, 1 = present.
3. Innermost tepals: 0 = free, 1 = connate. *Mollinedia* was scored as inapplicable for this character because its pistillate flowers have a calyptra while the staminate flowers have free tepals.
4. Fixed stamen number: 0 = absent, 1 = present.
5. Basal filament glands: 0 = absent, 1 = present.
6. Anther dehiscence: 0 = longicidal, 1 = valvate with 2 pollen sacs, 2 = valvate with four pollen sacs. The last is an autapomorphy of Lauraceae, which ancestrally are thought to have had four pollen sacs per anther. We opted for this coding to reflect the possibility that valvate anther dehiscence in Lauraceae is not directly homologous to that in the other lauralean groups.
7. Apertures: 0 = monosulcate, 1 = meridionosulcate or dicolpate (autapomorphic for Atherospermataceae), 2 = disulculate (autapomorphic for Calycanthaceae), 3 = inaperturate, 4 = porate (autapomorphic for Winteraceae). Data for Atherospermataceae and Monimiaceae from Sampson (1993, 1996, in press), Foreman and Sampson (1987), and Sampson and Foreman (1990); data for Lauraceae, Hernandiaceae, Gomortegaceae, and Calycanthaceae from Hesse and Kubitzki (1983), van der Merwe et al. (1990), and Kubitzki (1993*a,* 1993*b,* 1993*c*).
8. Carpels: 0 = several free, 1 = one, 2 = syncarpous. The last is an autapomorphy of *Gomortega keule,* which has syncarpous ovaries of 2–3 carpels (Leinfellner 1968); the character is included here because of our interest in its possible origin. *Tambourissa* and *Hennecartia* were scored inapplicable for this character because the former has numerous carpels embedded in the receptacle tissue while the latter has 1 (-2) carpels.
9. Epigyny: 0 = absent, 1 = present.
10. Chromosome base number: 0 = 19, 1 = 11–12, 2 = 21–22, 3 = 39 (*Peumus*), 4 = 44 (*Monimia*), 5 = 96 (*Hennecartia*). Hernandiaceae and Winteraceae, which have variable chromosome numbers, were coded as "?." Data from Morawetz (1986).
11. Ovules: 0 = several ventral, 1 = one apical, 2 = one basal, 3 = two ventral (autapomorphic for Calycanthaceae). The syncarpous Gomortegaceae were scored inapplicable for this character because of unclear homologies.
12. Number of integuments: 0 = two, 1 = one. Data for *Gomortega* from P. K. Endress and A. Igersheim (personal communication).
13. Fruit type: 0 = drupe (endozoochorous, buoyant, or winged, that is, dispersed in different ways), 1 = achene, 2 = capsule (autapomorphic for Magnoliaceae), 3 = enclosed druplets (inside a fleshy receptacle that usually tears open at maturity), 4 = berry.
14. Endosperm: 0 = present, 1 = absent.
15. Nodes: 0 = tri- or multilacunar, 1 = unilacunar. Data from Money et al. (1950).

Parsimony analyses of morphological data were performed with PAUP 3.1.1 using Multiple Parsimony (MULPARS), Accelerated Transformation, and the branch-and-bound option.

Field observations on floral function and experiments on pollen tube growth were made by D. Schulz-Burck (as part of his MSc project, Schulz-Burck 1997) in the Ducke Forest Reserve near Manaus, Brazil (59°58′W, 2°53′S). Controlled pollinations were performed on *Siparuna cristata* (Poeppig & Endl.) A. DC. by rubbing entire fresh male flowers onto receptive stigmatic areas. Receptiveness of stigmas was assessed by the fresh, glossy green appearance of stigmatic papillae; postanthetic papillae appear brown. Treated flowers were collected after 15 h, together with other flowers in different stages of development. In addition, flowers from four other species (*S. decipiens* [Tul.] A. DC., *S. depressa* Jangoux, *S. guianensis* Aublet, and *S. poeppigii* [Tul.] A. DC.) were collected from tagged trees already vouchered and identified by SSR as part of an ongoing floristic project currently being carried out at the Instituto Nacional de Pesquisas da Amazônia and the Royal Botanic Gardens Kew. Voucher specimens (collected by project staff) are deposited in the herbarium of the Instituto Nacional de Pesquisas da Amazônia. All flowers were preserved in FAA (formalin–acetic acid–alcohol) and later stored in 70% alcohol to be dissected and/or trimmed and sectioned to reveal stages in floral development. For comparison, spirit-preserved flowers of *S. aspera* (Ruiz & Pavón) A. DC., *S. muricata* (Ruiz & Pavón) A. DC., and *S. thecaphora* (Poeppig & Endl.) A. DC. from Ecuador were also sectioned. For sectioning, flowers were embedded in Historesin (Leica); sections were stained with Toluidine blue.

Species concepts follow a monograph of *Siparuna* (Renner and Hausner, in press; Renner, in prep.).

Results and discussion

Phylogenetic relationships of the monimiaceous groups

The *rbc*L maximum parsimony analysis found six equally parsimonious trees (L = 403 steps; CI [Consistency Index, informative characters only] = .722; RI [Retention Index] = .576), the strict consensus of

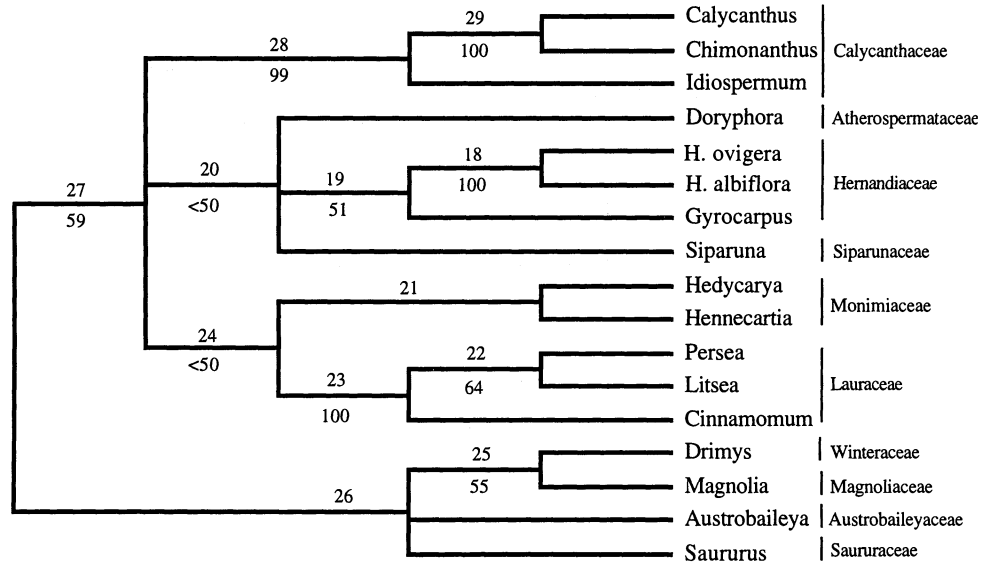

Fig. 3 Strict consensus of the six shortest trees obtained from the *rbc*L analysis (L = 403 steps; CI = .722; RI = .576). The number of character-state transitions supporting each node is given above the horizontal lines, percentage bootstrap replicability below the lines. Inclusion of an *rbc*L sequence of the monotypic Chilean family Gomortegaceae in the analysis places *Siparuna* as sister to *Hernandia* and *Gyrocarpus,* while *Gomortega* becomes sister to *Doryphora* (Ueda et al. 1997; K. Ueda, personal communication).

which is shown in figure 3. *Siparuna* forms a trichotomy with Atherospermataceae and Hernandiaceae. Inclusion of an unpublished *rbc*L sequence of the monotypic Chilean family Gomortegaceae in the analysis resolves the trichotomy and places *Siparuna* as sister to Hernandiaceae, while *Gomortega* is sister to Atherospermataceae (K. Ueda, personal communication, July 1997). The Monimiaceae s.str. (*Hedycarya* and *Hennecartia*) are sister to the Lauraceae, a relationship that is not affected by the presence or absence of *Gomortega* in the data set. The *rbc*L data thus indicate that the Monimiaceae in the wide sense are polyphyletic: their representatives *Siparuna, Doryphora, Hennecartia,* and *Hedycarya* come out in three different places in the Laurales.

Parsimony analysis of the *trn*L-*trn*F sequences resulted in 16 equally parsimonious trees (L = 191 steps; CI = .838; RI = .875), the 50% majority rule consensus of which is shown in figure 4. In the strict consensus, the Hernandiaceae-Lauraceae-Monimi-

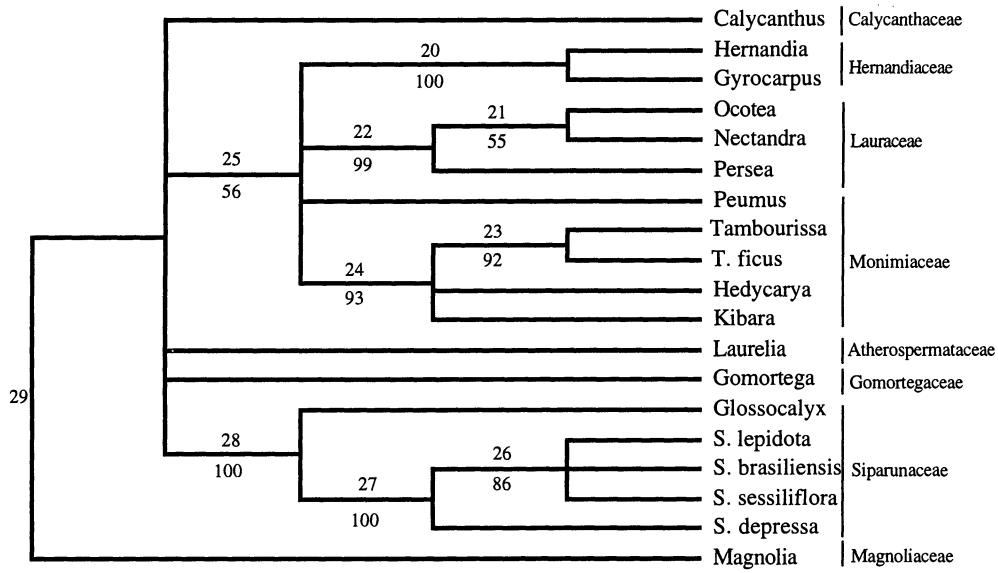

Fig. 4 Phylogenetic hypothesis for Laurales based on the majority rule consensus of 16 equally parsimonious trees obtained from the *trn*L-*trn*F analysis (L = 191 steps; CI = .838; RI = .875). The number of character-state transitions supporting each node is given above the horizontal lines, percentage bootstrap replicability below the lines. In the strict consensus, the Hernandiaceae-Lauraceae-Monimiaceae clade collapses.

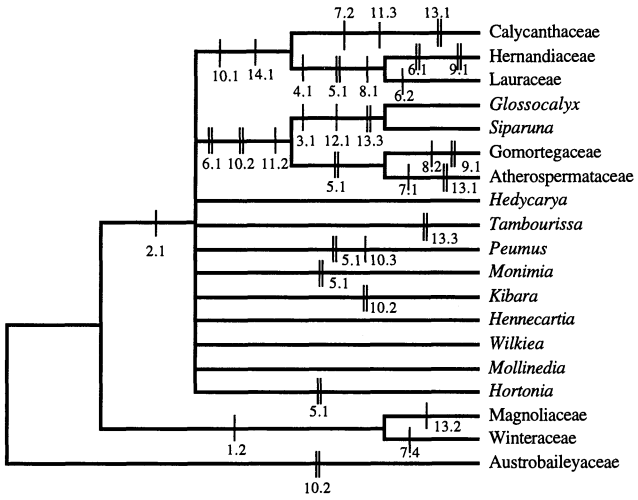

Fig. 5 Strict consensus of the 38 shortest trees obtained from the morphological analysis (L = 38 steps; CI = .789; RI = .778). Numbers refer to characters as discussed in the text and are followed by character states as listed in appendix; equivocal states not shown. See text for discussion.

aceae clade collapses. Exclusion of the calycanthaceous sequence resulted in an almost completely resolved single most parsimonious tree (L = 168; CI = .863; RI = .903), indicating that Calycanthaceae and outgroups need to be sampled more broadly. In all trees, *Siparuna* and *Glossocalyx* form a clade supported by a bootstrap value of 100% and the highest number of nucleotide changes (22) found on the tree. As in the *rbc*L data, there is no support for a monophyletic Monimiaceae.

Parsimony analysis of the morphological matrix yielded 38 equally parsimonious trees (L = 38 steps; CI = 0.789; RI = 0.778), the strict consensus of which is shown in figure 5. The only synapomorphy we could find for Laurales is the presence of a well-developed cup-like receptacle (character [char.] 2.1 in fig. 5). A doubling of the chromosome number from n=11 (char. 10.1) to n=22 (10.2) appears to distinguish two major clades within the order, and staminal glands (fig. 5.1) appear to have arisen several times. The sister group relationship between *Siparuna* and *Glossocalyx* is supported by char. 3.1 (innermost tepals connate and forming a floral roof) and 12.1 (one integument). In addition, they share disporangiate anthers in which the two thecae open not by two, but by a single or incompletely separated flap, a unique state in the Magnoliidae (Endress and Hufford 1989).

The results of all three analyses (*rbc*L, *trn*L-*trn*F, morphology) thus support the conclusion of Schodde (1969, 1970) who suggested that the Atherospermataceae, which he monographed, as well as the Siparunaceae (*Glossocalyx, Siparuna,* and an enigmatic close relative of *Siparuna, Bracteanthus* with two species in Brazil) should be recognized at the family level because there was no evidence that either shared a unique common ancestor with the Monimiaceae or each other. Although Schodde's approach was phenetic, his view of the Atherospermataceae as close to the Gomortegaceae is supported by parsimony analyses of *rbc*L data (Ueda et al. 1997).

Miniaturization of Ovules

Endress (1990) identified a trend toward miniaturization of ovules in Laurales. Based on the phylogenetic hypotheses (figs. 3–5), it apparently occurred with different morphological manifestations in several unrelated clades: once with a loss of endosperm (char. 14.1 in fig. 5) in the Calycanthaceae, Hernandiaceae, and Lauraceae; a second time with a loss of one of the two integuments (char. 12.1) in *Siparuna* and *Glossocalyx.*

Different species of *Siparuna* have between three and 35 carpels, or potential seeds, and the tight packing of these numerous carpels in the floral cups may have selected for diminutive carpels and ovules. (Male flowers have 2–72 stamens immersed in a fleshy receptacle of the same general shape as that of female flowers. There are no consistent patterns in terms of size differences or numbers of reproductive organs among male and female flowers.) *Siparuna* ovule morphology has been studied in detail by Heilborn (1931, *S. eggersii*), Endress (1972, 1980*b; S. thecaphora* as *S. nicaraguensis*), and Schulz-Burck (1997; esp. *S. thecaphora, S. cristata, S. guianensis*). All investigated species have ovules with a single integument. Sections of liquid-preserved flowers of *Glossocalyx longicuspis* Benth., the closest relative of *Siparuna* based on flower morphology (below) and *trn*L-*trn*F spacer sequences (fig. 4), showed that *Glossocalyx,* too, has ovules with a single integument (contra Philipson 1987, 1993). This provides a synapomorphy for these two genera because all other lauralean groups have ovules with two integuments (P. K. Endress and A. Igersheim, personal communication). Together with Atherospermataceae, *Siparuna* and *Glossocalyx* are among the few angiosperms that have plicate carpels with marginal closure and basal insertion of the ovules.

Glossocalyx consists of a single species of straggling shrubs and is quite common along forest margins in Cameroon (D. Thomas and R. Condit, personal communication). It differs from *Siparuna* in having strongly asymmetric leaf pairs and perigons, but shares with that genus at least three uniquely derived features: disporangiate anthers in which the two thecae open by a single or incompletely separated flap; the possession of a floral roof (below); and ovules with a single integument. Because of the first two features, *Glossocalyx* has long been interpretated as being closely related to *Siparuna* (Bentham 1880, pl. 1301; contra Philipson 1987, 1993), a hypothesis supported by the data reported here.

Enclosure of Reproductive Organs and Function of Flowers in Pollination

An unusual feature of *Siparuna* flowers is a membrane, called the floral roof or velum. This membrane covers the sexual organs except for a small pore in the

center. At anthesis, the styles or anthers emerge through this pore. In male flowers, the membrane is thin and sometimes tears. In female flowers, it forms a thick collar that surrounds the emerging style tips tightly, and it does not tear (figs. 1, 2). There are no diurnal movements of floral parts. Based on his study of *S. thecaphora* (as *S. nicaraguensis*), Endress suggested that the floral roof evolved from an inner whorl of tepals that have become completely fused: "the velum is initiated in a ring-like zone of nearly nonmeristematic tissue between the . . . tepal primordia and the highly meristematic floral apex in the centre of the floral cup" and "its position between the tepals and the sporophylls and its very early initiation . . . suggest that it may have evolved from an inner whorl of tepals" (Endress 1980*b*).

Pollination in *Siparuna* has been studied in Ecuador (Feil and Renner 1991; Feil 1992) and in Manaus (Schulz-Burck 1997). *Siparuna* is pollinated at night by cecidomyiids (*Asynapta, Clinodiplosis,* and *Dasineura,* Oligotrophini, Porricondylinae). These gall midges are ca. 3 mm long and capable of directed flight (although usually poor fliers; R. Gagné, personal communication). They are nocturnal and find food, mates, and oviposition sites by scent. Receptive *Siparuna* flowers, like the rest of the plant, are strongly lemon-scented. Female midges alight on flowers, insert their abdomens into the pore in the floral roof, and then move it rhythmically for ca. 1–2 min during which they deposit an egg into the flower. Gall midges have long hairs on their abdomens (Gagné 1989), and pollen grains become lodged between these hairs when the abdomen is inserted into male flowers. Plant-feeding midges distribute their numerous eggs onto different flowers and plants (Gagné 1989). Sixty of the 72 species of *Siparuna* are dioecious and can only set fruit if they are visited and pollinated by insects, which implies that pollen-covered midges visit both sexes regularly. Tropical midge species produce a new generation every 10–20 d (Gagné 1994), and flowers of different species of *Siparuna* last 3–24 d (Feil 1992; Schulz-Burck 1997). Larvae may therefore complete development in flowers on the tree, or they may hatch from flowers that have fallen to the ground. In populations studied in Ecuador (Feil and Renner 1991; Feil 1992), male flowers regularly contain at least two cecidomyiid larvae (which can be identified by their characteristic morphology) while female flowers and mature fruits almost never do. Thus, either female flowers are less suitable as egg-laying sites or female flowers with larvae abort.

Of the other lauralean lineages, Atherospermataceae are pollinated by flies and bees (Schodde 1969), Calycanthaceae by beetles (summary in Kubitzki 1993*a*), Lauraceae by small bees, flies, and sometimes thrips (Kubitzki and Kurz 1984; Norton 1984; D. Roubik, personal communication), and Monimiaceae s.str. by flies and small beetles (Lorence 1985). Pollination in Hernandiaceae and *Gomortega keule* remains unstudied, but based on the size and shape of the flowers and the presence of large nectary glands at the filament bases it is likely to be by small flies or bees. Phylogenetically, Siparunaceae may have evolved from ancestors that had stamens with nectar glands and rewarded their pollinators with liquid food. With an evolutionary switch from small bee or fly pollination to gall midge pollination and oviposition sites as a reward, nectar glands may have become unnecessary (these large convex glands also seem unsuited for nectar-uptake by gall midge mouthparts).

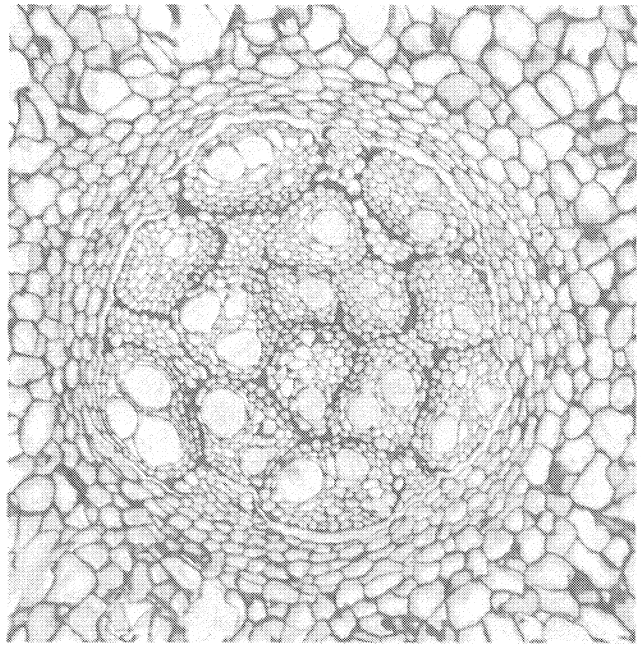

Fig. 6 *Siparuna thecaphora.* Transverse section of flower at the height where the styles (in this case 14) pass through the pore in the floral roof. Note the small-celled collar formed by the floral roof around the stylar complex and the stylar exudate (stained dark blue). Abundant yellow oil inclusions are typical of Laurales.

Gall midge pollination, which after all involves the presence of plant-feeding larvae in flowers, may also have provided the selective pressure for floral closure, at least of the female flowers, to prevent excessive damage. When a midge visits a female flower, she tries to insert her ovipositor into the pore of the floral roof, but different from the situation in staminate flowers, the pore in pistillate ones is smaller and moreover closed by the densely packed styles (figs. 1, 2, 6). We have never found larvae or eggs inside stylar tissue, supporting the idea that egg laying into the stylar complex is rare. Apparently, the contact between pollen-carrying midge abdomens and styles during (unsuccessful) oviposition attempts is enough to ensure pollination. Over evolutionary time, an increasing closure of flowers to protect them against egg-laying midges would have left less and less space for glandular structures on the stamens, but it is difficult to disentangle the sequence of these ecological and morphological changes.

Functional syncarpy

The third floral-morphological trend identified by Endress (1979, 1980*b*, 1982) in lower angiosperms concerns the aquisition of functional syncarpy in apocarpous gynoecia. Two forms of this have evolved in the Laurales, all of them in lineages with strictly unisexual flowers. In the Monimiaceae s.str., styles and/or the upper rim of the floral cup may secrete large amounts of mucilage (*Faika, Hedycarya, Hennecartia, Tambourissa, Wilkiea*). Pollen loads germinate in this mucilage and may be distributed onto different carpels (each with one ovule). In *Siparuna* and *Glossocalyx,* by contrast, functional syncarpy is achieved by fusion of the styles, starting just below the point at which they emerge through the pore in the floral roof (figs. 1, 2, 6). The tight packing of the styles apparently sets the stage for their postgenital fusion and thus the development of a joint pollen transmission track. In the eight species of which flowers were sectioned (see "Material and Methods" for species names), stylar complexes of various lengths (0.3 mm long in the very small flowers of *S. depressa,* ca. 1 mm long in *S. poeppigii*) and complexity were observed, with the identity of individual styles more or less lost in the center portion of the stylar complexes. In *S. cristata,* Schulz-Burck (1997) was able to reconstruct the path individual pollen tubes had taken in the stylar complex in consecutive cross sections from the stigmatic areas down to where tubes entered the carpel and then micropyle. Lateral growth of pollen tubes in the middle portion of stylar complex, and thus switching between "styles," was observed in a flower that had been experimentally pollinated and thus had received (unusually?) large pollen loads. Nothing is known about the size of pollen loads normally deposited on stigmas by midges. Based on field and herbarium observations, seed set in *Siparuna* seems regular, with young fruiting flowers containing only slightly fewer fruitlets than they have carpels.

Based on *rbc*L data (fig. 3), Siparunaceae are closest to Hernandiaceae, Gomortegaceae, and Atherospermataceae, while morphologically they are closest to the Atherospermataceae and Gomortegaceae (fig. 5); the *trn*L-*trn*F data provide no resolution at this level (fig. 4). *Gomortega keule* is the only species in the Laurales with a syncarpous and inferior gynoecium. Gynoecia in Hernandiaceae are inferior but not syncarpous, while Siparunaceae and Atherospermataceae have perigynous flowers and apocarpous gynoecia. Because of its pollen exine structure *Gomortega* has traditionally been placed near Lauraceae and Hernandiaceae (Kubitzki 1993*b;* contra Schodde 1969, 1970), but in terms of wood anatomy it is closest to Atherospermataceae (H. G. Richter, personal communication). Its chromosome number of n=21 (Goldblatt 1976) fits well with those of both Atherospermataceae, n=22, and Siparunaceae, n=22, while Hernandiaceae are strikingly variable in chromosome number (Morawetz 1986).

Conclusions

Reconstruction of phylogenetic relationships within Laurales is still in its initial phase. This investigation has shown how the topological position of *Siparuna* near *Glossocalyx,* the Hernandiaceae, Gomortegaceae, and Atherospermataceae, and away from the Monimiaceae s.str., affects the interpretation of the evolution of floral-morphological trends in this genus. However, floral morphological trends, such as the loss of nectary glands in the androecium of *Siparuna* and the evolution of its closed flowers and stylar complexes, need to be placed in an ecological as well as phylogenetic context. Thus, the peculiar pollination of *Siparuna* by ovipositing gall midges is what set the stage for loss of nectar and divergence between male and female flowers in the extent to which they are sealed off and protected. Postgenital stylar fusion may have arisen as a means to completely close the central pore, the only opening in the floral roof and later may have assumed a role in interstylar exchange of pollen tubes between basally free carpels.

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Appendix

DATA SET USED IN MORPHOLOGICAL CLADISTIC ANALYSIS OF LAURALES

	1					6					11				
Austrobaileyaceae	1	0	0	0	0	0	0	0	0	1	0	0	4	0	1
Magnoliaceae	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Winteraceae	2	0	0	0	0	0	4	0	0	?	0	0	4	0	0
Calycanthaceae	1	1	0	0	0	0	2	0	0	1	3	0	1	1	1
Hedycarya	1	1	0	0	0	0	3	0	0	0	1	0	0	0	1
Tambourissa	1	1	0	0	0	0	3	?	0	0	1	0	3	0	1
Peumus	1	1	0	0	1	0	3	0	0	3	1	0	0	0	1
Monimia	1	1	0	0	1	0	3	0	0	4	1	0	3	0	1
Kibara	1	1	0	0	0	0	3	0	0	1	1	0	0	0	1
Hennecartia	1	1	0	0	0	0	3	?	0	5	1	0	3	0	1
Wilkiea	1	1	0	0	0	0	3	0	0	0	1	0	0	0	1
Mollinedia	1	1	?	0	0	0	3	0	0	0	1	0	0	0	1
Hortonia	1	1	0	0	1	0	3	0	0	0	1	0	0	0	1
Glossocalyx	1	1	1	0	0	1	3	0	0	?	2	1	3	0	1
Siparuna	1	1	1	0	0	1	3	0	0	2	2	1	3	0	1
Gomortegaceae	1	1	0	0	1	1	3	2	1	2	?	0	0	0	1
Atherospermataceae	1	1	0	0	1	1	1	0	0	2	2	0	1	0	1
Hernandiaceae	0	1	0	1	1	1	3	1	1	?	1	0	0	1	1
Lauraceae	(0/1)	1	0	1	1	2	3	1	0	1	1	0	0	1	1

Note. Inapplicable data and missing data are indicated as "?" and polymorphisms are enclosed in brackets.

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