



Phylogeny, Molecular and Fossil Dating, and Biogeographic History of Annonaceae and Myristicaceae (Magnoliales)

Author(s): James A. Doyle, Hervé Sauquet, Tanya Scharaschkin, and Annick Le Thomas

Source: *International Journal of Plant Sciences*, Vol. 165, No. S4, Tropical Intercontinental Disjunctions (July 2004), pp. S55-S67

Published by: [The University of Chicago Press](#)

Stable URL: <http://www.jstor.org/stable/10.1086/421068>

Accessed: 17/09/2015 20:34

Your use of the JSTOR archive indicates your acceptance of the Terms & Conditions of Use, available at <http://www.jstor.org/page/info/about/policies/terms.jsp>

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact support@jstor.org.



The University of Chicago Press is collaborating with JSTOR to digitize, preserve and extend access to *International Journal of Plant Sciences*.

<http://www.jstor.org>

PHYLOGENY, MOLECULAR AND FOSSIL DATING, AND BIOGEOGRAPHIC HISTORY OF ANNONACEAE AND MYRISTICACEAE (MAGNOLIALES)

James A. Doyle,* Hervé Sauquet,¹† Tanya Scharaschkin,‡ and Annick Le Thomast

*Section of Evolution and Ecology, University of California, Davis, California 95616, U.S.A.;

†Ecole Pratique des Hautes Etudes, Muséum National d'Histoire Naturelle, 16 rue Buffon,

75005 Paris, France; and ‡School of Life Sciences, University of Queensland,

Brisbane, Queensland 4072, Australia

Annonaceae and Myristicaceae, the two largest families of Magnoliales, are pantropical groups of uncertain geographic history. The most recent morphological and molecular phylogenetic analyses identify the Asian-American genus *Anaxagorea* as sister to all other Annonaceae and the ambavioids, consisting of small genera endemic to South America, Africa, Madagascar, and Asia, as a second branch. However, most genera form a large clade in which the basal lines are African, and South American and Asian taxa are more deeply nested. Although it has been suggested that *Anaxagorea* was an ancient Laurasian line, present data indicate that this genus is basically South American. These considerations may mean that the family as a whole began its radiation in Africa and South America in the Late Cretaceous, when the South Atlantic was narrower, and several lines dispersed from Africa-Madagascar into Laurasia as the Tethys closed in the Tertiary. This scenario is consistent with the occurrence of annonaceous seeds in the latest Cretaceous of Nigeria and the Eocene of England and with molecular dating of the family. Based on distribution of putatively primitive taxa in Madagascar and derived taxa in Asia, it has been suggested that Myristicaceae had a similar history. Phylogenetic analyses of Myristicaceae, using morphology and several plastid regions, confirm that the ancestral area was Africa-Madagascar and that Asian taxa are derived. However, Myristicaceae as a whole show strikingly lower molecular divergence than Annonaceae, indicating either a much younger age or a marked slowdown in molecular evolution. The fact that the oldest diagnostic fossils of Myristicaceae are Miocene seeds might be taken as evidence that Myristicaceae are much younger than Annonaceae, but this is implausible in requiring transoceanic dispersal of their large, animal-dispersed seeds.

Keywords: Annonaceae, Myristicaceae, phylogeny, biogeography, paleobotany, molecular dating.

Introduction

Magnoliales as defined in the Angiosperm Phylogeny Group (APG 1998; APG II 2003) system are a strongly supported clade of six families. Three are Australasian and consist of one genus with two species each—*Degeneria* (= Degeneriaceae) on Fiji, *Eupomatia* (= Eupomatiaceae) and *Galbulimima* (= Himantandraceae) in Australia and New Guinea—considered to be of Gondwanan origin (Raven and Axelrod 1974; Schuster 1976). The larger family Magnoliaceae shows a classic Asian–North American disjunct distribution, with an abundant fossil record all around Laurasia (Azuma et al. 2001). In contrast, Annonaceae and Myristicaceae are still larger pantropical families with more controversial geographic histories.

Although Magnoliales were long considered the most primitive angiosperm group (Cronquist 1981) and were the sister group of all other angiosperms in the morphological cladistic analysis of Donoghue and Doyle (1989), large-scale

molecular and combined morphological and molecular analyses (Mathews and Donoghue 1999; Qiu et al. 1999, 2000; Doyle and Endress 2000; Graham and Olmstead 2000; Soltis et al. 2000; Zanis et al. 2002) nest the order well within the angiosperms, usually in a clade referred to as eumagnoliids, which also includes Laurales, Winterales, and Piperales. These analyses and the more focused study of Sauquet et al. (2003) link Annonaceae with *Eupomatia* and place Myristicaceae at the base of the Magnoliales (fig. 1).

Annonaceae are the largest family of “basal” angiosperms, with about 2500 species. Sinclair (1955) and Takhtajan (1969) took the great number of species in the Asian tropics as evidence that Annonaceae originated in that region. The family was therefore thought to support the view that angiosperms as a whole originated “between Assam and Fiji” (Takhtajan 1969; Smith 1973) and dispersed into Africa and the Americas. However, the rise of the theory of plate tectonics led to consideration of a Gondwanan origin. In a study emphasizing pollen morphology, Walker (1971) proposed trends from the presumed primitive monosulcate pollen type to tetrads and inaperturate grains. He was impressed that monosulcate taxa are most diverse in South America, whereas most Asian taxa, although rich in species, have inaperturate pollen. He therefore concluded that the family originated in South America, or Africa–South America when the two

¹ Current address: Department of Palaeobotany, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, Sweden.

Manuscript received July 2003; revised manuscript received March 2004.

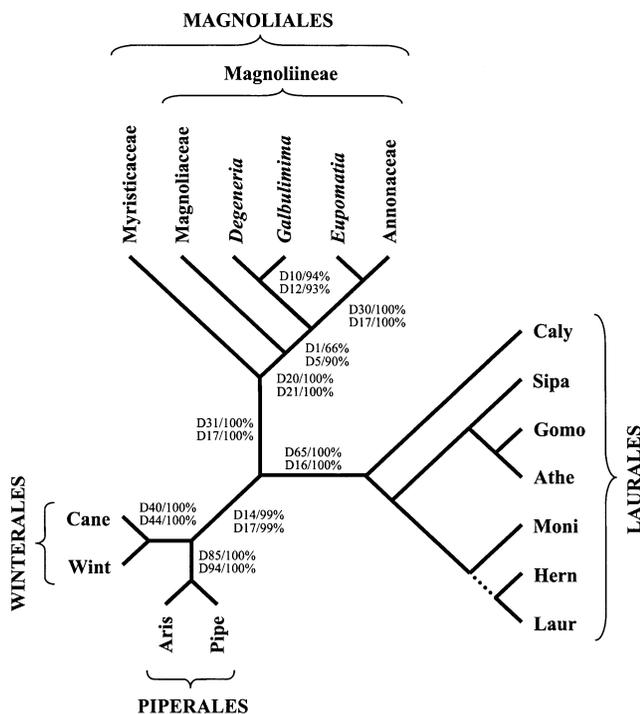


Fig. 1 Unrooted tree of Magnoliales, Laurales, Winterales, and Piperales found in parsimony analyses of all molecular and combined morphological and molecular data by Sauquet et al. (2003). Branch support is indicated by decay and bootstrap values for the molecular analysis (above) and the morphological and molecular analysis (below). Dotted line indicates a branch that was not found in the molecular analysis. Cane = Canellaceae; Wint = Winteraceae; Aris = Aristolochiaceae; Pipe = Piperaceae; Caly = Calycanthaceae; Sipa = Siparunaceae; Gomo = *Gomortega*; Athe = Atherospermataceae; Moni = Monimiaceae; Hern = Hernandiaceae; Laur = Lauraceae.

continents were still joined, and later spread into Asia. Raven and Axelrod (1974) cited Annonaceae as evidence for their view that the angiosperms as a whole originated in western Gondwana. Based on TEM studies (Le Thomas and Lugardon 1974, 1975, 1976; Lugardon and Le Thomas 1974), Le Thomas (1980–1981) shifted attention toward Africa or Africa–South America as the area of origin based on the recognition that many African genera are not only monosulcate but also have granular exine structure, which was considered more primitive than columellar structure, the type found in most American monosulcates.

Doyle and Le Thomas (1994, 1996) undertook to test these hypotheses with cladistic methods, using morphological data. Their results confirmed that granular monosulcate pollen is ancestral in Annonaceae and that columellar structure, inaperturate pollen, and tetrads are derived. Successive branches in their trees were *Anaxagorea*, the only genus with an Asian and American distribution, which is plesiomorphic in having granular monosulcate pollen and laminar rather than peltate stamens; the ambavioid clade, including *Ambavia* in Madagascar, *Cleistopholis* in Africa, and *Tetrameranthus* in South America; several lines with monosulcate and disulcate pollen (malmeoids, piptostigmoids, miliusoids, and two smaller lines); and a large clade with inaperturate pollen, often in

tetrads, which includes the well-known genera *Annona*, *Asimina*, *Xylopi*a, and *Uvaria*, called the inaperturates.

Le Thomas and Doyle (1996b) and Doyle and Le Thomas (1997) considered implications of these results for biogeography by plotting geographic distribution as a cladistic character on one of their most parsimonious trees (fig. 2). Africa and Madagascar were treated under one state, and Asia and North America were combined into a Laurasian state. Parsimony optimization indicated that the ancestral area for the family could be any of the areas recognized, but within the family the inferred ancestral area for *Anaxagorea* was Laurasia or South America, while the original area for the line leading to the rest of the family was Africa–Madagascar. Doyle and Le Thomas suggested that this pattern reflects an ancient split into a Laurasian and a Gondwanan line, probably in the mid-Cretaceous, when Laurasia and Gondwana were still almost connected between Europe and Africa and Magnoliales appear in the fossil record (Dilcher and Crane 1984; Ward et al. 1989). They suggested that *Anaxagorea* entered South America much later, from Central America, as the two continents moved together. The other line radiated in Africa and South America in the Late Cretaceous, when the South Atlantic was still narrow enough for exchange in both directions. Except for *Anaxagorea*, Laurasian taxa were all nested within African–South American clades. These included several groups found in both Africa and Asia: *Uvaria* and related lianas, *Artabotrys*, and *Xylopi*a, which is pantropical. These were interpreted as lines that spread from Africa or India into Laurasia as these continents collided in the Early Tertiary. Le Thomas and Doyle (1996a) suggested that India may have acted as a “raft” that transported some taxa from Madagascar to Asia. The one Australian group, *Ancana*, is nested within an Asian clade, indicating that it may have reached Australia from Asia as Australia moved north.

This scenario needs reexamination in the light of molecular data. A combined analysis of *rbcL* sequences and the morphological data set of Doyle and Le Thomas (1996) by Doyle et al. (2000) confirmed many of the morphological results of Doyle and Le Thomas (1994, 1996), such as the basal position of *Anaxagorea* and the existence of a large inaperturate clade. However, the several lines between the ambavioids and the inaperturates were consolidated into a single malmeoid-piptostigmoid-miliusoid (MPM) clade, *Cananga* moved from near *Xylopi*a to the base of the ambavioids, and the uvaroid lianas moved from the base of the inaperturate clade to a position nested within it. The *rbcL* data supported a different position of the ambavioids, linked with the inaperturates rather than basal to both the MPM and inaperturate clades, but both positions were equally parsimonious in the combined analyses.

Support for most of these shifts increased with molecular analyses of Magnoliales as a whole by Sauquet et al. (2003; fig. 3), which included fewer taxa of Annonaceae but sequences of many more genes and noncoding regions. These analyses contradict one aspect of the *rbcL* trees, namely, the position of the ambavioids, supporting instead the arrangement found with morphology, in which the ambavioids are basal to the MPM and inaperturate clades. In addition, analyses of relationships within *Anaxagorea* (Scharaschkin 2003, 2004), based on several plastid regions and morphology,

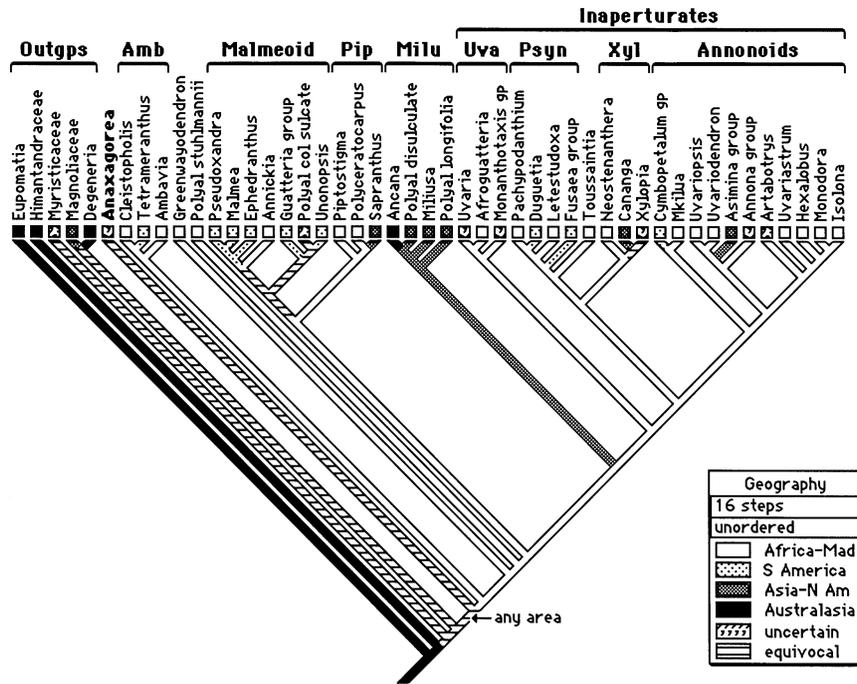


Fig. 2 Representative tree of Annonaceae and related families found in the morphological cladistic analysis of Doyle and Le Thomas (1996) with geography plotted as an unordered multistate character (Doyle and Le Thomas 1997). Outgps = outgroups; Amb = ambavioids; Pip = piptostigmoids; Milu = miliusoids; Uva = uvarioids; Psyn = pseudosyncarpis; Xyl = xylopioids; Polyal = Polyalthia.

require reassessment of the concept of an original split into Laurasian and Gondwanan lines because they imply that the ancestral area for this genus was South America rather than Laurasia.

Ideas on the biogeography of Myristicaceae, which contain about 500 species, have paralleled those on Annonaceae, but they are less developed because of less attention to the phylogeny of Myristicaceae. Warburg (1897) suggested that the most primitive member of the family was the Malagasy genus *Mauloutchia* because of its numerous stamens with nearly free filaments, and he divided the rest of the family into groups of genera limited to one continent or another, but he did not attempt to infer an area of origin for the family. Sinclair (1958) assumed that Myristicaceae originated in Asia. However, an Asian origin was questioned by Walker (1971), who suggested that Myristicaceae, like Annonaceae, originated in Africa or South America based on indications that the Asian genera are advanced in their floral morphology, and this concept was supported by Raven and Axelrod (1974) and Leroy (1978). Walker and Walker (1981) further supported this view based on palynological studies, in which they interpreted *Mauloutchia* and another Malagasy genus, *Brochoneura*, as the only Myristicaceae with granular exine structure, then considered primitive in angiosperms.

This interpretation too needs reexamination because phylogenetic analyses of Myristicaceae and other Magnoliales (Sauquet et al. 2003) indicate that *Mauloutchia* and *Brochoneura* are not basal in Myristicaceae and that their granular exine structure is derived (actually within *Mauloutchia*) rather than ancestral (Sauquet and Le Thomas 2003). Closer examination of *Mauloutchia*, including new species from

Madagascar, showed a remarkable diversity in androecial morphology, ranging from the numerous, apparently spiral stamens of *M. chapelieri*, emphasized by Warburg (1897) and Walker and Walker (1981), to only six sessile anthers in other species (Sauquet 2003). The morphological analyses of Sauquet et al. (2003) associated *Mauloutchia* with *Brochoneura*, the new Malagasy genus *Doyleanthus* (Sauquet 2003), and two African genera, *Staudtia* and *Cephalosphaera*, and nested *M. chapelieri* within *Mauloutchia*. Even after amassing ca. 7 kB of new sequences from rapidly evolving plastid regions, the rooting and internal relationships of the family were not conclusively resolved due to a surprisingly low level of molecular divergence. Parsimony analyses often identified the American genus *Compsoeura* as sister to the rest of the family, but Sauquet et al. (2003) presented evidence that this rooting is a result of long-branch attraction. First, parsimony analyses of all characters but only taxa with less than 30% missing data yielded a rooting nearer the mauloutchioids but with the African genera *Pycnanthus* and *Coelocaryon* (pycnanthoids) linked to them (fig. 4). Second, the same rooting of Myristicaceae was obtained with maximum likelihood analyses of those molecular data sets with reduced taxon sampling that showed the *Compsoeura* rooting when analyzed with parsimony. Despite these uncertainties, all these analyses confirmed that *Mauloutchia* and the associated genera form a clade, called the mauloutchioids, supported not only by substitution data but also by a 43-bp insertion in the *trnL-trnF* spacer in all genera except *Staudtia* (favoring a basal position for this genus), and that *Mauloutchia* is nested within this clade. In most analyses the remaining genera form a large clade, called the myristicoids.

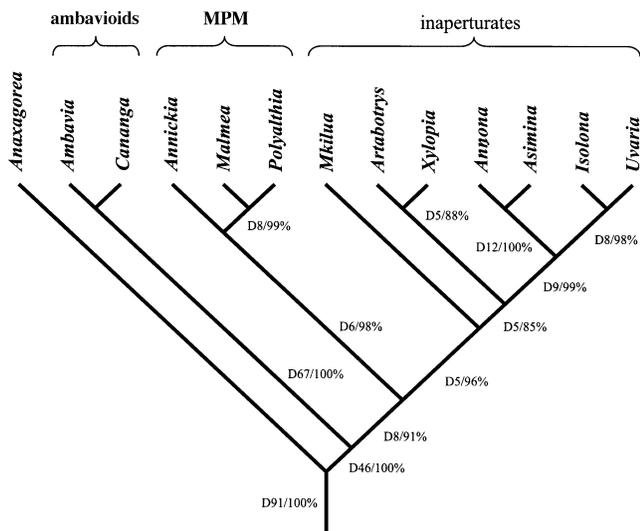


Fig. 3 Relationships within Annonaceae found in the parsimony analysis of all molecular data by Sauquet et al. (2003). Branch support is indicated by decay and bootstrap values. MPM = malmeoid-piptostigmoid-miliusoid clade.

In this article we evaluate the implications of these improved data for the geographic history of Annonaceae and Myristicaceae by synthesizing the results of optimization of geographic distributions on trees, molecular estimates of divergence times, and fossil evidence.

Material and Methods

For phylogenetic relationships of Annonaceae, we used results of the analyses of Doyle et al. (2000), based on *rbcL* and the morphological data set of Doyle and Le Thomas (1996). For relationships among Magnoliales as a whole and in Myristicaceae, we used results of Sauquet et al. (2003), which were based on 115 morphological characters; published data for 18S rDNA, *rbcL*, *atpB*, *atp1*, and *matR*; and original sequences of *ndhF*, the *trnK* 5' intron, *matK*, the *trnK* 3' intron, *trnT-trnL*, the *trnL* intron, and *trnL-trnF*. Some analyses were restricted to genes that were found to be relatively rapidly evolving (*ndhF* through *trnL-trnF*), informally designated as "fast," and to taxa with less than 30% missing data.

We used MacClade (Maddison and Maddison 2001) to reconstruct geographic history on the published trees, with different distribution areas coded as states of an unordered multistate character; this program optimizes states across the tree on the basis of parsimony. This approach treats vicariance (split of a continuous range by a new external barrier), dispersal, and extinction as equally probable events, comparable to character-state changes in a morphological character. Thus when sister clades occur in two different areas (0, 1) and parsimony optimization indicates that the ancestral area is uncertain (0/1), this could be a result of vicariance, in which the ancestor existed in a combined area that later split, or it could mean that the line originally existed in one area

and then dispersed to the other. However, vicariance would be favored if two or more related clades showed the same pattern, which might be explained by the same vicariance event. On the other hand, when a taxon restricted to one area (1) is nested within a clade in which the other lines are in another area (0), the reconstructed ancestral area of the nested taxon and its sister group is (0), and dispersal from (0) to (1) is favored. Such a pattern could come about by vicariance but only if each of the more basal lines once had relatives in the other area (1) that became extinct, which would be less parsimonious in terms of total events.

In defining geographic states and scoring taxa, we have made three main changes from the scheme of Doyle and Le Thomas (1997). First, we have split the "Laurasian" state into a North American state (3) (including Central America and the West Indies) and an Asian state (4) in order to introduce fewer prior assumptions concerning the historical relationships of these areas. Second, we have scored *Anaxagorea* in two different ways: first, as either South American, North American, or Asian (2/3/4), corresponding to the situation (as before) in which there was no evidence from internal relationships of which of these areas was ancestral; and second, as South American (2), based on the phylogenetic results of Scharaschkin (2003, 2004). Third, we have rescored the *Annona* group, previously scored as either African or South American, as African, South American, or North American, based on the fact that the supposedly primitive and potentially basal sections of *Annona*, namely *Atta* and *Euannonia*, are primarily West Indian and South American, respectively (Fries 1959; H. Rainer, personal communication). Doyle and Le Thomas (1997) scored several taxa that occur in both South and Central America as South American (*Cymbopetalum* group, *Duguetia*, *Guatteria*, *Malmea*, *Unonopsis*, *Xylopia*) because of their far greater diversity in South America

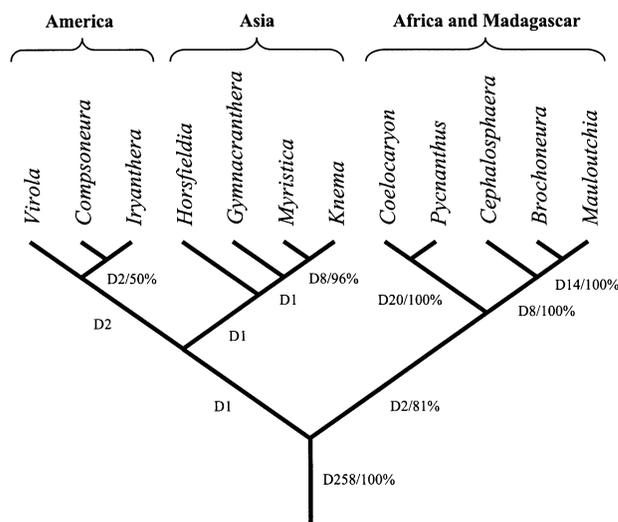


Fig. 4 Relationships within Myristicaceae found with parsimony analysis of combined morphological and molecular data with taxon sampling reduced to taxa with less than 30% missing data (Sauquet et al. 2003). Branch support is indicated by decay and bootstrap values (not given when less than 50%).

(cf. Fries 1959); in the case of *Malmea*, the systematic relationships of the Central American species imply that they represent recent immigrants to this region following emergence of the Panama land bridge (Chatrou 1997). This contrasts with the situation in *Sapranthus*, which is limited to Central America. We have retained this treatment, but the assumption that the Central and South American groups were originally South American needs testing by means of phylogenetic studies of relationships within these genera.

For molecular dating experiments, we calculated ages for nodes based on the combined “fast” gene data set with reduced taxon sampling (Sauquet et al. 2003) using three methods. All analyses began with branch lengths estimated by maximum likelihood in PAUP* (Swofford 2002), assuming a GTR + Γ + I model of molecular evolution (the same data that were used to construct the phylogram in Sauquet et al. 2003, fig. 11). We transferred these data to the program r8s of Sanderson (2003), with which we calculated ages using (1) the assumption of a molecular clock (“Langley-Fitch”); (2) nonparametric rate smoothing (NPRS; Sanderson 1997); and (3) penalized likelihood (Sanderson 2002), which estimates the appropriate level of rate smoothing from the branch length data by a cross-validation procedure before estimating ages. The resulting “chronograms” were calibrated in r8s by specifying two alternative ages, 100 and 120 m.yr., for the most recent common ancestor of Magnoliaceae and Annonaceae, based on fossil evidence discussed below.

Results and Discussion

Annonaceae

In order to evaluate the biogeographic implications of the more recent phylogenetic results, we optimized geographic distribution on one of the most parsimonious trees of Doyle et al. (2000) with the changes in scoring described above (fig. 5). The tree selected is one of those in which the ambavioids are basal to the MPM and inaperturate clades, consistent with both morphological data (Doyle and Le Thomas 1996) and analyses of additional molecular sequences (Sauquet et al. 2003; fig. 3). One important change for biogeography is the shift of the Asian genus *Cananga* (and presumably the related Asian genus *Cyathocalyx*) to the ambavioid clade, the other members of which appear to be Gondwanan (*Ambavia* in Madagascar, *Cleistopholis* in Africa, *Tetrameranthus* in South America). However, implications of this tree vary depending on interpretation of the original area of *Anaxagorea*.

When the ancestral area of *Anaxagorea* is treated as uncertain (fig. 5A), the inferred ancestral area for the family could be any of the areas recognized except Australasia, or any of these combined, while the ancestral area for *Anaxagorea* could be either South America, North (including Central) America, and/or Asia. If outgroups are added in the currently preferred arrangement (fig. 3), in which the closest outgroups (*Degeneria*, *Galbulimima*, and *Eupomatia*) are Australasian,

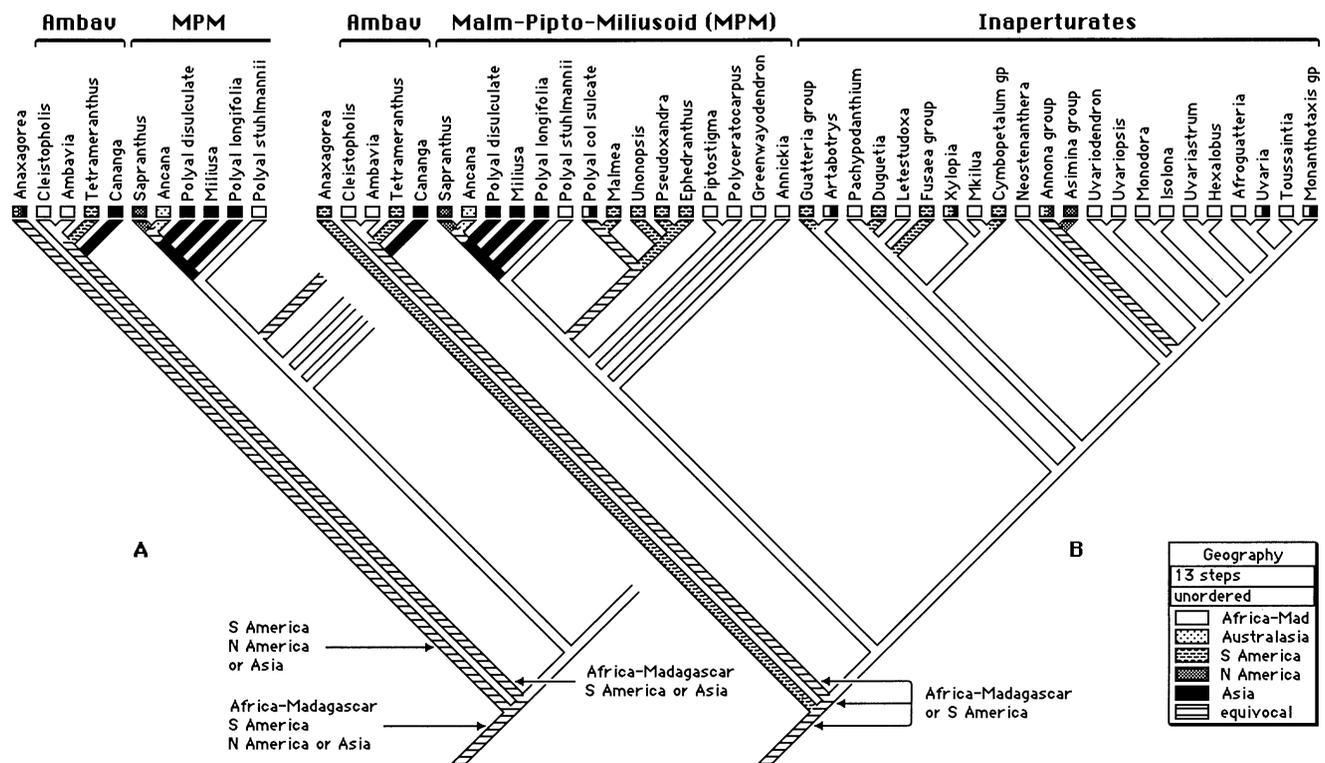


Fig. 5 Representative tree of Annonaceae found in the combined analysis of morphology and *rbcl* sequences by Doyle et al. (2000) with geographic distribution plotted as an unordered multistate character. **A**, With *Anaxagorea* scored as either South American, North (including Central) American, or Asian; **B**, with *Anaxagorea* scored as South American, based on the phylogenetic analyses of Scharaschkin (2003, 2004). Ambav = ambavioids.

all five areas are potentially ancestral. These results are similar to those of Doyle and Le Thomas (1997). However, because of the presence of *Cananga* at the base of the ambavioids, the ancestral area of the clade comprising all Annonaceae except *Anaxagorea* is not Africa but rather Africa, South America, and/or Asia, and the same is true for the ambavioids. Still, above this point the ancestral area of the MPM-inaperturate clade is Africa, as inferred previously.

This scheme is still consistent with the concept of Doyle and Le Thomas (1997) that the basal split in the family represents a Laurasian-Gondwanan vicariance event in which a Laurasian line leading to *Anaxagorea* was separated from a Gondwanan line that gave rise to the rest of the family. The addition of *Cananga* to the ambavioids raises the possibility that the ancestor of this clade also occurred in both Laurasia and Gondwana before splitting into a line leading to *Cananga* in Laurasia and a line leading to the other ambavioids in Gondwana. However, to interpret both the Laurasian distribution of *Anaxagorea* and the basal split in the ambavioids as due to the same vicariance event would require that *Anaxagorea* and the MPM-inaperturate clade once had sister groups in Gondwana and Laurasia, respectively, that later became extinct. This is certainly possible but less parsimonious than a scenario involving dispersal across the Tethys. Actually, there may be an additional Laurasian-Gondwanan disjunction in the ambavioids, given that molecular analyses by P. Bygrave (personal communication) indicate that the Asian genus *Mezzettia* (not included by Doyle and Le Thomas 1994, 1996) also belongs to the ambavioids, consistent with its two lateral ovules, three integuments, thick endosperm ruminations, and chromosome number of $n = 7$ (Okada and Ueda 1984; van Setten and Koek-Noorman 1992; Doyle and Le Thomas 1996).

A very different and less ambiguous picture emerges when *Anaxagorea* is scored as South American (fig. 5B), based on phylogenetic analyses of the genus by Scharaschkin (2003, 2004). In this case the ancestral area for the family is reconstructed as South America and/or Africa, as are the ancestral areas for the remaining Annonaceae and for the ambavioid line. Above the ambavioids, the ancestral area for the MPM-inaperturate clade is Africa, as inferred before.

This scheme would clearly restrict the ancestral area of the family to Gondwana. Rather than a Laurasian-Gondwanan split, *Anaxagorea* could be the product of an African-South American split caused by opening of the South Atlantic in the Aptian-Albian or by dispersal across the new narrow ocean. Again, relationships among the remaining Annonaceae conflict with the notion that a single vicariance event was responsible for both the divergence of *Anaxagorea* and the existence of South American and African taxa in the ambavioids, favoring instead dispersal of several lines across the South Atlantic rift and differentiation of some lines (notably *Anaxagorea* but presumably also *Tetrameranthus*) in South America and others in Africa. Because the inferred ancestral area of the ambavioids is Africa and/or South America, the Asian distribution of *Cananga* (and *Cyathocalyx*) is most parsimoniously interpreted as a result of dispersal from Africa or South America to Laurasia rather than fragmentation of a continuous distribution by widening of the Tethys.

Under either interpretation of *Anaxagorea* (fig. 5A, 5B), the trees indicate a similar history for the MPM-inaperturate clade and one more consistent with the scenario of Doyle and Le Thomas (1997). In particular, the radiation of the rest of the family appears to have been centered in Africa. In terms of parsimony, it is difficult to interpret the partition of taxa into Africa and South America as a result of vicariance. There are many American-African pairs, such as the *Cymbopetalum* group in America and *Mkilua* in Africa, or the *Gutteria* group in America and *Artabotrys* in Africa and Asia, but there are more African branches below them. A more parsimonious interpretation is that the South American taxa represent several transatlantic dispersals from Africa, presumably in the Late Cretaceous, when the South Atlantic was narrower. To reconcile the observed pattern with one vicariance event, one would have to assume that there were once many lines in South America that later became extinct. However, this is the opposite of what would be expected based on the species diversity and climatic history of the two continents (Raven and Axelrod 1974; Morley 2000). Africa was much more affected by Late Tertiary aridization and Pleistocene climatic fluctuations than South America, and this is reflected in the smaller number of species in the African genera of Annonaceae.

As in the scenario of Doyle and Le Thomas (1997), most Laurasian lines within the MPM-inaperturate clade appear to be derived from Africa-Madagascar. This is consistent with the view that they entered Laurasia as a result of the collision of Africa and/or India with Eurasia in the Late Cretaceous or Early Tertiary. The ancestral area of *Asimina* and the *Annona* group is reconstructed as Africa and/or North America (including the West Indies, where *Annona* is diverse). This could mean that the lines leading to *Asimina* and *Annona*, or their common ancestor, dispersed from Africa to Laurasia but survived in North America rather than Asia (or never extended to Asia). The Central American genus *Sapranthus* belongs to the predominantly Asian miliusoid group, indicating that it may be a relict of a formerly more widespread Laurasian line within the miliusoids.

This scenario is also consistent with the fossil record. Some Late Cretaceous and Tertiary leaves have been identified as Annonaceae (e.g., Hollick 1906; Wolfe 1977), but leaves are less diagnostic than the seeds of the family. These have testal ruminations, like the three most closely related families of Magnoliales (but unlike Magnoliaceae, which lack ruminations, and Myristicaceae, which have tegminal rather than testal ruminations), and they are perichalazal, with the raphe running around the seed (Corner 1976; van Setten and Koek-Noorman 1992). Elsewhere among basal angiosperms, this combination is found only in *Austrobaileya*, where the seed coat anatomy and the form of the ruminations are different (Endress 1980). The ruminations in Annonaceae are usually of two types: spiniform, with radial spines penetrating the endosperm, like pins in a pincushion; and lamelliform, consisting of four plates (which are sometimes subdivided) intruding from the sides. The phylogeny (fig. 6) implies that Annonaceae originally had thicker, irregular ruminations, as in related Magnoliales, which are found in *Anaxagorea* and the ambavioids, including *Cananga*. Spiniform ruminations are restricted to the MPM clade, while the inaperturates have

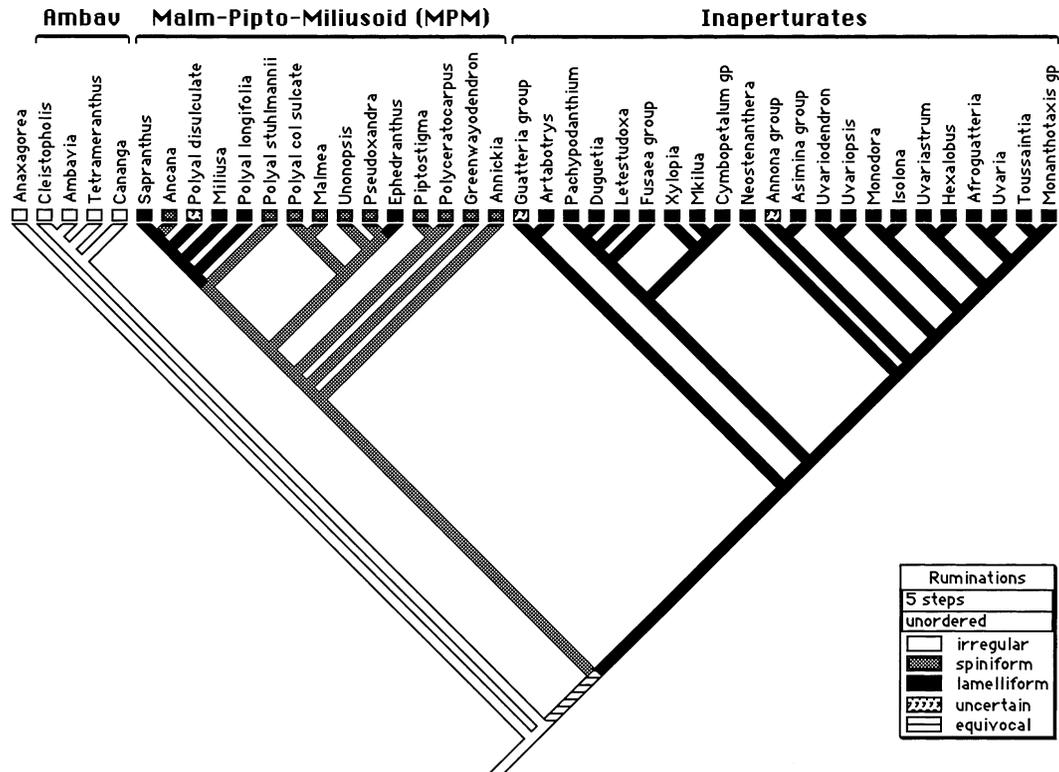


Fig. 6 Representative tree of Annonaceae found in the combined analysis of Doyle et al. (2000), showing inferred evolution of the endosperm ruminations character. Ambav = ambavioids; Malm-Pipto-Miliusoid (MPM) = malmeoid-piptostigmoid-miliusoid.

only lamelliform ruminations, which also arose independently from spiniform within the MPM clade.

Ruminate, perichalazal seeds are known from the Maastrichtian of Nigeria (Chesters 1955), confirming that Annonaceae occurred in Africa at that time. These seeds have lamelliform ruminations as in the inaperturate clade. This does not necessarily indicate that the inaperturate clade had evolved by this time, because without a basis for ordering the ruminations types, it is equivocal whether spiniform and lamelliform were both derived separately from the irregular type or one from the other. Thus the presence of these seeds can only be used to infer that the split between the ambavioids and the combined MPM-inaperturate clade had occurred by the Maastrichtian. However, seeds with all three kinds of ruminations occur in the Early Eocene London Clay (Reid and Chandler 1933, pl. 5), indicating that the MPM and inaperturate clades had definitely diverged by the Eocene and that a variety of Annonaceae had invaded Laurasia. Annonaceae are one of many tropical groups found in the London Clay that later contracted to Southeast Asia.

Fossil pollen and wood data, although less critically evaluated from a phylogenetic perspective, are also consistent with this geographic history. Reticulate monosulcate pollen similar to that of the malmeoids has been reported from the Maastrichtian of Colombia (Sole de Porta 1971; Muller 1981) as have tetrads like those of *Annona* from the Oligocene of Cameroon (Salard-Cheboldaeff 1978). Reports of fossil woods of Annonaceae from the Paleocene-Eocene of England (Crawley 2001) and the Eocene of Oregon (Wheeler and

Manchester 2002) confirm the presence of Annonaceae in Laurasia in the Early Tertiary.

Myristicaceae

Despite conflicts among subsets of the data and different methods of analysis, the molecular and morphological analyses of Myristicaceae by Sauquet et al. (2003) confirm the biogeographic parallels between Myristicaceae and Annonaceae noted by Walker (1971) and Raven and Axelrod (1974).

Implications of the best-supported rooting can be evaluated by rerooting the majority-rule consensus tree based on parsimony analysis of all taxa and characters (which was rooted on *Compsooneura*) between the myristicoid and pycnanthoid clades, as found in parsimony and maximum likelihood analyses with reduced taxon sampling (figs. 7, 8). We have treated geographic distribution as a character with three states: Africa and Madagascar, America, and Asia and Australasia. We eliminated the North American state recognized for Annonaceae because there are relatively few Myristicaceae in Central America, and (in contrast to *Asimina* and *Sapranthus*) these all belong to genera that are much more diverse in South America, consistent with the assumption that they are recent immigrants from South America. We combined Asia and Australasia because all the Asian genera (except the monotypic genus *Paramyristica*, which is endemic to New Guinea) extend from Southeast Asia to New Guinea and sometimes farther east (de Wilde 2000). In this tree, one basal branch consists of the African-Malagasy mauloutchioids

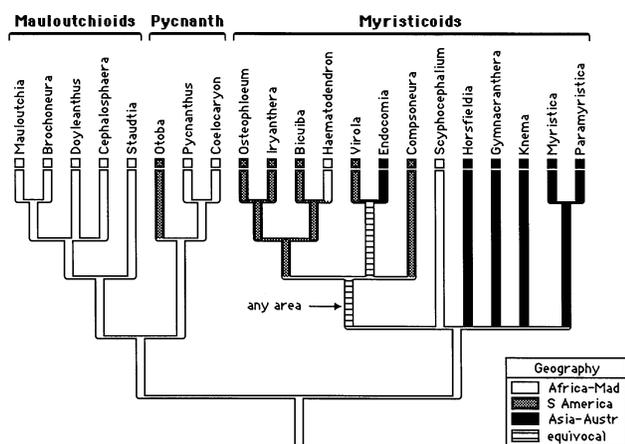


Fig. 7 Majority-rule consensus tree of relationships in Myristicaceae found in the analysis of all morphological and molecular characters by Sauquet et al. (2003), rerooted as in fig. 4, with geographic distribution plotted as a multistate character. Pycnanth = pycnanthoids.

and the African–South American pycnanthoids. The other branch, the myristicoids, shows an unresolved basal polytomy consisting of a predominantly American clade (with the African genus *Haematodendron* and the Asian genus *Endocomia* nested within it), the African genus *Scyphocephalum*, and five Asian lines. These five Asian lines form a clade in some most parsimonious trees (Sauquet et al. 2003).

With this arrangement, the inferred ancestral area for the family is Africa–Madagascar. The placement of the American genus *Otoba* in the otherwise African pycnanthoid clade and the African genus *Haematodendron* in the primarily American subclade of myristicoids is reminiscent of the mixture of African and South American taxa in Annonaceae, which we have attributed to Late Cretaceous dispersal across the South Atlantic—in this case, apparently in both directions. With the relationships among the five Asian genera *Horsfieldia*, *Gymnaecranthera*, *Knema*, *Myristica*, and *Paramyristica* unresolved and *Scyphocephalum* in the same polytomy, it is most parsimonious to assume that the whole myristicoid clade is basically African, implying that these five genera reached Asia from Africa or Madagascar. In the majority rule consensus (fig. 7), the Asian genus *Endocomia* is also nested in the basically American subclade of the myristicoids, indicating a different dispersal history, but in some trees it is associated with the other Asian genera. These results are therefore consistent with either several dispersals from Africa to Asia or as few as one if all the Asian genera form a clade.

Trees based on morphology alone, with the mauloutchioids basal and other African taxa at the next nodes, also imply that the ancestral area was Africa–Madagascar. With the rooting on *Compsonaura*, found with parsimony analyses of molecular and combined data, the inferred ancestral area was South America, but again the relationships seem to indicate a period of exchange between South America and Africa, followed by dispersal to Asia.

These results would fit a timetable similar to that proposed here for Annonaceae, assuming that *Anaxagorea* was originally South American: exchange between Africa and South

America in the Late Cretaceous, followed by dispersal into Laurasia in the Tertiary, whether from Africa or India. There is no evidence for initial connections between Laurasia and Gondwana of the sort suggested when *Anaxagorea* was scored as uncertain. The seed morphology of Myristicaceae may also indicate that the transatlantic disjunctions are old. Myristicaceae have large and animal-dispersed seeds (Sinclair 1958; Ayensu 1974; Alexandre 1978; Howe and Vande Kerckhove 1980; Howe 1981, 1993; Howe et al. 1985; Forget and Milleron 1991; Beehler and Dumbacher 1996; Julliot 1997; Sabatier 1997; Galetti et al. 2000; Russo 2003), so it is hard to imagine how they could disperse across the South Atlantic unless it was much narrower.

Molecular Dating of Annonaceae and Myristicaceae

These arguments for a Cretaceous age of Myristicaceae conflict with molecular age estimates for nodes within Magnoliales. As a framework for this analysis, we used the tree of Magnoliales and other eumagnoliids found by Sauquet et al. (2003) in their maximum likelihood analysis of the combined “fast” genes with reduced taxon sampling. This analysis gave the same relationships outside Myristicaceae as parsimony analyses of the molecular and combined molecular and morphological analyses of all taxa (fig. 1) and the same relationships within Myristicaceae found in parsimony

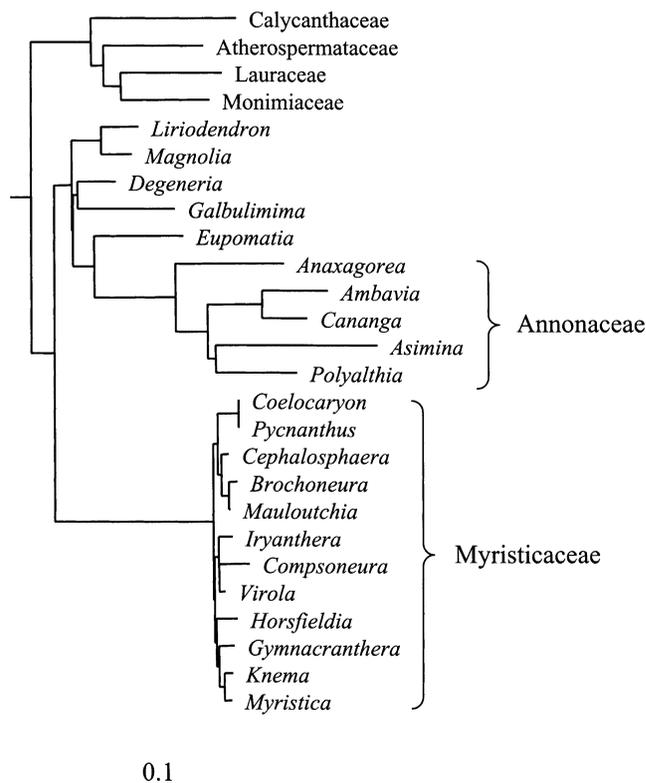


Fig. 8 Tree derived from maximum likelihood analysis of the “fast” loci with reduced taxon sampling (Sauquet et al. 2003), presented as a phylogram. Branch lengths are proportional to the amount of molecular evolution inferred from a GTR + Γ + I model.

analyses of all characters with the reduced taxon set (fig. 4). This tree is presented as a phylogram (fig. 8), with branch lengths proportional to the amount of molecular evolution in the “fast” genes. Within Magnoliales, many molecular analyses have grouped Magnoliaceae with *Degeneria* and *Galbulimima* (and *Eupomatia* with Annonaceae). However, our molecular and combined analyses (fig. 1) both place Magnoliaceae below the other four taxa, as in the combined morphological and molecular analysis of Doyle and Endress (2000), supported by a shift to single prophylls, granular exine structure, and testal ruminations. The difficulty in resolving these relationships appears to be the result of the extremely small amount of molecular evolution on the branch subtending the four core taxa (fig. 8).

Even on casual inspection, this phylogram reveals radical differences between Annonaceae and Myristicaceae, either in age or in rate of molecular evolution. Annonaceae show more divergence from the common ancestor of the order than other Magnoliales, with about half the divergence within the crown group, because either their molecular evolution accelerated or that of other taxa slowed down. Although the stem lineage leading to Myristicaceae is also long, reflecting somewhat more divergence from the ancestor of Magnoliales than occurred from that point to Annonaceae, their crown group forms a “rake” of remarkably short branches. This is what caused so much difficulty in resolving relationships within the family (Sauquet et al. 2003). Assuming a constant rate of molecular evolution, this would indicate that the radiation of crown group Myristicaceae was much more recent than that of Annonaceae, calling into question the biogeographic analogies between the two groups. However, it seems clear from inspection that these data are not clocklike (fig. 8), and this is confirmed by a χ^2 test for a clock (Sanderson 2003) conducted by r8s ($\chi^2 = 1393.5$, $df = 29$, $P \ll 0.001$). This raises the possibility that the two families originated at about the same time, but Myristicaceae underwent a deceleration in molecular evolution; in other words, Myristicaceae are molecular “living fossils.” There is evidence for a similar slowdown in other ancient groups, such as Winteraceae (Suh et al. 1993), *Nelumbo*, *Platanus*, and Fagales (Sanderson and Doyle 2001).

We tested these intuitions by applying three molecular dating methods to the tree and the branch length estimates used to construct the phylogram in figure 8 based on maximum likelihood analysis of the combined “fast” genes of Sauquet et al. (2003), with Laurales as the first outgroup of Magnoliales and Winterales plus Piperales as the second outgroup. The relationships of these four orders are not conclusively resolved, but most recent large-scale analyses have grouped Laurales and Magnoliales (Qiu et al. 2000; Soltis et al. 2000; Zanis et al. 2002), and this is supported over the most commonly encountered alternative relationship (Winterales and Magnoliales; Chase et al. 1993; Doyle and Endress 2000) by the unrooted tree of Sauquet et al. (2003; fig. 1). The relationships of Piperales and Winterales are poorly supported in larger analyses and are not addressed by the unrooted tree of Sauquet et al. (2003), but most analyses have grouped these two orders in a clade, and we used this arrangement for our analyses. However, for simplicity we removed Winterales and Piperales after construction of the chronograms.

Estimation of absolute dates requires a fossil calibration point inside or outside the study group. The best-dated node in the present trees is the most recent common ancestor of Magnoliaceae and Annonaceae (fig. 9), i.e., Magnoliineae in the sense of Sauquet et al. (2003). The most conclusively related fossil is *Archaeanthus* from the early Cenomanian of Kansas (Dilcher and Crane 1984), which appears to be related to the stem lineage of Magnoliaceae based on the combination of the distinctive derived stipules and elongate receptacle and the apparently more plesiomorphic fruits, which consist of numerous well-spaced (rather than closely aggregated) follicles. This gives a minimum age of 98 m.yr. for the key node (using the timescale of Palmer and Geissman 1999). However, there is evidence that this node may be older. One potentially relevant fossil is *Lethomasites* (Ward et al. 1989), a monosulcate pollen type with granular exine structure from the lower Potomac Group, or early Aptian, about 120 m.yr. ago. If *Lethomasites* is magnolialian, which is somewhat speculative in the absence of other organs, it should be in the clade consisting of Annonaceae and the three Australasian genera or on the line leading to them, where exine structure changed from columellar to granular (Doyle and Endress 2000; Sauquet et al. 2003).

Another possibly magnolialian fossil is a laminar stamen with smooth monosulcate pollen described by Crane et al. (1994, dispersed stamen type 1, fig. 11a, 11b) from the upper Potomac Group, or Albian, about 110 m.yr. ago. Its morphology supports a relationship to Magnoliineae because laminar stamens with sunken pollen sacs appear at this point in the phylogeny (Doyle and Endress 2000; Sauquet et al. 2003). Unfortunately, the infratectal structure is unknown; depending on whether it was granular or columellar, the fossil could be above or below the basal node of Magnoliineae. Thus it may provide a minimum age for Magnoliales but not for Magnoliineae.

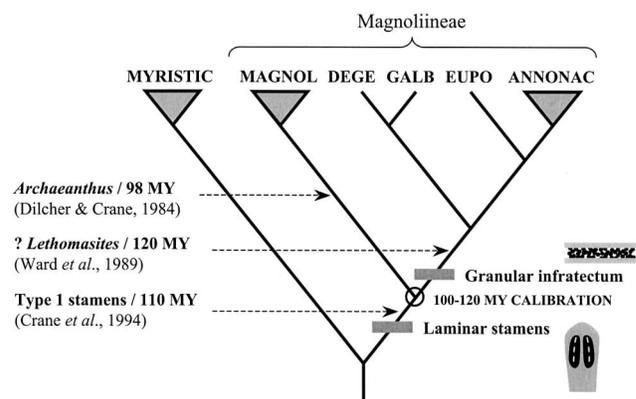


Fig. 9 Calibration of the tree of Magnoliales for molecular dating estimates (fig. 10) showing putative positions of fossil taxa that provide minimum ages. The arrows indicate only the lowest possible positions of the fossils; a given fossil may have diverged from the branch indicated or from some point in the tree above this branch. MYRISTIC = Myristicaceae; MAGNOL = Magnoliaceae; DEGE = *Degeneria*; GALB = *Galbulimima*; EUPO = *Eupomatia*; ANNONAC = Annonaceae.

There is little basis for assigning a maximum age to any of these nodes. This would require a sufficiently dense fossil record to state that the node in question is unlikely to be older than some date. Use of *Archaeanthus* or *Lethomasites* for calibration of ages within Magnoliineae assumes that this clade is unlikely to be much older than these fossils, but strictly speaking they provide only minimum age estimates for the internal nodes.

The first dating method that we used, based on the assumption of a molecular clock, gives plausible ages for Annonaceae, but as expected it indicates that Myristicaceae are much younger. Using the “fast” genes and two alternative dates for the calibration point, 100 and 120 m.yr. (late Albian, early Aptian), the clock-based age of Annonaceae is 69 or 82 m.yr. (fig. 10A), and the age of the split between the ambavioids and the MPM-inaperturate clade is 60 or 72 m.yr. At least the 72-m.yr. date, based on the older calibration for Magnoliineae, is consistent with the occurrence of annonaceous seeds with lamelliform ruminations, representing the MPM-inaperturate clade, in the Maastrichtian, 65–70 m.yr. The split into the MPM clade, with basically spiniform ruminations, and the inaperturate clade, with lamelliform ruminations, is dated 57 or 68 m.yr., consistent with the pres-

ence of seeds with both kinds of ruminations in the London Clay, at about 50 m.yr. However, the inferred ages for crown group Myristicaceae are much younger: 15 or 18 m.yr., or Early–Middle Miocene. These dates would imply that the Africa–South American distribution of Myristicaceae came about by long-distance dispersal across oceans nearly as wide as they are today and that the biogeographic parallels between Myristicaceae and Annonaceae are illusory.

There has been much hope that problems such as these may be solved with methods of molecular dating that do not assume a clock (Sanderson 1998). The first such method that we used, NPRS (Sanderson 1997), gave anomalous results, with ages for Magnoliales in the Precambrian. This appears to be an example of an artifact sometimes encountered with NPRS that is apparently caused by the combination of an internal calibration point and near zero-length branches within the tree (M. J. Sanderson, personal communication). The second method, penalized likelihood (Sanderson 2002; fig. 10B), with an inferred optimal rate-smoothing value of 10, gave slightly older ages for Myristicaceae, 17 or 21 m.yr. (Early Miocene), but younger ages for Annonaceae, 57 or 69 m.yr. (Late Paleocene or Maastrichtian), and the split between the ambavioids and the MPM-inaperturate clade, 46 or 56 m.yr. (Middle Eocene or Late Paleocene). The latter ages are actually too young, considering the presence of seeds with lamelliform ruminations in the Maastrichtian (65–70 m.yr.), which implies that the MPM-inaperturate and ambavioid lines had diverged by this time (assuming the true age of the Magnoliineae is not much older than 120 m.yr.). Thus the penalized likelihood method does not remove the conflict between molecular data and the biogeographic evidence for a greater age of Myristicaceae. These results could mean either that the large seeds of Myristicaceae did indeed disperse across the South Atlantic in the Miocene or that the assumptions of the methods are no better than those of a clock; for example, if rates of molecular evolution in Magnoliales changed abruptly rather than gradually (the situation in which NPRS is most appropriate and to a lesser extent penalized likelihood).

Fossil evidence could help test these hypotheses, as it did in Annonaceae, but surprisingly Myristicaceae have almost no recognized fossil record. Some Early Tertiary leaves have been identified as Myristicaceae (Wolfe 1977), but these are difficult to distinguish from leaves of Annonaceae, except based on their distinctive hairs, which have not been studied. There are reports of Early Tertiary pollen of Myristicaceae (Frederiksen 1973; Jan du Chêne et al. 1978), but these too are not convincingly diagnostic. Poinar and Poinar (1999) illustrated a flower identified as *Virola* from Tertiary amber of the Dominican Republic, but although its external appearance is myristicaceous, the more diagnostic fertile structures are not visible.

Boureau (1950) described *Myristicoxylon princeps* from the earliest Paleocene (Danian) of the Sahara as wood of Myristicaceae. Comparison with the OCPN wood identification database (LaPasha and Wheeler 1987) indicates that Myristicaceae are the closest extant match for this wood, with the combination of marginal (circummedullary) parenchyma (a feature of most Magnoliales) and both scalariform and simple vessel perforations being most suggestive (E. A. Wheeler, personal communication). However, *M. princeps*

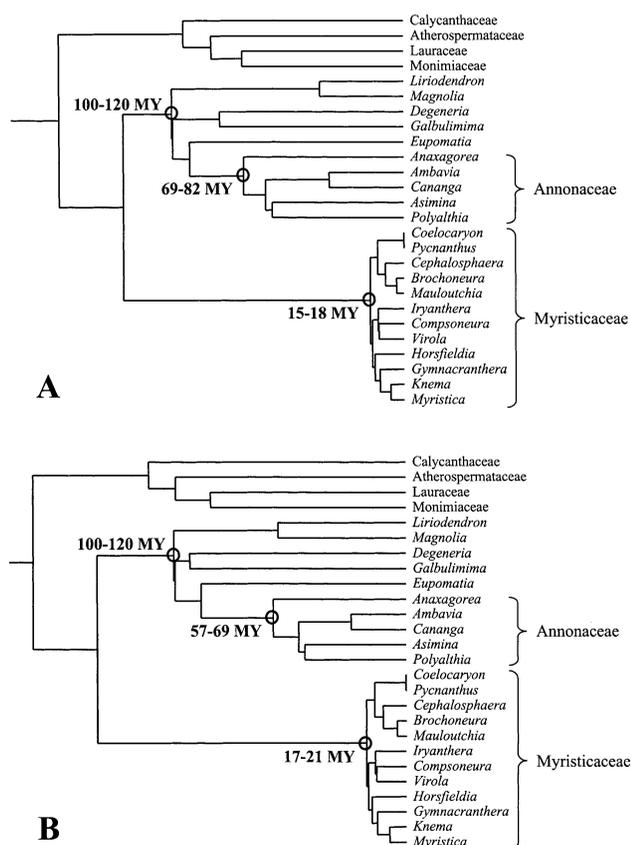


Fig. 10 Molecular dating estimates for Magnoliales, based on the “fast” genes (Sauquet et al. 2003), assuming alternative ages of 100 m.yr. (late Albian) and 120 m.yr. (early Aptian) for the basal node of Magnoliineae. *A*, Using the assumption of a molecular clock; *B*, using penalized likelihood (Sanderson 2002).

cannot be related to any particular subgroup of Myristicaceae, implying that it could be a stem relative of the family. Thus it does not bear directly on the age of the crown group, although it is significant in confirming that either Myristicaceae or the line leading to them existed in Africa at the beginning of the Tertiary.

The often dehiscent berry-like fruits and large seeds of Myristicaceae, most of which have tegminal ruminations (in contrast to the testal ruminations of Annonaceae and related families) and/or a prominent aril, are more distinctive and would seem likely to be preserved as fossils, but they too are almost unknown. If Myristicaceae had a history similar to that of Annonaceae, their seeds would be expected in the London Clay, but none has been recognized (Reid and Chandler 1933; Collinson and Cleal 2001). Berry (1924) described putative seed and fruit casts called *Myristica cataboulensis* from the Late Eocene of Texas, which would be substantially older than the molecular dates. These fossils have grooves that superficially resemble myristicaceous ruminations. However, examination of the specimens by S. R. Manchester (personal communication) indicates that they are molds of a longitudinally ribbed endocarp, favoring instead a relationship to Cornales, such as *Nyssa* or *Mastixioideae*. The best record is *Myristicacarpum miocaenicum* from the Middle Miocene of Germany (Gregor 1977), consisting of lignitized seeds with ruminations of the appropriate type and a dehiscent pericarp.

The absence of pre-Miocene records of Myristicaceae might be taken as evidence that the family is unexpectedly young, as implied by the molecular data. However, it could

also mean that their seeds have, for some unknown reason, a low capacity for preservation and that their other organs do occur as fossils but lack sufficient diagnostic characters to be identified without more detailed study (e.g., of epidermal structure). In any case, a Miocene age for Myristicaceae remains difficult to reconcile with their combination of pan-tropical distribution and low dispersibility.

Conclusion

Annonaceae and Myristicaceae present a case of conflict lurking below the surface of apparent analogy. In Annonaceae, phylogenies, fossils, and molecular dates converge on a fairly coherent story involving both plate tectonics and long-distance dispersal. However, although phylogenies seem to imply that Myristicaceae had a similar history, both molecular dates and the fossil record are problematic. If nothing else, this case illustrates the need for caution, whether in using molecular data to date geographic disjunctions or in assuming that similar geographic patterns on cladograms reflect similar histories.

Acknowledgments

We thank Susanne Renner for inviting us to participate in the symposium on which this article is based, Steve Manchester for unpublished observations, Tom Near and Mike Sanderson for advice and assistance in the use of programs, and Eli Wheeler for help in locating and evaluating literature on fossil wood.

Literature Cited

- Alexandre DY 1978 Le rôle disséminateur des éléphants en forêt de Taï, Côte d'Ivoire. *Terre Vie* 32:47–72.
- APG (Angiosperm Phylogeny Group) 1998 An ordinal classification for the families of flowering plants. *Ann Mo Bot Gard* 85:531–553.
- APG II (Angiosperm Phylogeny Group II) 2003 An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Bot J Linn Soc* 141:399–436.
- Ayensu ES 1974 Plant and bat interactions in West Africa. *Ann Mo Bot Gard* 61:702–727.
- Azuma H, JG García-Franco, V Rico-Gray, LB Thien 2001 Molecular phylogeny of the Magnoliaceae: the biogeography of tropical and temperate disjunctions. *Am J Bot* 88:2275–2285.
- Beehler BM, JP Dumbacher 1996 More examples of fruiting trees visited predominantly by birds of paradise. *Emu* 96:81–88.
- Berry EW 1924 The Middle and Upper Eocene floras of southeastern North America. *US Geol Surv Prof Pap* 92:1–206.
- Boureau E 1950 Étude paléoxylologique du Sahara (IX): sur un *Myristicoxylon princeps* n. gen., n. sp., du Danien d'Asselar (Sahara soudanais). *Bull Mus Natl Hist Nat Ser 2*, 22:523–528.
- Chase MW, DE Soltis, RG Olmstead, D Morgan, DH Les, BD Mishler, MR Duvall, et al 1993 Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. *Ann Mo Bot Gard* 80:528–580.
- Chatrou LW 1997 Studies in Annonaceae. XXVIII. Macromorphological variation of recent invaders in northern Central America: the case of *Malmea* (Annonaceae). *Am J Bot* 84:861–869.
- Chesters KIM 1955 Some plant remains from the Upper Cretaceous and Tertiary of West Africa. *Ann Mag Nat Hist Ser 12*, 8:498–504.
- Collinson ME, CJ Cleal 2001 Early and Early-Middle Eocene (Ypresian-Lutetian) palaeobotany of Great Britain. Pages 185–226 in CJ Cleal, BA Thomas, DJ Batten, ME Collinson, eds. *Palaeobotany of Great Britain*. Joint Nature Conservation Committee, Peterborough.
- Corner EJM 1976 The seeds of the dicotyledons. Cambridge University Press, Cambridge.
- Crane PR, EM Friis, KR Pedersen 1994 Paleobotanical evidence on the early radiation of magnoliid angiosperms. *Plant Syst Evol Suppl* 8:51–72.
- Crawley M 2001 Angiosperm woods from British Lower Cretaceous and Palaeogene deposits. *Spec Pap Palaeontol* 66:1–100.
- Cronquist A 1981 An integrated system of classification of flowering plants. Columbia University Press, New York.
- de Wilde WJJO 2000 Myristicaceae. *Flora Malesiana: Series I, Spermatophyta*. Vol 14. Nationaal Herbarium Nederland, Leiden.
- Dilcher DL, PR Crane 1984 *Archaeanthus*: an early angiosperm from the Cenomanian of the Western Interior of North America. *Ann Mo Bot Gard* 71:351–383.
- Donoghue MJ, JA Doyle 1989 Phylogenetic analysis of angiosperms and the relationships of Hamamelidae. Pages 17–45 in PR Crane, S Blackmore, eds. *Evolution, systematics, and fossil history of the Hamamelidae*. Vol 1. Clarendon, Oxford.
- Doyle JA, P Bygrave, A Le Thomas 2000 Implications of molecular data for pollen evolution in Annonaceae. Pages 259–284 in MM Harley, CM Morton, S Blackmore, eds. *Pollen and spores: morphology and biology*. Royal Botanic Gardens, Kew.

- Doyle JA, PK Endress 2000 Morphological phylogenetic analysis of basal angiosperms: comparison and combination with molecular data. *Int J Plant Sci* 161(suppl):S121–S153.
- Doyle JA, A Le Thomas 1994 Cladistic analysis and pollen evolution in Annonaceae. *Acta Bot Gall* 141:149–170.
- 1996 Phylogenetic analysis and character evolution in Annonaceae. *Bull Mus Natl Hist Nat Sect B Adansonia* 18: 279–334.
- 1997 Phylogeny and geographic history of Annonaceae. *Geogr Phys Quat* 51:353–361.
- Endress PK 1980 The reproductive structures and systematic position of the Austrobaileyaceae. *Bot Jahrb Syst* 101:393–433.
- Forget PM, T Milleron 1991 Evidence for secondary seed dispersal by rodents in Panama. *Oecologia* 87:596–599.
- Frederiksen NO 1973 New mid-Tertiary spores and pollen grains from Mississippi and Alabama. *Tulane Stud Geol Paleontol* 10: 65–86.
- Fries RE 1959 Annonaceae. Pages 1–171 in H Melchior, ed. *Die natürlichen Pflanzenfamilien*. 2d ed. Vol 17aII. Duncker & Humblot, Berlin.
- Galetti M, R Laps, MA Pizo 2000 Frugivory by toucans (Ramphastidae) at two altitudes in the Atlantic Forest of Brazil. *Biotropica* 32: 842–850.
- Graham SW, RG Olmstead 2000 Utility of 17 chloroplast genes for inferring the phylogeny of the basal angiosperms. *Am J Bot* 87: 1712–1730.
- Gregor HJ 1977 Subtropische Elemente im europäischen Tertiär II (Fruktifikationen). *Palaeontol Z* 51:199–226.
- Hollick A 1906 The Cretaceous flora of southern New York and New England. *US Geol Surv Monogr* 50:1–219.
- Howe HF 1981 Dispersal of a neotropical nutmeg (*Virola sebifera*) by birds. *Auk* 98:88–98.
- 1993 Aspects of variation in a neotropical seed dispersal system. *Vegetatio* 107/108:149–162.
- Howe HF, EW Schupp, LC Westley 1985 Early consequences of seed dispersal for a Neotropical tree (*Virola surinamensis*). *Ecology* 66: 781–791.
- Howe HF, GA Vande Kerckhove 1980 Nutmeg dispersal by tropical birds. *Science* 210:925–927.
- Jan du Chêne RE, MS Onyike, MA Sowunmi 1978 Some new Eocene pollen of the Ogwashi-Asabe Formation, south-eastern Nigeria. *Rev Esp Micropaleontol* 10:285–322.
- Julliot C 1997 Impact of seed dispersal by red howler monkeys *Alouatta seniculus* on the seedling population in the understorey of tropical rain forest. *J Ecol* 85:431–440.
- LaPasha CA, EA Wheeler 1987 A microcomputer-based system for computer-aided wood identification. *IAWA (Int Assoc Wood Anat) Bull New Ser* 8:347–354.
- Leroy JF 1978 Composition, origin, and affinities of the Madagascar vascular flora. *Ann Mo Bot Gard* 65:535–589.
- Le Thomas A 1980–1981 Ultrastructural characters of the pollen grains of African Annonaceae and their significance for the phylogeny of primitive angiosperms. *Pollen Spores* 22:267–342; 23:5–36.
- Le Thomas A, JA Doyle 1996a Geographic relationships of Malagasy Annonaceae. Pages 85–94 in WR Lourenço, ed. *Biogéographie de Madagascar*. ORSTOM, Paris.
- 1996b Implications d'une analyse cladistique dans l'histoire géographique des Annonaceae: famille d'Angiospermes primitives. Pages 171–180 in JL Guillaumet, M Belin, H Puig, eds. *Phytogéographie tropicale: réalités et perspectives*. ORSTOM, Paris.
- Le Thomas A, B Lugardon 1974 Quelques types de structure grenue dans l'ectexine de pollens simples d'Annonacées. *C R Hebd Seances Acad Sci Ser D Sci Nat* 278:1187–1190.
- 1975 Ultrastructure d'un pollen original parmi les Annonacées (*Ambavia*). *Bull Soc Bot Fr* 122:109–111.
- 1976 De la structure grenue à la structure columellaire dans le pollen des Annonacées. *Adansonia* 15:543–572.
- Lugardon B, A Le Thomas 1974 Sur la structure feuilletée de la couche basal de l'ectexine chez diverses Annonacées. *C R Hebd Seances Acad Sci Ser D Sci Nat* 279:255–258.
- Maddison DR, WP Maddison 2001 MacClade 4: analysis of phylogeny and character evolution, version 4.03. Sinauer, Sunderland, Mass.
- Mathews S, MJ Donoghue 1999 The root of angiosperm phylogeny inferred from duplicate phytochrome genes. *Science* 286:947–950.
- Morley RJ 2000 Origin and evolution of tropical forests. Wiley, New York.
- Muller J 1981 Fossil pollen records of extant angiosperms. *Bot Rev* 47:1–142.
- Okada H, K Ueda 1984 Cytotaxonomical studies on Asian Annonaceae. *Plant Syst Evol* 144:165–177.
- Palmer AR, J Geissman 1999 Geologic time scale. Geological Society of America. <http://www.geosociety.org/science/timescale/timescl.htm>.
- Poinar G Jr, R Poinar 1999 The amber forest: a reconstruction of a vanished world. Princeton University Press, Princeton, N.J.
- Qiu YL, J Lee, F Bernasconi-Quadroni, DE Soltis, PS Soltis, M Zanis, EA Zimmer, Z Chen, V Savolainen, MW Chase 1999 The earliest angiosperms: evidence from mitochondrial, plastid and nuclear genomes. *Nature* 402:404–407.
- 2000 Phylogeny of basal angiosperms: analyses of five genes from three genomes. *Int J Plant Sci* 161(suppl):S3–S27.
- Raven PH, DI Axelrod 1974 Angiosperm biogeography and past continental movements. *Ann Mo Bot Gard* 61:539–673.
- Reid EM, MEJ Chandler 1933 The London Clay flora. British Museum (Natural History), London.
- Russo SE 2003 Responses of dispersal agents to tree and fruit traits in *Virola calophylla* (Myristicaceae): implications for selection. *Oecologia* 136:80–87.
- Sabatier D 1997 Description et biologie d'une nouvelle espèce de *Virola* (Myristicaceae) de Guyane. *Adansonia* 19:273–278.
- Salard-Chebouldaëff M 1978 Sur la palynoflore maestrichtienne et tertiaire du bassin sédimentaire littoral du Cameroun. *Pollen Spores* 20:215–260.
- Sanderson MJ 1997 A nonparametric approach to estimating divergence times in the absence of rate constancy. *Mol Biol Evol* 14: 1218–1231.
- 1998 Estimating rate and time in molecular phylogenies: beyond the molecular clock? Pages 242–264 in DE Soltis, PS Soltis, JJ Doyle, eds. *Molecular systematics of plants. II. DNA sequencing*. Kluwer, Boston.
- 2002 Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Mol Biol Evol* 19:101–109.
- 2003 r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. *Bioinformatics* 19:301–302.
- Sanderson MJ, JA Doyle 2001 Sources of error and confidence intervals in estimating the age of angiosperms from *rbcL* and 18S rDNA data. *Am J Bot* 88:1499–1516.
- Sauquet H 2003 Androecium diversity and evolution in Myristicaceae (Magnoliales), with the description of a new Malagasy genus, *Doyleanthus*, gen. nov. *Am J Bot* 90:1293–1305.
- Sauquet H, JA Doyle, T Scharaschkin, T Borsch, KW Hilu, LW Chatrou, A Le Thomas 2003 Phylogenetic analysis of Magnoliales and Myristicaceae based on multiple data sets: implications for character evolution. *Bot J Linn Soc* 142:125–186.
- Sauquet H, A Le Thomas 2003 Pollen diversity and evolution in Myristicaceae (Magnoliales). *Int J Plant Sci* 164:613–628.
- Scharaschkin T 2003 Biogeographic history and character evolution of *Anaxagorea* (Annonaceae) based on phylogenetic analyses of

- morphological characters and non-coding chloroplast regions. Botany 2003 (Mobile, Alabama) Abstracts, p 92.
- 2004 Phylogeny of *Anaxagorea* (Annonaceae): implications for biogeographic history and character evolution. PhD diss. University of California, Davis.
- Schuster RM 1976 Plate tectonics and its bearing on the geographical origin and dispersal of angiosperms. Pages 48–138 in CB Beck, ed. Origin and early evolution of angiosperms. Columbia University Press, New York.
- Sinclair J 1955 A revision of the Malayan Annonaceae. Gard Bull 14: 149–516.
- 1958 A revision of the Malayan Myristicaceae. Gard Bull 16: 205–472.
- Smith AC 1973 Angiosperm evolution and the relationship of the floras of Africa and South America. Pages 49–61 in BJ Meggers, ES Ayensu, WD Duckworth, eds. Tropical forest ecosystems in Africa and South America: a comparative review. Smithsonian Institution, Washington, D.C.
- Sole de Porta N 1971 Algunos géneros nuevos de polen procedentes de la Formación Guaduas (Maastrichtense-Paleoceno) de Colombia. Stud Geol Salamanca 2:133–143.
- Soltis DE, PS Soltis, MW Chase, ME Mort, DC Albach, M Zanis, V Savolainen, et al 2000 Angiosperm phylogeny inferred from 18S rDNA, *rbcL*, and *atpB* sequences. Bot J Linn Soc 133:381–461.
- Suh Y, LB Thien, HE Reeve, EA Zimmer 1993 Molecular evolution and phylogenetic implications of internal transcribed spacer sequences of ribosomal DNA in Winteraceae. Am J Bot 80: 1042–1055.
- Swofford DL 2002 PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer, Sunderland, Mass.
- Takhtajan AL 1969 Flowering plants: origin and dispersal. Smithsonian Institution, Washington, D.C.
- van Setten AK, J Koek-Noorman 1992 Fruits and seeds of Annonaceae: morphology and its significance for classification and identification. Studies in Annonaceae XVII. Bibl Bot 142:1–101.
- Walker JW 1971 Pollen morphology, phytogeography, and phylogeny of the Annonaceae. Contrib Gray Herb Harv Univ 202:1–131.
- Walker JW, AG Walker 1981 Comparative pollen morphology of the Madagascan genera of Myristicaceae (*Mauloutchia*, *Brocho-neura*, and *Haematodendron*). Grana 20:1–17.
- Warburg O 1897 Monographie der Myristicaceen. Nova Acta Leopold 68:1–680.
- Ward JV, JA Doyle, CL Hotton 1989 Probable granular magnoliid angiosperm pollen from the Early Cretaceous. Pollen Spores 33: 101–120.
- Wheeler EA, SR Manchester 2002 Woods of the Middle Eocene Nut Beds flora, Clarno Formation, Oregon, USA. IAWA J 3(suppl): 1–188.
- Wolfe JA 1977 Paleogene floras from the Gulf of Alaska region. US Geol Surv Prof Pap 997:1–108.
- Zanis MJ, DE Soltis, PS Soltis, S Mathews, MJ Donoghue 2002 The root of the angiosperms revisited. Proc Natl Acad Sci USA 99: 6848–6853.