

UC Davis

UC Davis Previously Published Works

Title

Integrating early Cretaceous fossils into the phylogeny of living angiosperms: Anita lines and relatives of Chloranthaceae

Permalink

<https://escholarship.org/uc/item/3bj1s569>

Journal

International Journal of Plant Sciences, 175(5)

ISSN

1058-5893

Authors

Doyle, JA
Endress, PK

Publication Date

2014

DOI

10.1086/675935

Peer reviewed

INTEGRATING EARLY CRETACEOUS FOSSILS INTO THE PHYLOGENY OF LIVING ANGIOSPERMS: ANITA LINES AND RELATIVES OF CHLORANTHACEAE

James A. Doyle^{1,*} and Peter K. Endress[†]

^{*}Department of Evolution and Ecology, University of California, Davis, California 95616, USA; and
[†]Institute of Systematic Botany, University of Zurich, 8008 Zurich, Switzerland

Editor: Patrick S. Herendeen

Premise of research. Discoveries of fossil flowers in Cretaceous rocks offer improved evidence for relationships with living clades, but for more secure inferences formal phylogenetic analyses are desirable. We extend previous analyses of magnoliids, monocots, and basal eudicots to Aptian, Albian, and Cenomanian fossils related to the basal “ANITA” lines and Chloranthaceae.

Methodology. We performed parsimony analyses of a morphological data set of Recent angiosperms and published fossils, with the arrangement of Recent taxa constrained to backbone trees based primarily on molecular data.

Pivotal results. Not only *Monetianthus* (as previously inferred) but also *Carpstellia* is nested within Nymphaeaceae, while *Pluricarpellatia* may be a stem relative of Cabombaceae or Nymphaeaceae. *Anacostia* (with *Similipollis* pollen) is nested within Austrobaileyales. The position of *Couperites* (with *Clavatipollenites* pollen) is ambiguous: it may be on the stem lineage of Chloranthaceae (and *Ceratophyllum*, if this extant aquatic is related to Chloranthaceae), nested in Chloranthaceae, or more basal. Plants with *Asteropollis* pollen and reduced tepals are related to the chloranthaceous genus *Hedyosmum*. *Zlatkocarpus*, which also has a reduced perianth, may be either a stem relative or a crown group member of Chloranthaceae. Plants that produced loosely reticulate *Pennipollis* pollen are more likely related to Chloranthaceae and/or *Ceratophyllum* than to monocots. We confirm that *Canrightia*, with bisexual flowers and a reduced perianth, is a stem relative of Chloranthaceae. Despite similarities to Piperales, *Appomattoxia* (with *Tucanopollis* pollen) is more likely near the base of the ANITA grade or related to Chloranthaceae and/or *Ceratophyllum*.

Conclusions. The Cretaceous rise of angiosperms involved the radiation not only of magnoliids, eudicots, and monocots but also of basal ANITA lines, including both aquatic Nymphaeales and woody groups. Our results reaffirm the early diversity of Chloranthaceae and clarify their floral evolution, in which a shift to unisexual flowers preceded loss of the perianth.

Keywords: angiosperms, Cretaceous, Nymphaeales, Chloranthaceae, paleobotany, phylogeny.

Online enhancement: data file.

Introduction

Since the 1960s and 1970s, studies of Cretaceous pollen and leaves have provided a broad-brush picture of early angiosperm evolution: an initial phase with monosulcate pollen and leaf types found in taxa that were formerly called magnoliids but are now recognized as a series of basal lines, plus a few monocots, followed by the rise of tricolpate pollen, diagnostic of the vast eudicot clade, and the appearance of leaf types comparable to modern tricolpate taxa (Doyle 1969, 1978, 2012; Muller 1970; Wolfe et al. 1975; Doyle and Hickey 1976; Hickey and Doyle 1977). This pattern was taken as evidence that the radiation of angiosperms (except perhaps its very earliest phases)

began in the Early Cretaceous, contrary to older views that angiosperms had diversified to a high level before they appeared in lowland basins of deposition (Axelrod 1952, 1970). However, most workers hesitated to assign pollen and leaf fossils to living taxa, which was probably wise in view of their relatively small number of characters and the resulting risk of incorrect conclusions on relationships due to homoplasy.

This situation improved with discoveries of lignitized and charcoaled fossil flowers (Friis 1983; Crane et al. 1986; Friis et al. 1986, 2006, 2011), mostly in the mesofossil (millimeter) size range, which often have in situ pollen in the stamens or on the stigma and can sometimes be associated with stems and leaves. These fossils have many more characters and therefore suggest more robust relationships with living clades. However, these paleobotanical advances have been only partially integrated with parallel improvements in the understanding of angiosperm phylogeny based on studies of living plants, which

¹ Author for correspondence; e-mail: jadoble@ucdavis.edu.

began in the 1980s with cladistic analyses of morphological characters (Dahlgren and Bremer 1985; Donoghue and Doyle 1989) and accelerated in the 1990s with analyses of molecular sequence data (summarized by Soltis et al. 2005). As a result, many systematic comparisons of fossil flowers can be questioned because they were not supported by formal phylogenetic analysis (Crepet et al. 2004).

With these considerations in mind, we undertook a project on integration of Cretaceous fossils into angiosperm phylogeny using a morphological data set amassed for living angiosperms. This data set was first presented in Doyle and Endress (2000), used with some modifications in Saarela et al. (2007), and substantially revised with more taxa and characters in Endress and Doyle (2009) and Doyle and Endress (2010). Theoretically, the ideal approach might be a “total evidence” analysis of a matrix containing molecular and morphological data for living taxa but only morphology for fossils (Springer et al. 2001; Hermsen and Hendricks 2008). However, because of theoretical and practical problems in compiling such a matrix, and because statistical support for most molecular relationships has become so strong, we have instead used a “molecular scaffold” approach, in which a morphological matrix of living and fossil taxa is analyzed with the arrangement of living groups constrained to a “backbone tree” based mainly on molecular data. Setting aside problems due to homoplasy, a fossil attaches to the stem lineage of a living clade, or crown group, if it has one or more synapomorphies of the living clade but ancestral states in other characters. It is nested within the crown group if it has all the derived states of the whole group in the characters preserved, plus at least one synapomorphy of some subgroup.

This approach does not address the possibility that fossils might change inferences on relationships among living taxa; it asks only what is the best position of a fossil if the backbone tree is correct and what the fossil says about the age and character evolution of the clade. There may be cases in which fossils would affect molecular results, but many relationships are now supported by so many DNA characters that they are unlikely to change with addition of fossils. Already in 1999 (Mathews and Donoghue 1999; Parkinson et al. 1999; Qiu et al. 1999; Soltis et al. 1999) there was strong statistical support (measured by bootstrap analysis) for the basal position of the “ANITA” lines (*Amborella*, Nymphaeales, Austrobaileyales) and for many clades in the remaining groups, named *Mesangiospermae* by Cantino et al. (2007). However, there are cases where support is weaker and different analyses give different results, for example, in the arrangement of the five main clades of mesangiosperms—eudicots, magnoliids in a restricted monophyletic sense (Magnoliales, Laurales, Canellales, Piperales), monocots, Chloranthaceae, and the rootless aquatic *Ceratophyllum*.

To take this uncertainty into account, we have used two backbone trees, which illustrate the range of currently viable hypotheses on relationships among the five mesangiosperm clades. The “D&E” tree is derived from an analysis of morphological data and sequences of 18S nrDNA, *rbcl*, and *atpB* by Doyle and Endress (2000), with several taxa added, Piperales moved into magnoliids, and some taxa rearranged within major clades, following more comprehensive molecular analyses (Endress and Doyle 2009). In this tree, Chloranthaceae and *Ceratophyllum* form a clade that is sister to all other

mesangiosperms, while monocots are linked with magnoliids. The Chloranthaceae-*Ceratophyllum* clade is most strongly supported by morphology (Endress and Doyle 2009), but it has also been found in analyses of chloroplast ITS sequences (Antonov et al. 2000); mitochondrial genes (Duvall et al. 2006, 2008; Qiu et al. 2010); chloroplast genes from the inverted repeat region (Moore et al. 2011), which show a combination of a high proportion of informative sites and low rates of substitution that suggests they may be more reliable than average; and low-copy nuclear genes (Zhang et al. 2012). In the “J/M” tree, based on analyses of nearly complete chloroplast genomes (Jansen et al. 2007; Moore et al. 2007), Chloranthaceae are linked with magnoliids and *Ceratophyllum* with eudicots, which together are sister to monocots. Relationships within the five mesangiosperm clades are assumed to be the same in both trees.

In previous articles, we analyzed the positions of the controversial aquatic fossil *Archaeofructus* (Endress and Doyle 2009), fossils relevant to the problem of Early Cretaceous monocots (Doyle et al. 2008), and fossil members of the magnoliid and eudicot clades (Doyle and Endress 2010). Most of these analyses confirmed systematic comparisons made by the original authors, with a few significant exceptions. In this study we extend this survey to fossils apparently related to the ANITA lines and Chloranthaceae. As in earlier analyses, we largely restrict our attention to the Early Cretaceous, in order to keep the number of taxa within limits and to avoid the necessity of further subdividing modern clades because fossils may be nested within terminal taxa. Except for fossils compared with the monocot family Araceae (Friis et al. 2004, 2010a), not yet analyzed formally, no known fossils of this age appear to be nested within terminal taxa in our data set. Two exceptions to the stratigraphic rule are the Cenomanian fossils *Couperites* (Pedersen et al. 1991) and *Zlatkocarpus* (Kvaček and Friis 2010), which are almost surely not nested within any of our terminal taxa. In four cases the original authors performed phylogenetic analyses of the fossils, using their own Recent data or ours (Saarela et al. 2007; Doyle and Endress 2010). In these cases our aim has been to update and test these analyses by rescoring taxa in terms of our current data set and character concepts and to explore implications for the evolution and geologic history of clades. Our analyses raise many general questions on integration of fossils with few characters into Recent data sets, a topic of much recent discussion (Manos et al. 2007), but because of time and space limitations, we have not explored these questions explicitly.

Material and Methods

Taxa and Characters

Fossil taxa analyzed in this study are listed in table 1. Recent taxa and definitions of characters and their states are listed in the appendix. The data matrix is presented as figure 1 and as a NEXUS file, available online, and at the Dryad Digital Repository (<http://www.datadryad.org>). For Recent taxa, this data set is the same as that of Doyle and Endress (2010), with one change discussed in the appendix (character 134). As in Doyle et al. (2008), Endress and Doyle (2009), and Doyle and Endress (2010), in order to avoid circularity when investigating

Table 1
Fossil Taxa Analyzed in This Study

Fossil taxon	Authors/sources of data	Locality and age	Characters scored (%)
<i>Monetianthus</i>	Friis et al. 2009	Vale de Agua, Portugal (early Albian)	37/142 (26.1)
<i>Carpstellia</i>	Von Balthazar et al. 2008	Puddledock, Virginia (middle Albian)	15/142 (10.6)
<i>Pluricarpellatia</i>	Mohr et al. 2008	Crato Formation, Brazil (late Aptian)	27/142 (19.0)
<i>Anacostia</i>	Friis et al. 1997a	Kenilworth, Maryland, Puddledock, Virginia (middle Albian); Buarcos, Famalicão, Vale de Agua, Portugal (early Albian)	39/142 (27.5)
<i>Couperites</i>	Pedersen et al. 1991	Mauldin Mountain, Maryland (early Cenomanian)	30/142 (21.1)
<i>Asteropollis</i> plant	Friis et al. 1994, 1997b, 1999, 2000b, 2006, 2011	Torres Vedras, Catefica (Aptian or early Albian), Vale de Agua, Buarcos (early Albian), Portugal	42/142 (29.6)
<i>Zlatkocarpus</i>	Kvaček and Eklund 2003; Kvaček and Friis 2010	Brník, Hloubětín-Hutě, Czech Republic (middle Cenomanian)	35/142 (24.6)
<i>Pennipollis</i> plant	Friis et al. 2000a	Vale de Agua, Buarcos, Portugal (early Albian)	41/142 (28.9)
<i>Canrightia</i>	Friis and Pedersen 2011	Catefica and other localities (Aptian or early Albian), Portugal	54/142 (38.0)
<i>Appomattoxia</i>	Friis et al. 1995, 2006, 2010a	Puddledock, Virginia (middle Albian), Torres Vedras, Portugal (Aptian or early Albian)	33/142 (23.2)

the position of fossils, all Recent taxa are defined as crown groups: they do not include potential fossil outgroups, and fossils were not considered in scoring their characters (in contrast to a few cases in Doyle and Endress 2000). References on the morphology of Recent taxa, studies on their internal relationships that we consulted in order to estimate ancestral states for variable characters, and arguments for treatment of problematic cases may be found in Doyle (2005) for pollen, Endress and Doyle (2009) for floral morphology, and Doyle and Endress (2010) for vegetative and other characters not used in Endress and Doyle (2009).

To facilitate comparison with earlier analyses, figure 1 includes fossil taxa treated in Doyle et al. (2008), Endress and Doyle (2009), and Doyle and Endress (2010). Sources of data on the fossils covered by this study and arguments for interpretation and scoring of their characters are presented in Results and Discussion, since we regard these considerations as results of this study as much as data.

Stratigraphy of Fossil Localities

The ages of mesofossil localities in Portugal, the source of six fossil taxa analyzed here, have been a topic of recent discussion. These localities were originally thought to range from Valanginian-Hauterivian to Barremian-Aptian (Friis et al. 1994), but this has required revision in light of sequence stratigraphy (Dinis et al. 2002, 2008) and palynological studies on coastal marine sections (Heimhofer et al. 2005, 2007). The Luz section in SW Portugal appears to be continuous from the earliest Aptian to the early Albian, but in the Cresmina section west of Lisbon, much of the Aptian is missing due to a regional unconformity, which cuts down to near the base of the Cretaceous in the area of the Vale de Agua, Buarcos, and Famalicão mesofossil floras farther north. These floras are from the lower part of the Figueira da Foz Formation, which overlies the unconformity. Dinis et al. (2008) assumed that deposition of this unit began in the late Aptian, based on dating of the unconformity in offshore wells, whereas Heimhofer et al. (2005, 2007) argued that it began in the early Albian, based on correlations with Cresmina. At Cresmina, the coarse clastic

unit above the unconformity (Rodízio Formation) yielded two pollen samples. The upper of these was dated by dinoflagellates as early Albian, but this does not rule out a late Aptian age for the oldest beds (Dinis et al. 2008). These samples contain the first tricolpate pollen in the section, while the upper one includes monosulcates (*Retimonocolpites* sp. 7) of the *Clavatipollenites rotundus* type, both of which enter in the dated early Albian at Luz (Heimhofer et al. 2007) and in England (Kemp 1968; Laing 1975).

Heimhofer et al. (2005, 2007) argued that in situ pollen reported from Vale de Agua, Buarcos, and Famalicão indicates a post-Aptian age, since it includes not only diverse monosulcates but also ~15% tricolpates. Friis et al. (2006, 2010a) and von Balthazar et al. (2005) moved toward this view by revising the age to late Aptian or early Albian. However, close examination of the data of Heimhofer et al. (2007, figs. 8, 9) suggests that the three localities are from the upper part of the interval that Heimhofer et al. (2007) considered early Albian. The tricolpates in the lower part of this interval are reticulate, but in the upper part these are joined by striate forms, and such pollen is known in flowers from Vale de Agua (Pedersen et al. 2007). Striate tricolpates occur earlier in Northern Gondwana, in the early Aptian of Egypt (Penny 1988b) and Gabon (Doyle et al. 1977; Doyle 1992), and in the late Aptian of Brazil (Heimhofer and Hochuli 2010), but they have not been reported from well-dated pre-Albian beds in Laurasia. Whether deposition of the Figueira da Foz began before or after the Aptian-Albian boundary, it seems most likely that the Vale de Agua, Buarcos, and Famalicão floras are securely above the base of the early Albian.

The age of the Torres Vedras and Catefica localities, in the continental Almagem Formation, nearer to but inland from Cresmina, is more problematic. The Torres Vedras flora is known in greater detail (Friis et al. 2010a). Heimhofer et al. (2005) assumed that it was the same age as the other floras, but Friis et al. (2010a) argued that it is significantly older, Barremian or early Aptian, based on the angiosperm flora, which contains less diverse monosulcates and only two reticulate tricolpates, and its position in the lower of two members

of the Almargem Formation. The boundary between these members has been equated with the regional unconformity (Dinis et al. 2008); if this is correct, Torres Vedras should be no younger than early Aptian. However, a Barremian age would conflict with the presence of *Pennipollis* (Friis et al. 2010a), which appears in early Aptian marine beds in England (Penny 1988a; Hughes 1994); statements that *Pennipollis* appears in the Barremian predate the present improved dating of the English section (see discussion of the *Pennipollis* plant). Other evidence suggests a younger age, after the regional unconformity. This includes the presence of tricolpates, which appear in the early Albian at Luz and in England (Kemp 1968; Laing 1975). However, this argument must be used with caution, since Hughes and McDougall (1990) reported isolated tricolpate grains in the latest Barremian and the middle Aptian of England, and the appearance of tricolpates is clearly diachronous at a global scale; they extend back to the probable latest Barremian in Northern Gondwana (Doyle 1992). It is possible that reticulate monosulcates with a distinct sulcus margin figured by Friis et al. (2010a, pl. IV) include *C. rotundus*, which also appears in the early Albian of England, but this is uncertain because early Albian *C. rotundus* has not been studied with SEM. Torres Vedras also yields flowers with typical *Asteropollis* pollen, with a four-branched sulcus; possibly related trichotomosulcate grains (with a three-branched sulcus) occur through the Aptian, but grains with a four-branched sulcus first appear in the early Albian of the coastal sequences and have not been confirmed from Aptian rocks elsewhere (see discussion of the *Asteropollis* plant).

Whether Torres Vedras is Aptian or Albian, it may be close in age to the upper part of Zone I in the Potomac Group (Brenner 1963), which includes the Fredericksburg, Baltimore, and upper Drewrys Bluff leaf localities. This interval has been considered Aptian (Brenner 1963; Doyle 1992) or early Albian (Doyle and Hickey 1976; Doyle and Robbins 1977; Hickey and Doyle 1977). More recently, based on palynological correlations with the marine sequence in Portugal, Hochuli et al. (2006) dated it as earliest Albian; notably, it contains at least two types of reticulate tricolpates, which are similar to those from Torres Vedras, and *C. rotundus* (aff. *Retimonocolpites dividuus* of Doyle and Robbins 1977; Hickey and Doyle 1977). Hochuli et al. (2006) argued that there is a significant hiatus between Potomac Zones I and II, based on the lack of floras like those from the late early Albian of Portugal, in which the angiosperm element is still dominated by diverse monosulcates but includes new tricolpate types, including striates. This would suggest that Zone II begins in the middle Albian, a date supported by the appearance of one of Brenner's (1963) most common Zone II index species, *Apiculatisporis babsae*, at the base of the middle Albian in England (Kemp 1970).

These correlations do not affect our previous dating of the Kenilworth (=Bladensburg, Subzone II-A) and Puddledock (lower Subzone II-B) localities as middle Albian or Mauldin Mountain (lower Zone III) as early Cenomanian (as in Pedersen et al. 1991). Friis et al. (1995, 1997a, 2011) and von Balthazar et al. (2008) considered Puddledock early or middle Albian, based in part on Doyle (1992), but the arguments of Hochuli et al. (2006) strongly favor a middle Albian age. Hochuli et al. (2006) suggested that Subzone II-B, considered by earlier authors to range from middle to early late Albian, may

instead be late Albian. However, this conclusion was based largely on broad quantitative comparisons, particularly the higher number of tricolpate species in Subzone II-B than in the Portuguese middle Albian, rather than the detailed species composition of the angiosperm flora. We have seen no new information to refute earlier species-level correlations with middle Albian floras (Doyle and Hickey 1976; Doyle 1977; Doyle and Robbins 1977; Hickey and Doyle 1977). For example, the species composition of the remarkably rich angiosperm pollen assemblage near the middle of Subzone II-B correlates especially well with the equally rich flora described by Hedlund and Norris (1968) from the well-dated late middle Albian of Oklahoma (Doyle 1977). Perhaps the quantitative discrepancies are a result of more complete sampling of angiosperms in Potomac fluvial facies than in marginal marine deposits in Portugal, and/or presence of only the lower part of the middle Albian in the Portuguese sections studied, if angiosperm diversity increased markedly later in the middle Albian.

Mohr et al. (2008) followed earlier authors in dating the Crato Formation of NE Brazil as late Aptian or early Albian. However, detailed palynological work by Heimhofer and Hochuli (2010) has favored a late Aptian age.

Analyses

As in our previous studies (Doyle et al. 2008; Endress and Doyle 2009; Doyle and Endress 2010), positions of fossil taxa were evaluated by analyzing the data set of Recent taxa plus one or more fossils using the parsimony program PAUP (Swofford 1990), with the arrangement of Recent taxa constrained to one of the two backbone trees (D&E, J/M) described in the Introduction and explained in detail in Endress and Doyle (2009), with random addition of taxa (100 replicates when adding more than one fossil) and tree-bisection-reconnection branch swapping. Alternatively, when investigating individual fossils, we moved the fossil to all possible positions on the backbone tree using MacClade (Maddison and Maddison 2003). The robustness of the relationships obtained and the relative parsimony of alternative arrangements were evaluated by searching for trees one, two, and three steps longer than the most parsimonious trees and by moving taxa manually with MacClade. We also conducted less exhaustive unconstrained analyses, with trees rooted on *Amborella*.

Character evolution and characters supporting particular relationships were investigated with MacClade. In the following sections, when characters are described as unequivocal synapomorphies, this means that the position of the character state change is unequivocal, not that it occurs only once on the entire tree. In discussing alternative less parsimonious relationships, statements that particular relationships are “*x* steps worse” mean “*x* steps less parsimonious than relationships in the most parsimonious tree(s).”

Results and Discussion

As in Doyle et al. (2008) and Doyle and Endress (2010), results of analyses of single fossils are shown in cladograms with the fossil placed at its most parsimonious position on the backbone tree or at one of its most parsimonious positions if

there are several. Thick lines indicate branches to which the fossil attaches in the set of most parsimonious trees, and successively thinner lines indicate branches to which it attaches in trees that are one and two steps less parsimonious.

The unconstrained analyses recovered many extant clades found with molecular data (e.g., the four magnoliid orders), and in most cases the fossils are associated with the same clades as in the constrained analyses and have similar positions within them (except *Canrightia*, linked with *Sarcandra* and *Chloranthus*, and *Appomattoxia*, sister to Chloranthaceae and *Ceratophyllum*). However, as in the morphological analysis of Doyle and Endress (2000), some higher-level relationships conflict with molecular data (most notably, Piperales, Nymphaeales, and monocots are nested within eudicots). Because these relationships were refuted by the total evidence analysis of Doyle and Endress (2000), we do not discuss the unconstrained analyses further.

Nymphaeales

Because the stem lineage of Nymphaeales diverges one node above the base of the angiosperms in most molecular trees, these trees predict that the nymphaealean line existed in the Early Cretaceous (this is not certain with trees in which *Amborella* and Nymphaeales form a clade). This by itself does not indicate whether the crown group had originated yet, and if it had not, the nymphaealean line might not yet have become aquatic. However, our results support proposals that three recently described Early Cretaceous fossils are crown group Nymphaeales (cf. Friis et al. 2011).

***Monetianthus*.** *Monetianthus mirus* is based on a single coalified flower from Vale de Agua, Portugal, of probable early Albian age (see Material and Methods). It was briefly reported as nymphaealean by Friis et al. (2001) and formally described by Friis et al. (2009) using SEM and X-ray microtomography (synchrotron radiation X-ray tomographic microscopy). It has an inferior ovary, a star-shaped ring of 12 fused carpels, and broken bases of tepals and stamens. Pollen adhering to the carpels and areas between the stamen bases is all of the same reticulate monosulcate type and was therefore assumed to be from this species (and probably this flower). The relationship of this fossil to Nymphaeales was questioned by Crepet et al. (2004) and Gandolfo et al. (2004), based on a phylogenetic analysis that showed its characters were equally compatible with Illiciaceae and other angiosperm families. However, the new observations of Friis et al. (2009) clarified several characters, including presence of several ovules per carpel and laminar placentation, a distinctive feature of most Nymphaeales. Friis et al. (2009) argued that the flower was at the anthetic stage because of the irregular height of the broken-off organ bases and the fact that the ovules had not matured into seeds. They distinguished 9–10 perianth parts and ~20 stamens by the elliptical versus rhombic shape of their bases and the different form of their vascular bundles. The only characters of ovule morphology that could be determined were anatropous curvature and presence of two integuments.

Friis et al. (2009) analyzed the phylogenetic position of *Monetianthus* using the data set of Saarela et al. (2007), with the addition of three characters observed in the fossil, namely, “star-shaped” carpel arrangement, septal slits, and ovules not

filling the carpel locule. In Doyle and Endress (2010), we adopted star-shaped carpel arrangement as a state of a character that includes both carpel number and arrangement, defined as more than five carpels in one whorl or series. This does not specify whether the carpel phyllotaxis is whorled or spiral, which may be difficult to determine in fossils; we use the term “ring” to describe this state. Among extant taxa, Friis et al. (2009) scored *Lactoris* as having the star-shaped state, like the related Aristolochiaceae, but because *Lactoris* has three carpels, rather than six in most Aristolochiaceae, it falls in our state for one whorl of 2–5 carpels, like most monocots. However, we have not added their new characters for septal slits, because these are restricted to *Nymphaea* subgenus *Anecphyta* (Conard 1905, as *Apocarpiae*), which is nested within *Nymphaea* in the phylogeny of Borsch et al. (2011), and are therefore presumably derived within the genus, or for ovule size, because this character may vary with developmental stage, which is difficult to assess in fossils, and appears to be correlated with ovule number (one vs. more).

New characters of Doyle and Endress (2010) that were not used by Friis et al. (2009) are (44) flowers not in heads (inferred from presence of a pedicel), (45) pedicel present, (49) short receptacle, (50) carpels not sunken in pits in the receptacle, (51) no cortical vascular system, (52) protruding floral apex, (65) more than two stamen whorls or series, (86) sulcus not branched, and (89) uniform reticulum. Other new characters were the result of splitting of older characters, such as (53) presence of a perianth, which was previously implicit in the character for number of whorls; (61) absence of a bract-derived calyptra, whose presence was a state of the character for outer perianth whorl; and (62) more than one stamen.

A potentially important character for placement of *Monetianthus* is floral phyllotaxis, which is whorled in Nymphaeales but spiral in *Amborella* and Austrobaileyales. Friis et al. (2001) interpreted all floral parts as whorled, but on more critical examination Friis et al. (2009) were unable to detect either orthostichies or clockwise and counterclockwise parastichies that are inclined at different angles, which would indicate whorled or spiral phyllotaxis, respectively (Endress 2006; Endress and Doyle 2007). Based on these observations and the absence of Fibonacci numbers, they concluded that phyllotaxis was probably not spiral, but whorled phyllotaxis could not be established either. They considered a third possibility, that phyllotaxis was irregular, to be unlikely because of the relatively small number of parts. They suggested that the flower had a whorled arrangement that was distorted during fossilization. Based on their figures, we suspect that the flower is not distorted and its phyllotaxis was truly irregular, a condition that we score as unknown (except when phyllotaxis varies between irregular and well defined, as may occur within species; Ren et al. 2010). We therefore follow Friis et al. (2009) in scoring phyllotaxis of the perianth (54) and androecium (63) as unknown. Given the uncertainty on phyllotaxis, we follow Friis et al. (2009) in scoring merism of the perianth (55) and androecium (64) as unknown. Friis et al. treated the number of perianth whorls (or series if spiral) as unknown, but based on the presence of tepal scars at two levels (Friis et al. 2009, fig. 1C), we score this number (56) as either two or more. The number of stamen whorls or series (65) was clearly more than two.

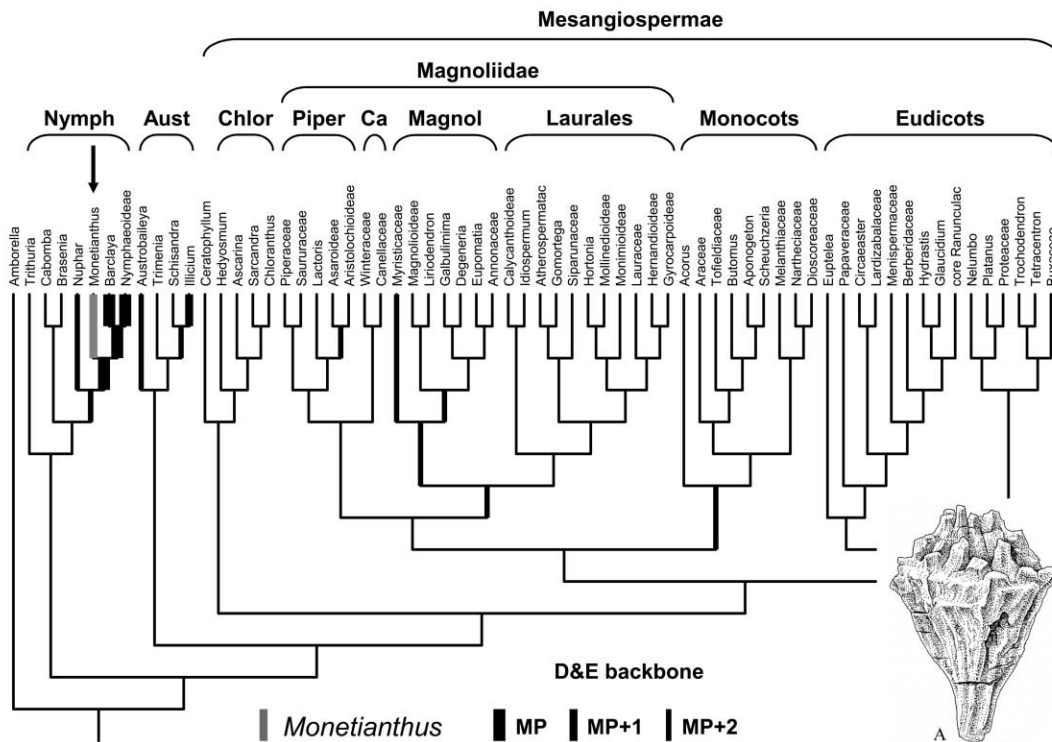


Fig. 2 One of three most parsimonious trees (1020 steps) obtained after addition of *Monetianthus* to the D&E backbone constraint tree. Thick lines indicate all most parsimonious (MP), one step less parsimonious (MP+1), and two steps less parsimonious (MP+2) positions for *Monetianthus*. Drawings of fossil flowers in this and subsequent figures reproduced from Friis et al. (2011), with permission of Cambridge University Press. Nymph = Nymphaeales, Aust = Austrobaileyales, Chlor = Chloranthaceae, Piper = Piperales, Ca = Canellales, Magnol = Magnoliales.

Considering more substantive differences in character analysis, Friis et al. (2009) scored pollen size (82), which is 18–20 μm , as small (<20 μm), but we score it as either medium (20–50 μm) or small (1/2) because pollen in fossil flowers studied with SEM is often smaller than dispersed pollen of the same type studied with light microscopy, suggesting the possibility that in situ pollen may undergo shrinkage (Doyle et al. 2008). Friis et al. (2009) scored carpel form (97) as ascidiate, but on page 1097 they compared carpels of the fossil with those of *Barclaya*, which has our intermediate state (plicate above and ascidiate below, with ovules in the ascidiate zone), versus ascidiate in other Nymphaeales. Schneider (1978) showed that carpels of *Barclaya* have a plicate zone that extends below the stigmatic area to the top of the area bearing ovules, so they are distinctly more plicate than carpels of *Nymphaea* and *Nuphar*, in which there is a shorter external slit surrounded by stigmatic tissue. In *Monetianthus* the carpel margin begins well above the level with ovules, so it may have had the condition seen in *Nymphaea* and *Nuphar*. *Monetianthus* has a ventral slit in the free part of the carpel (fig. 2B of Friis et al. 2009), but what happened below this is unclear. Because it is often impossible to distinguish plicate and ascidiate structure without developmental data (Endress 2005), we have scored this character as unknown. Friis et al. (2009) scored the character for stigmatic papillae, which Doyle and

Endress (2010) split into two characters (103, 104), as smooth or with unicellular papillae, but they (Friis et al. 2009, p. 1092) stated that the stigma “appears papillate with short, perhaps unicellular, papillae.” This would correspond to the state of character 104 for papillae unicellular or with one emergent cell, but because the nature of the papillae is too uncertain, we score this character as unknown. Friis et al. (2009) scored septal nectaries (111) as absent, but because of the possibility that the septal slits between the carpels were nectaries, we score this character as unknown.

Using the data set of Saarela et al. (2007), Friis et al. (2009) found that the most parsimonious position of *Monetianthus* was nested within the family Nymphaeaceae, as the sister group of *Barclaya* and Nymphaeoidae (*Nymphaea*, *Euryale*, and *Victoria*). Its next-best positions, which were one step less parsimonious, were sister to *Nuphar*, *Barclaya*, or Nymphaeoidae alone.

Our analysis using the D&E backbone tree (fig. 2) gave very similar results, despite the differences in character scoring. Results using the J/M backbone are virtually identical. *Monetianthus* has three most parsimonious positions, all nested within Nymphaeaceae: as the sister group of *Barclaya* plus Nymphaeoidae, of *Barclaya*, and of Nymphaeoidae. Considering unequivocal synapomorphies that support these results (derived states unambiguously placed on the branch in-

dicated), *Monetianthus* is linked with Cabombaceae plus Nymphaeaceae by more than two ovules per carpel (112), with Nymphaeaceae by a ring of more than five carpels (96) and eusyncarpy (106), and with *Barclaya* and Nymphaeaceae by inferior ovary (48) and globose pollen (83) versus superior ovary and boat-shaped pollen in *Nuphar* (for sources of data on Recent taxa, see Material and Methods). Another synapomorphy of *Monetianthus* and Nymphaeales is laminar placentation (113), in which we include both the typical laminar placentation of Nymphaeaceae and related conditions (such as “dorsal”) in Cabombaceae, but where this feature arose is equivocal, because the position of the single apical ovule in *Trithuria* (=Hydatellaceae) is uncertain (Rudall et al. 2007) and was therefore scored as unknown. The positions with *Barclaya* and Nymphaeaceae alone conflict with the monosulcate pollen of *Monetianthus*, since the two living groups are united by zonosulcate pollen (84), but the extra step in this character is balanced by the fact that *Monetianthus* shares medium-sized pollen (82) with *Barclaya* and a protruding floral apex (52) with Nymphaeaceae. Other positions in Nymphaeales are at least two steps less parsimonious.

As noted by Friis et al. (2009), two features of *Monetianthus* that are anomalous for Nymphaeaceae are reticulate pollen tectum (88) and ascendent rather than pendent ovule orientation (114). If *Monetianthus* is nested in Nymphaeaceae, it is most parsimonious to interpret these features as autapomorphies at the scale of Nymphaeales and as convergences with other groups. Friis et al. (2009) cited Moseley (1971) and Igersheim and Endress (1998) as showing that ovules may be horizontal or ascendent in *Nuphar* and *Barclaya*, but Moseley’s figures of *Nuphar* show that ovules are initially pendent, with some becoming horizontal or ascendent at anthesis. Based on Igersheim and Endress (1998, fig. 27), ovule direction in *Barclaya* appears to be irregular, possibly related to the fact that the ovules are orthotropous versus anatropous in other Nymphaeales. Ascidiate carpel form tends to be correlated with pendent ovules, so the ascendent orientation in *Monetianthus* might be explained if its carpels were more plicate than those of modern Nymphaeales, although ovules in multiovulate plicate carpels are more commonly horizontal.

A position of *Monetianthus* with *Illicium* in the Austrobaileales is only one step less parsimonious, in part because the tectum (88) is reticulate, as in Austrobaileales, rather than continuous, as in Nymphaeales. In addition, *Illicium* is as much like *Monetianthus* as Nymphaeaceae in having a ring of carpels (96). All other positions outside Nymphaeales are at least two steps less parsimonious. Determination of the floral phyllotaxis in *Monetianthus* could affect this picture. If the relevant characters (54, 63) of *Monetianthus* are scored as spiral, a relationship with *Illicium* (which is spiral) becomes one step more parsimonious than the position in Nymphaeaceae (which are whorled), but if these characters are scored as whorled, the position with *Illicium* becomes three steps worse.

Other characters not included in our data set could favor a relationship with Nymphaeales or clarify its position within the order if shown to be valid. Two are characters of Friis et al. (2009) that we rejected: ovules not filling the locule, as in *Monetianthus*, Cabombaceae, and Nymphaeaceae (vs. *Illicium*), which appears to be correlated with ovule number, and

presence of septal slits between the carpels, as in *Monetianthus* and *Nymphaea* subgenus *Anecphyra* (Conard 1905), which is nested in *Nymphaea* (Borsch et al. 2011). Unless *Monetianthus* is also nested in *Nymphaea*, which is unlikely in view of its small size and pollen morphology, septal slits are best interpreted as a convergence in the two taxa. Friis et al. (2009) noted that *Monetianthus* is like those Nymphaeales that have a relatively low number of ovules per carpel (e.g., *Nuphar*), since the ovules appear to be linear on the septa, rather than scattered over the surface, as in taxa with higher ovule numbers (e.g., *Nymphaea*, *Barclaya*); this character could favor a position below the *Barclaya*-Nymphaeaceae clade. Among characters that Friis et al. (2009) did not include in their data set, they suggested that the lowest appendage on the flower may be a closely associated floral subtending bract, a feature of many Nymphaeaceae (Chassat 1962; Endress and Doyle 2009). Friis et al. (2009) also noted that *Monetianthus* was like Nymphaeales and unlike *Illicium* in lacking resin bodies, assumed to be remnants of oil cells, in the carpels. Oil cells are absent in *Amborella* and Nymphaeales, and their origin is a presumed synapomorphy of Austrobaileales and mesangiosperms (Doyle and Endress 2000).

From a broader evolutionary perspective, one of the most interesting features of *Monetianthus* is its small size. Living Nymphaeaceae have large flowers, but like most Early Cretaceous flowers (Friis et al. 2011) *Monetianthus* was much smaller (~3 mm long and 2 mm across without stamens or perianth parts, estimated to be ~1 cm across with these parts; Friis et al. 2011), more like flowers of Cabombaceae, and *Carpestella* (treated next) was even smaller (~0.65 mm). In terms of morphological parsimony, this could mean either that small flowers were ancestral in Nymphaeaceae and there was a parallel size increase trend in *Nuphar* and other living Nymphaeaceae or that flower size increased on the line to Nymphaeaceae and secondarily decreased in *Monetianthus*. However, it could be argued that the age of *Monetianthus* and the fact that *Carpestella* was also small might shift the balance toward the first scenario, which would bring Nymphaeales in line with the broader picture of early floral evolution. Discovery of fossils situated elsewhere in Nymphaeaceae could resolve this problem.

Reticulate monosulcate pollen like that associated with *Monetianthus* was the predominant dispersed angiosperm type prior to the rise of tricolpates, but it contrasts with the pollen of living Nymphaeales, which has a continuous tectum and is usually larger (except in *Barclaya* and *Trithuria*). As already noted, pollen characters are partly responsible for the fact that it is only one step less parsimonious to place *Monetianthus* in Austrobaileales. Parsimony optimization unambiguously indicates that the reticulate tectum of *Monetianthus* was derived within Nymphaeales and not homologous with the same state in other groups, which originated at the node connecting Austrobaileales and mesangiosperms (Doyle 2005). This scenario could change if reticulate pollen is found to be more widespread in early Nymphaeales.

This discussion depends on the assumption that the pollen adhering to the flower was from *Monetianthus*, but this is uncertain. Although Friis et al. (2009) included pollen characters in their analysis, E. M. Friis (personal communication, 2012) cautioned that the association of the pollen was not

demonstrated, as only stamen bases are preserved and therefore no pollen was actually found in situ in anthers. If the pollen was not from *Monetianthus*, the anomaly would disappear. To address this issue, we analyzed the data set with pollen characters (81–95) scored as unknown. In this analysis the most parsimonious position of *Monetianthus* is sister to Nymphaeaceae, since this is no longer contradicted by the aperture character and is favored by the protruding floral apex, and a position with *Illicium* is three steps less parsimonious. Clearly, discovery of *Monetianthus* specimens with pollen in the anthers could resolve these problems.

***Carpestella*.** *Carpestella lacunata* is based on a single charcoalified flower from the middle Albian Puddledock locality in Virginia, described by von Balthazar et al. (2008) using SEM and X-ray microtomography. *Carpestella* is like *Monetianthus* in having an inferior ovary, a star-shaped ring of fused carpels with septal slits, and numerous tepals and stamens (represented by ~15 larger oval and ~60 smaller quadrangular scars, respectively). However, it is smaller (0.65 mm long, 0.45 mm wide) and less well preserved, with the top of the 13-carpellate gynoecium missing and no adhering pollen. One carpel contains a poorly preserved structure that von Balthazar et al. (2008) interpreted as a possible seed, but this provides no reliable evidence on ovule characters.

Von Balthazar et al. (2008) examined the phylogenetic position of the fossil using the data set of Saarela et al. (2007), with the addition of characters for star-shaped carpel arrangement and septal slits. As with *Monetianthus*, there are several new characters in our data set that can be scored: (44) flowers not in heads, (45) pedicel present, (49) short receptacle, (50) carpels not sunken in pits, (53) presence of a perianth, (61) absence of a bract-derived calyptra, (62) more than one stamen, and (65) more than two stamen whorls or series. The gynoecium has a “central bump” suggestive of the protruding floral apex of Nymphaeaceae and *Illicium* (52), but because the top of the carpels is missing, it cannot be established that the apex protruded. An existing character not scored by von Balthazar et al. (2008) is (47) bisexual flowers.

Von Balthazar et al. (2008) and Friis et al. (2009) interpreted phyllotaxis of the perianth and androecium as spiral, but we score both characters as unknown. In their figure 1C, von Balthazar et al. marked one set of parastichies in the androecial zone (slanting to the left as seen in surface view), but in order to distinguish spiral from whorled, two sets must be considered. A second set (slanting to the right) is visible in their figure; assuming there was no deformation during fossilization, it is at a slightly higher angle than the first set, indicating that the phyllotaxis was not perfectly whorled, but this angle is not as much higher than the first angle as would be expected with a Fibonacci spiral (the type almost always found in flowers with spiral phyllotaxis; Endress and Doyle 2007). Parastichies with similar angles were observed by Wolf (1991) in the androecium (though not the perianth) of *Nymphaea alba*, along with rarer whorled and Fibonacci conditions, so whether this sort of phyllotaxis is interpreted as spiral or whorled, it is consistent in *Carpestella* and *Nymphaea*. Von Balthazar et al. (2008) scored perianth and androecium merism as irregular, but Endress and Doyle (2009) eliminated this state because it is redundant with spiral phyllotaxis; we score both characters (55, 64) as unknown. As in *Monetianthus*, von Balthazar et al. (2008) scored

septal nectaries (111) as absent, but we treat this character as unknown.

When von Balthazar et al. (2008) analyzed the phylogenetic position of *Carpestella*, they obtained highly ambiguous results, but in discussion they emphasized similarities to Nymphaeaceae and *Illicium*. With the greater number of characters in our current data set and both backbone trees, we found the same three most parsimonious positions for *Carpestella* as for *Monetianthus* (fig. 3)—nested within Nymphaeaceae, as the sister group of *Barclaya*, Nymphaeaceae, or both. There are no unequivocal synapomorphies that associate *Carpestella* with Nymphaeales as a whole or with Cabombaceae plus Nymphaeaceae, but it is linked with Nymphaeaceae by the ring of carpels (96) and eusyncarpy (106) and with *Barclaya* and/or Nymphaeaceae by the inferior ovary (48). There are no characters that support or contradict a relationship with *Barclaya* or Nymphaeaceae alone. Remarkably, a position linked with the eudicot *Trochodendron* is only one step less parsimonious, supported by more than two whorls (series) of stamens (65) and the ring of carpels (96), plus the inferior ovary (48), as in both *Trochodendron* and its sister group *Tetracentron*. This illustrates how lack of pollen in fossil flowers can be a serious handicap, since *Trochodendron* and related eudicots differ sharply from Nymphaeales in having reticulate tricolpate pollen. Other positions outside Nymphaeales are at least two steps worse, including one with *Illicium*, which has a similar gynoecium. If floral phyllotaxis is assumed to be spiral, a relationship with *Illicium* becomes as parsimonious as one with Nymphaeaceae but not more so. Von Balthazar et al. (2008) stressed the septal slits as a feature shared with *Nymphaea* but not *Illicium*, but as discussed in connection with *Monetianthus*, this is unlikely to be homologous in the fossil.

Despite their identical most parsimonious positions on the tree, *Carpestella* and *Monetianthus* are clearly not the same taxon. *Carpestella* has a few more perianth scars and carpels and many more stamen scars than *Monetianthus*, but the two taxa do not differ in the scoring of any of our characters that can be determined in both, except more than two perianth whorls or series (56), rather than either two or more than two in *Monetianthus*. As a result, our data provide no evidence on whether they form a clade or two lines located at different points in Nymphaeaceae. If it could be shown that they formed two lines, this would add support for the hypothesis of a parallel size increase trend within Nymphaeaceae.

***Pluricarpellatia*.** *Pluricarpellatia peltata* is based on impression fossils of rhizomes with attached roots, leaves, and flowers in the fruit stage, locally permineralized with iron oxide, from the Crato Formation of NE Brazil (late Aptian). These remains were figured by Mohr and Friis (2000) and formally described by Mohr et al. (2008). *Pluricarpellatia* resembles Cabombaceae in having slender stems and more or less peltate leaves but differs in having more carpels (6–12), while it differs from Nymphaeaceae in being apocarpous. Whereas there is no direct evidence on the ecology of *Monetianthus* and *Carpestella*, the vegetative morphology of *Pluricarpellatia*, with rhizomes bearing roots, leaves with long petioles, and flowers with long pedicels, clearly indicates an aquatic habit, consistent with the lacustrine origin of the sediments.

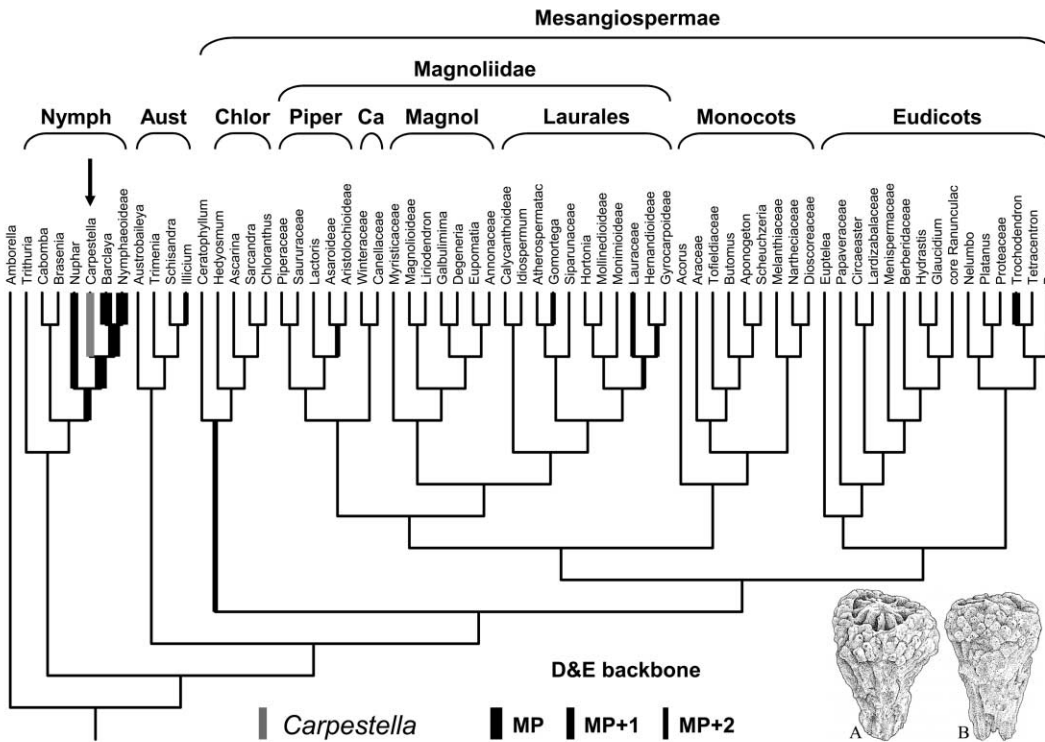


Fig. 3 One of three most parsimonious trees (1016 steps) obtained after addition of *Carpestella* to the D&E tree. Conventions and abbreviations as in fig. 2.

Using a data set of vegetative characters only (Taylor 2008), Mohr et al. (2008) and Taylor et al. (2008) found two most parsimonious positions for *Pluricarpellatia*, depending on how they scored the leaves, which they described as varying between eccentrically and centrally peltate: sister to Cabombaceae when the leaves were scored as centrally peltate but sister to Nymphaeales as a whole (not including *Trithuria*) when the leaves were scored as eccentrically peltate. However, Taylor (2008) used the term “peltate” to describe attachment of the petiole to the plane of the blade at a high angle (D. W. Taylor, personal communication, 2012), rather than in the well-established sense of extension of the blade around the adaxial side of the petiole. Taylor (2008) scored all living Nymphaeales as having one of four peltate states, but in most Nymphaeaceae (except *Euryale* and *Victoria*) the petiole is located at the top of a narrow notch between the basal lobes and there is no adaxial extension of the blade; we score this condition as nonpeltate. Because we define the peltate state as including cases in which some but not all leaves of the plant are peltate (32), we score *Pluricarpellatia* as peltate.

Mohr et al. (2008) described the vegetative phyllotaxis (21) as “unclear,” but the leaves were certainly not regularly opposite, and most were clearly alternate. However, whether they were spiral or distichous (22) is unknown. The angle and shape of the petiole attachment and the lack of thickening of the node indicate a nonsheathing base (25) with no stipules (26).

Single flowers with long pedicels were borne along the stem, with no visible subtending leaves or bracts or bracts on the

pedicel, but the preservation is not good enough to establish whether such appendages were absent or had been shed. Because we interpret lateral flowers as solitary if they have more than two bracts on the pedicel and borne in racemes if they have two or fewer bracts (Endress and Doyle 2009; Endress 2010), we score the inflorescence character (42) as either solitary or raceme (0/2) and floral subtending leaves or bracts (46) as unknown. By contrast, we score extant Cabombaceae and Nymphaeaceae, which have single flowers borne along a rhizome that also bears vegetative leaves, as having racemes (Endress and Doyle 2009). Rudall and Bateman (2010, p. 405) implied that this interpretation was due partly to our inclusion of the Early Cretaceous fossil *Archaeofructus*, which has more obvious racemes, in Nymphaeales, but this is incorrect. Rather it followed from the morphological analysis of branching in Nymphaeales by Chassat (1962) and the general principles of inflorescence classification summarized in Endress (2010).

In the flowers, only characters of the gynoeical zone are preserved. Mohr et al. (2008) thought that the carpels were most likely attached in a spiral, but we consider this uncertain. The number of carpels (6–12) suggests that they may have been borne in more than one whorl or series, as in *Brasenia*, but because it is possible that they were in one whorl, as in Nymphaeaceae, we score carpel number/arrangement (96) as uncertain (2/3). Mohr et al. (2008) described the carpels as slightly ascidiate when young, whereas Friis et al. (2009, p. 1098) said that they “appear to be plicate.” Many carpels show a dark line running down the middle, but it is uncertain

whether this was the ventral suture of a plicate carpel, a stigmatic crest like that of the ascidiate carpel of *Brasenia* (Endress 2005), or a vascular bundle, so we score carpel form (97) as unknown. Mohr et al. (2008) suggested that the fruits dehiscid along this line, but in the absence of actual dehiscid carpels we treat fruit dehiscence (125) as unknown. Mohr et al. described the stigmatic area as “not modified”; it is possible that a style was present but fell off in the fruit stage, but because the style does persist and is conspicuous in fruits of most ANITA lines and magnoliids that have a style (including *Brasenia* and *Cabomba*: Takhtajan 1988), we score style (101) as absent. Mohr et al. (2008) described the ovules as “most likely with laminar attachment,” but because this cannot be determined from their figures, we score placentation (113) as unknown. The seeds were clearly anatropous (115), but the only other ovule and seed characters that can be scored are presence of an operculum (134) and a palisade exotesta (128), which is somewhat degraded but appears to be comparable to that in better-preserved dispersed seeds from Portugal described by Friis et al. (2010a).

In our analysis (fig. 4), *Pluricarpellatia* has three most parsimonious positions, with both backbone trees: attached to the stem lineage of Cabombaceae, before origin of an elongate style; to *Brasenia*; or to the stem lineage of Nymphaeaceae, before the origin of syncarpy. It is associated with Nymphaeales as a whole by palisade exotesta (128) and operculum (134). Two additional synapomorphies that cannot be localized precisely, because *Trithuria* is too modified to score, are

palmate venation (30) and entire leaf margin (35). It is linked with Cabombaceae plus Nymphaeaceae by more than two ovules per carpel (112). The position with Cabombaceae is supported by peltate leaves (32), while the two other positions correspond to different assumptions on the carpel number character (96): with Nymphaeaceae if the carpels are in a whorl (series) of more than five (state 2), with *Brasenia* if they are in more than one whorl (state 3). The presumed absence of a style results in an extra step in this character (101) when the fossil is sister to *Brasenia*. Positions sister to Cabombaceae plus Nymphaeaceae, to *Cabomba*, to *Nuphar*, and to *Barclaya* plus Nymphaeaceae are one step less parsimonious.

The tentative nature of these results is underlined by the fact that it is only one step worse to link *Pluricarpellatia* with *Nelumbo*, in the eudicot order Proteales, which also has entire-margined (31) and peltate (32) leaves, carpels in more than one series (96), and a sessile stigma (101). Discovery of pollen of *Pluricarpellatia* could strengthen or refute this alternative, since *Nelumbo* has reticulate tricolpate pollen. This problem is all the more relevant because peltate leaves thought to be related to *Nelumbo*, called *Nelumbites*, are a conspicuous element in Albian floras of Virginia (Berry 1911; Hickey and Doyle 1977; Upchurch et al. 1994), Kazakhstan (Vakhrameev 1952), and Siberia (Samylina 1968). In Virginia, Upchurch et al. (1994) associated *Nelumbites* with floral receptacles that resemble those of *Nelumbo* in having carpels borne in pits but differ in being round rather than flat topped, and our phylogenetic analysis (Doyle and Endress 2010) supported a rela-

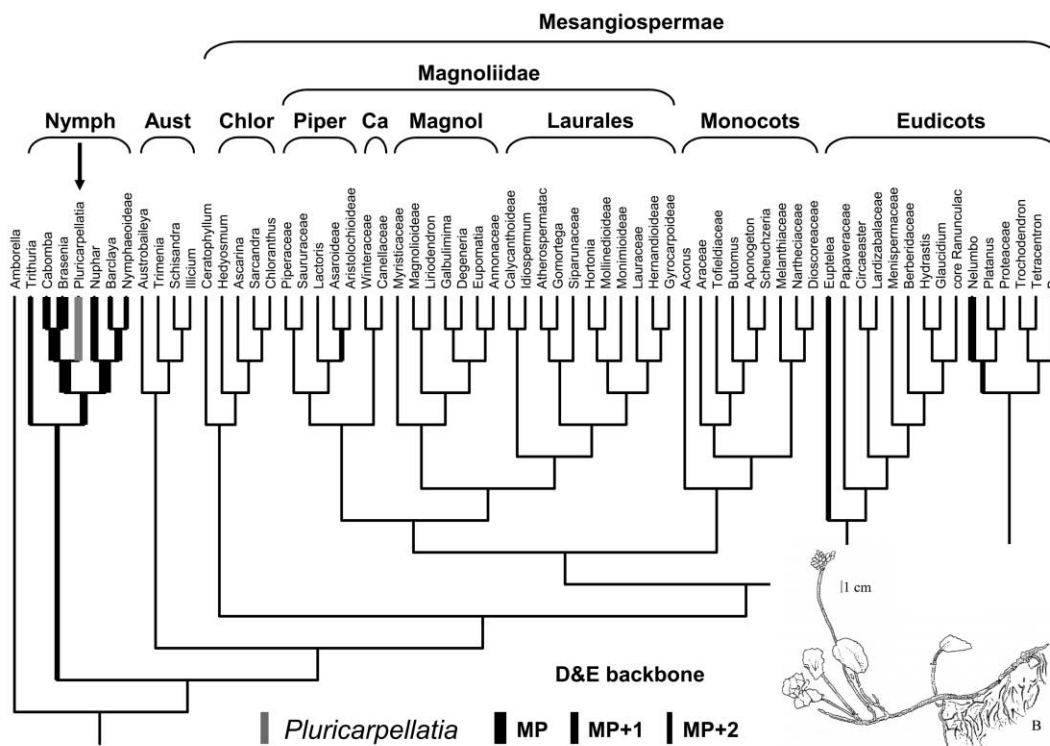


Fig. 4 One of three most parsimonious trees (1017 steps) obtained after addition of *Pluricarpellatia* to the D&E tree. Conventions and abbreviations as in fig. 2.

tionship to *Nelumbo*. Mohr et al. (2008) discussed similarities and differences between *Pluricarpellatia* and *Nelumbites*, but they confused the picture by referring to *Nelumbites* as nymphaealean and not mentioning the evidence that it was related to *Nelumbo*. However, there are other peltate leaves in the Albian of Jordan (*Scutifolium*; Taylor et al. 2008) and Kansas (*Brasenites*; Wang and Dilcher 2006) that are more like *Brasenites* than *Nelumbo* and *Nelumbites* in tending to be longer than wide, rather than round or wider than long, and in other characters used by Taylor et al. (2008).

Archaeofructus. Another fossil that has been associated with Nymphaeales is *Archaeofructus*, from the Barremian-Aptian Yixian Formation of China (Sun et al. 1998, 2001, 2002), an aquatic plant with finely dissected leaves and reproductive axes bearing pairs of stamens, single or paired carpels, and no accessory parts. A phylogenetic analysis by Sun et al. (2002) concluded that *Archaeofructus* was a stem relative of all living angiosperms, but Friis et al. (2003) argued that its proposed primitive features could be the result of reduction in an aquatic habitat, while the ternate leaf organization could support a relationship to basal eudicots and/or the Early Cretaceous fossil *Vitiphyllum*. The Friis et al. interpretation was in turn criticized by Crepet et al. (2004) and Crepet (2008). However, analyses by Endress and Doyle (2009) with the D&E backbone nested *Archaeofructus* in Nymphaeales with the highly reduced Hydatellaceae (now treated as one genus, *Trithuria*; Sokoloff et al. 2008), while analyses with the J/M backbone linked it with *Ceratophyllum*. An analysis in the context of seed plants as a whole (Doyle 2008) found that it was five steps more parsimonious to link *Archaeofructus* with *Trithuria* than to place it below the angiosperm crown group.

There have been several more recent observations on *Archaeofructus*; none of these are well enough corroborated to justify a new analysis, but we review them briefly because of the proposed relationship of *Archaeofructus* to Nymphaeales. Critical support for this relationship came from presumed in situ pollen grains studied with SEM by Sun et al. (2001, 2002), namely, their monosulcate aperture, boatlike shape, and continuous tectum. However, Friis et al. (2003, 2011) questioned whether these structures were indeed pollen, because of their irregular size and shape. Without pollen characters, Endress and Doyle (2009) found that the most parsimonious position of *Archaeofructus* with the D&E backbone was nested among basal eudicots, with *Euptelea*; with the J/M backbone, the connection with *Ceratophyllum* was strengthened. Eudicot affinities might be supported by the fact that the stamens are borne in pairs, suggesting the dimerous condition of Papaveraceae and other basal eudicots (Drinnan et al. 1994; Endress and Doyle 2009), and by the ternate leaf dissection. However, ternate dissection is inferred to have evolved within Ranunculales (Doyle 2007), which have tricolpate pollen, but there are only doubtful reports of tricolpate pollen before the Albian in this paleolatitudinal belt (see Doyle 2012). Endress and Doyle (2009) scored ovule curvature in *Archaeofructus* as unknown, but Ji et al. (2004) interpreted the ovules of *Archaeofructus eoflora* as orthotropous; if this is confirmed and holds for *Archaeofructus* as a whole, it would strengthen a relationship to *Ceratophyllum*. Wang and Zheng (2012) interpreted the ovules as “dorsal” (attached to the midrib), combined with laminar in our placentation character (113), rather than ven-

tral as assumed by Sun et al. (2002), which would strengthen a relationship to Nymphaeales. More work is needed to determine whether the seeds have a palisade exotesta (as assumed by Endress and Doyle 2009) or an operculum, a nymphaealean character previously scored as unknown.

Despite the remarkable preservation of *Archaeofructus*, there are so many questions concerning critical aspects of its morphology that we consider its systematic position highly ambiguous. It should certainly not be used to set a minimum age for crown group Nymphaeales in molecular dating analyses.

Implications for the history of Nymphaeales. Although the position of Nymphaeales in most molecular trees implies that the nymphaealean line existed in the Early Cretaceous, other evidence is needed to determine whether the crown group existed then. This question was addressed by a molecular dating analysis of Yoo et al. (2005). Under the assumption that the angiosperm crown group is not much older than the first definite angiosperm fossils (131.8 Mya, Hauterivian), this study concluded that crown group Nymphaeales (not including *Trithuria*) did not originate until the Eocene (44.6 ± 7.9 Mya). Yoo et al. recognized that this result conflicted with the reports of *Monetianthus* (Friis et al. 2001) and *Microvictoria*, from the late Turonian (~90 Mya) of New Jersey, which an analysis by Gandolfo et al. (2004) linked with *Victoria* or *Victoria* plus *Euryale*. Yoo et al. therefore suggested that these fossils were not crown group Nymphaeales but rather stem relatives.

These conclusions were criticized by Nixon (2008), who reaffirmed the crown group position of *Microvictoria* and argued that the conflict was more likely due to failure of the dating method due to rate heterogeneity—e.g., if a rapid radiation of Nymphaeales during invasion of aquatic habitats was followed by stasis. However, there is reason to doubt that *Microvictoria* belongs in Nymphaeales (Endress 2008, p. 855): it has spiral rather than whorled floral phyllotaxis and outermost tepals that did not enclose the flower, and the shape of the floral base is inconsistent with the supposed presence of an inferior ovary. Furthermore, as noted by Yoo et al. (2005), the analysis of Gandolfo et al. (2004) included only Nymphaeales and could not test the hypothesis that *Microvictoria* belonged elsewhere.

Whatever the status of *Microvictoria*, our results concerning *Monetianthus*, *Carpstellata*, and *Pluricarpellatia* strengthen the view that crown group Nymphaeales, and in fact Nymphaeaceae, are much older than inferred by Yoo et al. (2005)—at least 110 Mya. This was also noted by Taylor et al. (2008), who concluded that *Pluricarpellatia* and *Scutifolium* were related to Cabombaceae, and by Friis et al. (2011). Characters of the best-understood fossil, *Monetianthus*, do not make sense for a stem relative of Nymphaeales, since the most recent common ancestor of the order can be reconstructed as having hypogynous flowers and a smaller number of free carpels containing one ovule each (Endress and Doyle 2009). Crown group angiosperms are almost surely older than Hauterivian (Doyle 2012), but as Yoo et al. (2005) noted, if the relative ages found in their analysis are correct, the existence of crown group Nymphaeales in the Aptian-Albian would imply that angiosperms are far older (~330 Mya, mid-Carboniferous). Given the broad consistency of the angiosperm fossil record with molecular phylogenies (Doyle 2012), it seems more likely that the conflicts between molecular and fossil dates for Nym-

phaeales are due to problems of molecular dating methods in dealing with rate heterogeneity.

Dispersed seeds with a palisade exotesta and an operculum from the Aptian-Albian of Portugal and the Potomac Group have been considered evidence for Nymphaeales (Friis et al. 2000b, 2006, 2010a, 2011). However, both features appear to be ancestral in Nymphaeales, including *Trithuria* (Hamann 1975), so they do not indicate whether these seeds are from stem relatives or crown group members. Furthermore, the report by Yamada et al. (2003) of a small operculum in *Trimenia* (see appendix) raises the possibility that this feature was once more widespread in the ANITA grade.

Because all extant Nymphaeales are aquatic, except for an obvious reversal to wet terrestrial habitats in the well-nested species *Barclaya rotundifolia* (Schneider and Carlquist 1995; Feild et al. 2004), it is most parsimonious to assume that the aquatic habit had originated by the time the crown group evolved. Thus, recognition of crown group fossils in the Aptian-Albian indirectly implies that these were aquatic plants, and this is confirmed by the vegetative morphology of *Pluricarpellatia*, *Scutifolium*, and *Brasenites* (Wang and Dilcher 2006; Taylor et al. 2008; Friis et al. 2011).

Austrobaileyles

Anacostia. *Anacostia* includes four species described by Friis et al. (1997a) based on isolated lignitized and charcoalified carpels at the fruit stage, each with a single enclosed seed, and adhering pollen: *Anacostia portugallica* and *Anacostia teixeirae* from Buarcos, Famalicão, and Vale de Agua, Portugal (early Albian); *Anacostia virginiana* from Puddledock, Virginia (middle Albian); and *Anacostia marylandensis* from Kenilworth, Maryland (middle Albian). We also include an axis bearing numerous spirally arranged immature carpels from Puddledock (Friis et al. 1997a, fig. 6A), which Friis et al. associated with *Anacostia* based on epidermal structure and adhering pollen.

The pollen, which varies from monosulcate to trichotomosulcate, is of a type once compared with monocots because its sculpture grades from coarsely reticulate to much finer on different parts of the grain, a pattern not known in other monosulcate angiosperms (Doyle 1973; Walker and Walker 1984). Such pollen was first identified as *Retimonocolpites* (Doyle 1973) or *Liliacidites* (Doyle and Hickey 1976; Doyle and Robbins 1977; Walker and Walker 1984), but because it differs from species originally assigned to *Liliacidites* in having finer sculpture at the proximal pole and around the middle of the sulcus, rather than at the ends of the grain, it was transferred to the new genus *Similipollis* by Góczán and Juhász (1984). Because Doyle and Hotton (1991) could find no reports of this pattern of grading in monocots, they questioned whether *Similipollis* was monocotyledonous. However, Harley (1997) and Dransfield et al. (2008) described similar grading in two derived genera of palms (*Chamaedorea*, *Pseudophoenix*).

Friis et al. (1997a) recognized that *Anacostia* shows no sign of monocot affinities. They noted that it has similarities to several “magnoliid” groups, particularly Canellaceae, Winteraceae, Illiciaceae (= *Illicium*), and Schisandraceae (= *Schisandra*), now assigned to Canellales and Austrobaileyles, but they hesitated to relate it to any particular group (cf. Friis et

al. 2011). Friis et al. (1997a) emphasized that the seed coat of *Anacostia* is like that of the taxa listed in having a palisade exotesta. However, this is underlain by a layer of cells with digitate anticlinal walls, which resembles the sclerotic mesotesta of Austrobaileyles (Corner 1976; Takhtajan 1988; Oh et al. 2003) but has no counterpart in Canellales.

Because *Similipollis* had been compared with monocots, Doyle et al. (2008) analyzed *Anacostia* as part of a review of Early Cretaceous monocots, using the data set of Endress and Doyle (2009). They used the Puddledock floral axis to score *Anacostia* as having a pedicel (45), superior ovary and no hypanthium (48), elongate receptacle (49), and apocarpous gynoecium (106). Among characters introduced or redefined since Doyle et al. (2008), this specimen also allows us to score flowers as not in heads (44), carpels not sunken in pits in the receptacle (50), and carpels in more than one whorl or series (96). No characters of the perianth and androecium can be scored. The Puddledock axis shows that the carpels had a distinctly spiral phyllotaxis, but there are few traces of other floral parts below these, so there is no convincing evidence on their identity or arrangement. Probable stamen bases apparently in the same spiral phyllotaxis as the carpels are preserved in another specimen from Puddledock (Crane et al. 1994, fig. 1a), but its relation to *Anacostia* is uncertain because it has no associated pollen.

As in Doyle et al. (2008), we score pollen size (82), measured by Friis et al. (1997a) as 12–18 μm , as either small or medium, because similar dispersed pollen is larger than 20 μm , suggesting the possibility of shrinkage in the in situ pollen, and nexine thickness (95), which appears thick in TEM sections (Friis et al. 1997a, fig. 3), as either thick or thin (1/2) because the sections are oblique and the nexine is thinner in dispersed pollen. One of the pollen grains figured by Friis et al. (1997a, fig. 8C) has a uniform reticulum and an extended sulcus, so it was probably from a different taxon.

Doyle et al. (2008) treated placentation (113) as unknown, but the basal position of the single seed in the laterally compressed fruits suggests that it had our “ventral” state (0), like the basal ovule of modern taxa such as *Illicium* and Myristicaceae. The cells with digitate anticlinal walls that make up the inner layer of the seed coat are very similar to those of the sclerotic mesotesta in *Illicium* (Oh et al. 2003), which supports scoring of the mesotesta character (129) as the same state (1; see appendix for different interpretations of the mesotesta in Austrobaileyles by Yamada et al. 2003). Because there is too little space between the two layers for a fleshy sarcotesta, we score this character (130) as absent.

As in Doyle et al. (2008), our analyses using both backbone trees nest *Anacostia* within Austrobaileyles (fig. 5), as the sister group of either *Illicium* plus *Schisandra* or *Schisandra* alone. Synapomorphies associating it with Austrobaileyles as a whole are more than one whorl or series of carpels (96) and sclerotic mesotesta (129); it is linked with Austrobaileyles other than *Austrobaileya* by palisade exotesta (128) and with *Illicium* plus *Schisandra* by ascendent ovule orientation (114; modified to horizontal in *Schisandra*, an autapomorphy in this context). When *Anacostia* is sister to *Illicium* plus *Schisandra*, the two living taxa are united by tri/hexacolpate pollen (84); when *Anacostia* is linked with *Schisandra* alone, there is an extra step in this character, but this is balanced by the fact

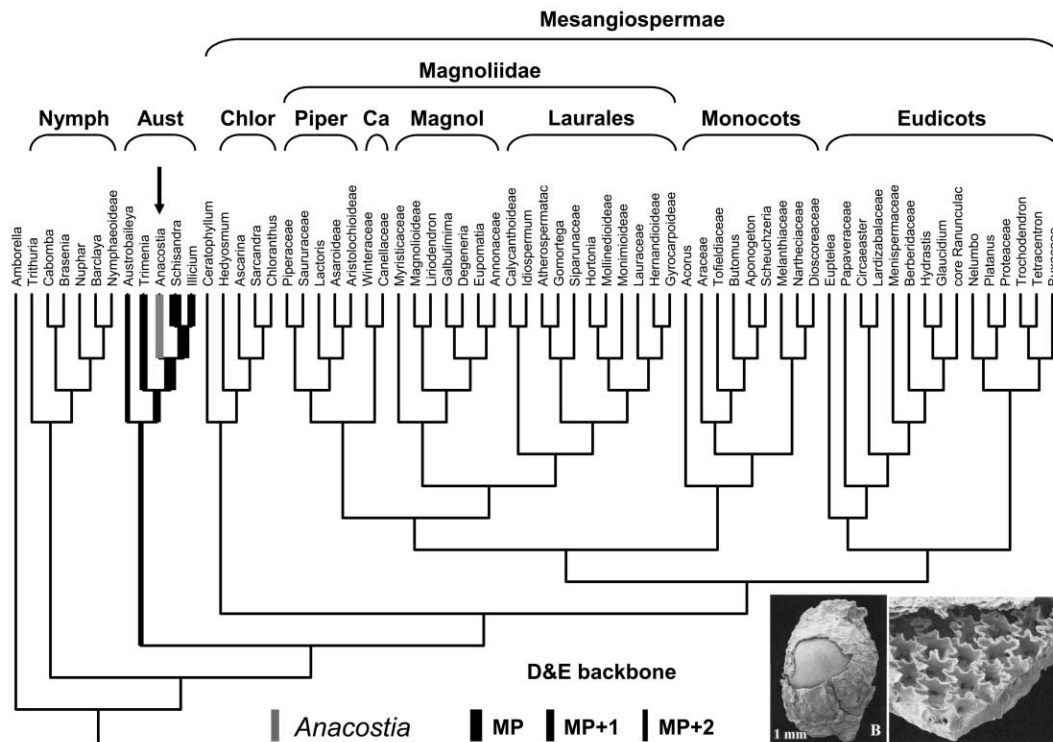


Fig. 5 One of two most parsimonious trees (1018 steps) obtained after addition of *Anacostia* to the D&E tree. Conventions and abbreviations as in fig. 2.

that *Anacostia* and *Schisandra* share an elongate receptacle (49). Other positions above *Austrobaileya* are one step worse, while those with *Austrobaileya* or on the stem lineage of the order are two steps worse. All positions outside Austrobaileales are at least three steps less parsimonious.

These results indicate that the crown group of the third ANITA line had originated by the early Albian. Demonstration that *Anacostia* had spiral phyllotaxis in the perianth and androecium, which is likely in view of the spiral carpel arrangement, would strengthen this conclusion. Fossil evidence for the *Amborella* line is discussed in connection with *Appomattoxia*.

Other mid-Cretaceous fossils have been compared with Austrobaileales, but these comparisons are based on fewer characters. Yamada et al. (2008) assigned a seed from the late Albian of Japan to Trimeniaceae, based on presence of a thick lignified layer that they interpreted as a multilayered exotesta (see appendix) and an operculum, a feature typical of Nymphaeales but not known in Austrobaileales before it was reported in *Trimenia* by Yamada et al. (2003; see appendix). Seeds resembling *Illicium* were described by Frumin and Friis (1999) from the Cenomanian-Turonian of Kazakhstan. Other potential Austrobaileales are leaves from the lower Potomac (Aptian–early Albian) that Upchurch (1984) compared with *Amborella* and *Austrobaileya* based on cuticle structure and leaves (*Longstrethia*) near the Albian–Cenomanian boundary in Nebraska that Upchurch and Dilcher (1990) assigned to “Illiciales” based on cuticles and venation. Periporate pollen from the late Albian to Turonian of Africa and Brazil, *Cre-*

taceiporites scabratus (Herngreen 1973), was compared with *Trimenia* by Muller (1981) and Friis et al. (2011), but its exine structure is very different (Sampson and Endress 1984; Ward and Doyle 1994). Pollen called *Trisetorites* from the Santonian to Paleocene of North America (Tschudy 1970) and the Aptian and early Albian of Brazil (Heimhofer and Hochuli 2010) is like *Illicium* in having three colpi that join at the poles, such that the grains usually split into three sectors. However, *Illicium* has uniformly reticulate sculpture, whereas the Early Cretaceous pollen has conspicuous longitudinal “costae” with reticulate sculpture. Pollen more like that of *Illicium* and *Schisandra* is known from the Maastrichtian of California (Chmura 1973; Muller 1981).

The variation between monosulcate and trichotomosulcate apertures in pollen of *Anacostia* (*Similipollis*) may provide evidence on the origin of the unusual pollen of *Illicium* and *Schisandra*, which has three colpi joined at one pole (plus three alternating colpi in most *Schisandra* species) but differs from the tricolpate pollen of eudicots in orientation of the colpi in the tetrad (Huynh 1976). The three fused colpi have been interpreted as the extended arms of a trichotomosulcus (Doyle et al. 1990; Friis et al. 1997a; Doyle 2005). *Similipollis* could support this view by representing an earlier state where the trichotomosulcate condition was not yet fixed. As noted by Friis et al. (1997a), the graded reticulum of *Similipollis* differs from the uniform reticulum of *Illicium* and *Schisandra*. However, this does not rule out a relationship, since the only extant taxa with the *Similipollis* sculpture pattern are palms (Harley

1997; Dransfield et al. 2008) and tricolpate eudicots with finer sculpture at the poles, both clearly unrelated based on other characters. In *Anacostia* this sculpture appears to be an autapomorphy of an extinct side line that has no direct bearing on relationships.

Chloranthaceae and Possible Relatives

Chloranthaceae are one of the living taxa most frequently mentioned in discussions of the early angiosperm record. The extant genera are known for their extremely simple flowers: unisexual flowers consisting of either one stamen or one carpel in *Ascarina*, or one stamen or one carpel with three tepals at the top (so the ovary is inferior) in *Hedyosmum*, or bisexual flowers consisting of one carpel and one stamen or a problematic three-lobed androecium, respectively, in *Sarcandra* and *Chloranthus* (Swamy 1953; Endress 1987). Doria et al. (2012) interpreted the ovary of *Hedyosmum* as superior, but as discussed in the section on the *Asteropollis* plant, this is incorrect. Most morphological and molecular phylogenetic analyses have inferred the same arrangement of the four genera, with *Hedyosmum* sister to the rest of the family and *Ascarina* sister to *Sarcandra* and *Chloranthus* (Eklund et al. 2004; Soltis et al. 2005).

The first indication that Chloranthaceae were an important early angiosperm group was Couper's (1958) comparison of *Clavatipollenites hughesii* from the upper Wealden of England (Barremian) with pollen of *Ascarina*. Many other workers have used the name *C. hughesii* for similar pollen worldwide, but Hughes et al. (1979, 1994) showed that the type sample contains several reticulate-columellar monosulcate types that differ at the SEM level and argued that it is not clear which of these corresponds to the holotype, which was studied with light microscopy only. Hedlund and Norris (1968) described Albian pollen with similar sculpture but a 4–5-branched sulcus from the middle Albian of Oklahoma as *Asteropollis asteroides*, which Doyle (1969) and Muller (1970, 1981) compared with pollen of *Hedyosmum*. Pollen from the same beds with several colpoid apertures, described by Hedlund and Norris (1968) as *Stephanocolpites fredericksburgensis* and transferred by Ward (1986) to the new genus *Hammenia*, was compared with the polycolpate pollen of *Chloranthus* by Walker and Walker (1984).

Subsequent studies confirmed the angiospermous affinities of pollen of the *Clavatipollenites* type and provided additional evidence for the early presence of Chloranthaceae. Using light microscopy, SEM, and TEM, Doyle et al. (1975) showed that pollen called *Clavatipollenites* cf. *hughesii* from the lower Potomac Group of Maryland (Aptian) is typically angiospermous in having columellar infratectal structure and lacking a laminated endexine, except under the sulcus, and has special features such as an unusually thick extra-apertural nexine composed of foot layer, a sculptured sulcus membrane, and supraterctal spinules on the muri that make up the reticulum. Walker and Walker (1984) showed that these features are shared with pollen of *Ascarina*, and they described SEM and TEM similarities between *Asteropollis* and pollen of *Hedyosmum*. It is important to note that the similarities between *C.* cf. *hughesii* and *Ascarina* do not necessarily indicate a special relationship of the fossils and the modern genus, since pollen

of *Ascarina* differs from that of the other genera mainly in its monosulcate aperture, which was presumably ancestral for the family as a whole.

More direct evidence that pollen of the *Clavatipollenites* type was produced by angiosperms came when Pedersen et al. (1991) associated such pollen with isolated carpels from the early Cenomanian of Maryland, named *Couperites*. Many more or less similar pollen types have been associated with stamens, carpels, and more complete reproductive structures from the Early Cretaceous of the Potomac Group and Portugal (Friis et al. 1994, 1999, 2011) and the Late Cretaceous (Eklund et al. 2004; Friis et al. 2011).

Similarities to Chloranthaceae have also been noted in Aptian-Albian leaves, particularly chloranthoid teeth (Hickey and Wolfe 1975) and cuticle features (Upchurch 1984; Upchurch and Dilcher 1990; Taylor and Hickey 1992). However, chloranthoid teeth alone are not necessarily evidence of Chloranthaceae, as they also occur in *Amborella*, Austrobaileyales (*Trimenia*, *Schisandra*), and basal eudicots, and parsimony optimization on molecular trees indicates that they may be ancestral for angiosperms as a whole (Doyle 2007).

Stimulated in part by fossil reports, there was much earlier speculation that Chloranthaceae might provide an alternative model for the ancestral flower (Meeuse 1972; Burger 1977; Leroy 1983), a view supported by some morphological phylogenetic analyses (Taylor and Hickey 1992; Nixon et al. 1994). This has been refuted by molecular analyses, which consistently place Chloranthaceae above the ANITA grade, as one of the five main lines in the mesangiosperm clade. However, their position among the mesangiosperms is not resolved. As already noted, in the combined morphological and molecular analysis of Doyle and Endress (2000) Chloranthaceae were sister to all other mesangiosperms, partly because they retain ascidiate carpels like those in the ANITA lines, and morphological data of Endress and Doyle (2009) grouped *Ceratophyllum* with them. The Chloranthaceae-*Ceratophyllum* clade has also been found in some molecular studies (Antonov et al. 2000; Duvall et al. 2006, 2008; Qiu et al. 2010; Moore et al. 2011; Zhang et al. 2012), although most indicate different relationships, such as analyses of whole chloroplast genomes (Jansen et al. 2007; Moore et al. 2007), in which Chloranthaceae are sister to the magnoliid clade and *Ceratophyllum* is sister to eudicots.

In the following sections, we consider both taxa that were compared with Chloranthaceae when first described and others not originally associated with the family. Four of these six “chloranthoid” taxa have been treated in previous phylogenetic analyses, using varying numbers of taxa and characters. Our goal in these cases is to update these analyses using our present data set and to consider their broader implications for evolution of the family.

In contrast to the analyses of ANITA-grade fossils, in most of these analyses the backbone trees used do make a difference for inferred relationships, as might be expected because they differ in the position of Chloranthaceae—sister to *Ceratophyllum* (D&E) or to magnoliids (J/M). Furthermore, the positions of the fossils often vary depending on whether they are added to the Recent trees individually or together with other fossils. Because we refer to trees derived from the latter analyses while discussing each of the fossils, it is most convenient

to figure them first: with *Canrightia*, *Zlatkocarpus*, the *Pennipollis* plant, and the *Asteropollis* plant, the four fossils most securely associated with Chloranthaceae (fig. 6), and with these four fossils plus *Couperites* (fig. 7) and *Appomattoxia* (fig. 8).

Couperites. *Couperites mauldinensis* is based on isolated carpels at the fruit stage with pollen of the *Clavatipollenites* type on the sessile stigma, described by Pedersen et al. (1991) from Mauldin Mountain, Maryland (early Cenomanian). Friis et al. (1997b) reported an isolated stamen with similar pollen from Puddledock, Virginia, but because it is possible that such pollen is systematically heterogeneous and Puddledock is ap-

preciably older (middle Albian), we base our scoring on the Mauldin Mountain material only.

Discovery of *Couperites* was a breakthrough in botanical understanding of pollen of the *Clavatipollenites* type. However, it is important to recognize that the associated pollen differs from the best-known dispersed material from the Aptian (Doyle et al. 1975; Walker and Walker 1984): it has a narrower sulcus with sharply defined, thickened, infolded margins and a tendency for the columellae to detach from the nexine. Pierce (1961) described apparently similar pollen from the Cenomanian of Minnesota as *Retimonocolpites dividuus*, but the

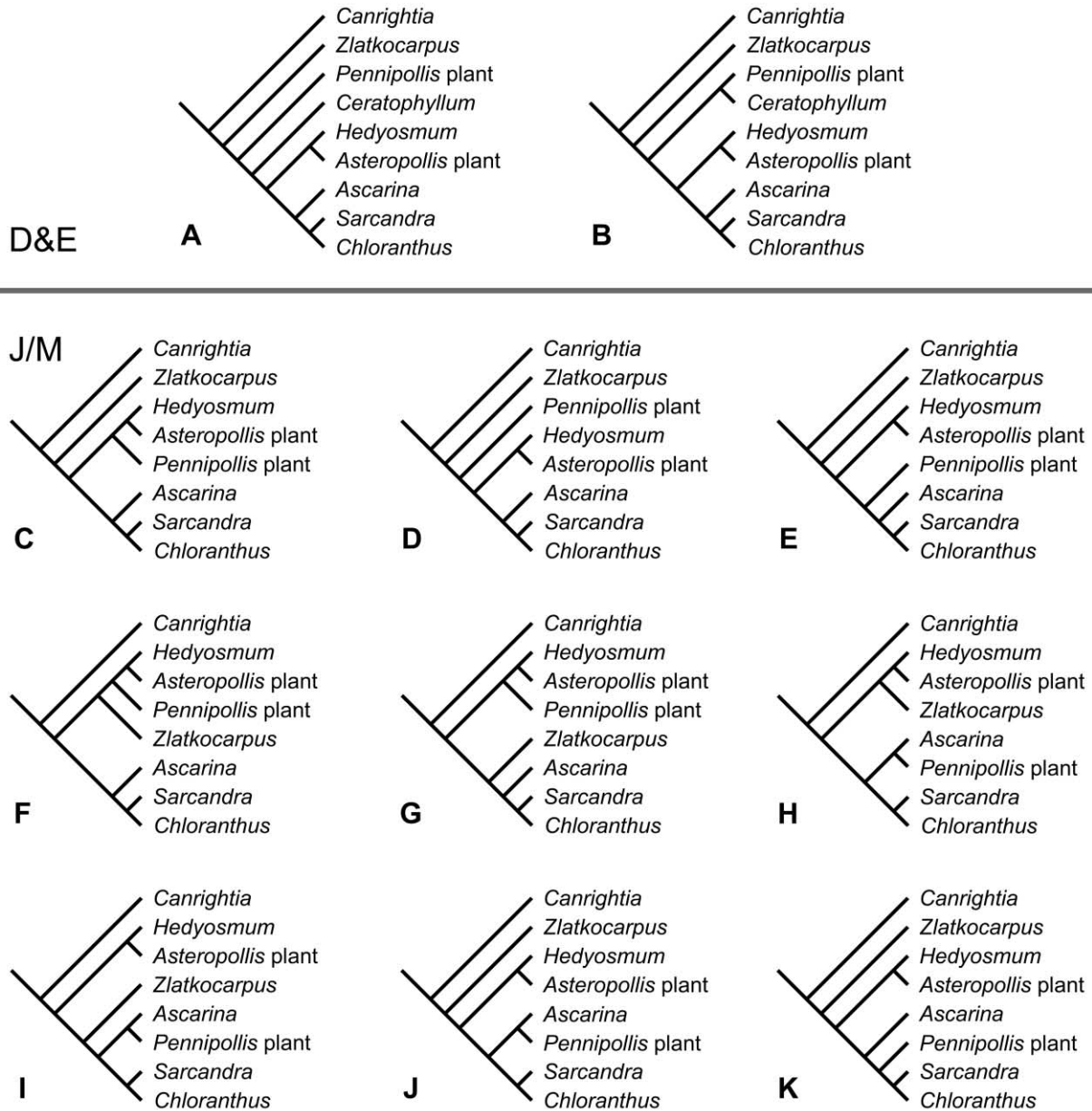


Fig. 6 Arrangements of relevant taxa in most parsimonious trees obtained after addition of *Canrightia*, *Zlatkocarpus*, the *Pennipollis* plant, and the *Asteropollis* plant to the backbone trees. A, B, Arrangements obtained with the D&E tree (1026 steps). C–K, Arrangements obtained with the J/M tree (1037 steps).

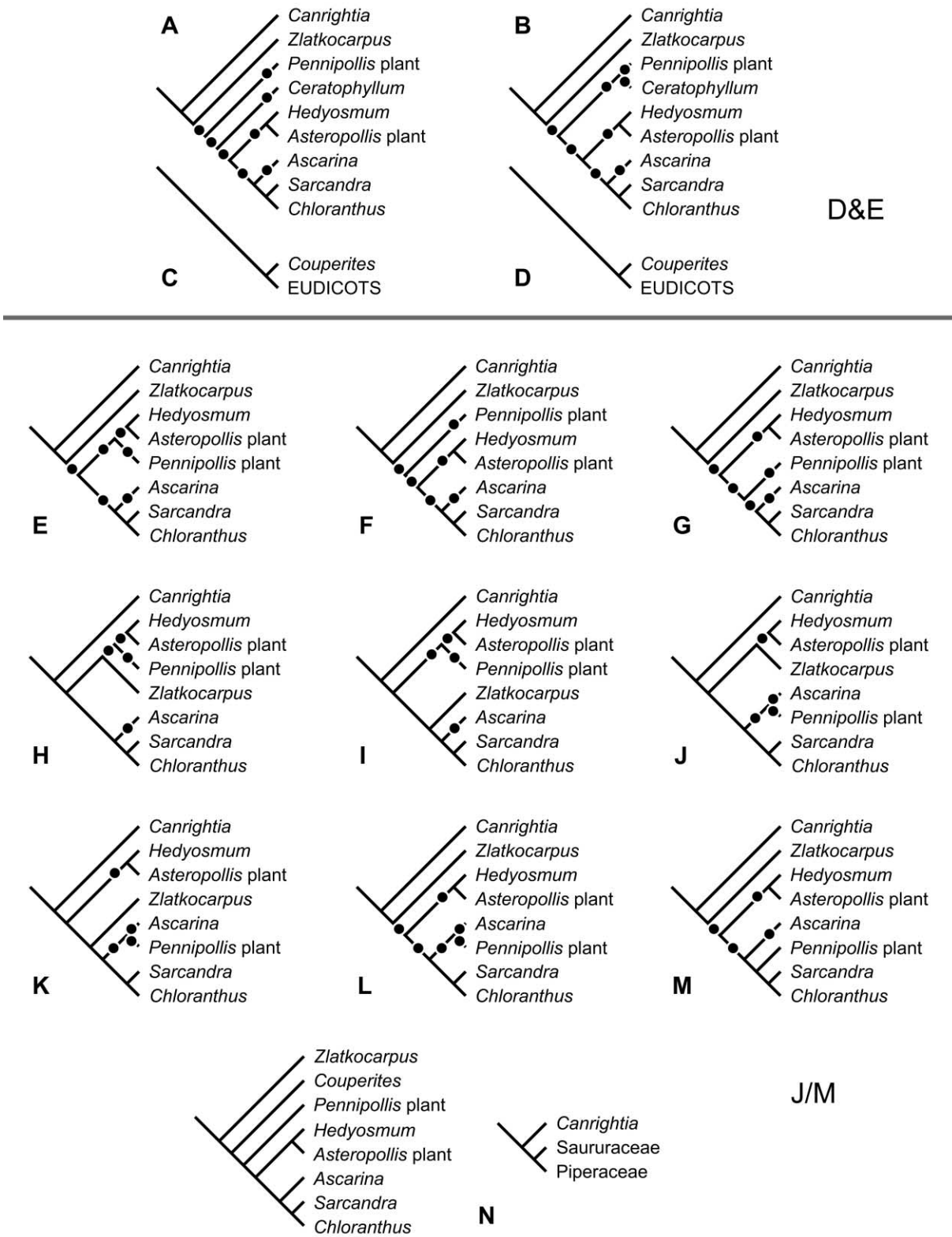


Fig. 7 Arrangements of relevant taxa in most parsimonious trees obtained after addition of *Canrightia*, *Zlatkocarpus*, the *Pennipollis* plant, the *Asteropollis* plant, and *Couperites* to the backbone trees. A–D, Arrangements with the D&E tree (1029 steps); A and B (same trees as in fig. 6A, 6B) show 16 most parsimonious positions of *Couperites* with dots, while C and D represent two trees where *Couperites* is associated with eudicots rather than Chloranthaceae (other relationships as in fig. 6A, 6B). E–N, Arrangements with the J/M tree (1040 steps), with most parsimonious positions of *Couperites* indicated with dots (same trees as in fig. 6C–6K), except in N, where *Canrightia* is nested in Piperales.

identity of this species is uncertain because of the low magnification of the figures; it may differ in having a sulcus that extends more than halfway around the grain. Brenner (1963) transferred Pierce's species to *Liliacidites*, as *Liliacidites dividuus*, which he reported throughout Zone II of the Potomac Group, but later workers (Doyle and Hickey 1976; Doyle and Robbins 1977; Hickey and Doyle 1977) reserved *Liliacidites* for pollen with graded sculpture and identified the Potomac pollen as *Retimonocolpites* cf. or aff. *dividuus*, which they extended downward into upper Zone I. Some Potomac pollen of this type is probably identical to *Clavatipollenites rotundus* of Kemp (1968), which enters in the early Albian of England (see Material and Methods). However, the few available EM data show variation in Potomac pollen of this type; a grain from upper Subzone II-B (late Albian) that Walker and Walker (1984) identified as *R. dividuus* has a thinner nexine than *Couperites* pollen and transverse ridges on the muri rather than microverrucae. Resolving whether these pollen types form a natural group may require new EM observations and/or discoveries of pollen in situ.

The carpels of *Couperites* are like those of Chloranthaceae in containing one pendent ovule, but they differ in other characters. The ovule is anatropous rather than orthotropous, which Pedersen et al. (1991) recognized might mean that *Couperites* was outside the crown group of Chloranthaceae, assuming that the orthotropous ovules of the family are derived (as inferred from molecular trees; Endress and Doyle 2009). The seed coat has two structural layers; the outer is probably a palisade exotesta, which occurs in Nymphaeales and most Austrobaileyales but not in Chloranthaceae.

The phylogenetic position of *Couperites* was analyzed by Eklund et al. (2004) in a morphological analysis that included 38 species of Recent Chloranthaceae and 10 outgroups, including the ANITA lines and three exemplars of the mesangiosperms. This treatment needs modification in light of the larger number of outgroups and different characters and character state definitions in our present data set, as well as more recent observations.

According to Pedersen et al. (1991), pollen size (82) is 22–25 μm , which is unambiguously medium. Eklund et al. (2004) did not include a character for sculpture on the sulcus membrane (93). However, although the sulcus was narrow, SEM and TEM (Pedersen et al. 1991, figs. 5D, 6D) show that it had at least some verrucae. Another character not used by Eklund et al. (2004) is extra-apertural nexine structure (94). Pedersen et al. (1991) reported thick endexine under the sulcus and thin endexine in other areas, but the latter is comparable to a very thin and discontinuous layer of questionable identity that occurs in *Ascarina* and other Chloranthaceae, which we include in the state (0) for foot layer only (Doyle 2005).

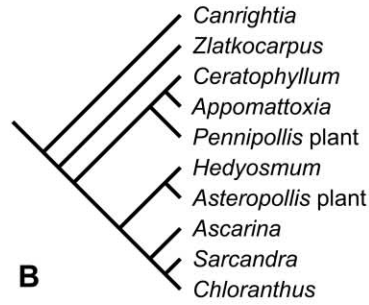
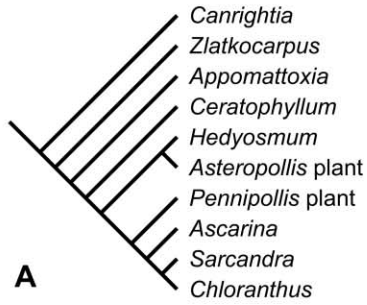
No characters of floral organization can be scored. If it could be assumed that the flower had other parts, the character for floral base/ovary position (48) could be scored as superior, either with or without a hypanthium (0/1), but because the flower may have consisted of a single carpel, with no other floral parts to define ovary position, we treat this character as unknown, as in living taxa with flowers that consist of one carpel. The carpels have a short stalk that could be the pedicel of a unicarpellate flower, but because it could equally well be the stipe of the carpel, we score floral pedicel (45) as unknown.

As in unicarpellate extant taxa and other fossils with isolated carpels, we score carpel fusion (106) as unknown, in order to avoid artifactual steps in cases where a syncarpous gynoeceum was reduced to one carpel (Endress and Doyle 2009). The shape of the carpel and the apical ovule attachment suggest that the carpel was ascidiate, but in the absence of developmental data or anatomical markers, we score carpel form (97) and placentation (113) as unknown. The stigma was sessile (101) but is too degraded for scoring of related characters (102–104). Pedersen et al. (1991) interpreted resin bodies in the carpel wall as probable altered oil cells (they are consistent in size, $\sim 50 \mu\text{m}$, to oil cells in the seed coat of *Magnolia* figured by Corner 1976), but these are not of the intrusive type visible at the surface (107).

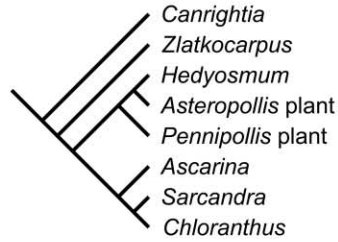
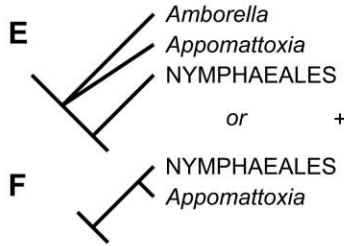
Pedersen et al. (1991, p. 588) stated that the seed of *Couperites* is like that of living Chloranthaceae (except *Hedyosmum*, which has no lignified layer in the seed coat) in having “cuboid and lignified palisade cells in the testa.” However, as they recognized, potentially related taxa have two sorts of testal palisade layers: Chloranthaceae have an endotesta (derived from the inner epidermis of the outer integument), whereas Nymphaeales and Austrobaileyales (except *Austrobaileya*) have an exotesta (from the outer epidermis). They left open the question of whether the palisade layer in *Couperites* was exo- or endotestal, but Friis et al. (2011) and Friis and Pedersen (2011) interpreted it as an exotesta (128), which we accept. In addition, the seed coat had an inner structural layer of longitudinally elongate cells. Although the nature of this layer is not fully established, we follow Pedersen et al. (1991) and Eklund et al. (2004) in interpreting it as a fibrous exotegmen (132), a feature known in *Ascarina* and *Chloranthus*.

The analysis of Eklund et al. (2004) found two most parsimonious positions for *Couperites*: sister to the crown clade of Chloranthaceae and nested within it as the sister group of *Ascarina*, *Sarcandra*, and *Chloranthus* (here designated the “ASC clade”). With the present data set, the inferred relationships of *Couperites* differ with the two backbone trees.

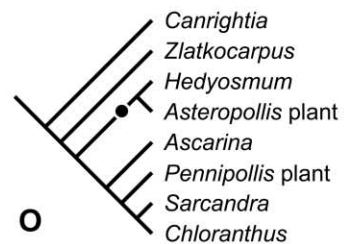
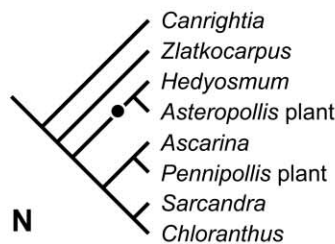
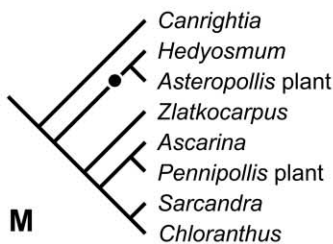
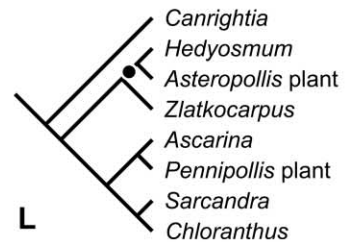
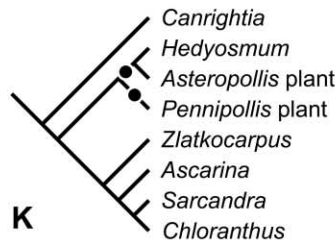
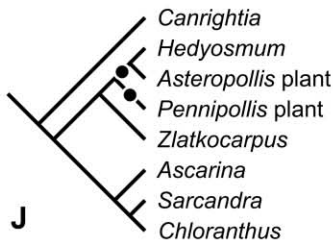
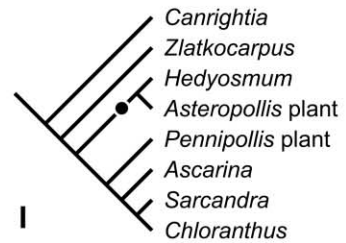
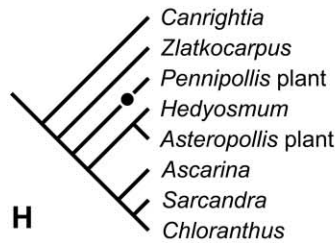
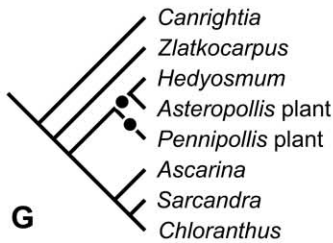
With the JM tree (fig. 9B), where *Ceratophyllum* is well separated from Chloranthaceae, *Couperites* has one most parsimonious position, as the sister group of Chloranthaceae as a whole. Synapomorphies that link it with the family are two of the pollen characters stressed by Walker and Walker (1984), supratectal spinules (91) and thick nexine (95), while its position below the crown group is a consequence of its anatropous ovule (115), which becomes orthotropous in living Chloranthaceae. Similarities such as sculptured sulcus membrane (93) and one pendent ovule (112, 114) are not evidence for a special relationship, since they are inferred to be ancestral for angiosperms (Endress and Doyle 2009). The palisade exotesta (128) and fibrous exotegmen (132) are convergences with other taxa where they occur. However, three positions within Chloranthaceae are only one step less parsimonious: sister to *Hedyosmum*, *Ascarina*, and the ASC clade. The third position is less parsimonious than it was in Eklund et al. (2004) because we rescored the tegmen character (132) in *Chloranthus* as uncertain (Doyle and Endress 2010): a fibrous exotegmen like that of *Ascarina* occurs in *Chloranthus erectus*, one of the two species studied, but not in *Chloranthus spicatus* (Corner 1976; Endress 1987). Positions outside the chloranthaceous line are at least two steps worse.



D&E



+ 8 other trees
(fig. 5D-K)



With the D&E tree, where *Ceratophyllum* is linked with Chloranthaceae, *Couperites* has four most parsimonious positions (fig. 9A): sister to the Chloranthaceae-*Ceratophyllum* clade, based on the combination of thick nexine and anatropous ovule; sister to the ASC clade, based on the sessile stigma (101), with the three modern genera united by one-layered endoreticulate endotesta (131); linked with *Ascarina* by fibrous exotegmen; and sister to mesangiosperms as a whole, supported by nexine consisting of foot layer only (94). The positions of *Couperites* within Chloranthaceae require a reversal from orthotropous to anatropous ovules (115) and convergent origin of the palisade exotesta (128), but because both *Ceratophyllum* and *Hedyosmum* share an elongate style, this is counteracted by one less step in the style character (101). If *Couperites* is located below *Ceratophyllum* and Chloranthaceae, the ancestral state of the style character in this line is equivocal and it undergoes two steps, but if *Couperites* is nested in Chloranthaceae, a style is ancestral and there is only one step, namely, loss of the style in the common ancestor of *Couperites* and the ASC clade (with the J/M tree, there is only one change in the chloranthaceous line, wherever *Couperites* is located, since the style of *Hedyosmum* is an autapomorphy). Nine positions outside the Chloranthaceae-*Ceratophyllum* clade are only one step less parsimonious, including six in the ANITA grade, such as on the stem lineage of Nymphaeales or Austrobaileyales, sister to *Trimenia* (which has a similar carpel with one pendent anatropous ovule), or sister to the eudicots. Most of these positions are more consistent with the palisade exotesta, which occurs in Nymphaeales, Austrobaileyales, and many basal eudicots.

Still more possibilities emerge when *Couperites* is added to the analysis together with the four fossils most securely associated with Chloranthaceae (*Canrightia*, *Zlatkocarpus*, *Pennipollis* plant, *Asteropollis* plant). With the D&E backbone tree (fig. 7A–7D), *Couperites* is located above *Canrightia* and *Zlatkocarpus* in 16 of the 18 most parsimonious trees (fig. 7A, 7B): either below the Chloranthaceae-*Ceratophyllum* crown clade, with or without the *Pennipollis* plant, in any of their three possible arrangements; with *Ceratophyllum*, with or without the *Pennipollis* plant; or sister to or nested within Chloranthaceae, as the sister group of *Hedyosmum* plus the *Asteropollis* plant, the ASC clade, or *Ascarina*. In most of these trees its anatropous ovule is best interpreted as a reversal (this is equivocal in the two trees where *Couperites* is just above *Zlatkocarpus*). Finally, in two trees (fig. 7C, 7D) *Couperites* is united with the eudicots by palisade exotesta. With the J/M backbone (fig. 7E–7N), *Couperites* is associated with Chloranthaceae in all 45 trees, but in eight trees it is a stem relative of the family, above *Canrightia* and/or *Zlatkocarpus*, with or without the *Pennipollis* plant, whereas in the other 37 it is nested at various points within the crown group.

In view of these results, a relationship of *Couperites* to Chlo-

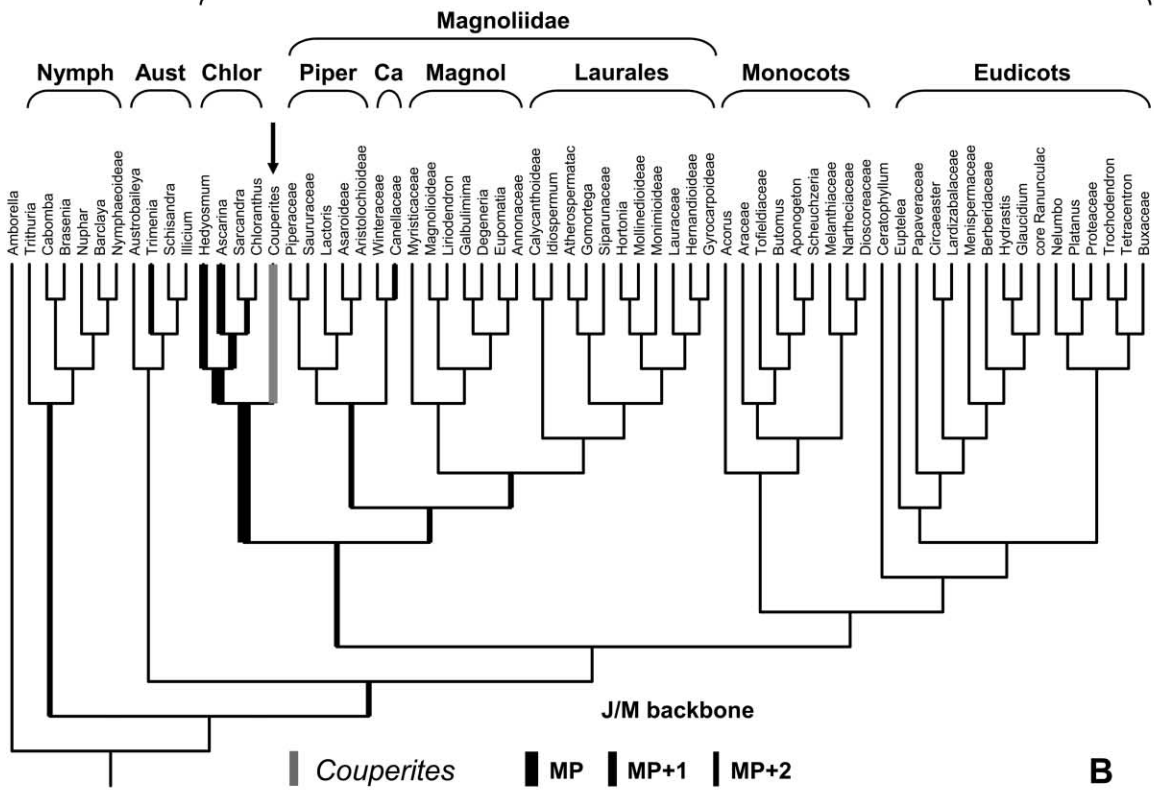
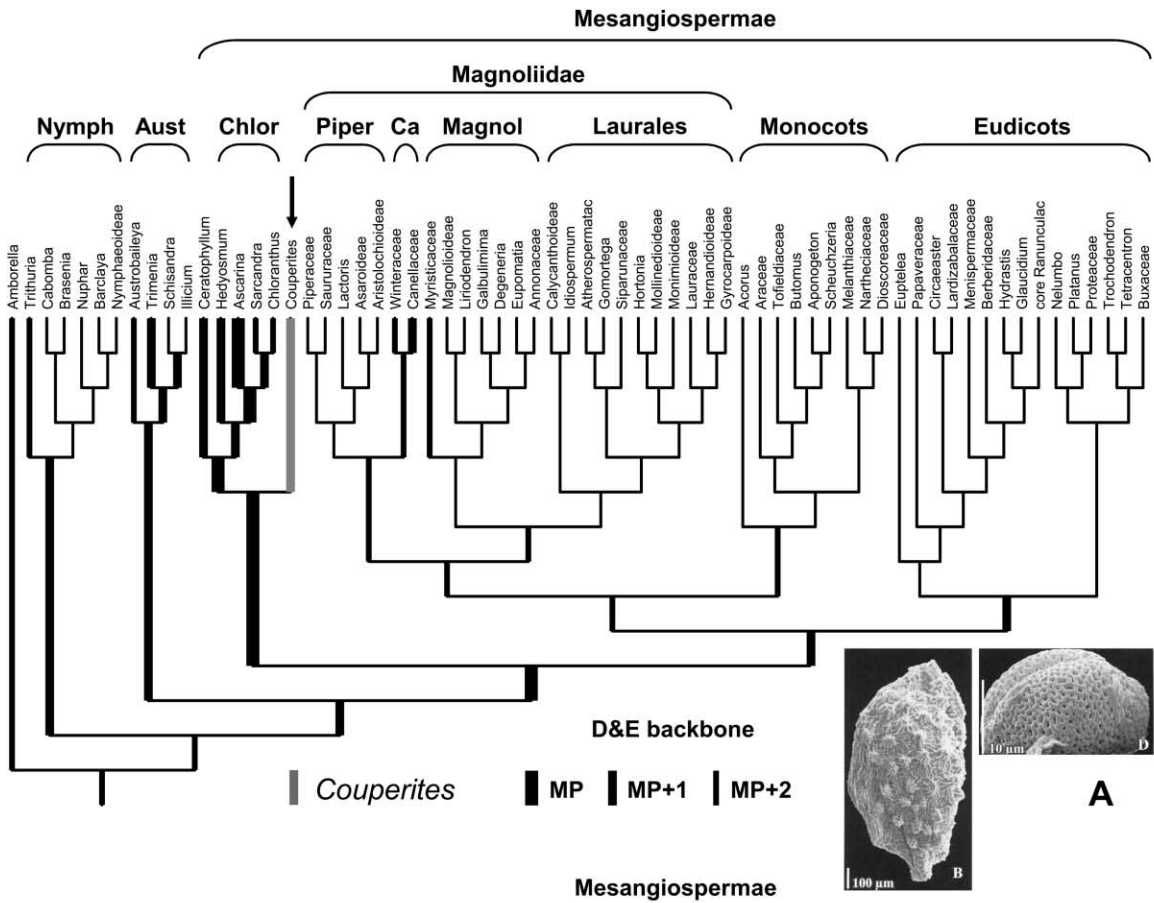
ranthaceae is possible but uncertain. It cannot be used as secure evidence for the chloranthaceous line and certainly not for the palynologically most similar genus, *Ascarina*, which it resembles largely in shared ancestral states. Information that might resolve this problem could include any indication on organization of the flowers that bore the carpels—whether they had a perianth or not, had one carpel or several, or were unisexual or bisexual. The carpels differ from those of *Hedyosmum*, *Zlatkocarpus*, and *Canrightia* in lacking an adnate perianth, but this could mean that they came from either a flower like that of *Ascarina* or a flower with several free carpels, which might be unrelated to Chloranthaceae. It should be borne in mind that these results do not necessarily apply to other dispersed pollen identified as *Clavatipollenites*, which may well be systematically heterogeneous, especially considering that most of its features are plesiomorphic.

***Asteropollis* plant.** This taxon, not yet formally described, is based on isolated female flowers with adhering pollen of the *Asteropollis* type from Torres Vedras, Catefica, Vale de Agua, and Buarcos, Portugal, figured by Friis et al. (1994, fig. 3c, 3d; 1997b, fig. 6.3; 1999, pollen type J.4, figs. 105–107; 2000b, fig. 3E; 2006, fig. 7F–7L; 2011, fig. 8.15), and axes bearing numerous stamens that contain similar pollen from the same localities (Friis et al. 1994, fig. 1; 2006, fig. 7A–7E; 2011, fig. 8.13). Torres Vedras may be either Aptian or earliest Albian, but the other localities are more securely dated as early Albian.

Asteropollis, first described by Hedlund and Norris (1968) from the middle Albian of Oklahoma, differs from *Clavatipollenites* in having a 4–5-branched sulcus. Similar pollen is known only in the chloranthaceous genus *Hedyosmum* (Doyle 1969; Muller 1970, 1981; Walker and Walker 1984). The flowers were first figured by Friis et al. (1994) and associated with the pollen by Friis et al. (1997b, 1999), who pointed out their similarity to *Hedyosmum*. As in *Hedyosmum*, the female flower consists of one carpel with three tepals on top and three window-like depressions on the sides, while the male axes are also like those of *Hedyosmum*, in which the individual stamens are interpreted as unistaminate flowers with no perianth or subtending bracts (Endress 1987; Eklund et al. 2004).

Eklund et al. (2004) analyzed the phylogenetic position of this plant based on the female flowers and pollen alone, because of uncertainty that the male structures were associated, but this association is now well established (Friis et al. 2006, 2011). The female flower illustrated by Eklund et al. (2004, fig. 2A) is from Torres Vedras (Friis et al. 1994, fig. 3c; 2006, fig. 7F), whereas the pollen figured by Eklund et al. (2004, fig. 2B), which had a verrucate sulcus with a distinct margin, is from a stamen at Buarcos (E. M. Friis, personal communication, 2004). According to E. M. Friis (personal communication), the pollen adhering to female flowers at Torres Vedras

Fig. 8 Arrangements of relevant taxa in most parsimonious trees obtained after addition of *Canrightia*, *Zlatkocarpus*, the *Pennipollis* plant, the *Asteropollis* plant, and *Appomattoxia* to the backbone trees. A–D, Arrangements with the D&E tree (1031 steps); C and D represent two trees where *Appomattoxia* is associated with Nymphaeales rather than Chloranthaceae (other relationships as in fig. 6A, 6B). E–O, Arrangements with the J/M tree (1042 steps); in E and F, all other arrangements in the chloranthaceous clade shown in fig. 6D–6K are equally parsimonious; in E, all three resolutions of the basal trichotomy are equally parsimonious, resulting in 27 trees; in G–O, most parsimonious positions of *Appomattoxia* are indicated with dots (same trees as in fig. 6C–6K).



differs in having a “fragmented” reticulum over the sulcus, but the two types are similar in characters in our data set.

The scoring of this plant by Eklund et al. (2004) requires revision along lines discussed for *Couperites*. In general, in taxa with unisexual flowers, we score inflorescence and flower characters based on the sex with more complex structures (Endress and Doyle 2009). Because female inflorescences are unknown, we score inflorescence type (42) based on the male structures, which we interpret as spikes of unistaminate flowers (as did Friis et al. 2006, 2011), but inflorescence partial units (43) as unknown, because the female flowers may or may not have been borne in cymose units, as in *Hedyosmum* (which has male spikes and female thyrses, both included in the same state of character 42). Contrary to earlier authors (Endress 1987; Todzia 1988; Eklund et al. 2004; Endress and Doyle 2009), Doria et al. (2012) interpreted the partial units in female inflorescences of *Hedyosmum* as spikes, rather than monochasial cymes. Under this hypothesis, the total number of bracts within and subtending each unit should be one more than the number of flowers. Doria et al. (2012) claimed this was true for the three species that they studied, but this is not clear from their figures, and in other species the number of bracts is the same as the number of flowers, as expected if each unit is a cyme (*Hedyosmum mexicanum*: Endress 1987, fig. 23; *Hedyosmum brenesii*: Todzia 1988, fig. 15A). We therefore continue to score the partial units in *Hedyosmum* as cymes. Fossil flowers of both sexes lack a pedicel (45). Friis et al. (1994) did not observe floral subtending bracts below the male flowers, but because there is no evidence on whether such bracts occurred in the female inflorescences (as they do in *Hedyosmum*), we score this character (46) as either present in female and absent in male flowers or absent in all flowers (1/2).

Doria et al. (2012) interpreted the ovary of *Hedyosmum* as superior rather than inferior, with three connate tepals free from the base of the ovary. However, as shown in their figure 5E and in figures 5, 8, and 16 of Endress (1971), the lobes that develop into the tepals are initiated above the level of the ovary. As support for their interpretation, Doria et al. (2012) cited the superior ovary position in “abnormal bisexual flowers” of *Hedyosmum orientale* figured by Yamazaki (1992), but these specimens appear to be misidentified flowers of a member of the Ulmaceae; they differ from *Hedyosmum* in having a papillate stigma, wide ovary locule, and short dorsifixed anthers. Doria et al. (2012) interpreted the distinctive “windows” in the ovary wall below the tepals as schizogenous, as suggested by D’Arcy and Liesner (1981). However, Endress (1971) showed that the windows form well after tepal initiation by morphogenetic differentiation of circular rims on the floral surface. A section in Doria et al. (2012, fig. 4J) that shows a discontinuity between the ovary and an outer layer appears to be a nonmedian section through the edge of a window “frame,” which is continuous with the rest of the flower just above the level of the ovule.

Scoring of perianth organization (53–56) is based on the female flowers. As in other taxa with one whorl of sepaloid tepals, we score tepal differentiation (57) as either all sepaloid or outer sepaloid and inner petaloid (Endress and Doyle 2009), on the assumption that the one whorl of sepaloid tepals could be derived from the outer whorl of either polycyclic type. Eklund et al. (2004) scored the tepals as free rather than connate, following terminology used by Todzia (1988) to describe variation within *Hedyosmum*, but Doria et al. (2012) argued that all *Hedyosmum* species have fused tepals. Closer examination shows that the variation noted by Todzia (1988) and Eklund et al. (2004) is between tepals that are fused only at the very base and much more fused. As illustrated by Friis et al. (1994, fig. 3d), the tepals in the fossil are basally fused into a shallow ring around the ovary, as in *H. orientale* (Yamazaki 1992, fig. 3), one of the species that Todzia (1988) and Eklund et al. (2004) described as free. Our present character (60), which is defined in a broader angiosperm context, treats this condition as fused, so we score both the *Asteropollis* plant and *Hedyosmum* as fused, thus bringing our data in line with Doria et al. (2012). As with other unistaminate flowers, we score all characters of androecial organization except single stamen (62) as unknown. Friis et al. (2011, p. 182) stated that the connective “extends apically into a short sterile extension,” but their figures show that the apex (72) was like that of taxa that Eklund et al. (2004) and Endress and Doyle (2009) scored as truncate or rounded. The stigma is usually broken off, but Friis et al. (2000b, fig. 3E; 2006, fig. 7I) figured one flower with a large, basally constricted stigma, which is more like the stigma of *Hedyosmum*, which we score as having a style (101), than the sessile stigma of other Chloranthaceae. Seeds have not been figured, but according to Friis et al. (2011, p. 183) the carpel contained a single orthotropous ovule (112, 115).

In Eklund et al. (2004), the *Asteropollis* plant was either sister to *Hedyosmum* or nested within the basal grade of the genus. The present analysis, which treats living *Hedyosmum* as a single taxon, confirms that the fossil is related to *Hedyosmum* (fig. 10). With the D&E backbone tree, synapomorphies that link it with Chloranthaceae and *Ceratophyllum* are sessile flower (45), one stamen (62), embedded pollen sacs (73), one carpel (96), and orthotropous ovule (115). Despite the remarkable similarity of the fossil and *Hedyosmum*, their only unequivocal synapomorphy is the branched sulcus (86). They share several other derived features, namely, absence of bracts subtending the stamens (46), inferior ovary (48), one perianth whorl (56), and basally fused tepals (60), but where these characters arose is equivocal, since they are uncertain or inapplicable in *Ceratophyllum*, which has female flowers that consist of a single carpel. The last three characters were also scored as unknown in *Ascarina*, which also has naked female flowers, and in *Sarcandra* and *Chloranthus*, where the single stamen or three-lobed androecium is attached to the back of the carpel, which might or might not mean that the ovary is inferior. As a result, positions sister to Chloranthaceae and/or

Fig. 9 Analyses of the position of *Couperites*. A, One of four most parsimonious trees (1019 steps) obtained after addition of *Couperites* to the D&E tree. B, Single most parsimonious tree (1029 steps) obtained after addition of *Couperites* to the J/M tree. Conventions and abbreviations as in fig. 2.

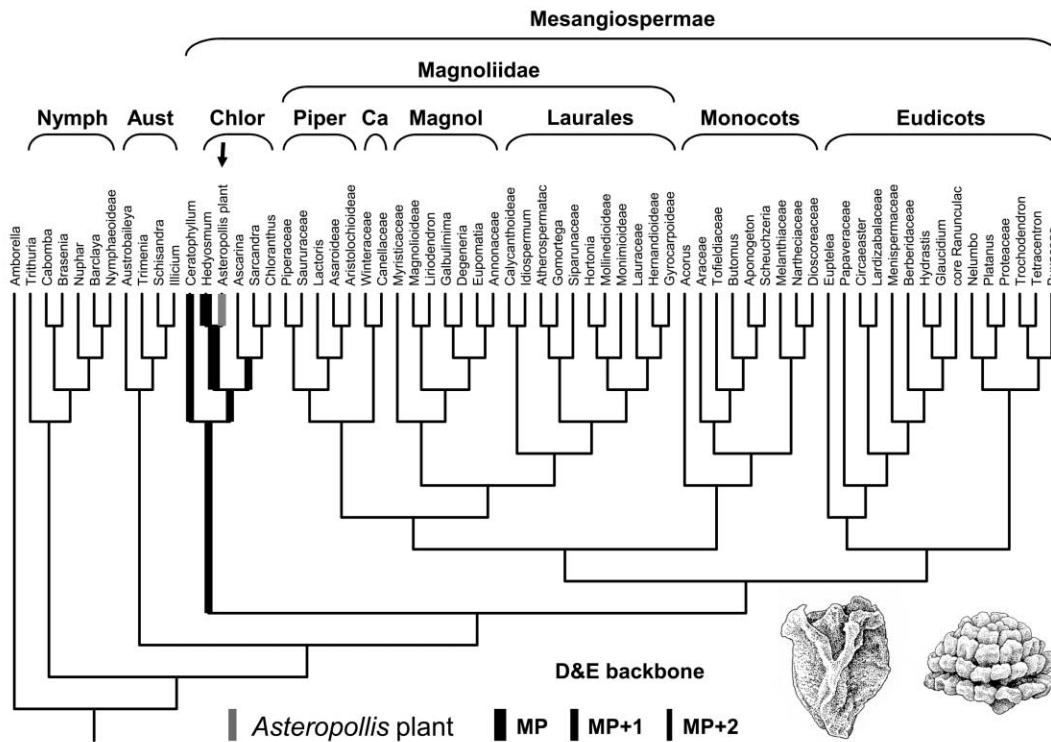


Fig. 10 Single most parsimonious tree (1017 steps) obtained after addition of the *Asteropollis* plant to the D&E tree. Conventions and abbreviations as in fig. 2.

Ceratophyllum and to the ASC clade are only one step less parsimonious, but the best position outside the whole line (with Myristicaceae) is eight steps worse. With the J/M backbone, a sister group relationship of the *Asteropollis* plant to *Hedyosmum* is two steps more parsimonious than the best alternatives (sister to Chloranthaceae and to the ASC clade). The fossil is associated with Chloranthaceae by the five synapomorphies listed above and with *Hedyosmum* by both the branched sulcus and the absence of bracts subtending the stamens. Its best position elsewhere, with *Ceratophyllum*, is six steps worse. It is also sister to *Hedyosmum* in all analyses that include several fossils (figs. 6–8).

The unique “windows” in the fruit wall (not included in our data set) would strengthen a link between the *Asteropollis* plant and *Hedyosmum* rather than *Canrightia* or *Zlatkocarpus*, which both have an inferior ovary but no windows. However, it would not affect the relative parsimony of other positions among living Chloranthaceae, as it would not be justifiable to score such a character in the ASC clade, where ovary position cannot be defined.

The *Asteropollis* plant presents one of several conspicuous cases in which fossils that closely resemble an extant clade are much older than ages of the crown clade inferred from molecular dating analyses. Another concerns Early Cretaceous fossils that resemble *Ephedra* (Rydin et al. 2004, 2010). This has led to views that the fossil and molecular data are in conflict (Rydin et al. 2004; Friis et al. 2005; Nixon 2008). However, this is not necessarily true if the fossils have no derived

features that arose within the crown group, in which case they could be stem relatives (Doyle and Donoghue 1993; Pirie and Doyle 2012); the problem becomes explaining the long period of morphological stasis between the time of the fossil and the radiation of the crown clade (cf. Friis et al. 2005). In the case of *Ephedra*, closer examination showed that the fossils are more plesiomorphic than living *Ephedra* in having more valves surrounding the ovule, supporting the stem relative hypothesis (Rydin et al. 2010). In the case of *Hedyosmum*, Eklund et al. (2004) found seven most parsimonious positions for the *Asteropollis* plant, one sister to *Hedyosmum* and six nested within the crown group. Of these, the latter would conflict with molecular dating analyses (Zhang and Renner 2003; Antonelli and Sanmartín 2011; Zhang et al. 2011), which have given Eocene to Oligocene ages for the crown group. Eklund et al. (2004) took these results as support for the view that the *Asteropollis* plant was a stem relative of *Hedyosmum*, rather than a crown group member. They noted one character (not used in their analysis) that might favor a stem position, namely, the fact that the sulcus in the *Asteropollis* grain in their figure 2B (from Vale de Agua) had a distinct margin and verrucate sculpture, as opposed to an indistinct margin and a fragmented reticulum in extant species. However, according to E. M. Friis (personal communication, 2004), the sulcus in grains from Torres Vedras has a fragmented reticulum.

Despite these problems, the *Asteropollis* plant does provide a secure minimum age of early Albian for the crown node of Chloranthaceae. Use of dispersed pollen for this purpose is

problematic, because many authors have used the name *Asteropollis* for trichotomosulcate pollen as well as pollen of the original type with more than three sulcus branches. Heimhofer et al. (2007) recorded *Asteropollis* throughout the Aptian and Albian in well-dated marine sections in Portugal, but the only figured specimen with a four-branched sulcus was early Albian, and U. Heimhofer (personal communication, 2009) confirmed that he observed no four-branched grains in the Aptian. *Asteropollis* has also been reported from the Aptian of Argentina (Archangelsky et al. 2009), but the aperture condition is trichotomosulcate (V. Barreda, personal communication, 2009), poorly defined and irregular (Llorens 2003; M. Llorens, personal communication, 2009), or polycolpoidate (Prámparo et al. 2007; M. Prámparo, personal communication, 2009). The trichotomosulcate pollen may well be related to *Asteropollis* and *Hedyosmum*, but because the several-branched type is unique to *Hedyosmum* today, whereas trichotomosulcate pollen is more widespread, we consider this uncertain (see also Friis et al. 2011). It therefore seems most prudent to base any calibrations on the flowers, but there are stratigraphic problems associated with these too. As discussed in Material and Methods, the Vale de Agua and Buarcos localities are probably early Albian, but Torres Vedras is older, either earliest Albian or Aptian. The conservative approach would therefore be to use the *Asteropollis* plant to set a minimum age of early Albian (~110 Mya) for crown group Chloranthaceae.

Zlatkocarpus. *Zlatkocarpus* is based on compressions of female inflorescences at the fruit stage, *Zlatkocarpus brnikensis* from Brník and *Zlatkocarpus pragensis* from Hloubětín-Hutě, Czech Republic (middle Cenomanian), with adhering pollen of the *Retimonocolpites* type. The latter species was first described by Kvaček and Eklund (2003) as *Myricantheum pragense* and segregated as the new genus *Zlatkocarpus* by Kvaček and Friis (2010).

Zlatkocarpus shows a combination of characters not seen in any living Chloranthaceae. It has spikes of female flowers that recall *Ascarina*, but it is like *Hedyosmum* in having a presumed reduced perianth adnate to the ovary. However, its pollen differs from that of *Hedyosmum* in having a normal sulcus, and the stigma is sessile, as in the other living genera. In its exine sculpture, it is unlike both *Hedyosmum* and *Ascarina* and like *Sarcandra* and *Chloranthus* in having smooth rather than spinulose muri.

The spikes (known to be compound in *Z. pragensis*, a character not included in our data set) bear sessile flowers in the axils of bracts. Because male inflorescences are unknown, we score floral subtending bracts (46) as either present in all flowers or present in female flowers but absent in male (0/1). The lower part of the carpel is surrounded by a cup, which has one abaxial and one or two adaxial tips (with reference to the inflorescence axis) in *Z. brnikensis*. Kvaček and Friis (2010) interpreted the cup as most likely a perianth of fused tepals, but Friis and Pedersen (2011, p. 24) described it as a hypanthium, two alternatives that are difficult to distinguish. Under either interpretation the fossil has the inferior ovary state in character 48, which covers both fully and partially inferior. Kvaček and Friis (2010) considered the possibility that the cup consisted of fused bracts of a reduced cyme, but in that case we would expect not one abaxial bract aligned with the subtending bract but two bracts, one on either side. Hence we

score the flower as having a perianth made up of one whorl of sepaloïd tepals. Because the number of tips on the cup is variable, we score perianth merism (55) as unknown. Because the rim of the cup could be formed by laterally connate tepals or the edge of a hypanthium bearing separate tepals, we score tepal fusion (60) as unknown.

With pollen size 12–18 μm in *Z. brnikensis* and 8–10 μm in *Z. pragensis*, we score pollen size (82) as either medium or small (1/2) to allow for possible shrinkage. Kvaček and Friis (2010) did not describe the membrane of the sulcus (93), which is usually strongly infolded, but verrucae are visible at the end of the sulcus in the obliquely flattened grain in their figure 3B.

The gynoecium can be scored as consisting of one carpel (96) with no style (101) and an extended stigma (102). Kvaček and Eklund (2003, fig. 5B) illustrated a macerated fruit of *Z. pragensis* containing one ovule, which they described as orthotropous, with a notch at the lower end that they identified as the micropyle. However, it is possible that this notch is a random fracture, so we follow J. Kvaček (personal communication, 2010) in treating ovule curvature (115) as unknown. No other ovule or seed characters can be determined, but the fruit is a berry (123–125).

With the D&E backbone tree (fig. 11A), *Zlatkocarpus* is associated with Chloranthaceae and *Ceratophyllum* by sessile flowers (45) and one carpel (96); two advances that it shares with *Hedyosmum* but are inapplicable in other Chloranthaceae and *Ceratophyllum* are inferior ovary (48) and one perianth whorl (56). Its most parsimonious position is sister to the ASC clade, based on a shift to sessile stigma (101) and retention of a perianth (53). However, it only “costs” one more step to attach *Zlatkocarpus* to all other branches within the Chloranthaceae-*Ceratophyllum* clade, except *Sarcandra* and *Chloranthus*, and to its stem lineage. With the J/M backbone too (fig. 11B), *Zlatkocarpus* may be sister to the ASC clade, but it has two other most parsimonious positions, sister to Chloranthaceae and to *Hedyosmum*, because polarity of the sessile stigma character is equivocal.

With both backbone trees, the most parsimonious position of *Zlatkocarpus* outside the chloranthaceous line, with Myristicaceae (which also have unisexual flowers with one perianth whorl and one carpel), is three steps less parsimonious. With the J/M backbone, a sister group relationship to *Ceratophyllum* is four steps worse. Kvaček and Friis (2010) noted that similar pollen occurs in Portuguese fossils assigned to Araceae (Friis et al. 2010b) and suggested that a monocot affinity for *Zlatkocarpus* cannot be excluded. However, its best positions in monocots, with *Acorus* and Araceae, are seven steps less parsimonious with the D&E tree and eight steps less parsimonious with the J/M tree.

The fact that *Zlatkocarpus* has a perianth is significant for floral evolution in Chloranthaceae. With the J/M tree, with or without *Zlatkocarpus*, the perianth of *Hedyosmum* is an ancestral feature retained from the base of the angiosperms (as in Doyle et al. 2003). By contrast, with the D&E tree and no fossils, because *Ceratophyllum* has no perianth, it is equally parsimonious to assume that a perianth was ancestral in the Chloranthaceae-*Ceratophyllum* line and lost twice, in *Ceratophyllum* and the ASC clade, or that it was lost on the stem lineage of the two groups and regained in *Hedyosmum*. How-

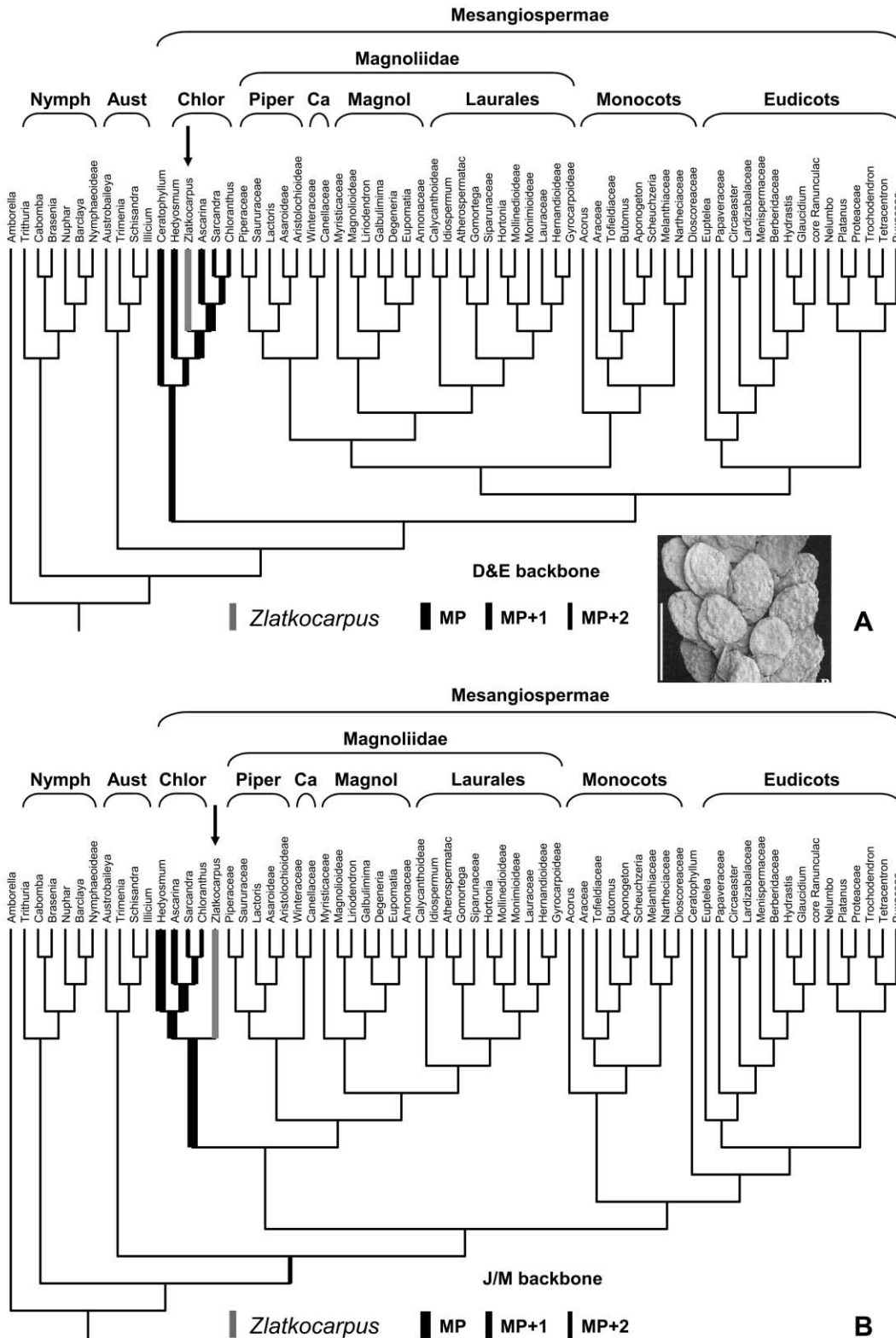


Fig. 11 Analyses of the position of *Zlatkocarpus*. **A**, Single most parsimonious tree (1016 steps) obtained after addition of *Zlatkocarpus* to the D&E tree. **B**, One of three most parsimonious trees (1027 steps) obtained after addition of *Zlatkocarpus* to the J/M tree. Conventions and abbreviations as in fig. 2.

ever, the former scenario is favored if *Zlatkocarpus* is nested within Chloranthaceae as the sister group of the ASC clade.

Although *Zlatkocarpus* is nested within Chloranthaceae when added to the D&E tree by itself, it is more basal in analyses that include other chloranthoid fossils. In both trees found when *Canrightia*, *Zlatkocarpus*, the *Pennipollis* plant, and the *Asteropollis* plant are added to the D&E backbone (fig. 6A, 6B), *Zlatkocarpus* is attached to the stem lineage of Chloranthaceae and *Ceratophyllum*, immediately above *Canrightia*. It has the same position when either *Couperites* or *Appomattoxia* is added as well (figs. 7A–7D, 8A–8D). In all these trees it is linked with the living taxa by unisexual flowers (47) and one carpel (96) but more basal because it lacks supracteal spinules (91), which unite the *Pennipollis* plant and living Chloranthaceae. However, in analyses with the J/M backbone and several chloranthoid fossils (figs. 6C–6K, 7E–7N, 8E–8O), *Zlatkocarpus* may be either attached to the stem lineage of Chloranthaceae or nested within the crown group, at the base of the line to either *Hedyosmum* or the ASC clade.

***Pennipollis* plant.** This plant was reconstructed by Friis et al. (2000a) based on association of *Pennipollis* pollen with isolated carpels (*Pennicarpus tenuis*) and stamens (*Pennistemon portugallicus*) and a fragment of a multistaminate axis, from Vale de Agua and Buarcos, Portugal (early Albian). They interpreted the staminate axis as a spike of male flowers that consist of a single stamen. The carpels contain a single orthotropous ovule.

Monosulcate pollen of the *Pennipollis* type, characterized by an unusually coarse reticulum that tends to detach from the nexine, is a conspicuous element in Aptian-Albian palynofloras worldwide (Penny 1988a; Friis et al. 2000a, 2011). Such pollen was first described by Brenner (1963), who assigned it to *Peromonolites*, a genus for monoete spores with a perispore, as *Peromonolites peroreticulatus* and *Peromonolites reticulatus*. However, Norris (1967) and Doyle (1969) suggested that it was monosulcate and angiospermous, and it was transferred to *Liliacidites* by Singh (1971) and *Retimonocolpites* by Doyle et al. (1975). Using SEM and TEM, Doyle et al. (1975) and Walker and Walker (1984) showed that this pollen resembles “*Clavatipollenites*” in having supracteal spinules and a thick nexine made up of foot layer, except for some endexine under the sulcus, but differs in having no columellae. It was transferred by Juhász and Góczán (1985) to the new genus *Brenneripollis*, in which they included species both with and without columellae. Because Juhász and Góczán (1985) described the type species of *Brenneripollis*, *Brenneripollis pelitus*, as having columellae, Friis et al. (2000a) transferred pollen of Brenner’s original type to the new genus *Pennipollis*, expressly defined as lacking columellae, as *P. peroreticulatus*. They noted that their in situ pollen had a thin layer of fine granules below the tectum.

Based on its exine characters, Doyle and Hotton (1991) suggested that *Pennipollis* was produced by extinct relatives of Chloranthaceae that had lost their columellae. By contrast, Friis et al. (2000a) argued that the combination of a reticulate tectum and granular infratectum is known only in some Alismatales, including some Araceae, and that the *Pennipollis* plant was therefore a monocot. The most similar modern pollen that we know is that of *Aponogeton* (=Aponogetonaceae, Alismatales), not cited by Friis et al. (2000a), which is mono-

sulcate and has a reticulum with supracteal spinules and infracteal granules (Thanikaimoni 1985). However, *Pennipollis* differs from *Aponogeton* and other Alismatales and resembles Chloranthaceae in having a thick nexine consisting of foot layer. Affinities of *Pennipollis* with Araceae were rejected by Wilde et al. (2005) and Hesse and Zetter (2007), who favored a relationship to Chloranthaceae, particularly *Ascarina*. However, the characters in which *Pennipollis* is most like *Ascarina* (unbranched sulcus, supracteal spinules) are probable sympleiomorphies that do not support a special relationship to the living genus.

Because Friis et al. (2000a) interpreted the *Pennipollis* plant as a monocot, Doyle et al. (2008) analyzed its position in their review of Early Cretaceous monocots, using the data set of Endress and Doyle (2009). However, new data and arguments require a few minor changes in character scoring.

As in Doyle et al. (2008), we assume that the stamen-bearing axis was a spike of naked, unistaminate flowers with no subtending bracts, as favored by Friis et al. (2000a, 2011), rather than a multistaminate flower. Because female inflorescences are not known, we score inflorescence units (43) as unknown and floral subtending bracts (46) as either present in female and absent in male flowers or absent in all flowers (1/2), as for the *Asteropollis* plant. Doyle et al. (2008) scored floral base/ovary position (48) as superior, either with or without a hypanthium (0/1), but as discussed for *Couperites*, because of lack of information on organization of the female flowers, we have rescored this character as unknown.

Friis et al. (2000a, 2011) described stamen dehiscence as extrorse, but this referred to orientation relative to the inflorescence axis, whereas for homology assessment it should be defined relative to the axis of the individual flower. If the stamen is abaxial relative to the inflorescence axis (anterior), it is extrorse, whereas if it is adaxial (posterior), it is introrse. Because stamen position in these highly reduced flowers is unknown and there are no clues such as orientation of the vascular bundle in the stamen, we score orientation of dehiscence (75) as either introrse or extrorse (0/2), as in Doyle et al. (2008). As discussed in Doyle et al. (2008), analogies with Chloranthaceae and other unistaminate taxa, where the stamen is abaxial, would favor the extrorse interpretation, but use of this argument would depend too much on the assumption that the fossil and Recent taxa were related. Doyle et al. (2008) did not include the character for food bodies on stamens or staminodes (69), but the stamens seem well enough preserved to score food bodies as absent.

Both the associated pollen and similar dispersed grains (Doyle et al. 1975; Walker and Walker 1984) measure less than 20 μm , so we score pollen size (82) as small. Following Friis et al. (2000a), Doyle et al. (2008) scored infracteal structure (87) as granular; Hesse and Zetter (2007) questioned this interpretation, but convincing granules are visible on the inner surface of the muri illustrated by Friis et al. (2010b, fig. 2d). Friis et al. (2000a) described the nexine (94) as consisting of a thick foot layer and a very thin endexine, the latter thickening under the aperture, but like the similar nexine with discontinuous endexine in *Couperites* and Chloranthaceae, we include this in the state for foot layer only (0).

Friis et al. (2000a) tentatively interpreted the carpel as having a sessile stigma, but to be cautious Doyle et al. (2008)

scored the style character (101) as unknown. However, because Friis et al. (2000a) reported not observing a style in over 100 fruits examined, we have rescored style as absent. Friis et al. (2000a, 2011) considered the single ovule to be most likely orthotropous (115) but cautioned that an anatropous curvature cannot be ruled out. However, as argued in Doyle et al. (2008), the shape of the seed suggests an orthotropous ovule with the chalaza displaced toward one side of the base, as in *Amborella* (Endress and Igersheim 2000; Tobe et al. 2000) and Chloranthaceae (Endress 1987; Yamada et al. 2001). Friis et al. (2000a) did not characterize ovule direction (114), but Friis et al. (2011) interpreted the seed as “apparently basally attached” (ascendent), because the micropylar end appeared to be directed toward the presumed stigmatic end of the fruit (E. M. Friis, personal communication, 2006). However, as discussed in Doyle et al. (2008) and Endress (2011), asymmetry of the base of the ovule of the sort seen here is generally correlated with apical and pendent ovule attachment (as in *Amborella* and Chloranthaceae), whereas basal and ascendent ovules (as in Piperaceae and some Araceae) have a symmetrical base. Furthermore, the outline of the enclosed seed in the carpel shown in figure 6A of Friis et al. (2000a) appears to be more asymmetrical toward the presumed stigmatic end of the carpel, like the chalazal end of the isolated seed in their figure 6G, which would support interpretation of the ovule as pendent. We therefore score ovule direction (114) as unknown.

Only the outer cuticle of the seed coat is preserved, so there is no evidence on the number of integuments (116) or cell layers. This precludes scoring of most seed coat characters, which are defined in terms of the integument (outer, inner) and original cell layer (outer or inner epidermis, mesophyll) from which each seed coat layer is derived. In many extant unitegmic angiosperms, it has been inferred that the single integument is derived from both original integuments (Kelley and Gasser 2009; Endress 2011). Under this hypothesis, the outermost cell layer in the seed coat can be interpreted as an exotesta and the innermost layer as an endotegmen, but intermediate layers cannot be identified (they may not even exist: in *Ceratophyllum* the integument is only two cells thick). Application of our tegmen character is also problematic, since it covers modifications of both the exo- and endotegmen. In living unitegmic taxa in our data set (Siparunaceae, *Ceratophyllum*, *Circaea-aster*) we therefore score only the exotesta character (128) and treat others (129–132) as unknown.

Consistent with this reasoning, Doyle et al. (2008) scored all seed coat characters of the *Pennipollis* plant except exotesta as unknown. They scored exotesta as either unspecialized or with tabular cells (0/2), but because the elongation of cells is not as marked as in those monocots scored as having tabular cells (*Aponogeton*, *Scheuchzeria*), we have rescored this character as unspecialized (0).

Both Doyle et al. (2008) and the present analysis strongly contradict the hypothesis that the *Pennipollis* plant was a monocot. When the fossil is added to the J/M tree (fig. 12B), its most parsimonious position is sister to Chloranthaceae; when it is added to the D&E tree (fig. 12A), it is sister to the Chloranthaceae-*Ceratophyllum* clade. With the J/M tree, synapomorphies uniting it with Chloranthaceae are unisexual flowers (47), one stamen (62), supracteal spinules (91), thick nexine (95), and orthotropous ovule (115); it is located below

the crown group because it has protruding rather than embedded pollen sacs (73) and either introrse or extrorse rather than latrorse anthers (75). Its next-best position, one step less parsimonious, is with *Hedyosmum*, supported by absence of bracts in the male inflorescences (46). Its best position outside the chloranthaceous line, with *Ceratophyllum*, is four steps worse, while its best positions in monocots, with *Acorus* and *Aponogeton*, are eight steps worse, despite the similar exine in *Aponogeton*, because these plants have such different multiparted flowers. With the D&E tree, the *Pennipollis* plant is linked with Chloranthaceae and *Ceratophyllum* by absence of bracts in the male inflorescence (46), a feature of *Ceratophyllum* and *Hedyosmum*, one stamen, thick nexine, and orthotropous ovule, while its protruding pollen sacs place it below the crown group. However, positions sister to *Ceratophyllum*, Chloranthaceae, and the ASC clade are only one step worse. Its best positions in monocots, again with *Acorus* and *Aponogeton*, are seven and eight steps worse, respectively.

The basal (ascendent) seed attachment favored by Friis et al. (2011) would contrast with the apical (pendent) condition in Chloranthaceae. However, if this character (114) is scored as ascendent rather than unknown, the relative parsimony of the positions in monocots improves by only one step or not at all for a relationship with *Acorus* in the J/M tree.

With the J/M backbone and the four most securely associated chloranthoid fossils, the position of the *Pennipollis* plant is more ambiguous: it is sister to Chloranthaceae, above *Canrightia* and *Zlatkocarpus* (fig. 6D); sister to *Hedyosmum* and the *Asteropollis* plant (fig. 6C, 6F, 6G), supported by loss of bracts in the male spikes (46) and/or supracteal spinules (91), depending on the position of *Zlatkocarpus*; sister to the ASC clade (fig. 6E), based on extended connective apex; sister to *Ascarina* (fig. 6H–6J), supported by supracteal spinules (equivocally in fig. 6J); or sister to *Sarcandra* and *Chloranthus* (fig. 6K). In the 45 trees found when *Couperites* is also added (fig. 7E–7N), the *Pennipollis* plant is either a stem relative of Chloranthaceae, above *Zlatkocarpus*, with or without *Couperites*, or nested within the crown group, on the line to *Hedyosmum* and the *Asteropollis* plant, the ASC clade, or *Ascarina*, with or without *Couperites*, or sister to *Sarcandra* plus *Chloranthus*. In the 48 trees found with the four main fossils and *Appomattoxia* (fig. 8E–8O), the *Pennipollis* plant is in one of the five positions found with four chloranthoid fossils (fig. 6C–6K); linked with *Appomattoxia*, as the sister group of either crown group Chloranthaceae or the *Asteropollis*-*Hedyosmum* clade; or sister to *Appomattoxia* plus the *Asteropollis*-*Hedyosmum* clade.

Analyses with the D&E backbone tree and other chloranthoid fossils raise the intriguing possibility that the *Pennipollis* plant is a stem relative of *Ceratophyllum*. In one of the two trees found with the four most securely associated fossils (fig. 6A), the *Pennipollis* plant is sister to the Chloranthaceae-*Ceratophyllum* clade, above *Canrightia* and *Zlatkocarpus*, based on its combination of supracteal spinules and protruding pollen sacs, but in the other tree (fig. 6B) it is linked with *Ceratophyllum* by a shift from latrorse to introrse or extrorse anthers (75). In the 18 trees found when *Couperites* is also added (fig. 7A–7D), where *Canrightia* and *Zlatkocarpus* are basal, the *Pennipollis* plant is either basal to the crown clade,

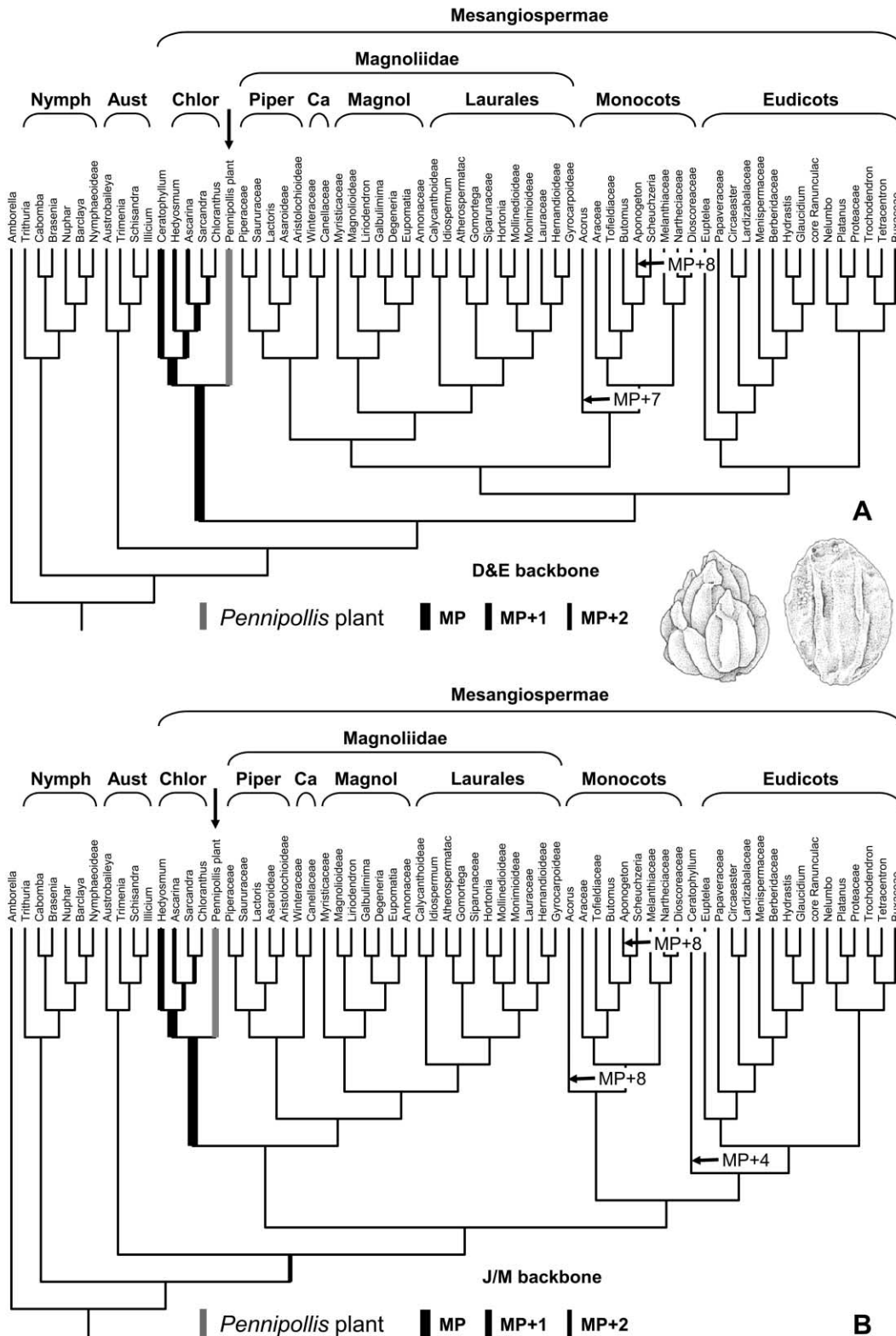


Fig. 12 Analyses of the position of the *Pennipollis* plant. *A*, Single most parsimonious tree (1020 steps) obtained after addition of the *Pennipollis* plant to the D&E tree. *B*, Single most parsimonious tree (1031 steps) obtained after addition of the *Pennipollis* plant to the J/M tree. Conventions and abbreviations as in fig. 2.

alone or with *Couperites*, in all three arrangements, or linked with *Ceratophyllum*, alone or with *Couperites*, in all three arrangements. When *Appomattoxia* is added, the *Pennipollis* plant may be in the same two positions found with the four fossils (fig. 8C, 8D); sister to the ASC clade (fig. 8A), based on extended connective apex (72) and a reversal to fleshy fruit wall (123), or sister to *Appomattoxia* and *Ceratophyllum* (fig. 8B), based on introrse or extrorse anthers and intermediate infractectum (87), as in *Appomattoxia* (counted as a step toward the granular structure of *Pennipollis* because the character is ordered). Implications of a relationship of the *Pennipollis* plant and *Ceratophyllum* are discussed in the section on *Appomattoxia*.

These analyses do not address the possibility that the *Pennipollis* plant was nested within Araceae, a family that is reported from the Aptian-Albian (Friis et al. 2004, 2010b), but as discussed in Doyle et al. (2008) this is unlikely. Although various Araceae have highly reduced unisexual flowers and exine similarities, these features do not occur in the same living clades, and in the context of current phylogenies (Cabrera et al. 2008; Cusimano et al. 2011) it is unparsimonious to assume that they occurred together in the past. The stamens of Araceae (illustrated in Mayo et al. 1997) are also very different from those of the *Pennipollis* plant.

Friis et al. (2010b, 2011) rejected a relationship of the *Pennipollis* plant to Chloranthaceae, but their main argument was the fact that the combination of reticulate tectum and granular infractectum is not known outside monocots. However, the spinulose muri are not fundamentally different from those of Chloranthaceae, and the granular infractectum is only one character out of many. As with any character, there is no reason to assume that it could not have originated by homoplasy in extinct relatives of a group where it does not occur today, especially considering the many shifts from columellar to granular structure elsewhere in angiosperms (Doyle 2009). Except for its coarser reticulum and absence of columellae, *Pennipollis* resembles Barremian-Aptian monosulcates that do have columellae (e.g., *Retisulc-dentat*; Hughes et al. 1979; Hughes 1994), and some *Pennipollis*-like grains in the earliest Aptian have rare columellae (*Retisulc-dubdent*; Hughes et al. 1979; Penny 1988a; Hughes 1994). These problems might be resolved easily if the floral remains were associated with vegetative parts.

Friis et al. (2000a, 2011) stated that *Pennipollis* ranges from the Barremian to the Cenomanian or Turonian, but the oldest well-dated records are early Aptian, confirming the use of *Pennipollis* as a guide fossil for Aptian and younger sediments (Penny 1988a; Doyle 1992; Heimhofer et al. 2007). Hughes et al. (1979) labeled the oldest grains of the *Pennipollis* type (*Retisulc-dubdent*) as Barremian-Aptian, but these were from the upper Vectis Formation, which has been redated by magnetostratigraphy as earliest Aptian (Kerth and Hailwood 1988; Allen and Wimbledon 1991; Hughes 1994). *Pennipollis* also appears above the base of the Aptian in Portuguese marine sections (Heimhofer et al. 2005, 2007).

Canrightia. *Canrightia resinifera* is based on lignitized and charcoalfied flowers and fruits, one with an attached stamen, and associated pollen of the *Retimonocolpites* type, from Cafeteca and other early Albian localities in Portugal, described by Friis and Pedersen (2011) using X-ray microtomography.

Some of these fossils were illustrated by Friis et al. (1999). A conspicuous feature is the presence of resin bodies in the fruit wall, assumed to be altered oil cells, which appear to be comparable to the intrusive oil cells of *Sarcandra* and *Chloranthus* (Endress and Igersheim 1997).

These flowers are of special significance for the origin of Chloranthaceae. They resemble *Hedyosmum*, the *Asteropollis* plant, and *Zlatkocarpus* in having a perianth adnate to the ovary, but they differ in being bisexual, with scars of approximately four stamens just above the ring of highly reduced tepals. As in Chloranthaceae the ovules are orthotropous and pendent, but the gynoecium consists of two to five fused carpels. As Friis and Pedersen (2011) noted, this means that in several respects *Canrightia* is more like flowers of Piperales, which are usually bisexual, with either one whorl of three tepals (*Lactoris*, Aristolochiaceae, Hydnoraceae) or no perianth (Saururaceae, Piperaceae), and with several carpels, which contain orthotropous ovules in Saururaceae and Piperaceae.

Friis and Pedersen (2011) analyzed the phylogenetic position of *Canrightia* using the data set of Doyle and Endress (2010). Here we make several minor changes in scoring, many following policies adopted in Endress and Doyle (2009) when parts are absent or hard to interpret.

Inflorescences are not known, but some flowers have a subtending bract (46) at the base. This suggests that the inflorescence units (43) were single flowers, but without direct evidence we follow Friis and Pedersen (2011) in scoring this character as unknown. We have scored some floral characters that Friis and Pedersen (2011) did not score: carpels not sunken in pits in the receptacle (50), floral apex not protruding (52), and tepals all sepaloïd or outer sepaloïd and inner petaloïd (57). Conversely, we have not scored other characters that Friis and Pedersen (2011) did score: presence of petals (58) and petal nectaries (59), treated as unknown in flowers with one perianth whorl; fusion of outer tepals (60), for reasons like those cited in *Zlatkocarpus*; and stamens not in double positions (66), which requires better evidence on the relation of the stamens to perianth parts. The perianth appears to be represented by four tips on the cup surrounding the ovary, but because this is usually unclear and the merism of the gynoecium is variable, we follow Friis and Pedersen (2011) in treating perianth merism (55) as unknown. The attached stamen seems too poorly preserved to score food bodies (69) and connective apex (72), which Friis and Pedersen (2011) described as prominently extended, but may be like the rounded apex in the *Asteropollis* plant, but stamen dehiscence (75) is latrorse rather than unknown.

Friis and Pedersen (2011, p. 24) did not score pollen aperture (84) and elongate versus round distal aperture (85), on the grounds that it is uncertain whether the elongated sulcus was polar. However, as discussed below, the fact that the furrow did not extend all the way around the grain strongly implies that it was polar. Friis and Pedersen (2011, p. 8) did not score nexine thickness (95), but because they stated that SEM of broken grains shows a “relatively thin” foot layer, we score it as thin.

We concur with Friis and Pedersen’s (2011) interpretation of the gynoecium as parasyncarpous (106), usually with four main vascular bundles alternating with four ovules, which were vascularized from the upper part of the ovary. This im-

plies that the bundles were most likely synlateral, but because the positional relationships of the carpels and ovules are uncertain, we follow Friis and Pedersen (2011) in scoring placentation (113) as unknown. Friis and Pedersen (2011) scored long hairs on or between the carpels (108), curved hairs on the carpels (109), and dorsal (110) nectaries as absent, but we question whether these characters would be preserved and therefore score them as unknown. They scored the chalaza (113) as unextended, but although it is theoretically possible for an orthotropous ovule to have an extended chalaza, the distinction between the two states can be made easily only in anatropous ovules (Periasamy 1962), so we follow our previous procedure (Endress and Doyle 2009) of scoring orthotropous ovules as unknown. We follow Friis and Pedersen (2011) in considering the endotesta (131) the only lignified layer of the seed coat. Friis and Pedersen (2011) scored mesotesta (129) as unlignified and sarcotesta (130) as absent, but these characters are inapplicable because the outer integument was only two cells thick (119). They identified the innermost seed coat layer of nonlignified, apparently tannin-filled cells as an endothelium, a character not included in either their or our data set; the most similar layer in our extant taxa is the endothelium of *Lactoris*, but this is collapsed at maturity (Takh-tajan 1988; Tobe et al. 1993).

When Friis and Pedersen (2011) added *Canrightia* to the Doyle and Endress (2010) data set, its best position was sister to Chloranthaceae with the J/M backbone tree and sister to Chloranthaceae and *Ceratophyllum* with the D&E tree. Friis and Pedersen (2011) stated that a position nested within Piperales, sister to Saururaceae and Piperaceae, was two or three steps less parsimonious, depending on the backbone tree, but it appears to be three or four steps less parsimonious in their figure 19. Despite our different scoring decisions, we obtained essentially the same results.

With the D&E backbone (fig. 13A), *Canrightia* is linked to Chloranthaceae and *Ceratophyllum* by sessile flowers (45), inferior ovary (48), one perianth whorl (56), and orthotropous ovule (115), while the crown clade shows a shift to unisexual flowers (47) and reduction to one stamen (62) and one carpel (96). Its next-best positions, which are two steps less parsimonious, are nested within Chloranthaceae, as the sister group of the ASC clade, supported by sessile stigma (101, a reversal) and one-layered endoreticulate endotesta (131), or linked with *Sarcandra* and *Chloranthus* by bisexual flowers (47), a reversal with this tree, and intrusive oil cells in the carpel wall (107). The best positions outside the Chloranthaceae-*Ceratophyllum* line are three steps worse: (a) sister to mesangiosperms as a whole and (b) linked with Saururaceae and Piperaceae by sessile flowers and orthotropous ovule, intrusive oil cells in the carpel wall, and latrorse stamen dehiscence (75).

With the J/M backbone (fig. 13B), *Canrightia* is sister to Chloranthaceae, based on the same four synapomorphies that link it with Chloranthaceae and *Ceratophyllum* with the D&E backbone, plus one stamen whorl (65), and the lack of synapomorphies of crown Chloranthaceae, which include not only one stamen and one carpel but also thick nexine (95). Again, a position sister to the ASC clade is two steps worse, but the position sister to *Sarcandra* and *Chloranthus* is three steps worse, rather than two; this is because bisexual flowers are not necessarily a shared derived state of the three taxa but

could equally well be ancestral in Chloranthaceae. A position sister to Saururaceae and Piperaceae is only two steps worse, supported by the same four synapomorphies listed above.

Canrightia is basal on the Chloranthaceae-*Ceratophyllum* or Chloranthaceae line in nearly all trees found in analyses that include other chloranthoid fossils, most often followed by *Zlatkocarpus*, which is associated with the remaining taxa by a shift to unisexual flowers and reduction to one carpel (figs. 6, 7A–7M, 8). The only exception is one tree found with the J/M backbone, *Canrightia*, *Zlatkocarpus*, the *Pennipollis* plant, the *Asteropollis* plant, and *Couperites*, in which *Canrightia* is nested within Piperales, as the sister group of Saururaceae and Piperaceae (fig. 7N).

These results imply that *Canrightia* diverged from the chloranthaceous line at an intermediate stage in reduction of the original multiparted angiosperm flower. *Canrightia* was clearly not directly ancestral to Chloranthaceae, since it coexisted with more derived forms, so some of its features may be autapomorphic rather than primitive. Parsimony optimization indicates that this is the case for outer integument with two cell layers (119) and intrusive oil cells in the carpel wall (107), plus one-layered endoreticulate endotesta (131) in trees that include several fossils. It may also be true for the syncarpous gynoecium; optimization of this character (106) is equivocal because other members of the clade have one carpel and such taxa were scored as unknown, on the grounds that it cannot be determined whether a single carpel originated by reduction from several free carpels or from a syncarpous gynoecium. The number of floral parts per whorl, which is most often four but was not scored because it is variable, may also be derived: the perianth and androecium at the node where *Canrightia* diverged are reconstructed as trimerous.

Friis and Pedersen (2011) compared the pollen of *Canrightia* to *Retimonocolpites* and *Dichastopollenites*; it resembles *Retimonocolpites dividuus* of Pierce (1961) in that the sulcus extends more than halfway around the grain, thus approaching *Dichastopollenites*, where it forms a ring. It differs from pollen of the *Clavatipollenites rotundus* type associated with *Couperites* in having smooth rather than sculptured muri and a thin rather than thick nexine. Friis and Pedersen (2011) suggested that the smooth muri of both *Canrightia* and *Zlatkocarpus* might be evidence for relationship, but on trees where these taxa are successive lines, it is a symplesiomorphy, like retention of a perianth. Friis and Pedersen (2011, p. 24) also suggested that the sulcus might not be distal but in “a derived, non-polar position as for instance in some Nymphaeales,” but this is unlikely in terms of the principle that the polar axis in pollen corresponds to its main axis of symmetry. The aperture of *Barclaya* and Nymphaeoidae forms a complete ring that is perpendicular to the axis of symmetry of the grain, which is known to correspond to the polar axis (Gabarayeva and Rowley 1994). By contrast, the sulcus of *Canrightia* does not form a complete ring, so the axis of symmetry runs through the midpoint of the sulcus, implying that it is polar.

Friis and Pedersen (2011) suggested that the similarities between *Canrightia* and both Chloranthaceae and Piperales support the older view that the two living taxa are related, which conflicts with molecular evidence that Piperales are nested within the magnoliid clade. We tested this hypothesis by comparing the morphological parsimony of the D&E and J/M trees

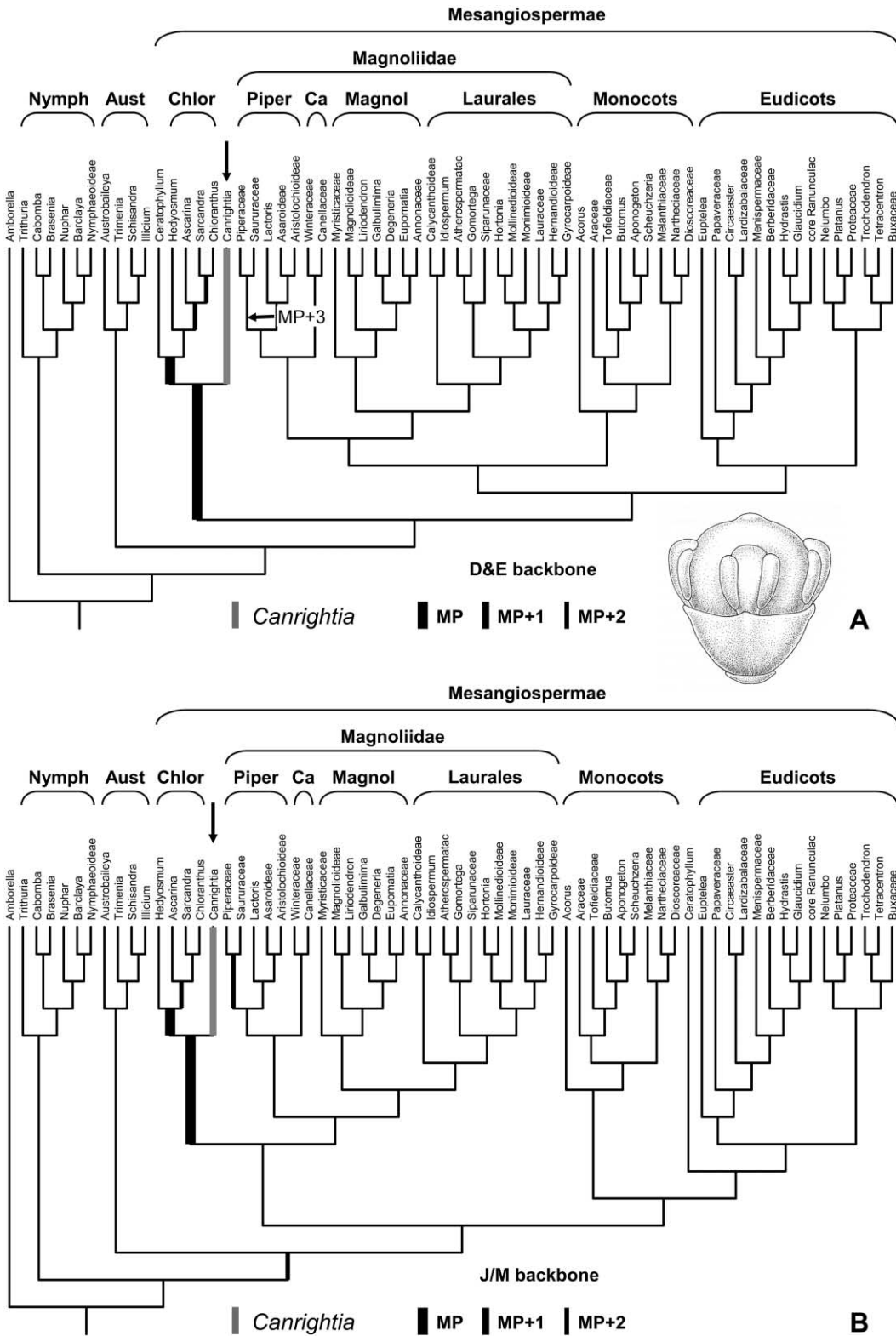


Fig. 13 Analyses of the position of *Canrightia*. **A**, Single most parsimonious tree (1021 steps) obtained after addition of *Canrightia* to the D&E tree. **B**, Single most parsimonious tree (1031 steps) obtained after addition of *Canrightia* to the J/M tree. Conventions and abbreviations as in fig. 2.

with each of two trees in which the chloranthoid line and Piperales are sister groups, with and without *Canrightia*: (a) with the Chloranthaceae-*Ceratophyllum* clade (D&E) or Chloranthaceae (J/M) moved into magnoliids and (b) with Piperales moved out of magnoliids as the sister group of Chloranthaceae-*Ceratophyllum* (D&E) or Chloranthaceae (J/M).

These experiments indicate that *Canrightia* provides only weak support for a closer relationship between Chloranthaceae and Piperales. With the D&E tree, addition of *Canrightia* has no effect on the morphological parsimony of this relationship. It is two steps less parsimonious to move the Chloranthaceae-*Ceratophyllum* clade into the magnoliids with Piperales both with Recent taxa only (1018 steps vs. 1016) and with *Canrightia* added (at any of its three possible positions relative to Piperales and the Chloranthaceae-*Ceratophyllum* clade or with Saururaceae and Piperaceae; 1023 steps vs. 1021). It is two steps more parsimonious to move Piperales out of magnoliids to the Chloranthaceae-*Ceratophyllum* line both with Recent taxa only (1014 steps vs. 1016) and with *Canrightia* added (on the Chloranthaceae-*Ceratophyllum* line; 1019 steps vs. 1021). With the J/M tree, addition of *Canrightia* does improve the parsimony of a relationship of Chloranthaceae and Piperales but only by one or two steps. It is one step more parsimonious to move Chloranthaceae into the magnoliids with Piperales with Recent taxa only (1026 steps vs. 1027) but three steps more parsimonious with *Canrightia* added (with Piperales; 1028 steps vs. 1031), an improvement of two steps. It is three steps more parsimonious to move Piperales out of magnoliids to Chloranthaceae with Recent taxa only (1024 steps vs. 1027) but four steps more parsimonious with *Canrightia* added (with Piperales or with Saururaceae and Piperaceae; 1027 steps vs. 1031), an improvement of one step. This modest increase in support for a closer relationship between Chloranthaceae and Piperales must be weighed against increasingly strong molecular evidence for the monophyly of magnoliids and the nested position of Piperales within them (cf. Soltis et al. 2005).

Friis and Pedersen (2011) suggested that the presence of an endothelium in seeds of *Canrightia* and *Lactoris* (Piperales) was evidence for relationship. However, since these are the only taxa with an endothelium, this feature would support a relationship only if *Canrightia* and *Lactoris* were sister groups, which is eight steps less parsimonious than a relationship of *Canrightia* with Chloranthaceae (J/M) or Chloranthaceae-*Ceratophyllum* (D&E). Friis and Pedersen (2011) suggested that an endothelium might be a basic feature of magnoliids that was lost in all lines except *Lactoris*, but this would require at least four losses, not a parsimonious scenario. Furthermore, an endothelium tends to occur in ovules with a thin nucellus (Endress 2011; *Lactoris* is weakly tenuinucellar: Tobe et al. 1993), which is unlikely to be ancestral in magnoliids.

***Appomattoxia*.** Our concept of this taxon is based primarily on *Appomattoxia ancistrophora*, known from isolated carpels at the fruit stage with adhering pollen of the *Tucanopollis-Transitoripollis* type, which were described by Friis et al. (1995) from Puddledock, Virginia (middle Albian). Additional characters are based on isolated stamens containing similar pollen from Torres Vedras, Portugal (Aptian or early Albian), figured by Friis et al. (2006, fig. 10C; 2010a, pl. II, fig. 2).

The most conspicuous feature of *Appomattoxia* is the presence of hooked hairs on the fruit wall. Friis et al. (1995) compared these with hairs of the eudicot *Circaeaster* (Ranunculales), but they rejected a close relationship because the pollen of *Appomattoxia* is monosulcate rather than tricolpate. Friis et al. (2010a) also reported fruits with similar pollen but without hairs at Torres Vedras; if these are related to *Appomattoxia*, they would tend to neutralize the hairs as evidence of relationship. The fruits contain a single pendent orthotropous ovule, as in Chloranthaceae, but the innermost layer of the seed coat around the micropyle consists of cells with thickened undulate walls. Friis et al. (1995) compared this with a similar layer in Piperaceae and Saururaceae (Piperales), which is known to be a sclerotic ecto- and endotegmen (Corner 1976; Takhtajan 1988). The pollen resembles *Clavatipollenites* and most Chloranthaceae in having a sculptured sulcus membrane, supracteal spinules, and an unusually thick nexine consisting of foot layer plus endexine under the aperture, but it differs in having a continuous tectum, again like Piperaceae and Saururaceae. Based on these characters, Friis et al. (1995, 2010a, 2011) favored a relationship with Piperaceae and Saururaceae. However, these taxa have a syncarpous gynoecium of 2–4 carpels (except in the derived genus *Peperomia*), with several ventral (horizontal) ovules per carpel in Saururaceae and one basal (ascendent) ovule per unilocular gynoecium in Piperaceae, rather than one pendent ovule per carpel, as in *Appomattoxia* and Chloranthaceae.

Dispersed pollen similar to that of *Appomattoxia* was described from the Barremian-Aptian of Brazil by Regali et al. (1974) as *Inaperturopollenites crisopolensis* (although it has a conspicuous sulcus, often widened into a large circular ulcus) and transferred to the new genus *Tucanopollis* by Regali (1989). Góczán and Juhász (1984) described similar but generally less sculptured pollen from the Albian of Hungary as *Transitoripollis*. Whether the two genera should be distinguished is unclear. Such pollen is a minor element in Southern Laurasia, extending back to the Barremian of England as the Barremian-ring group of Hughes (1994), but one of the most abundant angiosperm pollen types in the Barremian and Aptian of Northern Gondwana (Doyle et al. 1977; Regali 1989). Based on the similarities noted above, Doyle and Hotton (1991) argued that *Tucanopollis* was produced by relatives of Chloranthaceae that were either more primitive or more derived in having a continuous tectum. However, they also noted similarities to Saururaceae, which lack supracteal spinules and have a thin endexine around the grain but which also have a more or less continuous tectum and a thick foot layer (Smith and Stockey 2007).

As noted by Friis et al. (1995), the oblique position of the carpel stipe suggests that the carpels were from a multicarpellate apocarpous flower, rather than a unicarpellate flower, which would mean that the ovary was superior, but because this is speculative, we have scored floral base/ovary position (48) and other characters of inflorescence and flower organization as unknown. The apical view of the stamen in Friis et al. (2006, 2010a) shows a truncated connective apex (72), protruding pollen sacs (73), four microsporangia (74), and longitudinal dehiscence (76), but no other characters can be determined because of the unsuitable orientation.

Pollen measures 16–19 μm (82), which we score as either

small (<20 μm) or medium to allow for possible shrinkage. As noted by Friis et al. (1995), the infratectum (87) appears to consist of granules that are often fused into irregular columellae, falling in our intermediate state. The nexine (94) consists of a thick foot layer and much thinner endexine, but the endexine is darker and more consistently present than the discontinuous and indistinctly staining inner layer of most Chloranthaceae (Doyle 2005), which we treat as having foot layer only, so we score endexine as present (1).

As in most fossils, carpel form (97) cannot be established. We score the stigma (102) as extended because of the large size of the area (above the hooked hairs) that Friis et al. (1995) interpreted as stigmatic; however, we score style (101) as absent because there is no visible constriction at the base of the stigmatic extension. The stigma bears papillae (104) assignable to state 1 (unicellular or with one emergent cell).

The ovule is pendent (114) and orthotropous (115). As in the *Pennipollis* plant, there is no evidence on the number of integuments (116), so we have scored characters of intermediate cell layers in the seed coat (129–131) as unknown. However, because the innermost layer is so distinctive (relative to the *Pennipollis* plant and living unitegmic taxa), we score it as a sclerotic ecto- and endotegmen (132, state 1), to express its similarity and potential homology with that layer in Piperaceae and Saururaceae. We assume that the fruit wall (123) was dry because of its thinness and the fact that fleshiness and hooks appear to be mutually exclusive in living plants.

Our analyses give two very different sorts of results for the relationships of *Appomattoxia*, depending on whether it is added alone to the Recent trees or with other fossils. Both sets of results suggest that *Appomattoxia* may be important for understanding early angiosperm evolution but in different ways.

When *Appomattoxia* is added alone to both backbone trees (fig. 14), its four most parsimonious positions are at or near the very base of the tree: sister to all angiosperms, *Amborella*, all other angiosperms, or Nymphaeales. These positions are favored by the continuous tectum (88), which becomes perforate below Austrobaileyales and mesangiosperms, and they are consistent with the single pendent ovule (as in *Amborella* and *Trithuria*) and sessile stigma. The first three positions are equally parsimonious because the backbone trees include no outgroups, so there are no unambiguously derived states that favor one arrangement over another. The orthotropous ovule (115) of *Appomattoxia* and *Amborella* favors these three positions over a relationship with Nymphaeales, but this is balanced by truncated connective apex (72), a derived state shared with Nymphaeales. The next-best positions (one step worse) are sister to Austrobaileyales plus mesangiosperms, Chloranthaceae (J/M) or *Ceratophyllum* and Chloranthaceae (D&E), and *Hedyosmum* (J/M only).

Of the three positions of *Appomattoxia* around the basal node, the one sister to *Amborella* would be favored if the orthotropous ovule of the two taxa was shown to be derived. This would be consistent with the hypothesis that *Caytonia* was the sister group of angiosperms and its anatropous cupule was homologous with the anatropous bitegmic ovule of angiosperms (Gaussen 1946; Doyle 1978, 2008). Another possible synapomorphy, not included in our data set, is a tendency for low verrucate pollen sculpture (Friis et al. 1995, figs. 20–

24), which could be a step toward the more prominent verrucae of *Amborella* (Sampson 1993; Hesse 2001). Similar pollen with larger verrucae is known from the Hauterivian of England (Hauterivian-*cactisulc*; Hughes and McDougall 1987; Hughes 1994) and has been compared with *Amborella* (Doyle 2001; Hesse 2001). If these pollen types were related to *Amborella*, they would imply that *Amborella* is a relict of a group that was far more widespread and abundant in the Early Cretaceous.

There are characters that could support the other two near-basal positions for *Appomattoxia*, but they are also difficult to polarize (and often highly homoplastic). Whereas *Amborella* has pluricellular stigmatic papillae, *Appomattoxia* has papillae with one emergent cell (104), the inferred basic state for all other angiosperms; if this state is derived, it would favor a position sister to other angiosperms. With the D&E tree, dry fruit wall (123) is another such character. *Appomattoxia* differs from the reconstructed common ancestor of all extant angiosperms in its intermediate infratectal structure, thick nexine, hooked hairs, and sclerotic tegmen; if any of these features are ancestral, they would support a position on the angiosperm stem lineage.

If *Appomattoxia* is in any of these positions, the fact that *Tucanopollis* pollen is so abundant in Northern Gondwana would be significant for the ecological evolution of angiosperms. The Early Cretaceous climate in this province has long been interpreted as hot and dry, based on sedimentary indicators (salt deposits, lack of coal) and characteristics of the flora (few ferns; abundance of *Classopollis*, representing the xeromorphic conifer family Cheirolepidiaceae, and Gnetales), except for presumed wetter areas in the Middle East and northern South America (Brenner 1976, 1996; Doyle et al. 1982; Doyle 1999; Mejia-Velasquez et al. 2012). By contrast, *Amborella* and Austrobaileyales are restricted to wet, shaded forest understory sites. Feild et al. (2004, 2009) reconstructed such habitats as ancestral for angiosperms and argued that the rarity of such environments in the Triassic and Jurassic could explain why angiosperms escaped detection if they existed at that time, as indicated by many molecular dating analyses (Magallón 2010; Smith et al. 2010; Clarke et al. 2011; see Doyle 2012). If *Appomattoxia* is near the base of the tree, it could challenge this view of ecology of the first angiosperms or else indicate that members of the basal grade were able to break out of the ancestral niche and adapt to dry climates more easily than might be expected.

By contrast, when *Appomattoxia* is added to the Recent data set along with *Canrightia*, *Zlatkocarpus*, and the *Pennipollis* and *Asteropollis* plants, it is near basal in some most parsimonious trees but associated with Chloranthaceae in others. In one of the four trees found with the D&E backbone (fig. 8A), it is sister to Chloranthaceae plus *Ceratophyllum*, based on supracteal spinules (91) and dry fruit wall (123; reversed within Chloranthaceae). In another (fig. 8B) it is linked with *Ceratophyllum*, above the *Pennipollis* plant; the three taxa are united by a shift from latrorse to extrorse anthers (75) and intermediate infratectal structure (87; see discussion of the *Pennipollis* plant), while *Appomattoxia* is linked with *Ceratophyllum* by dry fruit wall. It should be noted that the exine features listed are not present in *Ceratophyllum*; its exine is reduced to a thin structureless layer (Takahashi 1995), so we

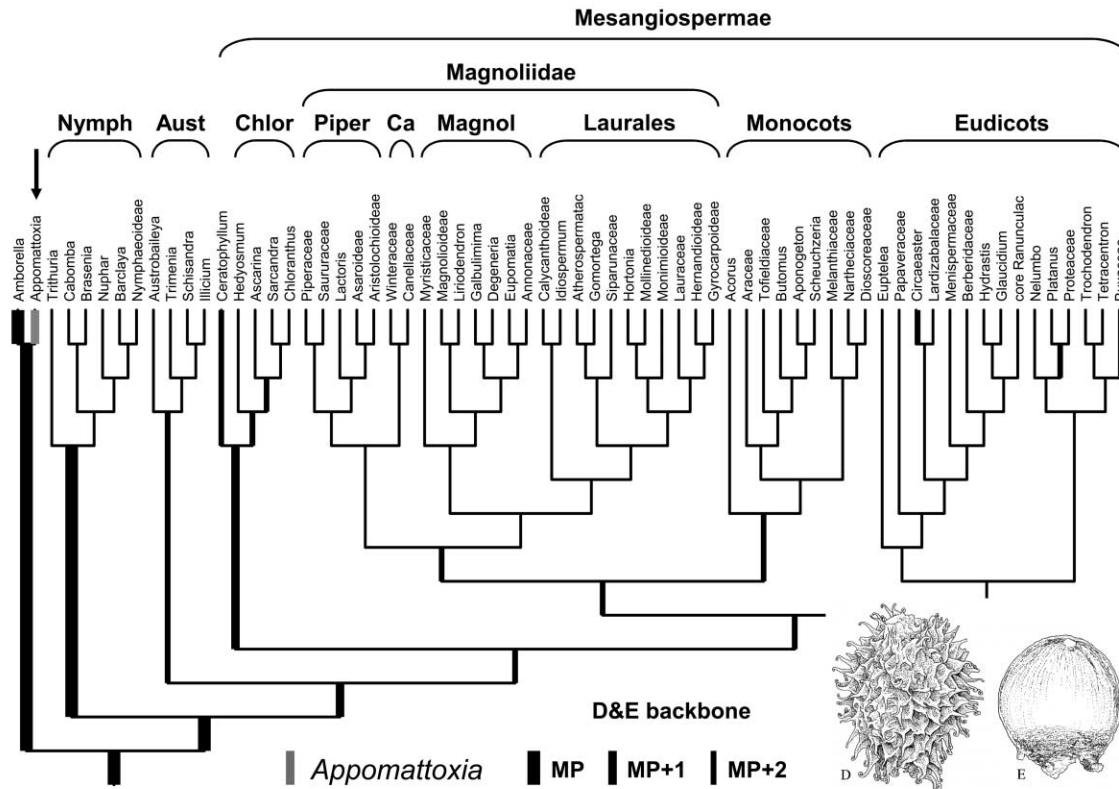


Fig. 14 One of four most parsimonious trees (1021 steps) obtained after addition of *Appomattoxia* to the D&E tree. Conventions and abbreviations as in fig. 2.

scored most of its pollen characters as unknown. Finally, in two trees (fig. 8C, 8D) it is sister to Nymphaeales. In 36 of the 48 trees found with the JM backbone, *Appomattoxia* is located in one of the three positions around the basal node or with Nymphaeales (fig. 8E, 8F), but it is related to Chloranthaceae in the remaining 12. In one of the latter trees (fig. 8H), it is linked with the *Pennipollis* plant by intermediate infractum, and the two are the sister group of crown Chloranthaceae, based on supratpectal spinules. In the other 11 trees (fig. 8G, 8I–8O), *Appomattoxia* is nested in Chloranthaceae, on the line to *Hedyosmum* and the *Asteropollis* plant: either by itself, with the three taxa united by dry fruit wall; linked with or above the *Pennipollis* plant, with the four taxa united by loss of bracts subtending the male flowers (46) and/or supratpectal spinules (depending on the position of *Zlatkocarpus*); or above *Zlatkocarpus*.

Association of *Appomattoxia* and/or the *Pennipollis* plant with vegetative remains could confirm or refute the hypothesis that these fossils are stem relatives of *Ceratophyllum*, and if they are related, they could clarify the origin of this enigmatic floating aquatic, which has whorled dichotomous leaves and no roots. There are several megafossils that merit investigation as possible relatives of *Ceratophyllum*. Dilcher and Wang (2009) explicitly related fruits (*Donlesia*) and associated leafy stems from the latest Albian of Kansas to *Ceratophyllum*. As in *Ceratophyllum*, the fruits are one-seeded achenes with

prominent spines, and the leaves are whorled and dichotomous, but the fruits differ in having a long “peduncle” and possibly basal (vs. apical) seed attachment and the leaves lack marginal denticles. *Montsechia*, from Barremian lake beds in Spain, has been interpreted as an aquatic with whorled undivided leaves (Martín-Closas 2003; Gomez et al. 2006). Krasilov (2011) reinterpreted it as a xerophytic marsh plant with long and short shoots and dimorphic opposite leaves, but because whorled phyllotaxis and opposite phyllotaxis are closely related, this would not contradict a relationship to *Ceratophyllum*. He interpreted *Montsechia* as a “proangiosperm” with “cupules” of scale leaves containing one ovule, based on the presence of pollen in the nucellar area. However, the pollen is poorly characterized, and the ovule appears to be orthotropous, with the chalaza displaced to one side, as in the *Pennipollis* plant and Chloranthaceae. Most intriguing is *Pseudoasterophyllites*, from the late Albian of France and the middle Cenomanian of Bohemia, which has pseudowhorls of opposite, linear, and apparently succulent leaves and was interpreted by Kvaček et al. (2012) as a halophyte. Kvaček et al. (2012) associated stamens containing *Tucanopollis*-like pollen with the plant, based on close co-occurrence and similar stomata. If *Pseudoasterophyllites* is related to *Appomattoxia*, it could support a relationship between *Appomattoxia* and *Ceratophyllum*. An aquatic habit for the *Pennipollis* plant would be consistent with the abundance of *Pennipollis* pollen

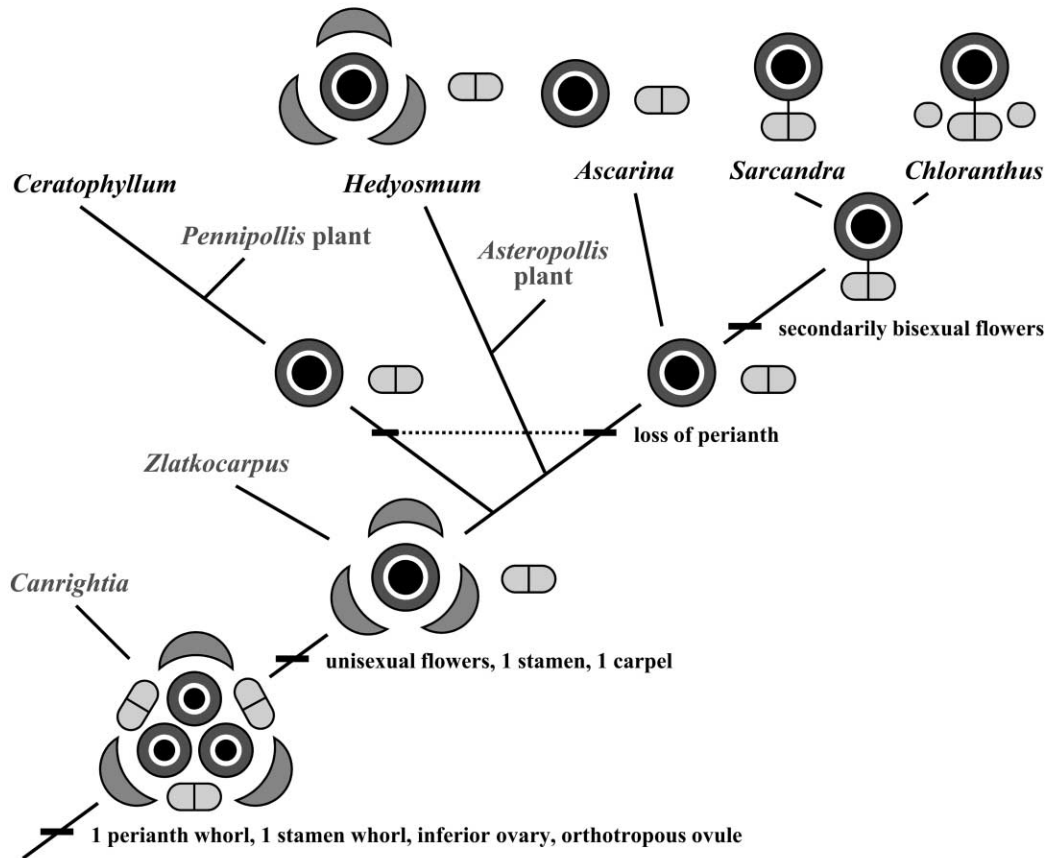


Fig. 15 Scenario for floral evolution in the chloranthoid line based on one of two trees (fig. 6B) found after addition of *Canrightia*, *Zlatkocarpus*, the *Pennipollis* plant, and the *Asteropollis* plant to the D&E backbone tree, with reconstructed floral diagrams placed at key nodes.

in the organic-rich Arundel Clay (e.g., at United Clay Mine; Brenner 1963; Doyle et al. 1975), a classic swamp deposit.

If *Tucanopollis* pollen came from relatives of *Ceratophyllum*, it would have little bearing on the original ecology of angiosperms, and its abundance in Northern Gondwana might reflect the presence of its parent plants in local wet and/or saline habitats. It is also possible that pollen referred to *Tucanopollis* is heterogeneous and was not all produced by plants related to *Appomattoxia*. However, the exine structure of *Tucanopollis* from the Barremian of Gabon (Doyle and Hotton 1991) is very similar to that of *Appomattoxia* pollen (Friis et al. 1995), even at the TEM level.

Appomattoxia again illustrates how having only isolated reproductive organs allows a wide range of hypotheses and how association with vegetative parts or evidence on floral organization could decide among them. For example, an ANITA-grade plant near *Amborella* would be expected to have a gynoeceium of several carpels and a perianth of several whorls or series of tepals (Endress and Doyle 2009), whereas a plant near *Ceratophyllum* and/or Chloranthaceae should have one carpel and either no perianth or one whorl of tepals.

Summary of evolution in the chloranthaceous line. In a synthesis based on a morphological cladistic analysis of living

Chloranthaceae, which included the ANITA lines and three magnoliid taxa as outgroups but not *Ceratophyllum*, Doyle et al. (2003) were unable to decide between two equally parsimonious scenarios for floral evolution in the family. In one scenario, flowers were still bisexual in the most recent common ancestor of Chloranthaceae and became unisexual independently in *Hedyosmum* and *Ascarina*. In the other, flowers were already unisexual in the most recent common ancestor and reversed to secondarily bisexual in the clade consisting of *Sarcandra* and *Chloranthus*. In both scenarios, a perianth was still present at the crown group node, as in female flowers of *Hedyosmum*, but was lost on the line to the *Ascarina-Sarcandra-Chloranthus* (ASC) clade.

Addition of fossils provides a better-resolved picture. Our discussion centers on a scenario (fig. 15) based on one of the two most parsimonious trees found when the four most securely associated fossils were added to the D&E backbone (fig. 6B), with remarks on variations seen in other trees. Because floral organization of *Appomattoxia* is largely unknown, its addition would have little impact on this scenario.

Since outgroup relationships imply that Chloranthaceae (and *Ceratophyllum*) were derived from ancestors that had bisexual flowers with many parts, the first inferred changes,

seen in *Canrightia*, were reduction of the pedicel, resulting in sessile flowers; reduction of the perianth to one whorl of tepals; adnation of the tepals and stamens to a gynoeceum of several carpels, resulting in an inferior ovary; and a shift from anatropous to orthotropous ovules. The carpels had a single pendent ovule, which is inferred to be a feature inherited from the first angiosperms (Endress and Doyle 2009). The androecium was also reduced to one whorl of stamens, but in the D&E tree, because of the arrangement of outgroups and treatment of this character as unordered, it is equally parsimonious to assume that this reduction occurred earlier, in the common ancestor of mesangiosperms.

The next major events were a shift to unisexual flowers and reduction to one carpel, still surrounded by adnate tepals, as in *Zlatkocarpus*, the *Asteropollis* plant, and *Hedyosmum*. This holds whether *Zlatkocarpus* is sister to the crown clade (figs. 6A–6E, 6J, 6K, 15) or nested within it (fig. 6F–6I), except that the number of shifts to unisexuality is ambiguous if *Zlatkocarpus* is attached to the *Hedyosmum* line (fig. 6F, 6H). Reduction to one stamen may have occurred on the same branch, but this is ambiguous if *Zlatkocarpus* is basal (as in fig. 15), since its male structures are unknown. In any case, stamen number had been reduced to one at the crown node.

These changes were followed by loss of the perianth, as in *Ascarina*, *Sarcandra*, *Chloranthus*, and *Ceratophyllum*. With the J/M backbone (fig. 6C–6K), this loss occurred once, on line to the ASC clade. However, with the D&E backbone (fig. 15), where *Ceratophyllum* is related to Chloranthaceae, it is equally parsimonious to assume that the perianth was lost twice, on the lines to *Ceratophyllum* and the ASC clade, or lost once in the common ancestor of *Ceratophyllum* and Chloranthaceae and regained in the *Asteropollis-Hedyosmum* line (perhaps a less plausible scenario). Information on whether female flowers of the *Pennipollis* plant had a perianth could affect these inferences (it clearly did not have an adnate perianth of the type seen in *Canrightia*, *Zlatkocarpus*, and *Hedyosmum*).

In most trees including the four fossils, it is most parsimonious to assume that the bisexual flowers of *Sarcandra* and *Chloranthus* were secondarily derived from unisexual; exceptions are two trees with *Zlatkocarpus* on the line to *Hedyosmum* (fig. 6F, 6H), where the course of evolution is ambiguous. This hypothesis might be consistent with the bizarre morphology of these flowers—a single carpel with one stamen attached to the back in *Sarcandra*, one carpel with a dorsal three-lobed androecium in *Chloranthus* and related Late Cretaceous fossils (Herendeen et al. 1993; Eklund et al. 1997), which is variously interpreted as three fused stamens or one subdivided stamen (Swamy 1953; Endress 1987; Eklund et al. 1997; Kong et al. 2002; Doyle et al. 2003). This raises the possibility that these bisexual structures are actually pseudanthia composed of extremely reduced unisexual flowers. Other reversals were increases in stamen number in male flowers of some *Ascarina* species (to 2–5; Jérémie 1980) and in *Chloranthus*, if it has three fused stamens.

Another reduction occurred in inflorescence morphology, namely, loss of the bracts subtending male flowers in *Hedyosmum*, the *Asteropollis* plant, the *Pennipollis* plant, and *Ceratophyllum*; the situation in *Zlatkocarpus* is unknown. In three trees found with the J/M backbone (fig. 6C, 6F, 6G), in which

the *Pennipollis* plant, the *Asteropollis* plant, and *Hedyosmum* form a clade, these bracts were lost once in Chloranthaceae. However, in trees with both backbones in which the *Pennipollis* plant is elsewhere, this character is homoplastic. In three trees (figs. 6B, 6D, 6E, 15), it is equivocal whether bracts were lost twice or lost once and regained in the ASC clade; the former scenario is favored in four trees (fig. 6H–6K), the latter in one (fig. 6A).

Pollen in the common ancestor of *Canrightia* and Chloranthaceae can be reconstructed as globose and monosulcate, with a reticulate-columellar exine, smooth muri (no suprategal spinules), and a sculptured sulcus membrane, as in *Canrightia* and *Zlatkocarpus*. All these features were inherited from lower in the tree. This was modified by origin of spinules on the muri, as in *Pennipollis*, *Asteropollis*, *Hedyosmum*, and *Ascarina* (as well as *Appomattoxia-Tucanopollis* and *Clavatipollenites*). In both trees with the D&E backbone (fig. 6A, 6B) and three with the J/M backbone (fig. 6D, 6E, 6K), spinules arose once in the common ancestor of the *Pennipollis* plant and living groups and were later lost in the *Sarcandra-Chloranthus* clade. However, in other J/M trees, spinules either originated twice (in the *Hedyosmum* and *Ascarina* lines, with the *Pennipollis* plant linked with one or the other; fig. 6F–6I) or the course of their evolution is ambiguous (fig. 6C, 6J). The distinctive thick nexine of *Pennipollis* and living Chloranthaceae (except some *Chloranthus* species), also seen in *Tucanopollis* and *Clavatipollenites*, originated after the divergence of *Canrightia*, but where exactly is uncertain if *Zlatkocarpus* is basal (fig. 6A–6E, 6J, 6K), since its nexine thickness is unknown. The ancestral sulcus was modified to a several-armed furrow in the *Asteropollis-Hedyosmum* clade and to scattered pores and several colpoid areas in *Sarcandra* and *Chloranthus*, respectively.

If both the *Pennipollis* plant and *Appomattoxia* are related to *Ceratophyllum* (fig. 8B), they show a picture of both progressive and markedly divergent pollen trends. From an ancestor with a reticulate-columellar exine and suprategal spinules, infrategal structure was modified to intermediate (as in *Appomattoxia*) and then granular (in *Pennipollis*). The original finely reticulate tectum was modified in opposite ways, becoming extremely coarse in *Pennipollis* but closed in *Appomattoxia-Tucanopollis*; however, both retained spinules and a thick nexine. *Ceratophyllum* neither supports nor contradicts this scenario; any signs of its earlier history were erased during reduction of its entire exine to a thin structureless layer (Takahashi 1995).

Implications for Pre-Cretaceous History of the Angiosperm Line

Our results relate indirectly to the age of the angiosperms, which has become a topic of renewed discussion as a result of molecular dating studies (see Doyle 2012). Such analyses address the age of the angiosperm crown group, i.e., the most recent common ancestor of all living angiosperms; diagnostic synapomorphies such as the flower, the carpel, or columellar exine structure could be significantly older. Most molecular dating analyses indicate that the angiosperm crown group extended back into the Jurassic (Sanderson and Doyle 2001; Bell et al. 2010) or even the Triassic or Permian (Magallón 2010;

Smith et al. 2010; Clarke et al. 2011). If these results are accepted, they raise the question of why convincing angiosperms have not been recognized in the fossil record until the Cretaceous. Rare reticulate-columellar monosulcate pollen has been reported from the Triassic of several areas (Cornet 1989; Doyle and Hotton 1991; Hochuli and Feist-Burkhardt 2004, 2013) and interpreted as angiospermous by some workers (Cornet 1989; Zavada 2007). However, TEM studies have shown that some of these fossils had a uniformly thick endexine, sometimes visibly laminated, as in gymnospermous seed plants, suggesting that they may represent either angiosperm stem relatives or some unrelated group (Doyle and Hotton 1991; Doyle 2005).

One hypothesis for resolving the conflict between molecular and fossil data (Feild et al. 2004, 2009), mentioned in connection with *Appomattoxia*, is based on the fact that the terrestrial ANITA lines are “xerophobic” plants restricted to wet, shaded forest understory habitats and evidence that such habitats were rare in the Triassic and Jurassic, when climates were relatively arid across the tropics (Ziegler et al. 2003). The ANITA lines make up less than 0.1% of living angiosperm species, and analyses by Magallón and Sanderson (2001) indicated that rates of angiosperm diversification were initially low and did not speed up until origin of the mesangiosperm clade. If angiosperms existed before the Cretaceous but were all in the ANITA grade, they might have escaped detection because they were geographically restricted and low in diversity, and their rapid rise in the Cretaceous could reflect the origin and radiation of mesangiosperms.

Our results challenge this scenario by showing that the ANITA lines were radiating in the Aptian-Albian, alongside Chloranthaceae and extinct relatives, magnoliids, monocots, and primitive eudicots. This argument could be questioned on the grounds that most of the definite ANITA-grade fossils recognized so far are Nymphaeales, which are a special case in being aquatic. However, the Aptian-Albian diversity of Nymphaeales would be consistent with low pre-Cretaceous diversity if the nymphaealean line was terrestrial in the Jurassic and diversified significantly only after it invaded aquatic habitats, which could have occurred in the Early Cretaceous. Furthermore, other ANITA lines are clearly represented by *Anacostia*, probably by leaves compared with Austrobaileyales (Upchurch 1984; Upchurch and Dilcher 1990), and possibly by *Appomattoxia*. This could mean that angiosperm diversification in general was being inhibited by external environmental factors before the Cretaceous, rather than by ecophysiological limitations of the first angiosperms, or that angiosperms are not as old as molecular dating implies.

Acknowledgments

We thank V. Barreda, E. M. Friis, U. Heimhofer, J. Kvaček, M. Llorens, M. Prámparo, and D. W. Taylor for discussion and unpublished information and two anonymous reviewers for valuable suggestions on organization and recognition of a problem in character scoring.

Appendix

Recent Taxa

1. *Amborella* (=Amborellaceae). 2. *Cabomba* (Cabombaceae). 3. *Brasenia* (Cabombaceae). 4. *Nuphar* (Nymphaeaceae). 5. *Barclaya* (Nymphaeaceae). 6. Nymphaeoidae (Nymphaeaceae; =*Nymphaea*, *Victoria*, *Euryale*). 7. *Trithuria* (including *Hydatella*; =Hydatellaceae). 8. *Austrobaileya* (=Austrobaileyaceae). 9. *Trimeria* (including *Piptocalyx*; =Trimeriaceae). 10. *Illium* (=Illiciaceae). 11. *Schisandra* (including *Kadsura*; =Schisandraceae). 12. *Hedyosmum* (Chloranthaceae). 13. *Ascarina* (Chloranthaceae). 14. *Sarcandra* (Chloranthaceae). 15. *Chloranthus* (Chloranthaceae). 16. *Liriodendron* (Magnoliaceae). 17. Magnolioideae (Magnoliaceae). 18. *Degeneria* (=Degeneriaceae). 19. *Galbulimima* (=Himantandraceae). 20. *Eupomatia* (=Eupomatiaceae). 21. Annonaceae. 22. Myristicaceae. 23. Calycanthoideae (Calycanthaceae). 24. *Idiospermum* (Calycanthaceae). 25. Atherospermataceae. 26. Siparunaceae. 27. *Hortonia* (Monimiaceae). 28. Monimioideae (Monimiaceae). 29. Mollinedioideae (Monimiaceae). 30. *Gomortega* (=Gomortegaceae). 31. Lauraceae. 32. Hernandioideae (Hernandiaceae). 33. Gyrocarpoideae (Hernandiaceae). 34. Winteraceae. 35. Canellaceae. 36. Saururaceae. 37. Piperaceae. 38. *Lactoris* (=Lactoridaceae). 39. Asaroideae (Aristolochiaceae). 40. Aristolochioideae (Aristolochiaceae). 41. *Euptelea* (=Eupteleaceae). 42. Papaveraceae. 43. Lardizabalaceae. 44. *Circaeaster* (Circaeasteraceae). 45. Menispermaceae. 46. Berberidaceae. 47. *Glaucidium* (Ranunculaceae). 48. *Hydrastis* (Ranunculaceae). 49. Core Ranunculaceae. 50. *Nelumbo* (=Nelumbonaceae). 51. *Platanus* (=Platanaceae). 52. Proteaceae. 53. *Tetracentron* (Trochodendraceae). 54. *Trochodendron* (Trochodendraceae). 55. Buxaceae. 56. *Acorus* (=Acoraceae). 57. Tofieldiaceae. 58. *Butomus* (=Butomaceae). 59. *Aponogeton* (=Aponogetonaceae). 60. *Scheuchzeria* (=Scheuchzeriaceae). 61. Araceae. 62. Nartheciaceae. 63. Dioscoreaceae. 64. Melanthiaceae. 65. *Ceratophyllum* (=Ceratophyllaceae).

Characters

See Doyle and Endress (2010) for synonymy with previous characters, sources of data, and discussion of decisions on scoring of all characters (except 134, operculum).

- Habit (0) tree or shrub, (1) rhizomatous, scandent, or acaulescent.
- Stele (0) eustele, (1) (pseudo)siphonostele, (2) monocot-type (atactostele).
- Inverted cortical bundles (0) absent, (1) present.
- Protoxylem lacunae (0) absent, (1) present.
- Pith (0) uniform, (1) septate (plates of sclerenchyma).

6. Cambium (0) present, (1) absent.
7. Storied structure (in tracheids and axial parenchyma, phloem) (0) absent, (1) present. Scored as unknown (?) when secondary growth is nearly or entirely lacking.
8. Tracheary elements (0) tracheids and elements with porose pit membranes, (1) vessel members with typical perforations.
9. Vessel perforations (end-wall pits in vesselless taxa) (0) scalariform, (1) scalariform and simple in the same wood, (2) simple.
10. Fiber pitting (lateral pitting of tracheids in vesselless taxa) (0) distinctly bordered, (1) minutely bordered or simple. Scored as unknown when secondary xylem is absent or fibers are replaced by pervasive parenchyma.
11. Vessel grouping (0) predominantly solitary, (1) mostly pairs or multiples.
12. Rays (0) narrow (generally not more than four cells wide), (1) wide.
13. Paratracheal parenchyma (0) absent or scanty, (1) well developed. Taxa with pervasive parenchyma scored as unknown.
14. Tangential apotracheal parenchyma bands (0) absent, (1) present. Taxa with pervasive parenchyma scored as unknown.
15. Secondary phloem (0) simple, (1) stratified (fibers in small tangential rows or bands several cells thick).
16. Sieve element plastids (0) S-type (starch), (1) PI-type, (2) PII-type.
17. Fibers or sclerenchyma in pericyclic area (including modified protophloem) of vascular bundles (0) present, (1) absent.
18. Pericyclic ring (0) separate fiber bundles with no intervening fibers or sclerenchyma, (1) more or less continuous ring of fibers and non-U-shaped sclereids, (2) ring of fibers alternating with U-shaped (hippocrepiform) sclereids, (3) continuous homogeneous ring of fibers. Taxa with no fibers or sclerenchyma scored as unknown.
19. Laticifers in stem (0) absent, (1) present.
20. Raphide idioblasts (0) absent, (1) present.
21. Phyllotaxis (0) alternate (spiral or distichous), (1) opposite or whorled.
22. Distichous phyllotaxis (0) absent, (1) on some or all branches.
23. Nodal anatomy (0) multilacunar, (1) unilacunar one-trace, (2) unilacunar two-trace, (3) trilacunar.
24. First appendage(s) on vegetative branch (0) paired lateral prophylls, (1) single distinct prophyll (adaxial, oblique, or lateral).
25. Leaf base (0) nonsheathing, (1) sheathing (half or more of stem circumference).
26. Stipules (0) absent, (1) adaxial/axillary, (2) interpetiolar, (3) paired cap.
27. Axillary squamules (0) absent, (1) present.
28. Leaf blade (0) bifacial, (1) unifacial.
29. Leaf shape (0) obovate to elliptical to oblong, (1) ovate, (2) linear.
30. Major venation (0) pinnate with secondaries at more or less constant angle, (1) palmate (actinodromous or acrodromous) or crowded (pinnate with crowded basal secondaries, upward decreasing angle), (2) parallel (lateral veins departing at low angles from the midrib and converging and fusing apically).
31. Fine venation (0) reticulate, (1) open dichotomous in some or all leaves.
32. Base of blade (0) not peltate, (1) peltate in some or all leaves.
33. Apex of blade (0) simple, (1) bilobed.
34. Leaf dissection (0) simple, (1) some or all leaves lobed or compound.
35. Marginal teeth (0) absent, (1) chloranthoid, (2) monimiod, (3) platanoid.
36. Stomata (predominant type on leaf) (0) paracytic, (1) laterocytic, (2) anomocytic, (3) stephanocytic (including tetracytic).
37. Midrib vasculature (0) simple arc, (1) arc with adaxial plate, (2) ring.
38. Palisade parenchyma (0) absent (mesophyll homogeneous), (1) present (mesophyll dorsiventral).
39. Asterosclerids in mesophyll (0) absent, (1) present.
40. Oil cells in mesophyll (0) absent, (1) present. *Trithuria* and *Ceratophyllum* scored as unknown because of the possibility that oil cells would be lost for functional reasons in submerged aquatics and the presence of tanniferous cells in *Ceratophyllum* (Metcalfe and Chalk 1950) that might be modified oil cells.
41. Mucilage cells in mesophyll (0) absent, (1) present. *Trithuria* and *Ceratophyllum* scored as unknown for reasons given for character 40.
42. Inflorescence (0) solitary flower (or occasionally with 1–2 lateral flowers), (1) botryoid, panicle, or thyrsoid (monotelic), (2) raceme, spike, or thyrse (polytelic).
43. Inflorescence partial units (0) single flowers, (1) cymes.
44. Inflorescence (or partial inflorescence) (0) not modified, (1) modified into globular head.
45. Pedicel (0) present in some or all flowers, (1) absent or highly reduced (flower sessile or subsessile).
46. Floral subtending bracts (0) present, (1) present in female, absent in male flowers, (2) absent in all flowers.
47. Sex of flowers (0) bisexual, (1) unisexual. Taxa with both bisexual and unisexual flowers scored as (0/1).
48. Floral base (0) hypanthium absent, superior ovary, (1) hypanthium present, superior ovary, (2) partially or completely inferior ovary.
49. Floral receptacle (female portion) (0) short, (1) elongate.
50. Pits in receptacle bearing individual carpels (0) absent, (1) present.
51. Cortical vascular system (0) absent or supplying perianth only, (1) supplying androecium, (2) supplying androecium plus gynoecium.
52. Floral apex (0) used up after production of carpels, (1) protruding in mature flower. Unicarpellate taxa scored as unknown.
53. Perianth (0) present, (1) absent.

54. Perianth phyllotaxis (0) spiral, (1) whorled.
55. Perianth merism (0) trimerous, (1) dimerous, (2) polymerous. Spiral taxa scored as unknown.
56. Perianth whorls (series when phyllotaxis is spiral) (0) one, (1) two, (2) more than two. Includes petals (character 58); taxa with no perianth scored as unknown.
57. Tepal differentiation (0) all more or less sepaloid; (1) outer sepaloid, inner distinctly petaloid; (2) all distinctly petaloid. Does not include petals (58).
58. Petals (0) absent, (1) present. Taxa with no perianth or only one whorl or series scored as unknown.
59. Nectaries on inner perianth parts (0) absent, (1) present.
60. Outermost perianth parts (0) free, (1) at least basally fused.
61. Calyptra derived from last one or two bracteate organs below the flower (0) absent, (1) present.
62. Stamen number (0) more than one, (1) one.
63. Androecium phyllotaxis (0) spiral, (1) whorled.
64. Androecium merism (0) trimerous, (1) dimerous, (2) polymerous. Spiral taxa scored as unknown.
65. Number of stamen whorls (series when phyllotaxis is spiral; includes inner staminodes) (0) one, (1) two, (2) more than two. Single stamens scored as unknown.
66. Stamen positions (0) single, (1) double (at least in outer whorl). Taxa with no perianth and/or single stamens scored as unknown.
67. Stamen fusion (0) free, (1) connate. Taxa with one stamen scored as unknown.
68. Inner staminodes (0) absent, (1) present. Taxa with one stamen or one whorl of stamens scored as unknown.
69. Glandular food bodies on stamens or staminodes (0) absent, (1) present.
70. Stamen base (0) short ($\frac{2}{3}$ or less the length of anther), (1) long ($>\frac{2}{3}$ length of anther) and wide ($>\frac{1}{2}$ width of anther), (2) long ($\frac{2}{3}$ or more length of anther) and narrow ($<\frac{1}{2}$ width of anther) (typical filament).
71. Paired basal stamen glands (0) absent, (1) present.
72. Connective apex (0) extended, (1) truncated or smoothly rounded, (2) peltate.
73. Pollen sacs (0) protruding, (1) embedded.
74. Microsporangia (0) four, (1) two.
75. Orientation of dehiscence (0) distinctly introrse, (1) latrorse to slightly introrse, (2) extrorse.
76. Mode of dehiscence (0) longitudinal slit, (1) H-valvate, (2) valvate with upward-opening flaps.
77. Connective hypodermis (0) unspecialized, (1) endothelial or sclerenchymatous.
78. Tapetum (0) secretory, (1) amoeboid.
79. Microsporogenesis (0) simultaneous, (1) successive.
80. Pollen nuclei (0) binucleate, (1) trinucleate.
81. Pollen unit (0) monads, (1) tetrads.
82. Pollen size (average) (0) large ($>50 \mu\text{m}$), (1) medium ($20\text{--}50 \mu\text{m}$), (2) small ($<20 \mu\text{m}$); ordered.
83. Pollen shape (0) boat-shaped, (1) globose, (2) triangular, angulaperturate (Proteaceae).
84. Aperture type (0) polar (including sulcate, ulcerate, and disulcate), (1) inaperturate, (2) sulcate, (3) (syn)tricolpate with colpi arranged according to Garside's law, with or without alternating colpi, (4) tricolpate.
85. Distal aperture shape (0) elongate, (1) round.
86. Distal aperture branching (0) unbranched, (1) with several branches.
87. Infratectum (0) granular (including "atectate"), (1) intermediate, (2) columellar; ordered.
88. Tectum (0) continuous or microperforate, (1) perforate (foveolate) to semitectate (reticulate), (2) reduced (not distinguishable from underlying granules).
89. Grading of reticulum (0) uniform, (1) finer at ends of sulcus (liliaceous), (2) finer at poles (rouseoid). Scored only in taxa with state (1) in character 88.
90. Striate muri (0) absent, (1) present.
91. Supratectal spinules (smaller than the width of tectal muri in foveolate-reticulate taxa) (0) absent, (1) present.
92. Prominent spines (larger than spinules, easily visible with light microscopy) (0) absent, (1) present.
93. Aperture membrane (0) smooth, (1) sculptured.
94. Extra-apertural nexine stratification (0) foot layer, not consistently foliated, no distinctly staining endexine or only problematic traces, (1) foot layer and distinctly staining endexine, or endexine only, (2) all or in part foliated, not distinctly staining.
95. Nexine thickness (0) absent or discontinuous traces, (1) thin but continuous, (2) thick ($\frac{1}{3}$ or more of exine); ordered.
96. Carpel number (0) one, (1) 2–5 in one whorl (series when phyllotaxis is spiral), (2) more than 5 in one whorl or series ("star-shaped" arrangement of von Balthazar et al. 2008), (3) more than one whorl or series.
97. Carpel form (0) ascidiate up to stigma, (1) intermediate (both plicate and ascidiate zones present below the stigma) with ovule(s) in the ascidiate zone, (2) completely plicate, or intermediate with some or all ovule(s) in the plicate zone.
98. Postgenital sealing of carpel (0) none, (1) partial, (2) complete.
99. Secretion in area of carpel sealing (0) present, (1) absent.
100. Pollen tube transmitting tissue (0) not prominently differentiated, (1) one cell layer prominently differentiated, (2) more than one cell layer prominently differentiated.
101. Style (0) absent (stigma sessile or capitate), (1) present (elongated, distinctly constricted apical portion of carpel).

102. Stigma (0) extended (half or more of the style-stigma zone), (1) restricted (above slit or around its upper part).
103. Multicellular stigmatic protuberances or undulations (0) absent, (1) present.
104. Stigmatic papillae (most elaborate type) (0) absent, (1) unicellular or with a single emergent cell and one or more small basal cells, (2) uniseriate pluricellular with emergent portion consisting of two or more cells.
105. Extragynoecial compitum (0) absent, (1) present.
106. Carpel fusion (0) apocarpous, (1) parasyncarpous, (2) eusyncarpous (at least basally). Taxa with one carpel scored as unknown.
107. Oil cells in carpels (0) absent or internal, (1) intrusive. Taxa with no oil cells in any tissue of the plant scored as unknown.
108. Long unicellular hairs on and/or between carpels (0) absent, (1) present.
109. Short curved appressed unligified hairs with up to two short basal cells and one long apical cell on carpels (0) absent, (1) present.
110. Nectary on dorsal or lateral sides of carpel or pistillode (0) absent, (1) present.
111. Septal nectaries or potentially homologous basal intercarpellary nectaries (0) absent, (1) present.
112. Number of ovules per carpel (0) one, (1) two or varying between one and two, (2) more than two.
113. Placentation (0) ventral, (1) laminar-diffuse or “dorsal.”
114. Ovule direction (0) pendent, (1) horizontal, (2) ascendent.
115. Ovule curvature (0) anatropous (or nearly so), (1) orthotropous (including hemitropous).
116. Integuments (0) two, (1) one.
117. Outer integument shape (0) semiannular, (1) annular. Orthotropous taxa scored as unknown.
118. Outer integument lobation (0) unlobed, (1) lobed.
119. Outer integument thickness (at middle of integument length) (0) two cells, (1) two and three to four, (2) four and five, or more; ordered.
120. Inner integument thickness (0) two cells, (1) two and three, or three, (2) three and more; ordered.
121. Chalaza (0) unextended, (1) pachychalazal, (2) perichalazal. Orthotropous taxa scored as unknown.
122. Nucellus (0) crassinucellar (including weakly so), (1) tenuinucellar or pseudocrassinucellar.
123. Fruit wall (0) wholly or partly fleshy, (1) dry.
124. Lignified endocarp (0) absent, (1) present. Taxa with dry fruit wall scored as unknown.
125. Fruit dehiscence (0) indehiscent or dehiscing irregularly, dorsally only, or laterally, (1) dehiscent ventrally or both ventrally and dorsally, (2) horizontally dehiscent with vertical extensions.
126. Hooked hairs on fruit (0) absent, (1) present.
127. Testa (0) slightly or nonmultiplicative, (1) multiplicative.
128. Exotesta (0) unspecialized, (1) palisade or shorter sclerotic cells, (2) tabular, (3) longitudinally elongated, more or less lignified cells.
129. Mesotesta lignification (0) unligified, (1) with sclerotic layer, (2) with fibrous layer. Yamada et al. (2003) described the middle layer of the outer integument in *Illicium* as parenchymatous, but Oh et al. (2003) reported one or two layers of sclerotic cells in all *Illicium* species that they studied. Yamada et al. (2003) argued that the supposed sclerotic mesotesta of *Trimenia* is part of a multilayered exotesta, but in the figure in Takhtajan (1988, p. 57) these cells do not line up with those of the outermost layer, which suggests that they have a subepidermal origin.
130. Mesotesta fleshiness (0) not juicy, (1) wholly or partly modified into a juicy sarcotesta.
131. Endotesta (0) unspecialized, (1) single layer of thin-walled cells with fibrous endoreticulum, (2) multiple layer of thin-walled cells with fibrous endoreticulum, (3) tracheidal, (4) palisade of thick-walled cells.
132. Tegmen (0) unspecialized, (1) both ecto- and endotegmen thick-walled, (2) exotegmen fibrous to sclerotic.
133. Ruminations (0) absent, (1) testal, (2) tegminal and/or chalazal.
134. Operculum (0) absent, (1) present. We previously scored operculum, i.e., a lid- or plug-like structure involving differentiation of the inner integument at the micropyle, as present only in Nymphaeales, but Yamada et al. (2003, 2008) reported a previously unrecognized “operculum” in the seeds of *Trimenia* as well but not in *Amborella*, *Austrobaileya*, *Illicium*, and *Schisandra* (Yamada et al. 2003). They also reported a “circular cap,” i.e., a differentiation of the outer integument, in the seeds of *Trimenia*, *Illicium*, *Schisandra*, Nymphaeaceae, and Cabombaceae but not in *Amborella* and *Austrobaileya* (Yamada et al. 2003). Because the putative operculum of *Trimenia* is smaller than that of Nymphaeales and apparently less visible in surface view, consistent with the fact it was not reported by earlier authors (Takhtajan 1988), which suggests that it might not be recognized in the fossil state, we have rescored this character in *Trimenia* as unknown rather than absent (0).
135. Aril (0) absent, (1) present.
136. Female gametophyte (0) four-nucleate, (1) eight- or nine-nucleate. Tetrasporic types in Piperaceae scored as unknown.
137. Endosperm development (0) cellular, (1) nuclear, (2) helobial.
138. Endosperm in mature seed (0) present, (1) absent.
139. Perisperm (0) absent, (1) from nucellar ground tissue, (2) from nucellar epidermis.
140. Embryo (0) minute (less than 1/2 length of seed interior), (1) large.
141. Cotyledons (0) two, (1) one.
142. Germination (0) epigeal, (1) hypogeal.

Literature Cited

- Allen P, WA Wimbledon 1991 Correlation of NW European Purbeck–Wealden (non-marine Lower Cretaceous) as seen from the English type-areas. *Cretac Res* 12:511–526.
- Antonelli A, I Sanmartín 2011 Mass extinction, gradual cooling, or rapid radiation? reconstructing the spatiotemporal evolution of the ancient angiosperm genus *Hedyosmum* (Chloranthaceae) using empirical and simulated approaches. *Syst Biol* 60:596–615.
- Antonov AS, AV Troitsky, TK Samigullin, VK Bobrova, KM Valiejo-Roman, W Martin 2000 Early events in the evolution of angiosperms deduced from cp rDNA ITS 2–4 sequence comparisons. Pages 210–214 in YH Liu, HM Fan, ZY Chen, QG Wu, QW Zeng, eds. Proceedings of the International Symposium on the Family Magnoliaceae. Science, Beijing.
- Archangelsky S, V Barreda, MG Passalia, M Gandolfo, M Prámparo, E Romero, R Cúneo, et al 2009 Early angiosperm diversification: evidence from southern South America. *Cretac Res* 30:1073–1082.
- Axelrod DI 1952 A theory of angiosperm evolution. *Evolution* 6:29–60.
- 1970 Mesozoic paleogeography and early angiosperm history. *Bot Rev* 36:277–319.
- Bell CD, DE Soltis, PS Soltis 2010 The age and diversification of the angiosperms re-visited. *Am J Bot* 97:1296–1303.
- Berry EW 1911 Systematic paleontology, Lower Cretaceous, Pteridophyta-Dicotyledonae. Pages 214–508 in WB Clark, ed. Lower Cretaceous. Maryland Geological Survey, Johns Hopkins Press, Baltimore.
- Borsch T, C Löhne, MS Mbaye, J Wiersema 2011 Towards a complete species tree of *Nymphaea*: shedding further light on subg. *Brachyceras* and its relationships to the Australian water-lilies. *Telopea* 13: 193–217.
- Brenner GJ 1963 The spores and pollen of the Potomac Group of Maryland. *Md Dep Geol Mines Water Res Bull* 27:1–215.
- 1976 Middle Cretaceous floral provinces and early migrations of angiosperms. Pages 23–47 in CB Beck, ed. Origin and early evolution of angiosperms. Columbia University Press, New York.
- 1996 Evidence for the earliest stage of angiosperm pollen evolution: a paleo-equatorial section from Israel. Pages 91–115 in DW Taylor, LJ Hickey, eds. Flowering plant origin, evolution and phylogeny. Chapman & Hall, New York.
- Burger WC 1977 The Piperales and the monocots: alternate hypotheses for the origin of monocotyledonous flowers. *Bot Rev* 43:345–393.
- Cabrera LI, GA Salazar, MW Chase, SJ Mayo, J Bogner, P Dávila 2008 Phylogenetic relationships of aroids and duckweeds (Araceae) inferred from coding and noncoding plastid DNA. *Am J Bot* 95:1153–1165.
- Cantino PD, JA Doyle, SW Graham, WS Judd, RG Olmstead, DE Soltis, PS Soltis, MJ Donoghue 2007 Towards a phylogenetic nomenclature of *Tracheophyta*. *Taxon* 56:822–846.
- Chassat JF 1962 Recherches sur la ramification chez les Nymphaeacées. *Mem Soc Bot Fr* 42:72–95.
- Chmura CA 1973 Upper Cretaceous (Campanian-Maastrichtian) angiosperm pollen from the western San Joaquin Valley, California, U.S.A. *Palaeontogr Abt B* 141:89–171.
- Clarke JT, RCM Warnock, PCJ Donoghue 2011 Establishing a time-scale for plant evolution. *New Phytol* 192:266–301.
- Conard HS 1905 The waterlilies: a monograph of the genus *Nymphaea*. *Publ Carnegie Inst* 4:1–292.
- Corner EJJH 1976 The seeds of the dicotyledons. Cambridge University Press, Cambridge.
- Cornet B 1989 Late Triassic angiosperm-like pollen from the Richmond rift basin of Virginia, U.S.A. *Palaeontogr Abt B* 213:37–87.
- Couper RA 1958 British Mesozoic microspores and pollen grains. *Palaeontogr Abt B* 103:75–179.
- Crane PR, EM Friis, KR Pedersen 1986 Lower Cretaceous angiosperm flowers: fossil evidence on early radiation of dicotyledons. *Science* 232:852–854.
- Crepet WL 2008 The fossil record of angiosperms: requiem or renaissance? *Ann Mo Bot Gard* 95:3–33.
- Crepet WL, KC Nixon, MA Gandolfo 2004 Fossil evidence and phylogeny: the age of major angiosperm clades based on mesofossil and macrofossil evidence from Cretaceous deposits. *Am J Bot* 91:1666–1682.
- Cusimano N, J Bogner, SJ Mayo, PC Boyce, SY Wong, M Hesse, WLA Hettterscheid, RC Keating, JC French 2011 Relationships within the Araceae: comparison of morphological patterns with molecular phylogenies. *Am J Bot* 98:654–668.
- Dahlgren R, K Bremer 1985 Major clades of angiosperms. *Cladistics* 1:349–368.
- D’Arcy WG, RL Liesner 1981 *Hedyosmum* (Chloranthaceae) in Panama. *Syst Bot* 6:74–86.
- Dilcher DL, H Wang 2009 An Early Cretaceous fruit with affinities to Ceratophyllaceae. *Am J Bot* 96:2256–2269.
- Dinis JL, J Rey, PP Cunha, P Callapez, R Pena dos Reis 2008 Stratigraphy and allogenic controls of the western Portugal Cretaceous: an updated synthesis. *Cretac Res* 29:772–780.
- Dinis JL, J Rey, PC de Graciansky 2002 Le Bassin lusitanien (Portugal) à l’Aptien supérieur–Albien: organisation séquentielle, proposition de corrélations, évolution. *C R Geosci* 334:757–764.
- 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.
- Doria MG, N Pabón-Mora, F González 2012 Reassessing inflorescence and floral morphology and development in *Hedyosmum* (Chloranthaceae). *Int J Plant Sci* 173:735–750.
- Doyle JA 1969 Cretaceous angiosperm pollen of the Atlantic Coastal Plain and its evolutionary significance. *J Arnold Arbor Harv Univ* 50:1–35.
- 1973 Fossil evidence on early evolution of the monocotyledons. *Q Rev Biol* 48:399–413.
- 1977 Spores and pollen: the Potomac Group (Cretaceous) angiosperm sequence. Pages 339–363 in EG Kauffman, JE Hazel, eds. Concepts and methods of biostratigraphy. Dowden, Hutchinson & Ross, Stroudsburg, PA.
- 1978 Origin of angiosperms. *Annu Rev Ecol Syst* 9:365–392.
- 1992 Revised palynological correlations of the lower Potomac Group (USA) and the Cocobeach sequence of Gabon (Barremian–Aptian). *Cretac Res* 13:337–349.
- 1999 The rise of angiosperms as seen in the African Cretaceous pollen record. *Palaeoecol Afr* 26:3–30.
- 2005 Early evolution of angiosperm pollen as inferred from molecular and morphological phylogenetic analyses. *Grana* 44:227–251.
- 2007 Systematic value and evolution of leaf architecture across the angiosperms in light of molecular phylogenetic analyses. *Cour Forschungsinst Senckenberg* 258:21–37.
- 2008 Integrating molecular phylogenetic and paleobotanical evidence on origin of the flower. *Int J Plant Sci* 169:816–843.
- 2009 Evolutionary significance of granular exine structure in the light of phylogenetic analyses. *Rev Palaeobot Palynol* 153:198–210.
- 2012 Molecular and fossil evidence on the origin of angiosperms. *Annu Rev Earth Planet Sci* 40:301–326.
- Doyle JA, P Biens, A Doerenkamp, S Jardiné 1977 Angiosperm pollen

- from the pre-Albian Cretaceous of Equatorial Africa. *Bull Cent Rech Explor-Prod Elf-Aquitaine* 1:451–473.
- Doyle JA, MJ Donoghue 1993 Phylogenies and angiosperm diversification. *Paleobiology* 19:141–167.
- Doyle JA, H Eklund, PS Herendeen 2003 Floral evolution in Chloranthaceae: implications of a morphological phylogenetic analysis. *Int J Plant Sci* 164(suppl):S365–S382.
- 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.
- 2010 Integrating Early Cretaceous fossils into the phylogeny of living angiosperms: Magnoliidae and eudicots. *J Syst Evol* 48:1–35.
- Doyle JA, PK Endress, GR Upchurch 2008 Early Cretaceous monocots: a phylogenetic evaluation. *Acta Mus Natl Pragae B* 64(2–4): 59–87.
- Doyle JA, LJ Hickey 1976 Pollen and leaves from the mid-Cretaceous Potomac Group and their bearing on early angiosperm evolution. Pages 139–206 in CB Beck, ed. *Origin and early evolution of angiosperms*. Columbia University Press, New York.
- Doyle JA, CL Hotton 1991 Diversification of early angiosperm pollen in a cladistic context. Pages 169–195 in S Blackmore, SH Barnes, eds. *Pollen and spores: patterns of diversification*. Clarendon, Oxford.
- Doyle JA, CL Hotton, JV Ward 1990 Early Cretaceous tetrads, zonosulculate pollen, and Winteraceae. II. Cladistic analysis and implications. *Am J Bot* 77:1558–1568.
- Doyle JA, S Jardiné, A Doerenkamp 1982 *Afropollis*, a new genus of early angiosperm pollen, with notes on the Cretaceous palynostratigraphy and paleoenvironments of Northern Gondwana. *Bull Cent Rech Explor-Prod Elf-Aquitaine* 6:39–117.
- Doyle JA, EI Robbins 1977 Angiosperm pollen zonation of the continental Cretaceous of the Atlantic Coastal Plain and its application to deep wells in the Salisbury Embayment. *Palynology* 1:43–78.
- Doyle JA, M Van Campo, B Lugardon 1975 Observations on exine structure of *Eucommiidites* and Lower Cretaceous angiosperm pollen. *Pollen Spores* 17:429–486.
- Dransfield J, NW Uhl, C Asmussen, WJ Baker, MM Harley, C Lewis 2008 *Genera Palmarum: the evolution and classification of palms*. Royal Botanic Gardens, Kew.
- Drinnan AN, PR Crane, SB Hoot 1994 Patterns of floral evolution in the early diversification of non-magnoliid dicotyledons (eudicots). *Plant Syst Evol Suppl* 8:93–122.
- Duvall MR, S Mathews, N Mohammad, T Russell 2006 Placing the monocots: conflicting signal from trigonometric analyses. *Aliso* 22:79–90.
- Duvall MR, JW Robinson, JG Mattson, A Moore 2008 Phylogenetic analyses of two mitochondrial metabolic genes sampled in parallel from angiosperms find fundamental interlocus incongruence. *Am J Bot* 95:871–884.
- Eklund H, JA Doyle, PS Herendeen 2004 Morphological phylogenetic analysis of living and fossil Chloranthaceae. *Int J Plant Sci* 165:107–151.
- Eklund H, EM Friis, KR Pedersen 1997 Chloranthaceous floral structures from the Late Cretaceous of Sweden. *Plant Syst Evol* 207:13–42.
- Endress PK 1971 Bau der weiblichen Blüten von *Hedyosmum mexicanum* Cordemoy (Chloranthaceae). *Bot Jahrb Syst* 91:39–60.
- 1987 The Chloranthaceae: reproductive structures and phylogenetic position. *Bot Jahrb Syst* 109:153–226.
- 2005 Carpels in *Brasenia* (Cabombaceae) are completely ascidiate despite a long stigmatic crest. *Ann Bot* 96:209–215.
- 2006 Angiosperm floral evolution: morphological developmental framework. *Adv Bot Res* 44:1–61.
- 2008 Perianth biology in the basal grade of extant angiosperms. *Int J Plant Sci* 169:844–862.
- 2010 Disentangling confusions in inflorescence morphology: patterns and diversity of reproductive shoot ramification in angiosperms. *J Syst Evol* 48:225–239.
- 2011 Angiosperm ovules: diversity, development, evolution. *Ann Bot* 107:1465–1489.
- Endress PK, JA Doyle 2007 Floral phyllotaxis in basal angiosperms: development and evolution. *Curr Opin Plant Biol* 10:52–57.
- 2009 Reconstructing the ancestral angiosperm flower and its initial specializations. *Am J Bot* 96:22–66.
- Endress PK, A Igersheim 1997 Gynoecium diversity and systematics of the Laurales. *Bot J Linn Soc* 125:93–168.
- 2000 The reproductive structures of the basal angiosperm *Amborella trichopoda* (Amborellaceae). *Int J Plant Sci* 161(suppl): S237–S248.
- Feild TS, NC Arens, JA Doyle, TE Dawson, MJ Donoghue 2004 Dark and disturbed: a new image of early angiosperm ecology. *Paleobiology* 30:82–107.
- Feild TS, DS Chatelet, TJ Brodribb 2009 Ancestral xerophobia: a hypothesis on the whole plant ecophysiology of early angiosperms. *Geobiology* 7:237–264.
- Friis EM 1983 Upper Cretaceous (Senonian) floral structures of juglandalean affinity containing Normapollis pollen. *Rev Palaeobot Palynol* 39:161–188.
- Friis EM, PR Crane, KR Pedersen 1986 Floral evidence for Cretaceous chloranthoid angiosperms. *Nature* 320:163–164.
- 1997a *Anacostia*, a new basal angiosperm from the Early Cretaceous of North America and Portugal with trichotomocolpate/monocolpate pollen. *Grana* 36:225–244.
- 1997b Fossil history of magnoliid angiosperms. Pages 121–156 in K Iwatsuki, PR Raven, eds. *Evolution and diversification of land plants*. Springer, Tokyo.
- 2011 Early flowers and angiosperm evolution. Cambridge University Press, Cambridge.
- Friis EM, JA Doyle, PK Endress, Q Leng 2003 *Archaeofructus*: angiosperm precursor or specialized early angiosperm? *Trends Plant Sci* 8:369–373.
- Friis EM, KR Pedersen 2011 *Canrightia resinifera* gen. et sp. nov., a new extinct angiosperm with *Retimonocolpites*-type pollen from the Early Cretaceous of Portugal: missing link in the eumagnoliid tree? *Grana* 50:3–29.
- Friis EM, KR Pedersen, PR Crane 1994 Angiosperm floral structures from the Early Cretaceous of Portugal. *Plant Syst Evol Suppl* 8:31–49.
- 1995 *Appomattoxia ancistrophora* gen. et sp. nov., a new Early Cretaceous plant with similarities to *Circaeaster* and extant Magnoliidae. *Am J Bot* 82:933–943.
- 1999 Early angiosperm diversification: the diversity of pollen associated with angiosperm reproductive structures in Early Cretaceous floras from Portugal. *Ann Mo Bot Gard* 86:259–296.
- 2000a Fossil floral structures of a basal angiosperm with monocolpate, reticulate-acolumellate pollen from the Early Cretaceous of Portugal. *Grana* 39:226–239.
- 2000b Reproductive structure and organization of basal angiosperms from the Early Cretaceous (Barremian or Aptian) of western Portugal. *Int J Plant Sci* 161(suppl):S169–S182.
- 2001 Fossil evidence of water lilies (Nymphaeales) in the Early Cretaceous. *Nature* 410:357–360.
- 2004 Araceae from the Early Cretaceous of Portugal: evidence on the emergence of monocotyledons. *Proc Natl Acad Sci USA* 101: 16565–16570.
- 2005 When Earth started blooming: insights from the fossil record. *Curr Opin Plant Biol* 8:5–12.
- 2006 Cretaceous angiosperm flowers: innovation and evolution in plant reproduction. *Palaeogeogr Palaeoclimatol Palaeoecol* 232:251–293.
- 2010a Cretaceous diversification of angiosperms in the west-

- ern part of the Iberian Peninsula. *Rev Palaeobot Palynol* 162:341–361.
- 2010*b* Diversity in obscurity: fossil flowers and the early history of angiosperms. *Philos Trans R Soc B* 365:369–382.
- Friis EM, KR Pedersen, M von Balthazar, GW Grimm, PR Crane 2009 *Monetianthus mirus* gen. et sp. nov., a nymphaealean flower from the Early Cretaceous of Portugal. *Int J Plant Sci* 170: 1086–1101.
- Frumin S, EM Friis 1999 Magnoliid reproductive organs from the Cenomanian-Turonian of north-western Kazakhstan: *Magnoliaceae* and *Illiciaceae*. *Plant Syst Evol* 216:265–288.
- Gabarayeva NI, JR Rowley 1994 Exine development of *Nymphaea colorata* (Nymphaeaceae). *Nord J Bot* 14:671–691.
- Gandolfo MA, KC Nixon, WL Crepet 2004 Cretaceous flowers of Nymphaeaceae and implications for complex insect entrapment pollination mechanisms in early angiosperms. *Proc Natl Acad Sci USA* 101:8056–8060.
- Gaussen H 1946 Les Gymnospermes, actuelles et fossiles. *Trav Lab For Toulouse Tome II Etude Dendrol*, sect 1, vol 1, fasc 3, chap 5, 1–26.
- Góczán F, M Juhász 1984 Monosulcate pollen grains of angiosperms from Hungarian Albian sediments. I. *Acta Bot Hung* 30:289–319.
- Gomez B, V Daviero-Gomez, C Martín-Closas, M de la Fuente 2006 *Montsechia vidalii*, an early aquatic angiosperm from the Barremian of Spain. Page 49 in Abstracts, 7th European Palaeobotany and Palynology Conference, Prague, September 6–11. National Museum, Prague.
- Hamann U 1975 Neue Untersuchungen zur Embryologie und Systematik der Centrolepidaceae. *Bot Jahrb Syst* 96:154–191.
- Harley MM 1997 Palm pollen and the fossil record. PhD diss. University of East London and Royal Botanic Gardens, Kew.
- Hedlund RW, G Norris 1968 Spores and pollen grains from Fredericksburgian (Albian) strata, Marshall County, Oklahoma. *Pollen Spores* 10:129–159.
- Heimhofer U, PA Hochuli 2010 Early Cretaceous angiosperm pollen from a low-latitude succession (Araripe Basin, NE Brazil). *Rev Palaeobot Palynol* 161:105–126.
- Heimhofer U, PA Hochuli, S Burla, JML Dinis, H Weissert 2005 Timing of Early Cretaceous angiosperm diversification and possible links to major paleoenvironmental change. *Geology* 33:141–144.
- Heimhofer U, PA Hochuli, S Burla, H Weissert 2007 New records of Early Cretaceous angiosperm pollen from Portuguese coastal deposits: implications for the timing of the early angiosperm radiation. *Rev Palaeobot Palynol* 144:39–76.
- Herendeen PS, WL Crepet, KC Nixon 1993 *Chloranthus*-like stamens from the Upper Cretaceous of New Jersey. *Am J Bot* 80:865–871.
- Hermesen EJ, JR Hendricks 2008 W(h)ither fossils? studying morphological character evolution in the age of molecular sequences. *Ann Mo Bot Gard* 95:72–100.
- Herngreen GFW 1973 Palynology of Albian-Cenomanian strata of borehole 1-QS-1-MA, state of Maranhão, Brazil. *Pollen Spores* 15: 515–555.
- Hesse M 2001 Pollen characters of *Amborella trichopoda* (Amborellaceae): a reinvestigation. *Int J Plant Sci* 162:201–208.
- Hesse M, R Zetter 2007 The fossil pollen record of Araceae. *Plant Syst Evol* 263:93–115.
- Hickey LJ, JA Doyle 1977 Early Cretaceous fossil evidence for angiosperm evolution. *Bot Rev* 43:1–104.
- Hickey LJ, JA Wolfe 1975 The bases of angiosperm phylogeny: vegetative morphology. *Ann Mo Bot Gard* 62:538–589.
- Hochuli PA, S Feist-Burkhardt 2004 A boreal early cradle of Angiosperms? angiosperm-like pollen from the Middle Triassic of the Barents Sea (Norway). *J Micropalaeontol* 23:97–104.
- 2013 Angiosperm-like pollen and *Afropollis* from the Middle Triassic (Anisian) of the Germanic Basin (northern Switzerland). *Front Plant Sci* 4:344.
- Hochuli PA, U Heimhofer, H Weissert 2006 Timing of early angiosperm radiation: recalibrating the classical succession. *J Geol Soc Lond* 163:587–594.
- Hughes NF 1994 The enigma of angiosperm origins. Cambridge University Press, Cambridge.
- Hughes NF, GE Drewry, JF Laing 1979 Barremian earliest angiosperm pollen. *Palaeontology* 22:513–535.
- Hughes NF, AB McDougall 1987 Records of angiospermid pollen entry into the English Early Cretaceous succession. *Rev Palaeobot Palynol* 50:255–272.
- 1990 Barremian-Aptian angiospermid pollen records from southern England. *Rev Palaeobot Palynol* 65:145–151.
- Huynh KL 1976 L'arrangement du pollen du genre *Schisandra* (Schisandraceae) et sa signification phylogénique chez les Angiospermes. *Beitr Biol Pflanzen* 52:227–253.
- Igersheim A, PK Endress 1998 Gynoecium diversity and systematics of the paleoherbs. *Bot J Linn Soc* 127:289–370.
- Jansen RK, Z Cai, LA Raubeson, H Daniell, CW dePamphilis, J Leebens-Mack, KF Müller, et al 2007 Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns. *Proc Natl Acad Sci USA* 104: 19369–19374.
- Jérémie J 1980 Notes sur le genre *Ascarina* (Chloranthaceae) en Nouvelle-Calédonie et à Madagascar. *Adansonia Ser* 2 20:273–285.
- Ji Q, H Li, LM Bowe, Y Liu, DW Taylor 2004 Early Cretaceous *Archaeofructus eoflora* sp. nov. with bisexual flowers from Beipiao, western Liaoning, China. *Acta Geol Sin* 78:883–896.
- Juhász M, F Góczán 1985 Comparative study of Albian monosulcate angiosperm pollen grains. *Acta Biol Szeged* 31:147–172.
- Kelley DR, CS Gasser 2009 Ovule development: genetic trends and evolutionary considerations. *Sex Plant Reprod* 22:229–234.
- Kemp EM 1968 Probable angiosperm pollen from British Barremian to Albian strata. *Palaeontology* 11:421–434.
- 1970 Aptian and Albian microspores from southern England. *Palaeontogr Abt B* 131:73–143.
- Kerth M, EA Hailwood 1988 Magnetostratigraphy of the Lower Cretaceous Vectis Formation (Wealden Group) on the Isle of Wight, southern England. *J Geol Soc Lond* 145:351–360.
- Kong HZ, AM Lu, PK Endress 2002 Floral organogenesis of *Chloranthus sessilifolius*, with special emphasis on the morphological nature of the androecium of *Chloranthus* (Chloranthaceae). *Plant Syst Evol* 232:181–188.
- Krassilov V 2011 On *Montsechia*, an angiospermoid plant from the Lower Cretaceous of Las Hoyas, Spain: new data and interpretations. *Acta Palaeobot* 49:181–205.
- Kvaček J, H Eklund 2003 A report on newly recovered reproductive structures from the Cenomanian of Bohemia (Central Europe). *Int J Plant Sci* 164:1021–1039.
- Kvaček J, EM Friis 2010 *Zlatkocarpus* gen. nov., a new angiosperm reproductive structure with monocolpate-reticulate pollen from the Late Cretaceous (Cenomanian) of the Czech Republic. *Grana* 49: 115–127.
- Kvaček J, B Gomez, R Zetter 2012 The early angiosperm *Pseudoasterophyllites cretaceus* from Albian-Cenomanian of Czech Republic and France revisited. *Acta Palaeontol Pol* 57:437–443.
- Laing JF 1975 Mid-Cretaceous angiosperm pollen from southern England and northern France. *Palaeontology* 18:775–808.
- Leroy JF 1983 The origin of angiosperms: an unrecognized ancestral dicotyledon, *Hedyosmum* (Chloranthales), with a strobiloid flower is living today. *Taxon* 32:169–175.
- Llorens M 2003 Granos de polen de angiospermas de la Formación Punta del Barco (Aptiano), provincia de Santa Cruz, Argentina. *Rev Mus Argent Cienc Nat*, NS, 5:235–240.
- Maddison WP, DR Maddison 2003 MacClade 4: analysis of phylogeny and character evolution, version 4.06. Sinauer, Sunderland, MA.
- Magallón S 2010 Using fossils to break long branches in molecular

- dating: a comparison of relaxed clocks applied to the origin of angiosperms. *Syst Biol* 59:384–399.
- Magallón S, MJ Sanderson 2001 Absolute diversification rates in angiosperm clades. *Evolution* 55:1762–1780.
- Manos PS, PS Soltis, DE Soltis, SR Manchester, SH Oh, CD Bell, DL Dilcher, DE Stone 2007 Phylogeny of extant and fossil Juglandaceae inferred from the integration of molecular and morphological data sets. *Syst Biol* 56:412–430.
- Martín-Closas C 2003 The fossil record and evolution of freshwater plants: a review. *Geol Acta* 1:315–338.
- Mathews S, MJ Donoghue 1999 The root of angiosperm phylogeny inferred from duplicate phytochrome genes. *Science* 286:947–950.
- Mayo SJ, J Bogner, PC Boyce 1997 The genera of Araceae. Royal Botanic Gardens, Kew.
- Meeuse ADJ 1972 Facts and fiction in floral morphology with special reference to the Polycarpicae. *Acta Bot Neerl* 21:113–127, 235–252, 351–365.
- Mejia-Velasquez PJ, DL Dilcher, CA Jaramillo, LB Fortini, SR Manchester 2012 Palynological composition of a Lower Cretaceous South American tropical sequence: climatic implications and diversity comparisons with other latitudes. *Am J Bot* 99:1819–1827.
- Metcalfe CR, L Chalk 1950 Anatomy of the dicotyledons. Clarendon, Oxford.
- Mohr BAR, MEC Bernardes-de-Oliveira, DW Taylor 2008 *Pluricarpellatia*, a nymphaealean angiosperm from the Lower Cretaceous of northern Gondwana (Crato Formation, Brazil). *Taxon* 57: 1147–1158.
- Mohr BAR, EM Friis 2000 Early angiosperms from the Lower Cretaceous Crato Formation (Brazil), a preliminary report. *Int J Plant Sci* 161(suppl):S155–S167.
- Moore MJ, CD Bell, PS Soltis, DE Soltis 2007 Using plastid genome-scale data to resolve enigmatic relationships among basal angiosperms. *Proc Natl Acad Sci USA* 104:19363–19368.
- Moore MJ, N Hassan, MA Gitzendanner, RA Bruenn, M Croley, A Vandeventer, JW Horn, et al 2011 Phylogenetic analysis of the plastid inverted repeat for 244 species: insights into deeper-level angiosperm relationships from a long, slowly evolving sequence region. *Int J Plant Sci* 172:541–558.
- Moseley MF 1971 Morphological studies of Nymphaeaceae. VI. Development of the flower of *Nuphar*. *Phytomorphology* 21:253–283.
- Muller J 1970 Palynological evidence on early differentiation of angiosperms. *Biol Rev Camb Philos Soc* 45:417–450.
- 1981 Fossil pollen records of extant angiosperms. *Bot Rev* 47:1–142.
- Nixon KC 2008 Paleobotany, evidence, and molecular dating: an example from the Nymphaeales. *Ann Mo Bot Gard* 95:43–50.
- Nixon KC, WL Crepet, D Stevenson, EM Friis 1994 A reevaluation of seed plant phylogeny. *Ann Mo Bot Gard* 81:484–533.
- Norris G 1967 Spores and pollen from the Lower Colorado Group (Albian–?Cenomanian) of central Alberta. *Palaeontogr Abt B* 120: 72–115.
- Oh IC, T Denk, EM Friis 2003 Evolution of *Illicium* (Illiciaceae): mapping morphological characters on the molecular tree. *Plant Syst Evol* 240:175–209.
- Parkinson CL, KL Adams, JD Palmer 1999 Multigene analyses identify the three earliest lineages of extant flowering plants. *Curr Biol* 9:1485–1488.
- Pedersen KR, PR Crane, AN Drinnan, EM Friis 1991 Fruits from the mid-Cretaceous of North America with pollen grains of the *Clavatipollenites* type. *Grana* 30:577–590.
- Pedersen KR, M von Balthazar, PR Crane, EM Friis 2007 Early Cretaceous floral structures and *in situ* tricolpate-striate pollen: new early eudicots from Portugal. *Grana* 46:176–196.
- Penny JHJ 1988a Early Cretaceous acolumellate semitectate pollen from Egypt. *Palaeontology* 31:373–418.
- 1988b Early Cretaceous striate tricolpate pollen from the Borehole Mersa Matruh 1, North West Desert, Egypt. *J Micropalaeontol* 7:201–215.
- Periasamy K 1962 The ruminant endosperm: development and types of rumination. Pages 62–74 in P Maheshwari, ed. *Plant embryology: a symposium*. CSIR, New Delhi.
- Pierce RL 1961 Lower Upper Cretaceous plant microfossils from Minnesota. *Minn Geol Surv Bull* 42:1–86.
- Pirie MD, JA Doyle 2012 Dating clades with fossils and molecules: the case of Annonaceae. *Bot J Linn Soc* 169:84–116.
- Prámparo MB, M Quattrocchio, MA Gandolfo, MC Zamaloa, E Romero 2007 Historia evolutiva de las angiospermas (Cretácico-Paleógeno) en Argentina a través de los registros paleoflorísticos. *Asoc Paleontol Argent Publ Espec* 11:157–172.
- 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.
- Qiu YL, L Li, B Wang, JY Xue, TA Hendry, RQ Li, Y Liu, GT Hudson, ZD Chen 2010 Angiosperm phylogeny inferred from sequences of four mitochondrial genes. *J Syst Evol* 48:391–425.
- Regali MSP 1989 *Tucanopollis*, um gênero novo das angiospermas primitivas. *Bol Geocienc Petrobras* 3:395–402.
- Regali MSP, N Uesugui, AS Santos 1974 Palinologia dos sedimentos meso-cenozóicos do Brasil. *Bol Tec Petrobras* 17:177–191, 263–301.
- Ren Y, HL Chang, PK Endress 2010 Floral development in Anemoneae (Ranunculaceae). *Bot J Linn Soc* 162:77–100.
- Rudall PJ, RM Bateman 2010 Defining the limits of flowers: the challenge of distinguishing between the evolutionary products of simple versus compound strobili. *Philos Trans R Soc B* 365:397–409.
- Rudall PJ, DD Sokoloff, MV Remizowa, JG Conran, JI Davis, TD Macfarlane, DW Stevenson 2007 Morphology of Hydatellaceae, an anomalous aquatic family recently recognized as an early-divergent angiosperm lineage. *Am J Bot* 94:1073–1092.
- Rydin C, A Khodabandeh, PK Endress 2010 The female reproductive unit of *Ephedra* (Gnetales): comparative morphology and evolutionary perspectives. *Bot J Linn Soc* 163:387–430.
- Rydin C, KR Pedersen, EM Friis 2004 On the evolutionary history of *Ephedra*: Cretaceous fossils and extant molecules. *Proc Natl Acad Sci USA* 101:16571–16576.
- Saarela JM, HS Rai, JA Doyle, PK Endress, S Mathews, AD Marchant, BG Briggs, SW Graham 2007 Hydatellaceae identified as a new branch near the base of the angiosperm phylogenetic tree. *Nature* 446:312–315.
- Sampson FB 1993 Pollen morphology of the Amborellaceae and Hortoniaceae (Hortonioidae: Monimiaceae). *Grana* 32:154–162.
- Sampson FB, PK Endress 1984 Pollen morphology in the Trimeniaceae. *Grana* 23:129–137.
- Samylina VA 1968 Early Cretaceous angiosperms of the Soviet Union based on leaf and fruit remains. *J Linn Soc Bot* 61:207–218.
- 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.
- Schneider EL 1978 Morphological studies of the Nymphaeaceae. IX. The seed of *Barclaya longifolia* Wall. *Bot Gaz* 139:223–230.
- Schneider EL, S Carlquist 1995 Vessels in the roots of *Barclaya rotundifolia* (Nymphaeaceae). *Am J Bot* 82:1343–1349.
- Singh C 1971 Lower Cretaceous microfloras of the Peace River area, northwestern Alberta. *Res Counc Alta Bull* 28:1–310.
- Smith SA, JM Beaulieu, MJ Donoghue 2010 An uncorrelated relaxed-clock analysis suggests an earlier origin for flowering plants. *Proc Natl Acad Sci USA* 107:5897–5902.
- Smith SY, RA Stockey 2007 Pollen morphology and ultrastructure of Saururaceae. *Grana* 46:250–267.
- Sokoloff DD, MV Remizowa, TD Macfarlane, PJ Rudall 2008 Classification of the early-divergent angiosperm family Hydatella-

- ceae: one genus instead of two, four new species and sexual dimorphism in dioecious taxa. *Taxon* 57:179–200.
- Soltis DE, PS Soltis, PK Endress, MW Chase 2005 Phylogeny and evolution of angiosperms. Sinauer, Sunderland, MA.
- Soltis PS, DE Soltis, MW Chase 1999 Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. *Nature* 402:402–404.
- Springer MS, EC Teeling, O Madsen, MJ Stanhope, WW de Jong 2001 Integrated fossil and molecular data reconstruct bat echolocation. *Proc Natl Acad Sci USA* 98:6241–6246.
- Sun G, DL Dilcher, S Zheng, Z Zhou 1998 In search of the first flower: a Jurassic angiosperm, *Archaeofructus*, from northeast China. *Science* 282:1692–1695.
- Sun G, Q Ji, DL Dilcher, S Zheng, KC Nixon, X Wang 2002 Archaeofructaceae, a new basal angiosperm family. *Science* 296:899–904.
- Sun G, S Zheng, DL Dilcher, Y Wang, S Mei 2001 Early angiosperms and their associated plants from western Liaoning, China. Shanghai Scientific and Technological Education Publishing House, Shanghai.
- Swamy BGL 1953 The morphology and relationships of the Chloranthaceae. *J Arnold Arb Harv Univ* 34:375–408.
- Swofford DL 1990 PAUP: phylogenetic analysis using parsimony, version 3.0. Illinois Natural History Survey, Champaign, IL.
- Takahashi M 1995 Development of structure-less pollen wall in *Ceratophyllum demersum* L. (Ceratophyllaceae). *J Plant Res* 108:205–208.
- Takhtajan AL, ed 1988 Sravintel'naya anatomiya semyan. 2. Dvudol'nyye. Magnoliidae, Ranunculidae. Nauka, Leningrad.
- Taylor DW 2008 Phylogenetic analysis of Cabombaceae and Nymphaeaceae based on vegetative and leaf architectural characters. *Taxon* 57:1082–1095.
- Taylor DW, GJ Brenner, SH Basha 2008 *Scutifolium jordanicum* gen. et sp. nov. (Cabombaceae), an aquatic fossil plant from the Lower Cretaceous of Jordan, and the relationships of related leaf fossils to living genera. *Am J Bot* 95:340–352.
- Taylor DW, LJ Hickey 1992 Phylogenetic evidence for the herbaceous origin of angiosperms. *Plant Syst Evol* 180:137–156.
- Thanikaimoni G 1985 Palynology and phylogeny. Pages 11–14 in HWE van Bruggen. Monograph of the genus *Aponogeton* (Aponogetonaceae). *Bibl Bot* 137:1–76.
- Tobe H, T Jaffré, PH Raven 2000 Embryology of *Amborella* (Amborellaceae): descriptions and polarity of character states. *J Plant Res* 113:271–280.
- Tobe H, TF Stuessy, PH Raven, K Oginuma 1993 Embryology and karyomorphology of Lactoridaceae. *Am J Bot* 80:933–946.
- Todzia CA 1988 Chloranthaceae: *Hedyosmum*. *Flora Neotrop Monogr* 48:1–139.
- Tschudy RH 1970 Two new fossil pollen genera (Late Cretaceous and Paleocene) with possible affinity to the Illiciaceae. *U S Geol Surv Prof Pap* 643-F:F1–F13.
- Upchurch GR 1984 Cuticular anatomy of angiosperm leaves from the Lower Cretaceous Potomac Group. I. Zone I leaves. *Am J Bot* 71:192–202.
- Upchurch GR, PR Crane, AN Drinnan 1994 The megaflores from the Quantico locality (upper Albian), Lower Cretaceous Potomac Group of Virginia. *Va Mus Nat Hist Mem* 4:1–57.
- Upchurch GR, DL Dilcher 1990 Cenomanian angiosperm leaf megafossils, Dakota Formation, Rose Creek locality, Jefferson County, southeastern Nebraska. *U S Geol Surv Bull* 1915:1–55.
- Vakhrameev VA 1952 Stratigrafiya i iskopaemaya flora melovykh otlozheniy Zapadnogo Kazakhstana. Regional'naya Stratigrafiya SSSR 1. Akademiya Nauk SSSR, Moscow.
- von Balthazar M, KR Pedersen, PR Crane, EM Friis 2008 *Carpotesta lacunata* gen. et sp. nov., a new basal angiosperm flower from the Early Cretaceous (Early to Middle Albian) of eastern North America. *Int J Plant Sci* 169:890–898.
- von Balthazar M, KR Pedersen, EM Friis 2005 *Teixeiraea lusitanica*, a new fossil flower from the Early Cretaceous of Portugal with affinities to Ranunculales. *Plant Syst Evol* 255:55–75.
- Walker JW, AG Walker 1984 Ultrastructure of Lower Cretaceous angiosperm pollen and the origin and early evolution of flowering plants. *Ann Mo Bot Gard* 71:464–521.
- Wang H, DL Dilcher 2006 Aquatic angiosperms from the Dakota Formation (Albian, Lower Cretaceous), Hoisington III locality, Kansas, USA. *Int J Plant Sci* 167:385–401.
- Wang X, XT Zheng 2012 Reconsideration on two characters of early angiosperm *Archaeofructus*. *Palaeoworld* 21:193–201.
- Ward JV 1986 Early Cretaceous angiosperm pollen from the Cheyenne and Kiowa formations (Albian) of Kansas, U.S.A. *Palaeontogr Abt B* 202:1–81.
- Ward JV, JA Doyle 1994 Ultrastructure and relationships of mid-Cretaceous polyforates and triporates from Northern Gondwana. Pages 161–172 in MH Kurmann, JA Doyle, eds. Ultrastructure of fossil spores and pollen. Royal Botanic Gardens, Kew.
- Wilde V, Z Kvaček, J Bogner 2005 Fossil leaves of the Araceae from the European Eocene and notes on other aroid fossils. *Int J Plant Sci* 166:157–183.
- Wolf M 1991 Blütenphyllotaxis von Nymphaeaceae: ist das Androecium von *Nymphaea*, *Nuphar* etc. spiralig? Symposium Morphology, Anatomy, and Systematics, University of Göttingen, Abstracts.
- Wolfe JA, JA Doyle, VM Page 1975 The bases of angiosperm phylogeny: paleobotany. *Ann Mo Bot Gard* 62:801–824.
- Yamada T, R Imaichi, N Prakash, M Kato 2003 Developmental morphology of ovules and seeds of Austrobaileyales. *Aust J Bot* 51:555–564.
- Yamada T, H Nishida, M Umebayashi, K Uemura, M Kato 2008 Oldest record of Trimeniaceae from the Early Cretaceous of northern Japan. *BMC Evol Biol* 8:135.
- Yamada T, H Tobe, R Imaichi, M Kato 2001 Developmental morphology of the ovules of *Amborella trichopoda* (Amborellaceae) and *Chloranthus serratus* (Chloranthaceae). *Bot J Linn Soc* 137:277–290.
- Yamazaki T 1992 Floral morphology of *Hedyosmum orientale* Merr. et Chun (Chloranthaceae) and phylogenetic significance of its perianth. *J Jpn Bot* 67:257–269.
- Yoo MJ, CD Bell, PS Soltis, DE Soltis 2005 Divergence times and historical biogeography of Nymphaeales. *Syst Bot* 30:693–704.
- Zavada MS 2007 The identification of fossil angiosperm pollen and its bearing on the time and place of the origin of angiosperms. *Plant Syst Evol* 263:117–134.
- Zhang LB, S Renner 2003 The deepest splits in Chloranthaceae as resolved by chloroplast sequences. *Int J Plant Sci* 164(suppl):S383–S392.
- Zhang N, L Zeng, H Shan, H Ma 2012 Highly conserved low-copy nuclear genes as effective markers for phylogenetic analyses in angiosperms. *New Phytol* 195:923–937.
- Zhang Q, A Antonelli, TS Feild, HZ Kong 2011 Revisiting taxonomy, morphological evolution, and fossil calibration strategies in Chloranthaceae. *J Syst Evol* 49:315–329.
- Ziegler AM, G Eshel, PM Rees, TA Rothfus, DB Rowley, D Sundelin 2003 Tracing the tropics across land and sea: Permian to present. *Lethaia* 36:227–254.